TISSUE ENGINEERING: Part A
Volume 14, Number 5, 2008

DOI: 10.1089/tea.2008.1504

MEETING ABSTRACTS

June 22–26, 2008
Porto Congress Center–Alfândega
PORTUGAL

Conference Chair:
Rui L. Reis

Organized by:
3B's Research Group, University of Minho, Braga, Portugal

Editorial

Welcome to the TERMIS-EU 2008 Porto Meeting

Rui L. Reis [1–3]

IT IS A REAL PLEASURE TO WELCOME you to Porto and to the special issue of *Tissue Engineering: Part A* that contains all the abstracts submitted and accepted for presentation at the 2008 Annual Tissue Engineering and Regenerative Medicine International Society–European Chapter (TERMIS-EU) Meeting.

As you might be aware, it was a tradition for *Tissue Engineering* to publish the abstracts of TESI and then, after merging with ETES, of the TERMIS World Congresses (Shanghai, Pittsburgh) and North American meetings. Last year in London we started doing the same with the TERMIS-EU meetings. This year, once again, Mary Ann Liebert and the editors of *Tissue Engineering* were kind enough to decide to publish the abstracts of the Porto meeting.

The present issue of *Tissue Engineering* compiles the entire range of nearly 750 abstracts accepted for presentation for the 2008 TERMIS-EU Meeting. These abstracts were submitted by researchers from all over the world and from different research groups and core research fields. This number is considered by the organizers as a great achievement, since we have set a goal to make this the largest ever TERMIS-EU meeting. We knew, however, that this was quite a difficult challenge. In fact, not only was the London meeting, as expected, a great success in attracting abstracts, but we knew beforehand that 2008 was the year of the World Biomaterials Congress, to be held in Europe just three weeks before our meeting.

We also set an objective of making sure that we would be able to organize a very international meeting, with many more participants from North America, Asia, and third world countries than was typical in ETES and in TERMIS-EU. We have tried to work for that, using our network of contacts. We are proud to conclude that it was, in fact, possible to achieve that goal. For instance, the United States is the fourth country, after Portugal, the United Kingdom, and Germany, with more abstracts accepted (9% of the total). Korea is the eighth country represented (with 5% of the total), and China and Japan, as well as Turkey, were also quite active on submissions. This Asian representation is particularly relevant as we are approaching the TERMIS World Congress in Dajaeon, South Korea, next year. Receiving abstracts from all over the world proves that it was feasible to reach the goal of having a multidisciplinary conference, enriched with participants from different cultures.

We also tried to organize the meeting agenda so that the scientific community could build their own program of scientific excellence. We have opened a call for submission of proposals for the organization of 90-minute symposia, typically with one or two organizers, a distinguished keynote speaker (30 minutes), and four general presentations (of 15 minutes each, either invited or selected from the abstracts submitted to that particular symposia). In some cases, the symposia have more than one session and/or are composed of three keynote invited talks of 30 minutes each. These thematic symposia, organized by the world's leading scientists and featuring recognized keynote speakers, constitute 41 of the meeting's sessions. All of the remaining parts of the program are made up of general oral sessions, organized around state-of-the-art scientific topics that are based on the submitted abstracts, as well as a very well attended poster session of around 400 attendees.

In addition, the program of the TERMIS-EU 2008 Meeting includes eight plenary lectures given by some of the very best scientists working in different cutting-edge areas that are relevant to TERMIS. This is one of the highlights of the meeting.

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TERMIS has as its main goal to try to get together specialized international communities in the field of tissue engineering and regenerative medicine in a way that enables it to promote education and research in this area through regular meetings, publications, and other forms of communication/announcement. TERMIS-EU is also an international forum for promoting an informed discussion toward the challenges and benefits of the therapeutic application of the technologies developed by tissue engineering and regenerative medicine. Therefore, the Porto program assures a scientific and industrial impact and encourages the participation of a high number of scientists and researchers from both academia and industry. In order to enhance education and outreach aspects, a special session has been organized within the conference program, being hosted by the Pittsburgh Tissue Engineering Initiative (PTEI). This is specially aimed for high school teachers in order to make them aware of significant aspects of tissue engineering and regenerative medicine.

The conference will be held in the very hospitable World Heritage city of Porto, Portugal, from June 22 to 26, 2008. Besides a cutting-edge scientific program in the field of tissue engineering and regenerative medicine, we added a wonderful social program that will be associated with the typical St. John (S. João), one of the major Porto and Portuguese festivities. The venue, Alfândega (Old Customs House) Congress Centre is located in the very heart of the Porto historical centre on the north bank of the River Douro, overlooking the Port Wine cellars. Porto is a city of wonderful people, and a dream for food and wine lovers. So, make sure you enjoy it as much as possible with your friends and colleagues. I have been enjoying it since I was born (not the wine in the first few years!) 41 years ago, and I could never leave the city to live anywhere else!

Finally, the meeting will also be associated with the great opening of the headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine (www.3bs.uminho.pt). This state-of-the-art facility with 3600 m^2 will also be the new home for the 3B's Research Group of the University of Minho and our spin-off company Stemmatters. It is a unique facility, fully planned from scratch, for research in the field of TERM. This building and its well equipped laboratories will be available for visits during the meeting. These facilities will allow research ranging from synthesis, processing, and modification of new materials to small animal testing, and of course, all biological and stem cell work can be carried out in rather good conditions.

The new institute results from the European Network of Excellence (NoE) on Tissue Engineering—EXPERTISSUES (http://www.expertissues.org). The NoE was funded, in a very competitive selection process, by the Sixth Framework program (FP6) of the European Commission (EC), being the only European network of excellence in the field of tissue engineering. The institute, formed as a European Economic Interest Group (EEIG), will be sustainable in time, with headquarters in Taipas, Minho, Portugal, and branches in more than 20 different locations in 13 countries all over Europe. This is, however, an open process, and other groups of excellence may join this European effort. Rui L. Reis, the coordinator of the NoE, will be the initial CEO of the institute, which has as one of its main aims to collaborate even more with groups of excellence in Asia, North America, the Pacific, and elsewhere.

However, the TERMIS-EU 2008 meeting and its program are mainly made up of the conference delegates who will present their new results and scientific approaches to different problems. All the abstracts of these contributions are published in this issue of *Tissue Engineering,* the official journal of TERMIS.

A word of acknowledgement is owed to TERMIS and its officers for allowing us to organize the meeting and supporting us whenever necessary. We would also like to thank all the institutions, foundations, and companies that have sponsored us and made the meeting possible. You may visit many of them in the exhibition area.

But I am especially grateful to all of my postdoctoral fellows, Ph.D. students, management and informatics team members, and staff colleagues who work daily on the 3B's Research Group—Biomaterials, Biodegradables and Biomimetics, which I have the pleasure of directing. We now include more than 110 people, from around 20 different nationalities, of which only five are staff members. All of the others are quite young, bright, and ambitious fellows and students. The organization of this TERMIS-EU meeting was mainly the result of the hard work, devotion, commitment, ambitions, and aspirations of many of these people. They have put a great number of hours into the enterprise of organizing the conference and realized that this was an important event for all of us. I cannot refer to all the relevant names herein, but when you find one of the members of the 3B's–U. Minho during the meeting, please just speak with her/him and you will see how fortunate I am for being able to advise and direct such a wonderful group of young and bright researchers!

Many people made rather important contributions to the organization, but in this case I really have to give a special word of appreciation to two individuals. They are Ariana Santos, my event organizer within the 3B's, and Catarina Alves, the postdoctoral fellow who has been leading, along with Ariana and myself, all this organization. Without them we would never have been able to organize this meeting and have this special issue out today. They have made this their main daily activity for months. Thank you very much, Ariana and Catarina.

From both a scientific and a social/cultural perspective, we really hope that this will be a meeting to remember. Enjoy the conference, Porto, and if possible, Minho and other areas of Northern Portugal!

Rui L. Reis and the Organizing Team

Oral Presentations

(OP 1) *In Vivo* Bone formation of 3-D Prevascularized Tissues Consisting of Primary Osteoblasts and Outgrowth endothelial Cells

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Introduction: Tissue engineering of complex and vascularized tissues retaining the functional phenotype of involved cell types is still a problematic issue. We have previously established 3-D co-culture systems consisting of primary osteoblasts and outgrowth endothelial cells (OEC) forming prevascular structures and retaining the phenotype of both endothelial and osteoblastic cells [1]. In this study we assessed the *in vivo* bone formation to gain further insight into the functional stability of primary osteoblasts in these constructs.

Materials and Methods: 3-D constructs were generated in a rotating cell culture system as previously described (1). After one week 3-D constructs were harvested and subcutaneously implanted in nude mice, divided into 3 groups: group 1 (OEC + pOB), group 2 (pOB alone) and group 3 (sham group). The constructs were explanted at day 14 after implantation for histological evaluation by Movat's Pentachrom, Alcian-PAS and Goldner-staining.

Results: Specific histochemical analysis (Movat's Pentachrom, Alcian-PAS as well as Goldner-staining) revealed osteoid and bone tissue in an early mineralisation stage along the implantation channel. In accordance with the expression of osteoblastic markers (alkaline phosphatase, osteocalcin) shown *in vitro*, these results indicate that primary osteoblasts retain their functional phenotype.

Conclusion: The present study showed the ability of the 3-D constructs to produce a bone-like matrix in the subcutaneous implantation model. The possibility that prevascular structures formed by OEC might contribute to the vascularization of such constructs is currently under investigation.

References:

[1]Fuchs *et al.* 2007. Tissue Engineering

(OP 2) Role of Paracrine Factors Released by Mesenchymal Progenitors from the Umbilical Cord in Neurons/Glial Cell Viability, Proliferation and Differentiation

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Umbilical Cord Stem Cells are able to integrate within the CNS upon injury and to improve the condition of animals suffering from neurodegenerative diseases. However the mechanisms by which such phenomena are mediated are unknown. Therefore the main objective of the present work was to understand how a population of umbilical cord mesenchymal progenitor cells isolated from the Wharton Jelly (HUCPVCs), regulates viability, proliferation and different ion of post-natal hippocampal neurons and cortical glial cells. Hippocampal neurons and glial cells were exposed to HUCPVCs conditioned media (CM) (obtained 24, 48, 72 and 96 after 3 days of culture of HUCPVCs) for 1 week. Cell viability (MTS test) experiments revealed that HUCPVCs CM obtained for all time points did not cause any deleterious effects on both cell populations when compared to the control condition. Immunocytochemistry and total cell counts revealed that HUCPVCs CM triggered an upregulation of the proliferation on astrocytes (GFAP), oligodendrocytes (O4) and the differentiation of hippocampal neurons (MAP-2). For the latter this was noticed even in the absence of neuronal supplements B27 and FGF-2. In direct contact co-culture systems the total numbers of hippocampal neurons, astrocytes and oligodendrocytes increased, in spite of the fact that the numbers of HUCPVCs were decreasing in this new environment. We believe that the phenomena here in described are related to the release of neuroregulatory molecules by HUCPVCs. Current work is focused on their identification as well as the consequent cross-talking mechanisms related to the phenomena herein presented.

(OP 3) A Bioresorbable Device in Combination with Bone Morphogenetic Protein-2 for Anterior Lumbar Interbody Fusion in Porcine Model

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Introduction: Spine fusion, a procedure commonly performed for patients with debilitating back pain, often involves implantation of metallic cages and autograft bone into intervertebral disc spaces. Several problems are associated with the use of both metallic cages and autograft bone including donor site morbidity and difficulties with radiographic assessment. Allograft bone proposed as an alternative also carries an inherent risk of infection and rejection. Bioabsorbable cages (biocage) offer distinct advantages including biological and mechanical compatibility with host bone. This study aimed to evaluate the efficacy of a novel mPCL-TCP biocage when used in combination with MSCs and rhBMP-2 for spine fusion.

Material and Methods: Twelve (12) pigs underwent anterior lumbar interbody fusion (ALIF) at L3/4 and L5/6 in four groups viz: Biocage; Biocage + MSCs; Biocage + rhBMP-2 (0.6 mg/level); and Autograft bone, implanted for 3 months.

Results: After 3 months, all animals receiving rhBMP-2 showed radiographic evidence of fusion. Autograft bone showed fusion only in 50% cases. Biocage and biocage + MSCs did not show any fusion. Micro-CT and histology confirmed these findings.

Discussion and Conclusion: Incorporating low dose rhBMP-2 onto mPCL-TCP promoted early fusion compared to autograft in this model. Absence of fusion in standalone biocage was not unexpected due to lack of an osteoinductive component. Failure of MSCs to stimulate early fusion is in variance with previously observed ectopic ossification in rats and could indicate a less than optimal environment for these cells. This is the first study reporting mPCL-TCP application in a multi-level, weight bearing, ALIF model.

(OP 4) A Biosynthetic Bandage for Corneal Wound Repair

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Ocular trauma and disorders that lead to corneal blindness account for over 2 million new cases of monocular blindness every year. The use of amniotic membrane to treat such wounds is a popular treatment but its biological nature presents many issues for use in the clinic. To address this, we proposed to bioengineer a novel synthetic material that would serve as a biomimetic corneal bandage. The base component of this bandage is a bioinert PLGA–PEG–PLGA triblock copolymer which gels *in situ* at body temperature. We investigated the thermoreversible gelling properties of this polymer at various compositions and blends. In addition, we assessed the biocompatible nature of this hydrogel both *in vitro* and *in vivo*. We have also shown that the hydrogel can act as a controlled release depot for both drug and protein molecules. In addition, controlled release microparticles were embedded within the hydrogel, illustrating the potential for using this system for dual release of wound healing promoters. This bench-to-clinic research program has developed a hydrogel drug delivery system that could provide clinicians with a user friendly, off-the-shelf treatment for the promotion of corneal regeneration. Such a treatment holds great potential to enhance the quality of life for patients and ultimately save sight.

(OP 5) A Modified Erythropoietin Affects Secondary Injury and Improves Neurological Recovery After Spinal Cord Injury

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Introduction: Spinal cord injury (SCI) provokes a cascade of proinflammatory cytokines and induces apoptosis and necrosis of neurons, oligodendrocytes and endothelial cells. Erythropoietin (EPO) is known for its neuroprotective and anti-inflammatory functions. Because of the influence on erythropoiesis, EPO cannot be administered in high dosage for this indication. By modifying the EPO molecule we could prolong the half-life and abolish the haematopoietic effect. This could reduce potential side effects, but also affects activity. Therefore we asked the question whether the modified EPO can be successfully administered as a neuroprotective agent after SCI in rats. METHODS In male Sprague Dawley rats a laminectomy was carried out at TH11. A contusion trauma was evoked using the IH®400 Impactor (150 kdyn). All animals were randomly divided into 5 groups: I: rhEPO (1000 units/kg); II: Placebo (NaCl); III: rhEPO-FP (1000 units/kg); IV: rhEPO-cFP (1000 units/kg), V: Methylprednisolon (30 mg/kg). Substances were administered intravenously 1 hour after contusion injury. Locomotion testing was performed weekly on all animals using the BBB locomotor rating scale and the CatWalk assisted Gait Analysis. RESULTS During the observation period a distinct improvement of the BBB-scoring of the animals treated with carbamylated Fusion-Protein-EPO was observed. After 4 weeks there is a significant difference compared to the placebo animals. An improved outcome could also be achieved by the application of the Fusion-Protein-EPO, whereas the treatment with methylprednisolon didn't show any beneficial effects.

Conclusion: Carbamylated Fusion-EPO would be a good candidate for exploiting EPO's neuroprotective and anti-inflammatory properties while avoiding the haematopoietic side-effects.

(OP 6) A Novel Animal Model for Studying the Bone Forming Capacity of Stem Cell Loaded Biomaterials

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In a previously study we showed that MSCs loaded onto biomaterial could be used to repair large bone defects. The purpose of this study was to develop a new *in vivo* model that enabled efficient screening of different material/cell combinations at the same time. An implant pouch model was developed for use in sheep.

Cylindrical polymethylmethacrylate spacers (25:×15 mm) were implanted into 18 paraspinal subcutaneous sites in each of 7 animals for 6 weeks. The spacers cause a reactive encapsulating membrane that formed a pouch for subsequent implantation. Autologous-derived bone marrow stem cells (MSCs) were expanded and loaded onto each of four different particulate materials (coral Porites, coral Acropora, β-TCP and bone graft) prior to implantation. At surgery each spacer was removed and the pouch was filled with one of the four biomaterials, with or without MSCs. After 8 weeks, the entire pouch was resected and analyse, with radiography and undecalcified histology for evidence of material resorption and bone formation.

Results: Compared with controls, large amounts of resorption of the different implant materials were observed to different extents. Addition of MSCs to the biomaterials was associated with varying degrees of new bone formation.

Conclusions: Despite the ectopic nature of the implant site, this model was effective for studying bone formation in conjunction

with stem cell therapy. The pouch model enables relatively inexpensive sampling different materials and cell combinations. It remains, however, to validate the model by implanting the more promising materials into the relevant large bone defect model we previously described.

(OP 7) A Novel Cell Source for Urologic Tissue Reconstruction

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In this study we explored the possibility of isolating cells from urine as an alternative autologous cell source for tissue engineering. We investigated whether the cells contained in urine possess normal cellular characteristics for their use in urologic reconstructive procedures. To this end, human urine samples were collected from 9 healthy male donors (23 to 45 years of age). Cells were isolated, expanded and characterized with progenitor and differentiated cell specific surface markers. The culture expanded cells were labeled with Lac Z, seeded on collagen based matrices and implanted subcutaneously in athymic mice. Primary cultures of urine cells stained positively for C-kit, SSEA-4, CD105+, CD73+, CD91+, CD133+, and CD44+, but not for CD31−, CD34−, and CD45−, indicating that the cells are progenitor cells. Two different types of progenitor cells were identified; urothelial and smooth muscle. The urothelial progenitor cells expressed cytokeratins 7, 13, 17, 19 and uroplakin Ia, and the muscle progenitor cells expressed vimentin, α-smooth muscle actin, desmin and myosin. The surviving graft cells were verified by human X/Y chromosome detection assay (FISH). The implanted cells formed multilayered tissue structures on collagen based matrices *in vivo*, which were confirmed phenotypically. This study demonstrates that urothelial and smooth muscle progenitor cells can be isolated, grown and expanded from urine. The cells maintained their phenotypic and functional cellular characteristics *in vitro* and *in vivo*. The use of urine derived cells might provide an excellent additional/alternative source for cell harvest for urinary tract tissue reconstruction.

(OP 8) A Novel *Ex vivo* Platform to Develop Fetoscopic Closure Techniques for Punctured Fetal Membranes

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Endoscopic fetal surgery may be a live-saving option to correct anomalies. However, operative interventions into the uterine cavity carry a high risk for postoperative preterm premature rupture of the fetal membranes (PPROM). Indeed, iatrogenic PPROM (iPPROM) presents a bottleneck for fetoscopic surgery. Prophylactic plugging of fetoscopic entry sites with tissue sealants, optimization of the needle penetration procedure, needle geometry and puncture location in the fetal membranes potentially could prevent iPPROM. To explore such routes, *ex vivo* model systems are necessary. Here we introduce a new dynamic test device that permits to develop and study fetoscopic closure techniques on experimentally wounded fetal membranes *ex vivo*. We created a mechanical inflation device that permits to deform fetal membranes under fluid pressure till rupture, monitored by a CCD camera. For the characterization of the mechanical properties of the fetal membrane, the inverse finite element procedure and the optimization procedure will be used. Mechanical tests and treatment tests, e.g. plugging of fetal membrane wounds with glues, are performed in the aqueous milieu and the physiological loading conditions of fetal membranes membranes inside the uterus. The validation of this new experimental platform is ongoing: First rounds of biomechanical testing have been performed in order to understand the fracture behavior of healthy or fetoscopically punctured fetal membranes.

(OP 9) A Porous Collagen Scaffold Wrapped with PLGA Mesh for Cartilage Tissue Engineering

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Collagen-based scaffolds have been widely used for tissue engineering because of their good biocompatibility and biodegradability. Their porous structure facilitates cell delivery, gas and nutrient exchange, and waste removal. On the other hand, the highly porous structure also results in leakage of cells. High efficiency of cell seeding is important for tissue engineering, because an available cell source from patients is limited. Collagen porous scaffolds with high cell seeding efficiency are desirable. In the present study, a hybrid porous scaffold was developed by covering the surfaces of a collagen sponge with a poly(L-lactic acid-co-glycolic acid) (PLGA, monomer ratio of L-lactic acid to glycolic acid, 10:90) mesh to protect cell leakage during cell seeding.

At first, a cylinder mold was prepared with VICRYL mesh. And then collagen aqueous solution was introduced into the cylinder mold, frozen at −80°C and freeze-dried. After that, the collagen sponge was chemically crosslinked at 37°C for 4 h with saturated glutaraldehyde vapor and immersed in glycine solution to inactivate residual aldehyde groups. After being washed with water and freeze-dried, the leak-proof hybrid scaffold was obtained.

The hybrid scaffolds were used for culture of human mesenchymal stem cell and chondrocyte. The cell seeding efficiency was higher than 85%. SEM observation demonstrated that the cells were distributed homogeneously in the scaffolds. Besides the cell leak-proof effect, the hybrid scaffolds showed higher mechanical strength than the non-wrapped collagen sponge. The hybrid scaffold will be useful for tissue engineering.

(OP 10) A Possible Role of Fibrin-E from Degradation of Fibrin-Based Scaffolds for Recruitment of Endothelial Progenitor Cells?

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Exploitation of extracellular matrix molecules and mechanisms is an obvious way to seek improved functionality of biomaterials for scaffolds. We previously identified fibrinogen based matrices as pro-angiogenic in an *in-vitro* 3D gel migration assay, compared to collagen. Specifically, endothelial cells interact with fibrinogen or fibrin-E degradation fragments via RGD motifs and alpha-v integrins. Therefore fibrinogen might be used to form a rapidly vascularised scaffold material. *In vivo* evaluation of fibrinogen-based scaffolds shows a rapid cellularisation and neovascularisation by a process of vasculogenesis within the scaffold. Collagen-based scaffold is populated more slowly and appears vascularised by angiogenesis.

Because endothelial progenitor cells have been found to accelerate dermal wound healing, fibrinogen or fibrin-E covalently coated surfaces were used as substrata for attachment and expansion of EPC clones from human umbilical cord blood, in comparison to gelatin. Initial results demonstrated effective attachment of EPC to fibrin-based surfaces and subsequently showed a more rapid rate of expansion than cultures on gelatin. By contrast, EPC fail to attach to native collagen. A phenotypic analysis of EPC isolated from each surface showed that cells on fibrinogen-based surfaces had a stronger expression of MMP9, which is required for migratory activity.

This is the first demonstration of specific effects of fibrinogen molecules on EPC recruitment. This data suggests a mechanism which could be involved in the rapid vasculogenesis within prototype fibrinogen-based scaffolds, supplemental to or orchestrating endothelial cell stimulation. Understanding and controlling EPC function in tissue regeneration could guide scaffold design for wound healing and tissue engineering.

(OP 11) A Tissue Engineered Osteochondral Composite: An *In Vitro* Study

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The purpose of this work is to create an *in vitro* model of tissue engineered osteochondral composite by combining a cylinder of calcium phosphate and a neocartilaginous tissue produced by isolated swine articular chondrocytes embedded in fibrin glue.

Swine articular chondrocytes were enzimatically isolated and embedded in a fibrin glue gel. Immediately before gel polymerization, fibrin glue was placed in contact with the calcium phosphate scaffold. Osteochondral composites were left in standard culture conditions and retrieved after 1 and 5 weeks. At the end of each experimental time, samples were macroscopically analyzed, processed for histological (ematoxylin-eosin, safranin-o), immunohistochemical (collagen type I, type II) and biochemical evaluation (DNA, GAGs).

Data showed a macroscopic integrity of the osteochondral samples. Histology showed cartilage like tissue maturing within the fibrin glue scaffold. GAGs seemed to penetrate microscopically into the scaffold, determining an interface area of microscopic integration between the porous of the scaffold and the cellular fibrin glue. Immunohistochemical analysis demonstrated the presence of type II collagen fibers. Biochemical assays confirmed the presence of vital cells, and production of GAG matrix.

The results of this study demostrate that isolated chondrocytes, seeded onto fibrin glue, produce cartilage-like matrix that integrates with a cylinder of calcium phosphate. We noticed microscopic penetration of the newly synthesized GAGs inside the structure of the calcium phosphate, confirming the importance of an *in vitro* maturation of the engineered tissue.

This tissue engineered osteochondral composite could represent a valuable model for further *in vivo* studies on the repair of osteochondral lesions.

(OP 12) A Tissue-Engineered Tendon Scaffold

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A tissue-engineered tendon has the potential to significantly improve the treatment of tendon and ligament injuries. The goal of this study was to employ a simple process that combines decellularization and chemical oxidation to decellularize and modify the dense architecture of the tendon allograft to produce a scaffold capable of efficient cell seeding *in vitro* and more rapid remodeling *in vivo*. Flexor digitorum profundus (FDP) tendons were harvested from chickens. Tendons were trypsinized, decellularized and oxidized using an aqueous solution containing Triton X-100 and peracetic acid, then extensively rinsed. Cellularity, DNA content, cell compatibility, mechanical properties, cell infiltration *in vitro* and *in vivo* were determined using samples pre- and post-treatment. Post-treated samples had less cellularity by H&E, less DNA content, and increased porosity by SEM. Cells were compatible with the post-treated samples as measured using the MTS and neutral red assays. The elastic modulus and maximum stress at break were decreased in post-treated scaffolds, but statistical significance was not reached. *In vitro*, cell penetration into the interfascicular regions of the post-treated scaffold was observed. Host cell infiltration was also observed as early as 3 days after subcutaneous implantation. An increase in host cell infiltration was observed at 21 days. No evidence of a gross inflammatory reaction or capsule formation surrounding the post-treated scaffold was observed at the time of explantation.

(OP 13) Adipose Derived Stem Cells can Enhance Bone Regeneration in a Rabbit Skull Critical Defect

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Repair of substantial cranial defects in adults and children may be compromised due to limitations in donor bone stocks for autologous grafts. We evaluated the capability of autologous adipose derived mesenchymal stem cells (ADSC) in combination with Poly-Lactic Acid (PLA) scaffolds to regenerate bone in a critical-sized skull defect. Thirty adult New Zealand White rabbits were divided into 6 groups of 5 animals each consisting of: 1) PLA alone (control); 2) Fibronectin-coated PLA; 3) PLA with ADSC; 4) Fibronectin-coated PLA with ADSC; 5) PLA with osteogenically induced ADSC (osADSC), and 6) Fibronectin coated PLA with osADSC. All the animals were sacrificed after 6 weeks. X-Ray, histology and histomorphometric analysis were performed in order to evaluate the new bone formation inside the PLA scaffold. Radiographically and histomorphopmetrically, the groups in which the PLA was not fibronectin coated showed no bone formation in contrast to the fibronectin coated groups; the group treated with osteo-induced ADSC and fibronectin showed significantly more bone formation than the group treated with undifferentiated ADSC and the group treated without cells. These data indicate that the surface treatment with fibronectin permits cell attachment and survival within the scaffold, and that autologous, osteo-induced adipose-derived stem cells can regenerate bone if seeded into a surface-treated PLA scaffold.

(OP 14) Alginate Film as a Tissue Adhesion Barrier

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Adhesion formation is a well-known complication of abdominal surgery, which not only renders future operations more difficult but also is the most common cause of small bowel obstruction, female infertility, and chronic debilitating pain. In order to reduce post-surgical adhesion formation, physical barrier to isolate the traumatized tissue from surrounding organ was commonly used. It should have adhesiveness in injured site, biodegradibility, mechanical strength and flexibility for handling. Various natural and synthetic polymer sheets or membranes have been developed as non-absorbable or absorbable physical barriers, however, their loose contact with applied tissue needed for sutural fixation. It has been also reported that a variety of polymer solutions or gels such as hyaluronic acid, dextran, polyvinylpyrolidone, carboxymethyl cellulose and polyethylene glycol were used to prevent tissue adhesion. However, their easy washing out before the healing of injured tissues are still remained as a critical limtation.In this study, we prepared an alginate film to estimate its potential use as an abdominal adhesion barrier. We expected that the non-crosslinked alginate film can have good anti-tissue adhesion property, owing to its good mucoadhesiveness with injury site (inhibition of film migration without suturing) and low tissue affinity of slowly solubilized alginate in the body (prevention of tissue adhesion). The *in vivo* animal study using a rat model was carried to evaluate the anti-tissue adhesion effect of the prepared alginate film. It was also compared with those of crosslinked alginate film, alginate solution, and commercialized anti-adhesion membrane (InterceedTM).

(OP 15) Amniotic Fluid-Derived Stem Cells for Regenerative Medicine Therapies

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Cell sourcing for tissue engineering remains a major challenge for clinical translation of regenerative medicine therapies. Stem cells represent an alternative source of cells in instances where differentiated cells can not be expanded *in vitro*. Human amniotic fluid has been used in prenatal diagnosis for more than 70 years. It has been demonstrated to be a safe, reliable, and simple screening tool for a variety of developmental and genetic diseases. A subset of cells in amniotic fluid has been shown to have stem cell properties and express stem cell markers. Amniotic fluid stem (AFS) cells are capable of maintaining prolonged undifferentiated proliferation as well as of differentiating into multiple tissue types that encompass the three germ layers (mesenchymal, ectodermal and endodermal). These cells show a high self renewal capacity (>300 population doublings) and clonal growth capability, but unlike embryonic stem cells, they do not require a feeder layer and do not form teratomas when injected *in vivo*. These findings indicate that amniotic fluid may have increased utility than as a diagnostic tool and may become a preferred therapeutic cell source for a multitude of congenital and adult disorders.

(OP 16) An Oscillatory, Perfused Bioreactor for Cell and Tissue Culture

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Bioreactors can: (i) maintain viability of harvested cells prior to transplant, (ii) seed cells onto porous 3D scaffolds, and (iii) enhance tissue regeneration, by providing physiologic culture conditions and convective-diffusive mass transport. A single device complying with these specifications is an oscillating perfused bioreactor wherein 1 to 12 gas-permeable chambers are stacked on an incubator–compatible base that provides bi-directional flow of culture medium at a controllable rate (~0.40 to 5.0 mL/min; ~0.20 to 2.7 mm/s). This bioreactor was validated for cells cultured in suspension and on 3D scaffolds (hyaluronic acid-based non-wovens or sponges; collagen sponges). Suspension culture was demonstrated by inoculating primary heart cells into chambers. Bioreactors yielded higher ($48 \pm 5\%$) cell viabilities than orbitally mixed or spinner flask controls on culture day 8. Perfusion seeding and culture was demonstrated by inoculating chondrocytes into chambers containing porous scaffolds. Bioreactors yielded more spatially uniform cell distributions and higher DNA/construct ($p < 0.001$) than static controls on day 4, and enchanced cartilaginous matrix production to yield thicker contructs than static or spinner flask controls ($p < 0.001$) on day 14. Perfusion culture after seeding was demonstrated after hydrogel entrapment of heart cells within porous scaffolds. Bioreactors yielded more aerobic cultures (lactate produced/glucose consumed, $p < 0.05$), higher cell viability ($p < 0.01$), and lower apoptosis ($p < 0.01$) than static controls. Moreover, heart cell elongation was observed only in bioreactors. In conclusion, an oscillatory perfused bioreactor

preserved viability of freshly harvested cells and enhanced *in vitro* regeneration of tissue engineered cartilage and myocardium in the context of a modular, scalable, customizable system.

(OP 17) Application of an Autologous Mixture of Platelet Rich Plasma, Endothelial Progenitor Cells and Keratinocytes Promotes Matrix organisation, Neo-vascularisation and Reepithelialisation in Porcine Full Thickness Wounds.

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In search for an autologous vascularised skin construct, we treated full thickness wounds (FTWs) with a mixture of basal cell keratinocytes (KCs) and endothelial progenitor cells (EPCs) embedded in platelet rich plasma (PRP). This autologous gel functions as a guiding scaffold and a source of growth stimulating factors.

We cultivated autologous basal KCs in low serum conditions and expanded autologous EPCs from the internal jugular vein. FTWs ($n = 55$) were created on the back of in total 4 pigs, covered with transparent flexible wound chambers and randomly assigned to: 1. Saline; 2. PRP; 3. PRP + KCs; 4. PRP + EPCs; 5. PRP + KCs + EPCs. All wounds were biopsied (day 8) to measure neovascularisation (lectin BS-I, aSMA and MT1-MMP), matrix deposition (fibronectin and collagen I/III) and reepithelialisation. Wound fluids, aspirated daily, were analysed for protein expression.

All EPC treated wounds showed 6–8 times more vascular structures compared to saline ($p < 0.001$). Addition of PRP to EPCs further improved neo-vascularisation, confirmed by higher lectin, αSMA and MT1-MMP. All PRP treated groups showed higher collagen I/III deposition ($p < 0.05$) and a higher fibronectin content ($p < 0.001$). PRP treated wounds exhibited higher concentrations of pro-angiogenic growth factors in wound fluid samples. Application of PRP+KCs resulted in highest reepithelialisation rates compared to saline ($p < 0.001$).

In this porcine FTW model, PRP acts as a supportive biomatrix, creating a more developed vascular network, improving extracellular matrix organisation and leading to accelerated reepithelialisation. Addition of EPCs further enhanced the pro-angiogenic properties of this matrix.

We currently use this template in our 'MilleFeuille' autologous tissue engineering protocols to create laminated vascularised tissue layers.

(OP 18) Application of Fluorescence Techniques to the Study of Protein Adsorption and Packing on Biomaterial Surfaces

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The ways proteins compete for the surface of biomaterials and change conformation are believed to be important for the host response to implants. It is possible to elucidate information on packing and any induced conformational change by making use of different fluorescence techniques on fluorescently labelled proteins. Employing probe-probe resonance energy transfer (RET) allows inter and intra protein interactions to be distinguished. Homo resonance energy transfer (hRET) avoids many problems with having two different probes and means that labelling and subsequent purification can be done in one step.

In this study we made use of both steady state and time-resolved fluorescence techniques and imaging to study FITC (fluorescein isothiocyanate) tagged BSA (bovine serum albumin) adsorption to various (fluorescent) polymeric biomaterials (poly-caprolactone, starch polycaprolactone and starch ethylenevinylalcohol) using titania coated glass as a control. With the combination of both steady state anisotropy and lifetime methods applied on different dilutions (labelled:unlabelled and label:protein) of FITC-BSA differences in packing on the surfaces was determined. The anisotropy data indicated inter protein hRET, more clearly on the control. Dilution of over-labelled proteins in unlabelled seems to be easiest way to make sure the signal is from intraprotein interactions, but polymer autofluorescence requires addressing.

Acknowledgement: Portuguese Foundation for Science and Technology, project PROTEOLIGHT (PTDC/FIS/68517/2006) and J.B. grant SFRH/BPD/17584/2004. European Union NoE EXPERTISSUES (NMP3-CT-2004-500283) and European Union FP6 STREP project HIPPOCRATES (NMP3-CT-2003-505758).

(OP 19) Articular Chondrocytes Culturing Conditions: Optimization and Drawbacks for Cartilage Resurfacing Attempts

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Cell-based cartilage resurfacing requires the *ex-vivo* expansion of autologous articular chondrocytes (ACI) and the maintenance of cell differentiation potentials. Culture conditions have been devised to minimise cellular phenotypic changes, but their clinical applications have to cope with outcomes-undermining aspects: a) cell-source biopsies display different physio-pathological conditions; b) defined growth media composition may not be optimal to establish cartilage repair. We hypothesized that non-frank osteoarthitic (OA) chondrocytes could affect ACI outcomes. To this purpose human cartilage specimens were prepared from 65–75 year-old donors. Normal and early grade I-OA samples were examined histologically and used to derive primary cell cultures. OA chondrocytes showed immunopositivity to MMP-3, negativity to type II collagen and reduced matrix components in micromass cultures. We showed that Sox9, a cartilage-specific transactivator, is prevented from binding its responsive elements on the promoter of the Cartilage Oligomeric Matrix Protein (COMP) gene, coding for an extracellular protein. Clearly, a reduced Sox9 availability minimizes the cell chondrogenic potential. To improve it, OA

cells were cultured in a serum-free medium (SF) but COMP expression remained drastically reduced. TGFß-1 supplementation restored COMP transcript levels, as demonstrated by microarray and quantitative PCR. Unfortunately it also reactivated the chondrocytes endochondral ossification commitment. TGF-expanded cells showed loss of matrix component; immunoresponsivness was evidenced for RAGE, IHH, type-X collagen, apoptotic cells, and mineralization, paralleling BCL-2 expression reduction. Thus peculiar upgrading of the culturing conditions may be successfully targeted to the rescue of specific deficiencies, but may be detrimental to the fate of the cell culture in a clinical situation.

(OP 20) Autologous Nasal Chondrocytes and a Cellulose-Based Hydrogel for the Repair of Articular Cartilage Defects.

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Articular cartilage has low capacities for spontaneous repair. To promote the repair of this tissue, amplification and transfer of autologous chondrocytes using a three-dimensional matrix appear promising. In this attempt, we have developed a self-setting and three-dimensional matrix consisting in a silanized cellulose derivative (Si-HPMC). In previous works, we have shown that our hydrogel enabled the proliferation of human and rabbit chondrocytes, the production of sulfated GAG and the expression of chondrocytic markers during a three-dimensional culture *in vitro*. In parallel, the capacity of our hydrogel to allow the formation of a cartilaginous tissue *in vivo* have been determined by transplantation of human nasal chondrocytes and Si-HPMC in subcutaneous pockets in nude mice. Therefore, we sought to evaluate the preclinical interest of autologous chondrocyte transplantation with Si-HPMC in rabbit articular cartilage defects.

After harvesting and amplification during 4 weeks, nasal autologous rabbit chondrocytes were transplanted with Si-HPMC in critical-size defects created in rabbit articular cartilage. Implants were histologically characterized for the presence of sulfated GAG (Alcian blue staining) and collagen (Masson's trichrome staining). The presence of type II collagen was investigated by immunostaining.

After a 6-week implantation, histological analysis of implants revealed the formation of a repair tissue exhibiting a histological organization similar to that of healthy articular cartilage with a positive staining for sulfated GAG and total collagen. Immunohistological analysis of type II collagen showed that the repair tissue was hyaline-like cartilage.

Our results indicate that Si-HPMC hydrogel appears a potential scaffold for the cellular therapy of articular cartilage.

(OP 21) Automated Culture of Umbilical Cord Blood Derived Progenitor Cells

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Umbilical cord blood (UCB) derived mesenchymal stem cells (MSC) can be differentiated into multiple cell lineages, and are potential candidates for cell based regenerative medicine approaches to mesenchymal tissue regeneration. These cells are particularly attractive due to high proportions of immature progenitors, low immunogenicity and the lack of ethical issues associated with their source. Autologous and allogeneic applications are already apparent for haematopoietic reconstitution and treatment of cardiovascular and neurological disorders. We have demonstrated the growth of UCB derived CD29+ and CD44+ adherent cells on a commercial manufacturing scale automated cell culture platform. Slow growth rates *in vitro* confirmed that the development of UC progenitor based cell therapy products may be problematic, this is exacerbated by absolute low number of progenitor cells in the UCB sample. Overcoming these challenges will require significant improvement of the cell culture environment and development of scaleable automated cell culture processes. The scale and reproducibility of culture on the platform will assist application of systematic process engineering methods to optimise UCB derived MSC culture protocols towards levels of production that are appropriate for therapeutic aims. A further goal will be to verify the differentiation capacity of these automatically cultured cells to tissues of mesenchymal origin which can then be used as a defining end point for the quality of the process.

(OP 22) Automation, Scale-up and Process Engineering: Essentials for Successful Translation of Cell Based Therapies

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The translation of experimental cell based therapies to volume produced commercially successful clinical products requires the development of automated manufacturing processes. Automation removes operator effects to achieve capable and scaleable processes that are economic, able to satisfy medicinal product regulators and able to meet the unpredictable demands of the market place. The authors have successfully transferred a number of manual human cell culture processes to a manufacturing scale automated cell culture platform. The automation has also been used as an experimental platform for the application of factorial process improvement experiments underpinned by a six sigma inspired quality engineering approach at manufacturing relevant scale. Cell lines successfully processed on the automation include an osteoblast cell line, embryonic carcinoma cell line, primary bone marrow derived mesenchymal stem cells, primary umbilical cord derived progenitor cells and human embryonic stem cells. The human embryonic stem cell culture has been validated over multiple passages for genetic stability and a panel of surface markers. Factorial experiments on human mesenchymal stem cells have shown that cell culture parameters, such as seeding density and serum concentration, are not independent in their effects on important markers of cell fate e.g. STRO-1 and ALP. The combination of systematic methodologies, established process engineering techniques, and the enabling scale and stability of the automation

are therefore essential to engineer optimised and cGMP compliant manufacturing processes for the transition of cell based therapies to industrial production.

(OP 23) Biaxial-Rotating Bioreactor for Bone Tissue Engineering Application—*In vitro* and *In vivo* Study

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Aim: The generation of effective tissue engineered bone grafts requires efficient exchange of nutrients and mechanical stimulus. Bioreactors provide a manner in which this can be achieved. We have recently developed a biaxial rotating reactor with improved fluidics on in-silicon simulation. Here we investigated the performance of this bioreactor in proliferation and osteogenic induction of human fetal mesenchymal stem cell (hfMSC) seeded onto polycaprolactone-tricalcium phosphate (PCL-TCP) 3D bioactive scaffolds.

Methods: hfMSC-scaffolds were seeded to 3D scaffold and cultured in osteogenic medium in our bioreactor or standard static culture, with assessment of cellular viability, proliferation and differentiation/mineralization properties. Furthermore, after two weeks *in-vitro* culture, hfMSC/scaffolds were implanted subcutaneously into NOD/SCID mice to investigate their ectopic bone formation capabilities.

Result: *In-vitro*, bioreactor induced hfMSC-scaffolds achieved confluence within 7 days (while it took 28 days for static culture), resulted in increased cellularity, cell viability and homogenous cellular distribution after 28 days culture. In addition, bioreactor induced hfMSC-scaffolds resulted in higher osteogenic differentiation and mineralization as demonstrated by Von Kossa staining, calcium deposition assay, alkaline phosphatase assay and micro-CT analysis. Interestingly, majority of hfMSC were fully differentiated into osteoblast and encapsulated inside mineralized ECM after 28 days bioreactor culture. *In-vivo*, two-week bioreactor culture induced more ectopic bone formation than static culture, as demonstrated by histological and micro CT analysis.

Conclusion: The use of biaxial rotating bioreactors allows shorter *in-vitro* culture, with more efficient osteogenic differentiation of bone tissue-engineered grafts compared to static culture conditions.

(OP 24) Bio-Molecular Interaction between Fibrin and Lipoplexes Results in Extended Release of Plasmid DNA

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Fibrin has many applications in the clinical field, especially as a topical delivery system of bio-molecules. The objective of this study was to develop an efficient, safe, non-toxic delivery system for sustained release of DNA. The hypothesis was that fibrin will act as a scaffold that binds to the lipoplexes and release them in a sustained fashion. Fibrin scaffold was utilized to deliver lipoplexes encapsulating reporter genes to fibroblasts. Fibrin scaffolds containing pre-formed lipoplexes were incubated at 37°C in static conditions and the supernatant was used to transfect fibroblast cell lines. The system released only about 1–2% of total DNA during 144 hours of incubation. The lipoplexes released from the scaffold were successfully utilized for transfections *in vitro* indicating absence of degradation of plasmid DNA. In order to understand the kinetics of this release, we investigated the interaction between the bio-molecular components of our fibrin-based delivery system using Surface Plasmon Resonance, a well established method to obtain kinetic binding profiles [1]. CM3 sensor was used for immobilizing the fibrin components. The binding and kinetic analyses were carried out according to the manufacturer's instructions. The results revealed a sufficiently strong binding between the fibrinogen and lipid components of the lipoplexes. In conclusion, a programmed controlled release of lipoplexes from the scaffold was achieved. Future experiments are aimed at optimizing transfection efficiencies.

Acknowledgements: Research Frontiers Program, Science Foundation Ireland

Baxter Healthcare, Vienna

Reference:

[1]Besenicar, M., Macek, P., Lakey, J. H. and Anderluh, G. Chemistry and Physics of Lipids, 140, 169–178, 2006

(OP 25) Bioactive Functionalised Porous Scaffolds as Supports for Tissue Engineering

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Scaffolds able to support and reinforce the growing cells are required for tissue engineering applications. These scaffolds are required to be biocompatible, bioactive and porous to be suitable for cell growth. In order to obtain optimum scaffolds for tissue regeneration we have prepared new cryogels based on chitosan.

Cryogels are macroporous gels with large interconnecting pores prepared by polymerizarion in semifrozen state. This structure enables rapid mass transfer ensuring efficient supply of nutrients and removal of waste products during cell culturing

Chitosan is a polysaccharide composed of linked D-glucosamine residues that structurally shares similarities with various GAGs present in the extra cellular matrix of cartilage. It has a significant potential as a material for tissue engineering due to its biodegradability, low immunological response and positive effect on wound healing. The cationic nature of chitosan enables ionic interactions while the amine and hydroxyl groups in its structure are well suited for modifications in order to improve cell adhesion.

This study investigates the preparation of chitosan cryogels crosslinked with glutaraldehyde. These chitosan cryogels will be

modified using anionic polymers and/or RGD-containing peptides in order to improve the properties of chitosan.

(OP 26) Bioactive Nanostructures for Regenerative Medicine and Cancer Therapies

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Bioactive nanostructures molecularly crafted to signal cells *in vitro* or *in vivo* have the potential to emerge as the elements of future therapies to regenerate tissues and cure disease. The chemistry of such nanostructures should allow them to interact specifically with cell receptors or intracellular structures. Ideally, they should also disintegrate into nutrients within an appropriate time frame. The organization of these nanostructures at larger length scales comparable to cells and large colonies of cells will also be critical to their function. Our laboratory has developed an extensive family of amphiphilic molecules that self-assemble into nanofiber architectures with capacity to display a large diversity of signals to cells[1–5]. This lecture will illustrate the use of nanoscale molecular features in these systems to regenerate axons in the central nervous system for spinal cord injuries and other brain disorders, bone, and blood vessels in cardiovascular therapies. With the appropriate supramolecular design, these nanostructures could also be used as the functional elements in cancer and gene therapies. The lecture will also demonstrate their future potential to create niches for stem cells in regenerative medicine as the nanostructures self-assemble across scales forming constructs with macroscopic dimensions.

References:
[1]Science 2001, 294 (5527), 1684–1688
[2]Proc. Natl. Acad. Sci. USA 2002, 99 (8), 5133–5138
[3]Science 2004, 303, 1352–1355
[4]Nano Letters 2006, 6 (9), 2086–2090
[5]Biomaterials 2007, 28(31), 4608–4618

(OP 27) Bioactive Supramolecular Membranes for Tissue Engineering of Kidney Tubules

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The quality of life of patients with chronic renal failure will improve by innovative therapies that can be applied as total kidney replacement or in adjunct to conventional dialysis. Bioengineered renal tubules may significantly ameliorate the patients' health by regulating removal of uremic toxins, reabsorption of water and salts, and production of essential hormones. The basement membrane provides essential cues for maintenance of epithelial cell polarity and tubule integrity. Our major goal is to tissue engineer a part of the kidney, the renal tubule, by mimicking the basement membrane using a supramolecular polymer membrane and synthetic extracellular matrix (ECM)-derived peptides. We propose that a modular, self-assembly approach using ureido-pyrimidinone (UPy) modified supramolecular building blocks is an exquisite way to produce such bioactive filters. Supramolecular oligocaprolactone UPy-membranes can be easily formed by electrospinning from solution. After processing, the materials become supramolecularly cross-linked by lateral association of the UPy-dimers. Moreover, bioactive membranes can be prepared without tedious synthetic procedures but simply by addition of UPy-modified ECM-peptides, derived from laminin and different collagens, to the UPy-polymer solution to be used for electrospinning. Viability and function of primary tubule epithelial cells on electrospun UPy-membranes with and without ECM-peptides were evaluated under static and perfusion culture conditions. Our findings show that these UPy-membranes provide excellent mechanical stability, and that formation of a cellular monolayer with polarized cells and maintenance of cell specific marker expression can be achieved. Moreover, viability of the cells could be further improved by incorporating the specific ECM-peptides into the material.

(OP 28) Biocompatibility of a Decellularised Ureteric Scaffold for Tissue Engineering Small Diameter Vessels.

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Introduction: Allogenic vein or artery is not always available for revascularization. This study investigated the biocompatibility of an acellular porcine ureteric scaffold (AU) for tissue engineering vessels.

Methods: Ureters were decellularized by sequential incubation in PBS, hypotonic Tris buffer [10 mM plus 0.1% (w/v) EDTA, aprotinin (10 KIU/ml) pH 8.0], 0.1%(w/v) SDS, in Tris buffer 0.1% (w/v) with proteinase inhibitors, nuclease (1 U/ml RNase & 0.5 U/ml DNase) solution and PBS.

Expression of the major xenoepitope α–gal was studied by immunohistochemistry and antibody absorption. The cytotoxicity of the scaffold was determined using standard contact and extract cytotoxicity tests with porcine endothelial (EC) and smooth muscle cells (SMC). In order to determine the host response to the scaffold, 0.5 cm2 sections of AU, fresh tissue, and AU treated with α-galactosidase were implanted subcutaneously into GTKO mice. Tissues were explanted and examined after one month.

Results: The AU was devoid of α–gal. In contact cytotoxicity assays SMC and EC grew up to and in contact with AU with no change in morphology. Following incubation with extracts of AU the ATP content of SMC and EC was not significantly different from DMEM controls whereas fresh tissue extracts were toxic.

Explanted fresh tissues showed a typical host response with thick capsules (242 μm), CD3+ve T-cells and macrophages. The explants were vacuolated. AU explants had thin capsules (74 μm) and only sporadic CD3+ve T-cells The tissue remained intact and was infiltrated with macrophages, endothelial cells and fibroblastic cells.

Conclusion: This study demonstrates proof of concept for AU scaffold in vascular tissue-engineering.

(OP 29) Biocompatible Macroporous Scaffolds can Serve as Stem Cell Carriers for The Treatment of Spinal Cord Injury

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Spinal cord injury is accompanied by cell death and glial scar formation that negatively affects regeneration in the injured region. Extensive research is being done to prevent scarring, to bridge the lesion and to create an environment permissive for the ingrowth of tissue elements, e.g. axons, neurons, glia and blood vessels. We found that biocompatible polymer hydrogels can eliminate scarring and facilitate regeneration within a spinal cord injury; however, the ingrowing cells comprise mostly connective tissue. We tested the growth of bone marrow stromal cells (BMSC) and human or rat olfactory ensheathing glia (OEG) *in vitro* in biocompatible macroporous hydrogels based on copolymers of 2-hydroxyethylmethacrylate (HEMA) or hydroxypropylmethacril amide (HPMA). The BMSC or OEG containing hydrogels were implanted into an acute spinal cord hemisection, and the extent of tissue regeneration was evaluated after 4 weeks by histology and immunohistochemistry. Hydrogels based on HEMA and HPMA copolymers were biocompatible, well tolerated by the spinal cord and permissive for the growth of both MSC and OEG *in vitro*. The tissue formed within both hydrogels was comprised of connective tissue elements; neural cell processes entered the implants from both the cranial and caudal borders. The presence of MSC or OEG increased the number of ingrowing neural cell processes and decreased the amount of connective tissue in the implants. Our results show that seeding polymer hydrogels with cells, such as MSC or OEG, may augment their regenerative properties within a spinal cord lesion.

Supported by 1M0538, AVOZ503905703, 309/06/1246 and 1A8697-5.

(OP 30) Biodegradable Poly(Ester-Urethane-Urea)s Based with Amino Acids for Tissue Engineering and Drug Release

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Non-toxic biodegradable poly(ester-urethane-urea)s with different hydrophilic and crystallinity character were synthesized from poly(ε-caprolactone) as macrodiol, L-lysine diisocyanate (LDI) and ethyl ester L-lysine or L-ornithine as chain extenders, with different properties. A human embrionary fibroblast primary culture is used to measure biocompatibility. Cytotoxicity, attachment and proliferation onto materials were tested using biochemical methods (MTT, Alamar Blue) and morphological assays (optical and scaning electronic microscopy-SEM). Low cell citotoxicity is found for all systems. Cell adhesion and proliferation degree were strongly dependent of the polyurethane composition, which conditioned their physical/chemical properties.

Hydrophilic/hydrophobic grade, hardness, polycaprolactone molecular weight; and different aminoacid as chain extender, or component molar ratio, modified the cell biological parameters. The cell adhesion and proliferation are dependent of the chemical composition, morphology and topography of the PUs membranes and in this sense, the stiffness of hard and soft blocks, controlled by the amount and size of PCL blocks affects the cell parameters noticeably.

In vitro biocompatibility results and physical-chemical characteristics showed that these polymers are good candidates as biodegradable materials for soft tissue engineering and control release applications.

The design of the polymer microstructure with the composition and size of PCL blocks and the application of LDI or ODI diisocianate and aminoacids extenders, often interesting possibilities for modulating of stiffness of polymeric membranes and scaffolds for tissue engineering applications in soft and hard tissues.

(OP 31) Bioerodible Polymeric Nanoconstructs for the Administration of Hydrophobic Bioactive Agents in Tissue Engineering

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Successful tissue regeneration cannot always be achieved by the combination of cells and their scaffold alone. In this cases growth factor or differentiating agents to promote tissues regeneration are required.

The goal of this work was to investigate the use of bioerodible nanoparticles as carrier of retinoic acid (RA), which is known to induce differentiation of several cell lines into neurons. Due to its high hydrophobicity and short half-lives in blood, RA parenteral administration is very difficult and oral formulations are characterized by uncertain drug bioavailability. An organic solvent-free method, called ''Colloidal-Coating,'' has been developed for the preparation of nanoparticles based on a copolymer of maleic anhydride and butyl vinyl ether (VAM41) loaded with RA. This method presents numerous advantages if compared with other nanoparticles preparation methods: does not entail the utilization of toxic organic solvents, allows for the preparation of small nanoparticles with narrow size distribution, it is easy to perform, it is based on a reversible process which avoid wastes of material in case of accidental errors during the preparation. The activity of the encapsulated RA was evaluated on SK-N-SH human neuroblastoma cells which are known to undergo inhibition of prolif-

eration and neuronal differentiation upon treatment with RA. The activity RA was not affected by the encapsulation and purification processes. Growth inhibitory effects and morphologic differentiation into neuronal cell was observed indicating the suitability of the developed formulation for neuronal regeneration purposes.

(OP 32) Biologic Scaffold Remodeling and Macrophage Response in a Rat Model

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Macrophages are characterized as having an M1 or M2 phenotype based on receptor expression, cytokine and effector molecule production, and function. Classically activated (M1) macrophages produce pro-inflammatory cytokines and reactive oxygen intermediates and efficiently present antigen. Alternatively activated (M2) macrophages produce minimal pro-inflammatory cytokines and possess the ability to facilitate tissue repair/regeneration. Effects of macrophage phenotype upon tissue remodeling in the context of regenerative medicine are largely unknown, but the phenotypic profile may provide a tool by which constructive tissue remodeling can be predicted/promoted.

The objectives of this study were to determine the effects of a cellular component (autologous or xenogeneic) within an implanted extracellular matrix (ECM) scaffold upon macrophage phenotype, and to determine the relationship between macrophage phenotype and tissue remodeling.

Partial-thickness defects in the abdominal wall musculature of Sprague-Dawley rats were repaired with cellular autologous body wall tissue, acellular allogeneic rat body wall ECM, cellular xenogeneic pig urinary bladder tissue, or acellular xenogeneic pig urinary bladder ECM. At 3, 7, 14, and 28 days the host tissue response was characterized using histologic, immunohistochemical, and RT-PCR methods.

Acellular test articles elicited a larger M2 response than cellular test articles and resulted in more constructive remodeling, while those containing a cellular component, even an autologous cellular component, elicited a more prominent M1 response and resulted in deposition of dense connective tissue and/or scarring.

We conclude that the presence of cells does affect macrophage polarization following implantation of an ECM scaffold, and that macrophage phenotype is associated with tissue-remodeling outcome.

(OP 33) Biological Performance of Natural-Based Polymers for Tissue Engineering Scaffolding

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One of the open issues in the Tissue Engineering area is whether scaffolds are needed at all to regenerate many tissue defects. This is a fundamental question that may be answered by providing clear evidence of success by scaffold-less tissue regeneration strategies. In its absence, many groups within this research community are focused in the development of scaffold-dependent strategies.

Natural-based biomaterials have been proposed and explored to meet the requirements of many applications in the Tissue Engineering and Regenerative Medicine. Among the important characteristics of those biomaterials it should be highlighted the easy biodegradation by normal metabolic pathways, the low cytotoxicity and low immunogenic reaction upon implantation and the myriad of properties obtained by combination of those materials with other biodegradable polymers. This later strategy has been pursued in our lab in the quest to obtain scaffolds that effectively help, assist and drive cells to regenerate connective tissues.

Many sources of cells have been considered for tissue engineering. Embryonic and adult stem cells are among the most promising to achieve the cell numbers required to have therapeutic relevance. The ethical and political constraints surrounding embryonic stem cell line derivation led most research efforts to concentrate in adult stem cells. We have been using adult stem cells from different sources for bone and cartilage tissue engineering applications.

This presentation will review our latest developments in tissue engineering of bone and cartilage using natural-based biodegradable scaffolds with relevant cells both *in-vitro* and *in-vivo*.

(OP 34) Bioreactor Strategies in Tissue Engineering

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Over the last decade, we have witnessed an increased recognition of the importance of 3D culture models to study various aspects of cell physiology and pathology, as well as to engineer implantable tissues. Given the crucial role that bioreactors play in establishing a comprehensive level of monitoring and control over specific environmental factors in 3D cultures, we review herein the manifold potentialities of bioreactor systems in the translational paradigm of Tissue Engineering (TE) from bench to bedside. In particular, we will highlight their functions as:

1) Pragmatic tools for tissue engineers, making up for limitations of conventional manual and static techniques (e.g., seeding and culture of cells and engineered constructs), enabling automation and allowing physical conditioning of the developing tissues;

2) 3D culture model systems, enabling to recapitulate specific aspects of the actual *in vivo* milieu and, when properly integrated with computational modelling efforts and sensing and control techniques, to address challenging scientific questions (e.g. elucidation of cell function and tissue development mechanisms);

3) Tissue manufacturing devices, implementing bioprocesses so as to support safe, standardized, scaleable, traceable and possibly cost-effective production of grafts for clinical use (e.g. automating conventional cell and tissue culture processes, streamlining graft manufacturing within centralized and de-centralized production

facilities, defining and facilitating "intraoperative engineering" approaches).

In conclusion, we will provide evidences that fundamental knowledge gained through the use of well-defined and controlled bioreactor systems at the research level will be essential to define, optimize, and moreover, streamline the key processes required for efficient manufacturing models.

(OP 35) Bioreactor Technology for Vascular Tissue Engineering

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Cardiovascular Tissue engineering is a fast evolving field of biomedical science and technology to manufacture viable blood vessels, heart valves, myocardial substitutes and vascularised complex tissues. In consideration of the specific role of the hemodynamics of human circulation, bioreactors are a fundamental of this field. The development of perfusion bioreactor technology is a consequence of successes in extracorporeal circulation techniques, to provide an *in vitro* environment mimicking *in vivo* conditions. The Bioreactor system should enable an automatic hydrodynamic regime control. Furthermore the systematic studies regarding the cellular responses to various mechanical and biochemical cues, guarantee the viability, bio-monitoring, testing, storage, and transportation of the growing tissue.

Tissue engineering of complex tissues and organs is limited by the need of a vascular supply to guaranty graft survival and render bioartificial organ function. To overcome this hurdle numerous strategies and bioreactor technologies have been developed. To induce vessel sprouting and endothelial cell differentiation *in vitro* and *in vivo* a pulsatile hemodynamic flow pattern mimicking physiological conditions are essential. We recently introduce, a unique biological capillarized matrix (BioCaM) and a PC controlled bioreactor system for the generation of complex vascularised tissues.

(OP 36) BMP-12 and BMP-13 Transduced MSCs in Collagen Type I Hydrogel for ACL Constructs

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Introduction: The anterior cruciate ligament (ACL) ruptures inevitably lead to knee instability and premature osteoarthritis. The current treatments replace the ACL with autologous tendon grafts, but cannot restore the complex architecture and biomechanics of the ACL. Therefore our research aims to develop biologically augmented approaches for primary ACL repair. In this study we investigate the ligamentogenic potential of human mesenchymal stem cells (MSCs) transduced with BMP-12 and -13 genes in collagen hydrogel cultures.

Materials and Methods: MSCs from bone marrow were transduced at confluency with 10, 50, or 100 vp/cell of adenoviral vectors encoding BMP-12 or -13. Afterwards, genetically-modified hMSCs were placed in collagen I hydrogels at 3×10^5 cells/200 μL. After 21 days in culture histochemical, immunohistochemical, and RT-PCR analyses of the constructs were performed. Control cultures were also maintained that were transduced with equal amounts of Ad.GFP or remained untransduced.

Results: Histochemical (H&E, Azan, Goldner) analyses of BMP-12 and -13 transduced hMSCs in hydrogels showed elongated fibroblasts embedded in a ligament-like matrix. BMP-12 overexpression resulted in weak positive stainings for collagen III, V and elastin, but strong stainings for tenascin and vimentin. Furthermore, RT-PCR analyses of BMP-12 and -13 cultures showed high expression of ligament markers such as biglycan, collagen III and V, decorin, elastin, tenascin, tenomodulin, and vimentin compared to controls.

Discussion and Conclusion: Gene transfer of BMP-12 and -13 induced a ligament-specific matrix in MSC hydrogel cultures. This technology might serve as an effective strategy to augment ACL repair.

(OP 37) Bone Marrow Stromal Cells Recuperate Hepatocyte Functions in an Acute Liver Injury Model

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Humans are subject to being exposed to acrolein through the degradation of a variety of compounds including cigarette smoke, automobile exhaust, and burning oils. In addition, acrolein is a metabolite of the anticancer drug cyclophosphamide. Allyl alcohol (AA) has been used in food flavorings and causes a periportal-specific hepatocellular lesion in animal livers. Liver injury is dependent upon AA's metabolism to the reactive aldehyde acrolein via alcohol dehydrogenase. Bone marrow comprising heterogeneous cell populations contains certain progenitors with the ability to differentiate into multiple mesenchymal cell lineages. To identify any differentiation plasticity of adult bone marrow mesenchymal stem cells (MSCs), we used AA treated rabbits for acute liver failure model.

The MSCs were aspired and expanded to a cellular concentration of 1×10^8 mL. The rabbits were injected AA intraperitoneally. Three days after the injection, the MSCs were injected via portal vein. The control group rabbits were injected with 4 ml of normal saline under the same condition. The postoperative recovery was closely monitored by hepatic function markers. Within one week, in the experimental group, the AST, A LP, Billirubin and rGT were reduced significantly. The ALT and albumin level recovered two weeks after. The BUN was better off one week later.

Whether extracorporeal devices or the transplantation of primary hepatocytes, stem cells or cells genetically engineered to over-express key metabolic functions, a proliferative phenotype or cytoprotective pathways will be best suited to meeting these demanding challenges.

(OP 38) Bone Morphogenetic Protein-2 Delivery Using Poly(Lactic-Co-Glycolic Acid) Nanosphere for Bone Regeneration

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Here, we tested two hypothesis; (1) a long-term delivery of bone morphogenetic protein-2 (BMP-2) would enhance ectopic bone formation compared to a short-term delivery at an equivalent dose, (2) human cord blood mesenchymal stem cells (hCBMSCs) that are not osteogenically differentiated prior to implantation would regenerate bone extensively *in vivo* once exogenous BMP-2 was delivered to the implantation site. Heparin-conjugated poly(L-lactide-co-glycolide) (PLGA) nanospheres (HCPNs) suspended in fibrin gel (group 1) were developed for a long-term delivery of BMP-2. Fibrin gel containing normal PLGA nanospheres (group 2) was used for short-term delivery of BMP-2. The *in vitro* release of BMP-2 from group 1 was sustained for four weeks with no initial burst release. In contrast, 83% of BMP-2 loaded in group 2 was released only for the first three days. Group 1 induced bone formation to a much greater extent than did group 2 upon implantation into rat hind limb muscle. On the other hand, undifferentiated hCBMSCs on BMP-2-loaded scaffolds induce far more extensive bone formation in mouse calvarial defects than either undifferentiated hCBMSCs or osteogenically differentiated hCBMSCs. These results show that long-term delivery of BMP-2 enhances *in vivo* osteogenic efficacy of the protein compared to short-term delivery at an equivalent dose, and the BMP-2 delivery could prove invaluable for *in vivo* regeneration of bone from undifferentiated hCBMSCs.

Acknowledgements: This work was supported by a grant (SC 3220) from the Stem Cell Research Center of the 21st Century Frontier Program, the Ministry of Science and Technology, Republic of Korea.

(OP 39) Bone, Cartilage and Osteochondral Tissue Engineering Strategies Using Natural Origin Polymers and Ceramics, Growth Factors and Progenitor Cells

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Tissue engineering (TE) has emerged in the last decade of the 20th century as an alternative approach to circumvent the existent limitations in the current therapies for organ failure or replacement. The EU HIPPOCRATES project joined academic and industrial partners to develop novel products and concepts that can be used for bone, cartilage or osteochondral TE strategies. Several issues were addressed including i) the choice and study of adequate human cell cultures, (ii) the development of culture technology with which human tissues can be grown *ex vivo* in three dimensional biodegradable matrices, (iii) the development of a material technology with which polymeric matrices can be produced, being suitable for cell culture (proliferation, differentiation), (iv) to assess the *in-vivo* functionality and clinical relevance of the tissue engineering strategies. In this context, we proposed the use of a series of natural-based biomaterials, namely polysaccharides (e.g. starch, chitin derivatives, alginate, carrageenan, gellan gum) compounded, or not, with synthetic polymers and ceramics, including calcium phosphates obtained from mineralized red algae. A series of processing techniques and surface modifications were used to produce scaffolds with a wide range of porosity and porous architecture and controlled surface features. Hydrogels, including thermo-responsive injectable systems, were produced as biodegradable supports and vehicles for cells and growth factors. Complementary studies were performed to use the same kind of materials in drug release systems; such information could be integrated in tissue engineering strategies where growth factors or differentiation agents could be delivered in a controlled way to progenitor cells.

(OP 40) Calcium Phosphate Bioceramic Derived from Mineralised Red Algae for Bone Tissue Application by Low Temperature Exchange

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There is great need to design functional bioactive substitute materials capable of surviving harsh and diverse conditions within the body. Hydroxyapatite (HA) is a well established substitute material for orthopaedic and dental applications. The literature suggests that porous HA displays better osteoconductivity. A recent study by Roy *et al* [1] concluded that meso-porous ceramic material significantly improved the percentage of new bone formation when compared to non-porous ceramic material. The purpose of our research was to develop a bioceramic from algal origins suitable for bone tissue application. Corallina officinalis was selected as the source material for two main reasons (1) it has a high surface area owing to its highly porous interconnective morphology and (2) it is a magnesium rich calcite.

Low-temperature hydrothermal synthesis achieved the conversion of Corallina to HA in a simple and cost-effective manner [2]. The resultant material contained a calcium phosphate apatite phase of approximately 90%, with a pore size of <10 microns and specific surface area of 124.1 m^2/g. This morphology provides an additional mechanism for cellular activity and offers the potential for use as a drug delivery system. Furthermore, cell studies show that the material can support cell growth, thus indicating its biocompatibility. These physiochemical properties make this material an ideal candidate for tissue engineering applications.

Acknowledgements: Supported by the European Union funded STREP Project HIPPOCRATES (NMP3-CT-2003-505758).

References:

[1]Roy TD *et al*, J. Biomed Mater Res. 2003; 66(2): 283–291.
[2]Walsh *et al*, ESB, Sorrento, 2005

(OP 41) Cardiac Commitment of Human Embryonic Stems Cells: Genetic and Epigenetic Mechanisms

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Human embryonic stem cells (HUESCs) are derived as their mouse counterpart from early embryos (i.e. blastocyst stage). Recent studies using stem cells derived from mouse epiblast suggest that HUESC represent a slightly later stage of development than mouse ESC, questioning the molecular mechanisms underlying both their self-renewal and their specification towards various cell fates.

Herein, we provide evidence that the main genetic and epigenetic mechanisms that we recently uncovered in mouse ESC and which drive ESCs toward a cardiac lineage are fully conserved in HUESCs.

Indeed, the BMP2-dependent canonical cardiogenic pathway is functional in HUESCs. As one of the earliest element of the genetic and epigenetic event of this pathway, Oct-4 level of expression is critical to maintain selfrenewal of ESC or to drive them toward a mesendodermal fate trough a Sox17 mediated event in both mouse ESCs and HUESCs. BMP2 switches on a similar pattern of gene expression in both mouse and HUESCs. A change in the methylation state of mesodermal and cardiac gene promoters, already poised to activation, precedes this genetic program. Thus, we report that most of the basic genetic and epigenetic transcriptional events are fully conserved in HUESCs. BMP2 ensures a tune regulation of these events to drive HUESCs toward a cardiogenic fate. Furthermore, activation of the BMP2 pathway allows for expression of a membrane marker, which turned out to be suitable to sort out cardiac progenitors, a key step toward the genreration of a cell product for a secure cell therapy of heart failure.

(OP 42) Cardiovascular Tissue Engineering: Impact of Mechanotransduction and Functionalised Electrospun Scaffolds

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In the field of cardiovascular tissue engineering, the need for functional bioartificial constructs at the time of implantation remains a driving factor for further development. This is due to the active role of a functional endothelial cell layer in mediating blood compatibility and preventing adverse reactions. Thus designing a scaffold to present the correct mechanical and biochemical clues to cells and mechanically stimulating the construct after seeding are vital factors to support physiological tissue formation and cell activity.

Electrospinning offers a unique opportunity to process both synthetic and biological materials, either in pure form or as a combination, to generate nanofibrous mats as tissue engineering scaffolds. The generated fibrous microstructure closely resembles structures in the native extracellular matrix and can be oriented to provide tactile clues to influence cell migration and behaviour. Furthermore, processing different materials in parallel to finetune the internal microstructure allows a wide range in which to modulate mechanical and biochemical properties.

To support and guide neotissue formation, mechanical stimulation of the seeded construct up to the point of implantation is required. This has been recognized by the advent of specialized bioreactors to reproduce the mechanical stimuli in the cardiovascular environment. However, the effects of individual factors (e.g. shear rate and oxygenation level) are still not fully understood especially with regard to their temporal kinetics. Real time microscopic imaging of cells and their response to changes in their micromechanical stimulation may help to better define parameters for stimulation of tissue engineering constructs.

(OP 43) Cartilage Repair with Chondrocytes and a Novel PLGA-Based Scaffold in a Full Thickness Goat Model

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Introduction: Porous scaffolds in combination with cultured chondrocytes have demonstrated the best ability to regenerate hyaline cartilage both experimentally and clinically. This study aims to investigate the cartilage repair response of a novel methoxypolyethyleneglycol-block-co-poly(lactide-co-glycolide (MPEG-PLGA) porous scaffold and a fibrin scaffold combined with chondrocytes in a goat model.

Methods: MPEG-PLGA scaffold was tested *in vitro* with respect to adhesion and gene expression. 20 adult goats were used for the *in vivo* study. Defects were created in both femoral condyles. Cartilage tissue was harvested for chondrocyte culturing. At secondary open surgery, defects were randomized to four treatment groups; 1. Empty defect 2. Microfracture 3. Fibrin scaffold with chondrocytes and 4. Fibrin/chondrocyte solution in a MPEG-PLGA scaffold. Evaluation of the cartilage regeneration in the four groups was done after 4 months by ICRS macroscopic scoring and histological analyses, using the O,Driscoll and Pinada scores.

Results: Chondrocytes adhered within the MPEG-PLGA scaffold *in vitro* and expressed collagen type II and aggrecan. A migration assay demonstrated the ability of chondrocytes to migrate within the scaffold.

In vivo scores demonstrated significant difference between groups. The cartilage regeneration in group 4 demonstrated high defect fill and tissue characteristic close to hyaline cartilage whereas no regeneration tissue was observed in empty defects. Fibrin/chondrocyte and microfracture group demonstrated limited tissue formation.

Conclusion: MPEG-PLGA scaffold containing chondrocytes demonstrates extensive cartilage regenerative response. Fibrin scaffold with chondrocytes and microfracture stimulated only limited cartilage repair. MPEG-PLGA scaffold in combination with chondrocytes seems to be a good technique for cartilage tissue engineering.

(OP 44) Cartilage Tissue Engineering Using a Flow Perfusion Bioreactor

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Joint diseases include several conditions that have great impact on society, such as rheumatoid arthritis or osteoarthritis. Tissue engineering is one of the most promising alternatives to overcome the low capacity of cartilage self-repair in those debilitating diseases context.

Chitosan-polybutylene succinate (C-PBS) scaffolds have been studied for their suitability for cartilage tissue engineering. Previous works have shown that these scaffolds support chondrocytes primary cultures, its growth and ECM production. C-PBS fiber meshes were produced by fiber extrusion, followed by hot compression, producing a 3D non-woven mesh of variable pore size. These fiber meshes were used for supporting human mesenchymal stem cells (hMSCs) chondrogenic differentiation.

hMSCs isolated from human bone marrow were expanded until passage 2. Afterwards, 1 million cells were statically seeded in each C-PBS fiber mesh, in a total of 20. Then, constructs were transferred to a flow perfusion bioreactor. Perfusion rate was 0,1 mL/h per scaffold. Constructs were cultured for 28 days, with chondrogenic differentiation medium. Differentiation was evaluated by SEM, histology and immunolocalisation of collagens. Preliminary results indicate that cells are able to proliferate and to differentiate into chondrocytes, at 14 days of culture. Results indicate that C-PBS fiber meshes are able to support hMSCs chondrogenic differentiation and extracellular matrix production.

Acknowledgments: Portuguese Foundation for Science and Technology (PhD Grant to M. Alves da Silva, SFRH/BD/28708/2006). European IP Genostem (LSHB-CT-2003-503161) and European NoE EXPERTISSUES (NMP3-CT-2004-500283).

(OP 45) Cartilage Tissue Engineering–Innovative Scaffold Design and New Structural Characterization Methods

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Aims: Modern tissue engineering solutions use scaffolds, cells and growth factors. We present an innovative oriented scaffold concept. For three dimensional characterization we used a new method and compared the data with histomorphological techniques.

Methods: Scaffold design: Scaffolds were synthesized using a freeze structuring procedure. Gelatine from porcine skin was dissolved and partly amended by previously synthesized PLGA microspheres for release of signalling molecules. The solutions were directionally frozen, freeze-dried and chemically cross-linked in aldehyde-saturated atmosphere followed by detoxification. Cell culture: Bovine chondrocytes were cultivated on different scaffold modifications. After 8 days histology and histochemistry were performed.

Three-dimensional characterization: The three dimensional structure as well as the cell and microsphere distribution were analyzed by synchrotron-generated X-rays (SR-μCT) without any metal labelling using the Berlin synchrotron facility BESSY.

Results: After 8 days the chondrocytes maintained a chondrocytic phenotype, with synthesis of extracellular matrix as shown by alcian blue staining. The three dimensional characterization of the scaffold by SR-μCT revealed the orientation of the scaffold fibres and the homogeneous distribution of PLGA microspheres in the scaffolds. Furthermore, the detection of single cells and their distribution in the scaffold could be demonstrated and was in correspondence with the histological data.

Conclusions: Our scaffold concept offers innovative opportunities for chondrocyte culture in an oriented structure, imitating the fibre arrangement in articular cartilage. SR-μCT represents a high resolution three-dimensional imaging method showing cells, scaffolds and microspheres, without disturbing the structural integrity of the cell-scaffold construct, and in correspondence with the histological data.

(OP 46) Cell Encapsulation in Thermoresponsive Chitosan Based Hydrogels for Cartilage Tissue Engineering

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The repair of articular cartilage is still a challenging medical problem and there is a need for the development of a clinically useful cartilage tissue engineering strategies. A variety of materials have been suggested for cartilage engineered repair, and injectable hydrogels are among the most promising alternatives.

This work aimed the exploration of the suitability of chitosan/glycerol-phosphate/hydroxyethyl cellulose (CGP) hydrogel as scaffold for cell encapsulation, using ATDC5 cells (murine chondrogenic cell line) to produce cartilage-like tissue trough tissue engineering approach.

A high cell density was encapsulated within the CGP hydrogel and cultured for up 28 days. The MTS and DNA assays suggest that CGP hydrogel neither altered cell viability nor proliferation in three-dimensional culture. The constructs were histologicaly characterized by H&E staining which revealed that the cells were uniformly distributed within the hydrogel, they adopted a rounded morphology, and formed characteristic lacunae around each cell in the hydrogel. Toluidine Blue and Safranin O stainings also suggest an abundant accumulation of GAG in the extracellular matrix surrounding the ATDC5 cells cultured in 3D into CGP hydrogels, after 4 weeks of culture. The results also evidence an increase in the synthesis of GAG observed by the DMB quantification assay.

The results confirm the potential of the CGP hydrogel as carrier and support for chondrocyte-like cells. CGP hydrogel induces and

supports a stable chondrocytic phenotype of ATDC5 cells *in vitro*, by promoting the expression of cartilage-specific markers and the accumulation of cartilage-specific extracellular matrix, and thus showing promising properties for cartilage regeneration.

(OP 47) Cell Sheet Engineering for Clinical Application

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Ideal tissue engineering therapy should be achieved by cell and cell sheet manipulation engineering that would be established with the following three core technologies: first, the noninvasive harvest of cultured cells and cell sheets are realized with our developed temperature-responsive culture dishes. The surfaces are hydrophobic at 37°C, but change to hydrophilic below 32°C. Various cell lines adhere, spread and proliferate on the surfaces similarly to those on commercial tissue culture dishes. Only by reducing temperature, cells spontaneously detached from the surfaces without the need for trypsin. Confluent cells are also recovered as a single contiguous monolayer sheet with deposited extracellular matrix. Second, the harvested viable cell sheets can be transferred to other surfaces (2D cell sheet manipulation) because the extracellular matrix associated with the basal side of cell sheets shows adhesion. Thus, tissue regeneration with cell sheet tissue engineering can be accomplished either by transplantation of single cell sheets, as with skin, cornea and periodontal ligaments. Finally, the recovered cell sheets can be stratified to reconstruct complex stratified tissue architectures such as liver lobule, kidney glomeruli, and cardiac patches (3D cell sheet manipulation). For example, layered cardiomyocyte sheets harvested from temperature responsive dishes pulsate simultaneously and show diffuse gap junction formation. When transplanted into the subcutaneous tissues of nude rats, spontaneous beatings could be macroscopically observed after 3 weeks and maintained for over 1 year. We believe that these 2D and 3D cell manipulations, cell sheet tissue engineering, will become new revolutionary tools for tissue engineering.

(OP 48) Characterization of Early Cardiac-Specific Transcripts in Embryonic Stem Cell-Derived Multilineage Progenitors—A Transcriptional Profiling Study

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During *in vitro* differentiation, embryonic stem (ES) cells recapitulate cellular developmental processes and gene expression patterns of early embryogenesis. Here, we characterize early stages of cardiac differentiation of mouse ES cells. ES cells were cultured as embryoid bodies (EBs) for 5 days (d). After plating EBs were spontaneously differentiated for 9d into a multilineage progenitor population representing cells of all three primary germ layers including early cardiomyocytes. To investigate this stage of multilineage progenitors and of early cardiac cell types at the transcript level, microarray analysis was performed using Affymetrix chips comparing undifferentiated ES cells and $5+9$d progenitors followed by RT-PCR analysis. Transcript levels of transcriptional regulators (e.g. Tbx20, GATA4, Pitx2), extra-cellular matrix components (e.g. procollagens I, III, IV, MMPs), and cytoskeletal proteins (troponins T2 and C, cardiac alpha actin) involved in cardiac differentiation and function were found to be up regulated. Specifically, we detected a subset of up regulated transcripts of genes known to be specifically expressed in the cardiac neural crest or its derivatives. mRNA levels of transcription factors Hand1 and Hand2, known to control cardiomyocyte-specific gene expression through interaction with other transcription factors, and the neural crest-specific genes Mef2c, Id2, Lbx1, Efnb1, and Sema3c were clearly up regulated at this early stage of cardiogenesis. Also, the presence of the signaling molecule TrkC in this population suggests the involvement of neural crest-specific genes at an early stage of cardiac differentiation. Immunocytochemistry is presently performed to analyse the potential role of cardiac neural crest cells in early ES-derived cardiogenesis.

(OP 49) Characterization of Novel Tissue Engineered Vascular Grafts in a Murine Model

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Cardiovascular disease is a leading cause of morbidity and mortality in developed countries. Surgical options remain restricted secondary to the limitations of prosthetic vascular grafts or inaccessibility of adequate autologous vessels. Although large animal studies have greatly facilitated the development of tissue-engineered vascular grafts, little is known about the molecular and cellular mechanisms underlying the remodeling processes of these grafts. Using sub-1 mm tissue engineered vascular grafts composed of biodegradable poly-L-lactic acid scaffolds statically seeded with human aortic smooth muscle cells and human endothelial cells which were implanted into immunodeficient female SCID/bg mice, we were able to characterize cellular (histologic) composition of the grafts at various time points and evaluate progression of graft structure throughout the graft remodeling phase. Thirteen mice received our tissue-engineered grafts as infra-renal aortic interposition grafts. The mice were followed to early time points (4–18 days) as well as later time points (8–30 weeks). Histologic evaluation of the grafts using species specific cellular markers revealed replacement of the human seeded endothelial cells with those of murine origin. By 8 weeks, there was evidence of sub-endothelial exracellular matrix protein deposition with active remodeling and layering of cellular deposits resembling those of native mouse aorta. Additionally, all grafts remained patent and without rupture throughout the study. Although residual polymer scaffold was present in all samples, progressive aneurysmal dilation was noted at the later time points. Further understanding of graft remodeling may enable us to target this process and optimize the grafts for clinical application.

(OP 50) Chitosan-Based Hydrogels as Extracellular Matrix for Cartilage Tissue Engineering: *In vitro* Biological Evaluation

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Cartilage reconstruction demands for biocompatible materials with controlled biodegradation. Hydrogels, based on natural polymers, fulfil these properties. Recently we have developed an injectable chitosan-based hydrogel and have studied its biocompatibility *in vitro*.

Chitosan was made water soluble by the covalent attachment of glycolic acid with a graft ratio of 43. Phloretic acid was covalently attached to the chitosan (graft ratio 10) and was used as the crosslinking moiety. The hydrogels were prepared by enzymatic crosslinking of the phenol moieties using hydrogen peroxide and horseradish peroxidase. To evaluate biocompatibility, the polymer was mixed with 5×10^6 mL bovine primary chondrocytes and gelation was induced. Gelation occurred within 1 minute, under mild conditions, and resulted in efficient encapsulation of the cells. The reaction was not associated with cytotoxicity. The gels were cultured for 21 days in standard chondrocyte culture medium and cell viability was evaluated using a live-dead assay at 1,7,14 and 21 days. There was no evidence for toxicity over time. Next, we assessed the cell morphology using SEM-analysis. Results show the presence of round chondrocyte-like cells and the production of extracellular matrix. Biodegradation of the constructs was assessed by weight loss quantification, up to 28 days in culture. Hydrogels with encapsulated chondrocytes degrade slower than control.

In conclusion, our data show efficient encapsulation of primary chondrocytes in a chitosan-based hydrogel. The gelation reaction was fast and not harmful for the cells. Cells retained a chondrocyte-like morphology and produced extracellular matrix upto 21 days in culture. This novel chitosan-based biodegradable matrix may have application in cartilage reconstruction.

(OP 51) Chitosan/Soy-Based Membranes Enhance Wound Reepithelialization in Partial Thickness Skin Wounds

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Chitosan-based biomaterials proved to have promising characteristics for wound dressing and skin regeneration. In the context of developing new natural-based biomaterials for these applications, chitosan and soybean-based biomaterials were proposed. These materials were shown to be non cytotoxic and to impair human leukocytes activation *in vitro*. Thus the goal of this study was to evaluate the *in vivo* performance of chitosan/soy-based membranes in the regeneration of partial thickness skin wounds. Excisional skin wounds were created on the backs of rats and the healing capacity of chitosan/soy-based membranes was assessed after 1 and 2 weeks. To promote impaired wound healing all rats were injected with a steroid. After one week, membrane-dressed wounds showed less granulation tissue formation and the wounds margins were thinner than in untreated wounds (negative control). Furthermore, in comparison to the positive control (Eppigard®), the chitosan/soy-based membranes were also able to reduce the wound size and the thickness of the wound limits. A similar trend was observed 2 weeks after the dressing. The membrane-dressed wounds were almost closed, although with some contraction, and with almost no granulation tissue. For both time periods there were no signs of infection. The observed reepithelialization and engraftment between the regenerated tissue and the original skin reinforces the suitability of the proposed chitosan/soy-based membranes to be used in skin wound dressing.

Acknowledgements: This work was partially supported by the European Union funded STREP Project HIPPOCRATES (NMP3-CT-2003-505758) and was carried out under the scope of the European NoE EXPERTISSUES (NMP3-CT-2004-500283).

(OP 52) Chondrogenic Differentiation of Human Embryonic Stem Cells: Effects of TGF-beta and BMP-2

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Background: Human embryonic stem cells (hESCs) have potential to become cell source in tissue engineering applications upon successful differentiation into specific cell types. With its limited regenerative ability, cartilage is a particular candidate for skeletal tissue engineering. This study investigates the differentiation of hESCs into cartilaginous progenitors and the effects of TGF-β, BMP-2 on the process at different time points.

Materials and Methods: Undifferentiated hESCs (HUES-9) were characterized microscopically and by IHC. Pellet cultures were established and differentiated for 28 days in hESC expansion medium (ES-EM) supplemented with (1) 10 ng/ml TGF-β, (2) 50 ng/ml BMP-2, and (3) their combination. Cell pellets cultured in ES-EM alone served as controls. At Day 1, 14 and 28, specimens were fixed and stained with Alcian Blue to detect glycosaminoglycans. IHC and RT-PCR were performed for Collagen I, Collagen

II and Aggrecan expression. Resultant tissue structures were visualized by SEM. One-way ANOVA was used to determine the expression difference for each marker between the groups.

Results and Discussion: Results demonstrate the feasibility to direct hESCs into chondrogenic lineage based on micropellet culture and the influence of TGF-β and BMP-2 both alone and in combination. It appears that the two growth factors have a synergistic effect on the expression of chondrogenic markers, particularly when TGF-β is withdrawn from Day14 onward, but BMP-2 is present throughout the experimental period. The successful directed differentiation of hESCs into cartilaginous tissue progenitors supports the notion that they could be utilized as cell source in prospective cartilage tissue engineering applications.

(OP 53) Co-Culture System of Osteoblasts and Endothelial Cells, an *In vitro* Strategy to Enhance Vascularization in Bone Regeneration

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For bone TE applications the formation of a prevascularized structure is not only required for the survival of seeded cells on scaffolds, but is also a fundamental process in bone formation and repair. In this work we describe an *in vitro* strategy consisting in the simultaneous culture of osteoblasts and endothelial cells (ECs) onto a starch-based fiber-mesh scaffold, aiming to accelerate the establishment of a vascular bed in the implanted construct.

In order to distinguish between cell populations, samples were stained after several time points for nuclei (both osteoblasts and ECs), PECAM-1 (endothelial specific) and visualized by confocal microscopy. After 21 days of culture the formation of tube-like structures with PECAM-1 expression at the cell-cell junctions was observed. The structural complexity was even higher after 35 days with ramified tube-like structures. Immunohistochemistry of co-culture/scaffold cross-sections revealed that the scaffold's void spaces were filled with a dense ECM positively stained for type I collagen. Inserted in this matrix were PECAM-stained ECs surrounding a patent lumen. Furthermore, these tube-like structures were stained for type IV collagen, a component of the vascular basal lamina. Cross-talk communication between the two cell types was another aspect under analysis. Osteoblasts in co-culture were producing higher amounts of VEGF than in monoculture. In addition, the expression of gap junction connexin43 in the co-culture demonstrates the heterotypic communication established between cells.

In short, the use of this co-culture system in conjunction with a starch-based 3D scaffold holds great potential for the formation of a prevascular network *in vitro*.

(OP 54) Cocultivation of Hemopoietic Stem Cells (HSCs) and Mesechymal Stem Cells (MSCs) Derived from Umbilical Cord Blood in Spinner Flasks

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Autogenetic mesenchymal stem cells (MSCs) not only can support hematopoietic stem cells (HSCs) expansion *in vitro* but also alleviate complications and lead to a speedy recovery of hematopoiesis during hematopoietic stem cell transplantation. In order to achieve this destination, expansion of HSCs with autogenetic MSCs simultaneously is required. In the present study, we investigated the feasibility of expanding umbilical cord blood (UCB) mononuclear cells (MNCs) with appearance of autogenetic MSCs in the 3-D dynamic system without serum support. The cells were cultured in spinner flask with microcarriers and IMDM medium, supported with the combination of cytokines, including SCF, TPO, FL, IL-3, GM-CSF and G-CSF, and cocultured with allogeneic UCB-MSCs encapsulated in the beads of calcium alginate-chitosan (AC). With the diluted-feeding protocol, the total cell number, CD34+CD45+ cells (HSCs) and CD34−CD45− cells (MSCs) could expand 2.7-fold, 9.1-fold and 8.4-fold respectively during primary culture within 6 days. After coculture, HSCs and MSCs can be easily separated by gravity sedimentation. At the same time, by inducing differentiation and flow cytometric analysis, the fibroblast-like cells appeared on the surface of microcarriers could differentiate into osteoblasts, chondrocytes and adipocytes and expressed MSCs surface markers of CD29CD44CD105CD166 positive and CD34CD45HLA-DR negative. In conclusion we have developed an efficient protocol to expand and harvest UCB-HSCs and UCB-MSCs simultaneously by adding cytokines and stromal cells together with microcarriers in the dynamic culture system.

(OP 55) Development of an Osteochondral Repair Therapy Utilizing BMP2-Loaded Collagen Scaffolds

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With more than 20,000 patients treated globally, Genzyme's MACI (Matrix-assisted Autologous Chondrocyte Implantation) implants and Carticel® cultured chondrocytes are used by orthopaedic surgeons to treat patients who have clinically significant articular cartilage lesions. These therapies employ the patient's own chrondrocytes to grow new cells for implantation. Genzyme has over 20 years of clinical experience in developing and producing autologous cell therapy products that meet the highest standards of quality and compliance. In 2007, Genzyme announced clinical data which demonstrated that Carticel cultured chondroncytes provided sustained and clinically meaningful improvements in knee function and reduction in knee pain. In another study, the MACI implant was demonstrated to have the ability to generate hyaline-like cartilage as early as six months after implantation. Despite the success of these cell-based therapies, alternative therapies that rely solely on biomaterial scaffolds, in some cases containing growth factors, would offer advantages in simplicity and reduced patient morbidity. Cell-free biomaterial approaches to cartilage repair have been the subject of a great deal of work in academia and in industry, yet a successful cell-free cartilage repair product remains an unmet goal. Beyond the dis-

covery of the ideal scaffold and/or formulation, great challenges must be overcome to successfully develop a product for cartilage repair. These include ambiguities in preclinical models, the logistics of conducting controlled surgical trials, and the lack of global harmonization. Finally, a successful business model must be found despite significant uncertainty in market size, time to achieve market penetration and difficulties in obtaining reimbursement after regulatory approval.

(OP 56) Comparative Study of the Multidifferentiation Potential of Human Wharton's Jelly and Amniotic Fluid Derived Stem Cells

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Mesenchymal stem cells (MSCs), potentially immune-privileged cells, have been isolated from bone marrow, umbilical cord, e.g. umbilical vein and Wharton's Jelly, and from amniotic fluid. Amniotic fluid cells were used for prenatal diagnosis since 1950. Although being a well established diagnostic technique, little is known about the origin and properties of those cells, its embryonic or foetal origin remaining unclear.

In this study, we compared the osteogenic and chondrogenic potential of human Wharton's Jelly derived cells (WJCs) and amniotic fluid stem cells (hAFSCs). We used MSCs isolated from around the blood vessels (perivascular zone) of umbilical cords collected in caesarean surgeries of full-term pregnancies, and hAFSCs from day 6 supernatant of the cultures of amniotic fluid were also obtained from amniocentesis. Flow cytometry analysis was performed to characterize both cell populations, by evaluating the presence of stem cell surface markers. Osteogenic and chondrogenic differentiation was assessed by immunocytochemistry and RT-PCR.

Acknowledgments: Portuguese Foundation for Science and Technology (PhD Grant to Ana Costa-Pinto SFRH/BD/24735/2005). European IP Genostem (LSHB-CT-2003-503161) and European NoE EXPERTISSUES (NMP3-CT-2004-500283).

Work developed under the cooperation agreement established between the 3B's Research Group-UM and the Hospital de São Marcos, Braga.

(OP 57) Comparison of Bone and Cartilage Gene Expression in Marrow Stromal Cells and Embryonic Stem Cells During Osteogenic Differentiation

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Embryonic stem cells (ESCs) are a potential cell source for tissue engineering applications because they have unlimited proliferative potential and the capacity to differentiate into all cells of the adult organism, including osteoblasts. Here we sought to test the hypothesis that ESCs follow similar patterns of gene and protein expression as marrow stromal cells (MSCs) using qPCR, immunocytochemistry (ICC) and Raman spectroscopy. ESCs and MSCs were grown for 28 days in the presence of 280 μM ascorbate, 10 mM β-glycerophosphate, with 1μM dexamethasone added at d14 (osteogenic medium). At various timepoints qPCR was performed on extracted RNA using primers to Osteocalcin, Col1a1 and Col2a1 with normalisation using a combination of 3 different housekeeping genes. Cells were fixed at d0, d14 and d28 and stained with antibodies to Osteocalcin, Runx2 and Collagen type II. Live cells were examined by Raman spectroscopy with a 785 nm laser and spectra were examined between wavenumbers 400 and 1900 cm^{-1}. Osteocalcin expression was not significantly different in ESCs and MSCs at d0, but had increased by 900-fold in MSCs by d14 compared to only 3-fold in ESCs. Col1α1 expression was 1000-fold greater in MSCs than in ESCs at d0 but by d14 had increased 500-fold in ESCs compared to 10-fold in MSCs. Col2α1 peaked at d14 in both cell types (5-10-fold), and was detectable by ICC and Raman spectroscopy only at this timepoint. These results demonstrate that osteo- and chondogenic markers follow similar patterns of expression in ESCs and MSCs but are expressed at lower levels in ESCs.

(OP 58) Complex Tissue Engineering with a Novel Poly(Lactic Acid-Co-Caprolactone)-Collagen Hybrid

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A biocompatible hybrid construct was fabricated by plastic compressing hyperhydrated collagen gels onto the surfaces of a flat slow degrading poly(lactic-acid-co-caprolactone) warp knitted mesh. This resulted in natural polymer cell carrying components supported by a synthetic polymer backbone. Fluid/weight loss was measured to establish a standardised compaction protocol. Constructs were characterised for matrix density. Living interstitial, epithelial and complex tissues were constructed by seeding human dermal fibroblasts inside and on the surface of the collagen layers. Cell viability was determined after compression and at various time points during a 7 day culture period using the AlamarBlue reduction assay. Fluorescent cell staining and confocal microscopy were used to visualise cell death and migration. It was found that in the range of 120 to 240 grams, and 5 to 10 minutes, the level of fluid loss and collagen compaction was dependent on the duration of the compression process rather than the loading weight. No significant cell death was observed post-compaction. After one week of static culture, the hybrid construct showed no macroscopic contraction. Cells inside as well as layered on top of the constructs proliferated. Cells inside the collagen matrix were homogenously distributed and there was no evidence of degradation.

(OP 59) Complexing DNA with Dendrimers to Enhance Gene Delivery from Collagen Scaffolds to Mesenchymal Stem Cells

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Collagen scaffolds have been investigated as *in vitro* DNA reservoirs, which allow sustained release of genetic information. These scaffolds have proved effective in viral gene-transfer, where viruses transfer genetic material across the cell membrane. However, collagen will not transport DNA across the cell membrane, so non-viral vectors such as liposomes and linear polymers are commonly used to improve transfection efficiency [1]. In this study, partially degraded polyamidoamine dendrimers (dPAMAM) are combined with collagen scaffolds for gene delivery. dPAMAM dendrimers, such as those found commercially in SuperFect (Qiagen), have found to be highly effective transfection reagents [2]. It is hypothesized that the addition of these dendrimers to the DNA reservoir will improve transfection efficiency as compared to collagen scaffolds impregnated with naked DNA. Collagen scaffolds were cross-linked with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) or a combination of EDC and dPAMAM. Plasmid DNA encoding firefly luciferase was complexed with dPAMAM or impregnated into the collagen scaffold in its naked form. Rat mesenchymal stem cells were seeded on the scaffold and assayed for luciferase after 72 hours. Transfection was seen in all samples, with significant transfection in the scaffolds conjugated with DNA-dPAMAM complexes. This conjugation platform may lead to an effective method of scaffold mediated gene delivery.

Acknowledgement: Research Frontiers Program, Science Foundation Ireland.

References:

[1]Dang, J.M., Leong, K.W., "Natural polymers for gene delivery and tissue engineering." Adv. Drug Delivery Reviews. 58(4) (2007) 487–499.
[2]Gebhart, C.L., Kabanov, A.V. "Evaluation of polyplexes as gene transfer agents." Cont. Release. 73 (2001) 401–416.

(OP 60) Composite Scaffolds for Bone Tissue Regeneration

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Numerous materials have been proposed for bone tissue regeneration. However, none has shown to be entirely satisfactory. Ideal scaffolds for bone tissue regeneration should be biocompatible, biodegradable, easily fabricated and configured to provide adequate structural support. We have developed a scaffolding system that meets these criteria. In this study we evaluated the biocompatibility and structural properties of the scaffolds for their use in bone tissue regeneration. Poly(D,L-lactide-co-glycolide) (PLGA), biodegradable synthetic polymer, and a naturally derived collagen matrix derived from porcine bladder submucosa (BSM) were fabricated into a composite scaffolding system using a solvent casting/particulate leaching process. The structural properties were examined by scanning electron microscopy (SEM), mercury intrusion porosimeter, hydrophilicity assay, and biomechanical testing. The biological properties of the scaffolds were evaluated by seeding with both human embryonic stem cells and bovine osteocytes. Cell adhesion, survival, proliferation and toxicity were assessed. The BSM-PLGA composite bone scaffolds possessed uniform porous structures with a consistent interconnectivity throughout the entire scaffold. The average pore size of the composite bone scaffolds was 121.84 ± 23.44 μm and had a porosity of $94.79 \pm 10.76\%$. The surface hydrophilicity of the BSM-PLGA composite scaffolds was significantly enhanced, when compared to the hydrophilicity of each material separately, resulting in uniform cell seeding and distribution. Cells seeded on the composite bone scaffolds readily attached, survived and proliferated, as confirmed by histological examination, cell viability and MTT assays. There was no evidence of toxic effects of the scaffolds.

(OP 61) Compressed Collagen-PLA Hybrids as Scaffold for Human Bladder Cells

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Collagen gels have proven to be adequate scaffolds for tissue regeneration as cell attachment is facilitated by the presence of cell-adhesion domain sequences. This specific cellular interaction could also play a role in preserving the phenotype and functional activities of many cell types. However, collagen matrices offer a limited mechanical strength. In this work we described a cell-independent way to increase mechanical strength of collagen based scaffolds and its potential for bladder tissue regeneration. Material strength was increased by the use of hybrid materials composed of knitted fabrics of poly(lactic acid-co-epsilon-caprolactone) (PLA) embedded in plastic compressed collagen gels. Human smooth muscle (SMCs) and urothelial cells (UCs), the two major cellular components of the urinary tract, were cultured on and inside collagen-PLA hybrid materials. Cells were analysed by scanning electron microscopy, conventional histology, immunohistochemistry and proliferation assay over a period of 14 days. Cellular distribution of smooth muscle cells seeded inside the gel was also evaluated during this time. We showed that both cell types proliferated on the hybrid surface forming dense cell layers after two weeks and that urothelial cells featured spontaneous stratification. However for SMCs that have been seeded inside the gel, proliferation rates were reduced as compared to surface seeded cells. Conventional histology as well as immunohistochemistry will be performed to determine cellular distribution inside the hybrid material.

The first data we obtained so far encourage the use of compressed collagen–PLA hybrid materials as scaffolds for bladder tissue regeneration.

(OP 62) Contrast Enhanced Micro-CT Imaging of Vascular and Cartilaginous Tissues

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Advanced imaging methods play an increasingly important role in the rigorous evaluation of tissue regenerative strategies. Micro-computed tomography (micro-CT) imaging offers excellent resolution for quantifying 3D morphology but has typically been restricted to analysis of x-ray attenuating tissues such as bone. Even with this limitation, micro-CT analysis has been a valuable evaluation technique for tissue engineering studies to monitor mineralized matrix synthesis *in vitro* and quantify functional bone regeneration *in vivo*. Unfortunately, soft tissues such as blood vessels and cartilage alone are not sufficiently radiodense relative to surrounding tissues to allow micro-CT analysis. However, vascular ingrowth into scaffolds or regions of tissue injury may be imaged nondestructively following perfusion of a radiodense contrast agent. Subsequent morphological analysis can provide quantification of 3D vascular volume, vessel thickness and density. We have also recently established a new technique called Equilibrium Partitioning of an Ionic Contrast agent μCT (EPIC-μCT), that provides direct *in situ* analysis of both bone and cartilage during joint development, degeneration, or repair. The high resolution provided by EPIC-μCT and ability to separately analyze both bony and cartilaginous tissues nondestructively makes it particularly well suited for small animal studies. We have validated and applied EPIC-μCT for assessment of 3D articular joint morphology and proteoglycan composition with high precision and accuracy in the rat model. The use of selective contrast agents has thus extended the application of micro-CT imaging in TE/RM to include not only bone but also non-mineralized tissues such as blood vessels and cartilage.

(OP 63) Controlled Angiogenesis by Rapid FACS-Purification of Transduced Progenitors Expressing Defined VEGF Levels

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Vascular Endothelial Growth Factor (VEGF) gene delivery is a powerful strategy to re-vascularize ischemic tissues or tissue-engineered grafts. However, its microenvironmental dose must be controlled, as VEGF remains bound around each producing cell (Ozawa, JCI 2004). To achieve stable and homogeneous controlled expression *in vivo*, we developed a FACS-based method to allow the rapid purification of genetically-engineered progenitors expressing predictable levels of VEGF.

Myoblasts were transduced with a retrovirus expressing $VEGF_{164}$ transcriptionally linked to a truncated CD8a marker, which could be quantified on individual live cells by FACS and reflect differences in VEGF expression.

The transduced population expressed very heterogeneous transgene levels, but VEGF and CD8 expression were tightly correlated ($R^2 = 0.897$) in individual clones (range = 2–142 ng VEGF/10^6 cells/day). A clone expressing the highest safe VEGF level (40 ng VEGF/10^6 cells/day), inducing only normal angiogenesis *in vivo*) was selected as reference. Cells expressing similar CD8a levels (and therefore of VEGF) were rapidly purified from the primary population using 2 different gate sizes. *In vivo*, the heterogeneous population always caused angioma growth. However, both purified populations caused robust normal angiogenesis and completely prevented angioma formation. The angiogenic response was stable after 4 months, without adverse effects. Safety in the presence of endogenous angiogenic activation was confirmed in a rat model of chronic hindlimb ischemia.

Therefore, rapid FACS-purification of engineered progenitors completely prevented angioma growth, allowing safe and efficient angiogenesis *in vivo*. We are currently applying this technology to mesenchymal progenitors to stimulate controlled vascularization of osteoinductive grafts *in vivo*.

(OP 64) Controlling Human Mesenchymal Stem Cell (MSC) Differentiation on PCL Substrates Using Silane Modification Techniques (SAMs)

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Introduction: The ability to produce substrates that can trigger and maintain a cellular response, without the need for exogenous biological stimuli is one of the ultimate aims of tissue engineers. In this study -OH, -COOH, -NH2 and -CH3 groups were grafted onto spun coated PCL films and their ability to influence MSC behaviour *in vitro* was analysed.

Materials and Methods: PCL films were spin-coated onto prepared chromium coated glass coverslips and activated by oxygen gas plasma. Subsequent ultraviolet (UV) induced grafting of different chemical groups in the monomer solution was performed to introduce -CH3,-COOH, -OH, -NH2 groups on the surface. All materials were characterised using contact angle, surface energy and XPS. Phenotype defined human MSC were cultured in contact with the surfaces in either basal, chondrogenic or osteogenic media for up to 28 days. Analysis included cell adhesion and real time-PCR to evaluate the expression of beta-actin, ornithine decarboxylase, collagen I, collagen II, collagen X, CHOP, sox-9, osteocalcin, osteopontin, osteonectin and cbfa1 over the 28 day time period. Expression of STRO-1, nucleostemin, collagen I, II, X, osteocalcin and CBFA1 was visualized using fluorescent immunohistochemistry and confocal microscopy.

Results: -NH2 modified surfaces were positive for osteocalcin and cbfa1, fluorescent immunohistochemistry and RT-PCR, in both basal and osteogenic mediums, these surfaces were osteoinductive. Modifying PCL with -OH and -COOH groups induced chondrogensis as demonstrated by increased production of collagen II

and a reduction in collagen I formation. SAMs modification enhanced the potential of PCL as a tissue engineering substrate.

(OP 65) Coordinated Co-Expression of VEGF164 and PDGF-BB Prevents Aberrant Angiogenesis and Increases Blood Flow in Ischemia

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VEGF regulates normal vessel growth in development and physiology. However, VEGF gene delivery can have serious dose-dependent side-effects, such as the induction of angiomas. We have recently found that the switch between induction of stable capillaries and angioma growth occurs at a threshold level in microenvironmental VEGF concentration and correlates with aberrant pericyte recruitment. Since pericytes are responsible for nascent vessel maturation and are recruited by PDGF-BB, we investigated the role of PDGF-BB signaling in modulating VEGF-induced angiogenesis.

Primary mouse myoblasts were retrovirally transduced to deliver either VEGF164 or PDGF-BB to skeletal muscle. Although PDGF-BB did not induce any angiogenesis, its co-expression significantly increased VEGF-induced vascular density and prevented aberrant vessels, yielding a network of homogeneous, mature capillaries. However, both factors required precise co-localization in the microenvironment at fixed relative levels, through co-expression from a single bicistronic retrovirus. In a model of hindlimb ischemia, coordinated co-expression greatly improved blood flow, stimulated collateral arteriogenesis and reduced muscle damage compared to VEGF or PDGF-BB alone. PDGF-BB did not influence the initial response to VEGF, but modulated subsequent remodeling, preventing circumferential enlargement and leading to capillary sprouting. Mechanistically, we found that the VEGF level, at which the threshold between normal and aberrant angiogenesis occurs, was not fixed, but depended on the balance between VEGF and PDGF-BB signaling. The induction of homogeneous normal angiogenesis, despite heterogeneous and uncontrolled VEGF levels, suggests that VEGF and PDGF-BB co-expression is suitable for gene therapy with traditional vectors, whereby the distribution of microenvironmental levels cannot be controlled.

(OP 66) Cryoprotectants for Vitrification of Corneal Endothelial Cell

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The objective of this work is to study the suitable cryoprotective agents (CPAs) for vitrification of corneal endothelial cell (CECs). Fresh bovine CECs were isolated and tested with a optimized vitrification protocol with multi-step CPA loading and removal. Three types of CPAs components, i.e. the pnentrating CPA component, sugars and high molecular weight compounds were experimentally evaluated using the viability measured by trypan blue. Dimethyl sulfoxide ($(CH3)_2SO$), ethylene glycol (EG), 1,2-propanediol (1, 2-PD), glycerol (Gly), 2,3-butanediol (2,3-BD), diethylene glycol (DEG), acetamide (Ace) and ethylene glycol monomethyl ether(EGMME) were chosen as the pnentrating CPA components, and sugars included xylose, fructose, mannose, glucose, maltose, sucrose and trehalose. Ficoll(wt. 7KD), dextran(wt. 7KD), chondroitin sulfate(CS, wt. 18~30KD), albumin bovine V(wt. 68KD), and polyethylene glycol(PEG, wt. 6KD, 10KD and 20KD) were the high molecular weight compounds. The results showed that EG was the most suitable penetraring CPA component and glucose the best sugar, and CS the best high molecular weight component. The optimized concentrations for each compoent in the vitrification solution were 52% EG, 8% glucose and 3% CS. The CEC survival rate of $86.4 \pm 6.1\%$ (mean $\pm$ S.D.) was obtained following the established protocol.

(OP 67) Cultivation of Human mesenchymal Cells in a Microcarrier-Based Bioreactor System

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For an effective application of primary mesenchymal cells such as chondrocytes and mesenchymal stem cells (MSC) as cell source for tissue engineering approaches, obstacles regarding the cost- and labor-intensive *in vitro* expansion phase have to be overcome. In order to develop a controlled, cost-effective and scalable process, a microcarrier-based cultivation system for human bone marrow-derived MSC and chondrocytes was established.

MSC and chondrocytes were isolated and expanded in conventional monolayer culture. Passage 3 cells were seeded on Cytodex 1, 2 and 3 microcarriers and the adherence phase was investigated. For different time points, the number of adherent cells on 60 carriers was counted. By comparison of the measured frequency of cell distribution with a Poisson distribution and subsequent regression analysis, the average cell count per carrier was determined showing the best results for Cytodex 1 with over 80% adherent cells after 3 hours of incubation. After the adherence phase, the cells were cultivated in a spinner flask bioreactor for 28 days and cell viability, morphology and cell distribution was analyzed revealing proliferation and cell-specific morphology. At day 14 we added unseeded carriers and analyzed the colonization status in the following. This way, we could show that the cells colonize freshly added carriers by bead-to-bead transfer. Finally, the cells were harvested and evaluated for their differentiation potential.

After showing the applicability of microcarrier cultures for expansion of primary mesenchymal cells, current research approaches comprise the transfer of established protocols for regu-

lar spinnerflask bioreactors on the parallel DASGIP bioreactor system.

(OP 68) Cyclic-Compressive Stimulation does not Alter the Stem Cell Character of Mesenchymal Stem Cells but their Functional Behaviour

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Mesenchymal stem cells (MSCs) are involved in bone regeneration, which is influenced by mechanical boundary conditions. On the cellular level, mechanical stimulation leads to differences in the expression profile of MSCs, e.g. an increased matrix metalloproteases (MMPs) secretion. Also the paracrine stimulation of angiogenesis is elevated. Still, it remains unknown whether these effects are mediated directly by MSCs or indirectly as a consequence of differentiation processes. This study aimed to analyse the stem cell character and behaviour of rat-MSCs after mechanical stimulation.

MSC populations were homogeneous in their characteristic cell surface marker profile (CD14− CD45−, CD34−, CD29+, CD44+, CD73+, CD90+, CD105+, CD166+) and multipotent as confirmed by osteogenic, adipogenic and chondrogenic differentiation assays. In comparison to monolayer cultures, upregulation of CD106 and downregulation of CD29, CD44, CD105 and CD166 were observed in 3D-cultures. Mechanical loading (10 kPa, 1 Hz, 72h) lead to an increased expression of CD166 and decreased expression of CD44 and CD73. Determination of Alkaline Phosphatase activity, degree of mineralisation, fat deposition and Collagen-II content revealed no effect of mechanical stimulation. However, migratory capacity decreased significantly after mechanical stimulation.

In summary, we could show that antigen expression of MSCs is differentially regulated by mechanical stimulation, but still maintains the typical MSC marker profile. Furthermore, mechanical loading does not alter the cells multipotency. Thereby, physiological loading does not change the stem cell character of MSCs. However, the migratory capacity of MSCs is dependent on mechanical conditions. Additional studies are necessary to clarify the relevance of decreased migratory potency to bone regeneration.

(OP 69) Delivery Strategy of Biosignaling Molecules for Tissue Engineering

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To realize cells-induced tissue regenerative therapy, there are two practical methods; cell therapy and tissue engineering. For the former approach, stem cells with the high potential of proliferation and differentiation are directly transplanted to induce tissue regeneration. However, only by the simple cell transplantation, successful tissue regeneration cannot be always expected because of the lack of oxygen and nutrients supply to cells transplanted. Therefore, a biomedical technology and methodology are highly required to create a local environment which enables cells to promote their proliferation and differentiation for tissue regeneration. One of the practically possible strategies is to make use of biosignaling molecules (growth factors and genes). Some molecules induce angiogenesis which can supply cells oxygen and nutrients, while others directly act on cells to maintain and activate their biological functions. We have explored biodegradable hydrogels for the controlled release of bioactive growth factors or genes and succeeded in regeneration and repairing of various tissues. The growth factor delivery was a promising strategy to significantly increase the grafting rate of cells transplanted, and consequently enhance the efficacy of cell therapy. Some clinical trials for the growth factor-induced regeneration of blood vessels, bone or periodontal tissues, and skin dermis have been started to demonstrate the superior therapeutic availability. In this paper, several research data of growth factor and gene-induced tissue regeneration are overviewed to emphasize scientific and clinical significance of delivery strategy of biosignaling molecules in tissue regenerative therapy.

(OP 70) Demonstration of the Potential of Iron Oxide Labelling of Chondrocytes for *In Vivo* Tracking

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Autologous chondrocyte implantation is becoming a common treatment modality for cartilage defects. To verify its safety and efficacy it is necessary to determine the fate of these transplanted cells. One approach is to pre label cells prior to implantation and track them using a variety of imaging techniques. The use of superparamagnetic iron oxide for tracking of cells with MRI is ideal for this purpose. It is non-radioactive, does not require viral transfection and is already approved for clinical use as a contrast agent. The purpose of this study was to assess the effect of SPIO labelling on the redifferentiation potential of adult human chondrocytes and determine the ability to track these cells by MRI. Chondrocytes from 4 individual donors were culture expanded and dedifferentiated for two passages, labelled with SPIO and cultured for 21 days in alginate beads in redifferentiation medium. Following this period, cells were analysed for expression of cartilage related genes, proteoglycan production and collagen protein expression in triplicate. SPIO labelling did not significantly affect collagen type II expression at gene or protein level ($P = 0.6187$ and $P = 0.9491$ respectively) or any of the other parameters relative to unlabelled controls. We also demonstrated SPIO retention within the cells for the duration of the experiment and the ability to visualise *ex vivo* implanted cells in a large animal cartilage defect by MRI. This work demonstrates for the first time the effects of SPIO labelling on chondrocyte redifferentiation capacity and its potential for *in vivo* tracking of implanted chondrocytes.

(OP 71) Dendritic Cells and Host Reponses in Tissue Engineering

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The host response to such a tissue engineered construct includes an innate immune response (inflammatory response) towards the biomaterial component and an adaptive immune response towards an immunogenic biological component. Since biomaterials are used as vehicles in such combination products it is important to clarify the role of the biomaterial component in potentiating the immune responses towards the biological component due to the adjuvant effect of the biomaterial. Maturation of dendritic cells (DCs) upon encountering a pathogen or 'danger signal' is the central means by which adjuvants act in the enhancement of an adaptive immune response to an associated antigen. DCs are important in the context of biomaterials when there is a specific immunogenic biological component present which is part of the combination product. We have shown that poly(lactic-co-glycolic acid) (PLGA) acts as an adjuvant in the immune response against co-delivered antigen. As adjuvants act through the maturation of DCs, we have determined a differential biomaterial effect on DC maturation. We hypothesize that DCs use analogous mechanisms to recognize and respond to biomaterials as they use to recognize and respond to pathogens. We have identified 'danger signals' associated with biomaterial implantation site and the significance of this is being evaluated using an animal model. We are examining the pattern recognition receptors which may be involved in mediating DC recognition of and response to biomaterials, particularly focusing on toll like receptor-4 (TLR4) and C-type lectin receptors and the biomaterial associated ligands, e.g. carbohydrate modifications of adsorbed proteins.

(OP 72) Dependence of Cell Proliferation on the Morphology of Starch Based Scaffolds for Tissue Engineering

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In tissue engineering (TE), several biological, mechanical and geometrical design constraints need to be addressed in order to create an adequate micro-environment for cells response. In this study, the correlation between architectural features of the scaffold construct and cellular behavior was investigated for a blend of starch and polycaprolactone (SPCL). The aim was to determine the effects of scaffold parameters on mechanical properties on cell seeding efficiency and proliferation of human osteoblast-like cells (SaOs-2) of scaffolds obtained via Rapid Prototyping (Bioplotter) and fiber bonding. For fiber bonding, a L8 design of experiment approach was used to produce different scaffold morphologies and properties by systematically varying processing parameters. For rapid prototyping, processing parameters such as filament orientation, offset, and building layers were also varied to obtain different structural configurations.

Fiber bonding method produced randomly oriented fiber mesh with porosity values up to 75%, whereas rapid prototyping method produced a precise 3D architectures with similar porosity levels. Correlation between the mechanical performance and scaffold architecture was determined. Scaffold architecture was demonstrated to have a profound effect on cell seeding efficiency and cell proliferation. SEM micrographs and HE staining indicated cells' preference during seeding towards points of geometric constraint that resulted in higher seeding efficiencies for low values of porosity. Conversely, high values of porosity revealed to be an important aspect to enhance cell proliferation for longer culture periods. These results are an indication that scaffold architecture can be optimized according to a specific envisaged cellular response and period of culture.

(OP 73) Design of a Novel Bioreactor Employing the InCor VAD for Dynamic Conditioning of Tissue Engineered Heart Valves

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Previous studies have shown that dynamic bioreactor conditioning of tissue engineered (TE) heart valves is beneficial to their growth and maturation. To the authors' knowledge, no bioreactor system has yet employed a ventricular assist device (VAD) to host TE valves. In this study, the InCor paediatric VAD (30 mL blood chamber volume) is used to this end. The pump of the VAD system, the artificial ventricle, has two chambers: a blood and a pneumatic chamber separated by an elastic membrane. A pneumatic driver provides pulsatile air-pressure to the ventricle's pneumatic chamber, ejecting the fluid in the blood chamber. A St. Jude HP Mitral mechanical heart valve is placed in the inflow position of the ventricle. The TE heart valve is placed in the outflow position. The ventricle is coupled in series with a glass Windkessel compliance chamber, throttle and glass reservoir, connected by silicone tubings. The reservoir sits on a metal labjack used to adjust ventricular filling pressure between 0 and 7.4 mmHg. Gaseous exchange with the reservoir interior was achieved using a 0.2 μm filter. The system fits a standard incubator, and all components which contact culture medium may be autoclaved except for the ventricle, which is sterilized by ethyleneoxide. Preliminary tests with a biological valve in the outflow position achieved physiologically relevant values for pulmonary artery pressures (med/min/max = 15.7/18.3/6.5 mmHg), frequency (70 bpm) and flow output (2.1 L/min). The use of this system may better reproduce physiological pressure and flow conditions, which may improve conditioning of tissue engineered valves.

(OP 74) Designing Silk-Based 3D Architectures with Controlled Lamellar Morphology

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The impressive mechanical properties, along with established biocompatibility and slow degradability, render silk as an interesting biomaterial for bone tissue engineering applications. New silk-based scaffolds resembling bone lamellar structure were successfully developed using a freeze-drying technique. The structure could be controlled directly by the solute concentration and freezing parameters. The lamellar scaffolds disclosed a controlled and regular morphology (lamellar thickness: ~3.5 μm; interlamelar distance: ~15 μm). In order to induce water stability, different post-treatments were studied including methanol treatment, water annealing and steam sterilization. The resulting structures exhibited significant differences in terms of morphological integrity, structural details and mechanical properties. Steam sterilization preserved the structural integrity of the lamellar features, while improving mechanical properties of the scaffolds. Human bone marrow-derived mesenchymal stem cells (hMSCs) were seeded on these silk fibroin lamellar scaffolds and grown under osteogenic conditions to assess the effect of the microstructure on cell behaviour. The water annealing treatment promoted significantly improved osteogenic outcomes based on elevated alkaline phosphatase (ALP) activity and the deposition of mineralized matrix. Two-photon excited fluorescence (TPEF) was used to detect the presence of collagen, based on Second Harmonic Generation. Collagen was aligned along the morphology of the lamellar architecture, demonstrating that the lamellar morphology constituted a patterned surface onto which hMSCs cells attached, proliferated and guided the formation of new ECM. These lamellar-based constructs have potential for use in tissue engineering, were appropriate surface environments are important in the control of biological outcomes.

(OP 75) Detection Techniques for the Analysis of Protein Adsorption

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Protein adsorption is a complex phenomenon, governed by material surface properties, working conditions, protein nature itself, time dependency; deep understanding of the process can provide correlation between material properties and host tissue responses, promoting the development of efficient biomedical devices for tissue replacement and regeneration.

In order to investigate exhaustively protein adsorption we have pursued two main tasks: the choice of proper system models and suitable detection techniques.

We have modeled the process taking into account the individual contribution of protein concentration, composition of protein solution, the working conditions (time, temperature and way of incubation) as well as material surface chemistry and topography. System complexity has been increased gradually, starting from static incubation in individual protein solutions (albumin, fibrinogen and fibronectin); new variables as protein competition and incubation under dynamic conditions have been introduced step by step. The model has been tuned on Self Assembled Monolayers (SAMs) before studying protein adsorption on silk fibroin substrates.

We have obtained qualitative and quantitative information on the amount, conformation and distribution of proteins, along with real time monitoring of adsorption, by immuno-modified Atomic Force Microscopy (AFM), immunogold labeling and Surface Plasmon Resonance (SPR).

At the end we have related adsorption isotherms (provided by SPR) with protein activity and distribution (obtained by AFM) to material surface chemistry and topography, in order to correlate material properties to protein adsorption events: chemistry was effective in controlling the amount and conformation of proteins while topography had a major influence on protein distribution.

The authors thank Expertissues NoE

(OP 76) Determination of the Cell Viability of Primary Cultures of Chondrocytes by Quantitative Electron Probe X-ray Microanalysis

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Introduction: Evaluation of the cell viability is very important before the cells can be used for construction of artificial tissues and organs by tissue engineering. In this study, we determined the cell viability of isolated rat chondrocytes kept in culture at different subcultures to identify microanalytical changes associated to each cell subculture.

Materials and Methods: Rat chondrocytes were isolated from the condilar surface of the temporomandibular joint of laboratory rats, by incubating small pieces of cartilage with collagenase I at 37°C for 2 h. Primary cell cultures were cultured in DMEM medium supplemented with 10% fetal serum and several growth factors. Subconfluent chondrocytes were subcultured using trypsin-EDTA. Cell viability was determined in the first 6 cell passages by quantitative electron probe X-ray microanalysis of cells cultured on gold grids.

Results and Discussion: Electron probe X-ray microanalysis revealed that the K/Na ratio rose from the first to the third passage, and progressively decreased later on (0.07 for the first passage, 2.69 for the second, 15.61 for the third, 4.65 for the fourth, 2.46 for the fifth and 1.48 for the sixth). Intracellular concentrations of S and Cl tended to increase from the first passage, showing the highest values at the sixth passage. These results show the usefulness of this technique to evaluate cell viability and imply that cells at the third cell passage should be used in tissue engineering.

Supported by CTS-06-2191 from Junta de Andalucia.

(OP 77) Development and Characterization of Natural-Origin Bilayered Scaffolds for Osteochondral Tissue Engineering Under the Scope of Hippocrates Project

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Osteochondral tissue engineering presents a challenge to research due to requirements' combination of bone and cartilage tissue engineering. It has been accepted that bilayered structures are more adequate to regenerate an osteochondral defect. These should be able to incorporate/induce different types of cells in a favourable environment requiring different biochemical and mechanical cues, leading to the growth of two different tissues. A potential strategy to be discussed, also developed under the scope of HIPPOCRATES, is based in a hydrogel and CaP coated fibres meshes. Nevertheless, focus will be on the development of bilayered chitosan-based scaffolds produced by particle aggregation. This methodology is proposed to achieve an improved integrative bone and cartilage parts' interface, since any discontinuity is likely to cause long-term failure. An extensive characterization was carried out. Dynamical-mechanical analysis has demonstrated that scaffolds are mechanically stable in wet state under compression solicitation. Micro-Computed Tomography was performed for accurate morphometric characterization quantifying porosity, interconnectivity and pore size. In composite scaffolds, surprisingly it was observed an *in-vitro* cytotoxicity when unsintered hydroxylapatite was used. This study reports the investigation that was conducted to overcome and explain this behaviour. It is suggest that the uptake of divalent cations may be inducing the cytotoxic behaviour. Sintered hydroxylapatite was further used showing no cytotoxicity. Attention was paid primarily to chondrogenic differentiation of ATCD5 cells by developing polymeric systems that provide insulin release.

Acknowledgements: FCT (SFRH/BD/11155/2002) and EU funded projects HIPPOCRATES and EXPERTISSUES. Ana L. Oliveira and Paula Sol for their contribution for CaP fibre meshes.

(OP 78) Development and Characterization of Osteogenic Cell Sheets in an *In vivo* Model

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Despite some successes in the tissue engineering field its evolution seems to be tampered by limitations such as cell sourcing and the lack of adequate scaffolds to support cell growth and differentiation. The use of stem cells combined with cell sheet engineering technology seems a promising way to overcome these limitations. In this work bone marrow cells were flushed from 3 weeks old Wistar rat femurs and cultured in basal DMEM medium until subconfluence. Cells were then transferred to thermo-responsive dishes (3×10^5 cells/dish) and cultured for 3 weeks in osteogenic medium. Cell sheets were recovered from the dishes by temperature reduction and subcutaneously transplanted into the dorsal flap of nude mice. The histological characterization was performed before and after implantation using haematoxylin/eosin (HE) and alizarin red staining. H&E staining showed that the cell sheets before implantation were composed by a multilayer of cells embedded in a collagen matrix while the alizarin red revealed extensive calcium deposition. A similar analysis of the implanted cell sheets showed a good integration with host tissues without visible inflammatory response. Calcium deposition was visible several cell layers above the flap's basal cells both after 3 and 6 weeks of implantation suggesting the induction of mineralization by the implanted cell sheets. The present work shows promising results concerning the application of cell sheet engineering for bone-related applications and future work may confirm the suitability of this powerful technique for bone tissue engineering purposes.

(OP 79) Development of a Patient-Specific Autologous Hydrogel Suitable for Cellular Delivery Across a Spectrum of Tissue Engineering Applications

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In this study an extracellular matrix hydrogel scaffold validated as a candidate for both *in vivo* and *in vitro* cell expansion was derived exclusively using the components of a patient's own blood, so as to overcome any histocompatability issues.

Blood was fractionated into platelet poor plasma and then was gelled by the addition of either basal or bespoke cell culture media, creating a stable hydrogel at 37°C. This hydrogel was demonstrated to be a suitable substrate for the culture of both lineage committed cells, including articular chondrocytes and dermal fibroblasts, and multipotent cells such as human dental pulp progenitor cells and mesenchymal stem cells derived from umbilical cord and bone marrow. The proliferation of the cells was greater than those cultured in Matrigel. The gel also permitted microvascular formation from blood-derived endothelial cells.

Cell-loaded hydrogels were implanted in rats and NOD/SCID mice using an injection strategy which allowed the components of the gellation system to mix at the point of implantation, allowing the precise delivery of the gel into a defect site using a minimally-invasive procedure. The gel supported the expansion and phenotypic maintenance of primary dermal fibroblasts and osteoblasts for extensive periods *in vivo*.

The hydrogel supported the proliferation of a broad range of clinically-relevant primary human cells *in vitro* and demonstrated translation into an *in vivo* model. This is an excellent candidate for use as a non-immunogenic, autologous scaffold enabling the precise localisation of cells into a defect using minimally-invasive surgery.

(OP 80) Development of Porous Scaffolds by Template Method

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Porous scaffold is an important factor for tissue engineering to accommodate cells, control their functions and guide new tissue

formation. Many methods have been developed for scaffold preparation. However, precise control of porous structures such as interconnectivity and open surface structure remains challengeable. In this study, we developed a template method for the preparation of porous scaffolds by using biodegradable polymer template and ice particulates. Two kinds of collagen scaffolds were prepared. One is collagen mesh that was prepared by using a synthetic poly(DL-lactic-co-glycolic acid) knitted mesh as a template. At first a hybrid mesh of PLGA knitted and collagen was prepared by forming web-like collagen microsponges in the openings of a PLGA knitted mesh. And then the PLGA knitted mesh was selectively removed. The interconnected porous structure of collagen mesh was confirmed by SEM observation. Another collagen scaffold is collagen sponge with open surface structure prepared by using embossing ice particulates as a template. Ice particulates were formed on a plate and collagen aqueous solution was poured onto them. Collagen sponge with a surface structure of open big pores and a bulk porous structure of small pores was prepared after freeze-drying. These collagen scaffolds were used for cell culture of human dermal fibroblasts. The fibroblasts adhered and proliferated in the collagen scaffolds. The open surface and interconnected porous structures facilitated cell seeding and homogeneous cell distribution. The template method is a useful technique for the preparation of precisely controlled porous scaffolds for tissue engineering.

(OP 81) Development of Regenerative Therapies Using Genetically Modified Cell Transplantation

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Cell therapy may provide novel approaches to aid tissue regeneration. Genetic modification of cells may enhance the therapeutic effect and cell therapy may be considered a form of gene delivery given the homing characteristics of these cells. Thus combined gene and cell therapy approaches hold great potential for future therapeutics. A large number of vector systems exist for genetic modification of cells. These include non-viral means such as liposomes, adenovirus, adenoassociated virus and lentivirus. Mesenchymal stem cells and endothelial progenitor cells may have therapeutic use in a broad variety of diseases such as myocardial infarction and peripheral vascular disease. We have demonstrated that adenoviral vectors are a highly efficient gene delivery system for both cell types. Lentivirus can efficiently transduce mesenchymal stem cells but are randomly integrated into the host cell genome.

Autologous cell transplantation has advantages in terms of avoidance of allogeneic immune responses but disadvantages as the cells may be dysfunctional when harvested from diseased individuals. We explored this in the context of using endothelial progenitor cells as an autologous therapy for diabetic vascular disease. Endothelial progenitor cells were found to be dysfunctional in patients with diabetes mellitus. We showed that genetic modification of endothelial progenitor cells harvested from diabetic animals with an adenoviral vector encoding eNOS improved the ability of these cells to enhance wound healing.

Thus genetic modification of stem cells may enhance the therapeutic effect especially in the context of autologous cell transplantation.

(OP 82) Dexamethasone-Loaded Carboxymethylchitosan/Poly(Amidoamine) Dendrimer Nanoparticles Enhances Bone Formation *In Vivo*

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Dexamethasone-loaded carboxymethylchitosan/poly(amidoamine) dendrimer nanoparticles, CMC/PAMAM-Dex were successfully synthesized to find applications as a controlled system of relevant molecules in Bone Tissue Engineering. These are aimed at modulating the proliferation and differentiation of stem cells, both *in vitro* and *in vivo*. In previous work, we have demonstrated that CMC/PAMAM-Dex nanoparticles are internalized with high efficiency by different cell types, namely osteoblastic-cells, SaOs-2 and rat bone marrow stromal cells, RBMSCs. The biocompatibility of HA and SPCL scaffolds was also assessed by means of seeding RBMSCs onto the materials and performing a luminescent cell viability assay, after 24 and 72 hrs.

In this work, the ability of the nanoparticles to induce the osteogenic differentiation of RBMSCs and bone formation was investigated by exposing the nanoparticles (dispersed in culture media) to RBMSCs during expansion period, then cells were trypsinized and seeded (cell number of 1×10^6 per scaffold) overnight onto the hydroxyapatite, HA and starch-polycaprolactone, SPCL scaffolds. Afterwards, the constructs were implanted subcutaneously on the back of F344 rats for the period of 4 weeks. After the implantation period, the animals were sacrificed and constructs retrieved. Micro-Computed Tomography (μ-CT) analysis, ALP activity, osteocalcin content (ELISA), and calcium content were performed to investigate new bone formation. For routine examination of undecalcified constructs, Haematoxylin & Eosin (H&E) and Toluidine blue staining were also carried out. Results have shown that CMC/PAMAM-Dex nanoparticles are biocompatible and enhanced new bone formation as compared to controls (RBMSCs/scaffolds that were not exposed to nanopartiles), *in vivo*.

(OP 83) Different Configurations of Membranes for Bioartificial Liver Application

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Fulminant hepatic failure and acute episodes in chronic liver failure are associated with high mortality due to a sudden loss of liver functions. Organ transplantation is the only treatment available to improve patient survival but shortage of donors worldwide

results in approximately 20% of mortality. A temporary liver support system is thus required to bridge patients to transplantation or to help them recover native liver functions.

BioArtificial Liver (BAL) is an extracorporeal system which permits to exploit the potential performances of hepatocytes immobilized into bioreactors to replace main liver functions. Cells cultured in 3D environment exhibit higher activity in comparison with monolayer culture. In addition, a mechanical barrier should be placed between cells and host to prevent immune rejection. This barrier can under different configurations: spherical membrane encapsulating the cells–alginate bead- or hollow fiber membranes where the cells are located outside.

Our goal is to compare both configurations in term of mass transfer phenomena in order to help for the best choice for future bioartificial liver. Several solutes with different molecular weight are considered for transfer such as ammonia, lactate, glucose, vitamin B12, albumin, alpha-foetoprotein with and without the presence of human hepatic C3A cells.

The effects of bead diameter and the porosity are also studied. The biological behaviours are then analyzed though mass transfer models taking into account the synthesis or consumption rates.

(OP 84) Differential Gene Expression of Transcription Factors During Multilineage Differentiation of Human MSC

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To characterize transcription factors involved in adipogenic, osteogenic or chondrogenic stimulation of MSC.

Methods: Human bone marrow MSC from 3 independent donors were induced for adipogenic (DEX, insulin, indomethacin IBMX), osteogenic (DEX) and chondrogenic (pellet culture with BMP2 or TGFß3 or fibrin/PLGA 3D-culture with TGFß3) differentiation. Cartilage and fat tissue was derived from hip surgery and osteoblasts by outgrowth from squamous bone. Hybridization was performed on HG-U133Plus2.0 GeneChips and analysis with GCOS 1.4 and the SiPaGene database (www.bioretis.de).

Results: Selection for more than 80% change increased revealed 2154 probesets for adipogenic, 690 for osteogenic and 2422 to 2748 for each of the three chondrogenic differentiations. 147 transcription factors according to gene ontology were found involved in adipogenic (e.g. C/EBPα, PPARγ2), 40 in osteogenic (e.g. MSX2) and 171 to 220 in chondrogenic differentiation (e.g. Sox5, Sox6, Sox9, Runx3). Comparative analysis of MSC, fat, bone and cartilage enabled to develop a tissue specific score for each gene including transcription factors. Differentially expressed genes as well as the signal intensity of the best marker genes were used to scale the quality of tissue specific differentiation. For the three chondrogenic differentiation assays, 3D-culture with PLGA revealed the best quality with highest levels of collagen type two expression comparable to native cartilage.

Discussion: Extensive comparative analysis is necessary to comprehensively describe the network of transcription factor activity in tissue specific development. Three different assays and qualities of chondrogenic differentiation demonstrated how to optimize gene selection for the most important candidates for functional validation.

(OP 85) Donor Lymphocyte Infusion in Patients with Haematological Diseases

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Donor lymphocyte infusion (DLI) after allogeneic haematopoietic transplantation (alloHT) is an effective therapy for some patients with recurrent haematological diseases; in high risk patients with mixed chimerism it has been used to prevent relapse. Delayed lymphocyte add-back after transplants with selection of CD34+ cells prevent graft failure, decrease risk of relapse and graft-versus-host disease (GVHD). The aim of this review was to study efficacy, toxicity and long-term outcome.

We studied 54 patients(35 male/19 female) between 1994 and 2007; median age:32.5(7-59) years old. Diagnosis: Chronic Myeloid Leukemia(CML) 20/Acute Leukemia(AL) 20/Multiple Myeloma (MM) 6/others 8. Graft source: peripheral blood 46/bone marrow 9 (one also 2 blood cords); 4 donors unrelated. Conditioning regimen: myeloablative 38/reduced intensity 16. T-cell depletion of the graft: 25. GVHD prophylaxis: single agent 20/double 34. Median CD34+ and CD3+ cells infused with the graft: 6.71(0.62–13.43) and 0.31(0.01–596) respectively.

DLI was therapeutic in 42, pre-emptive in 5 and as T cell add-back in 7. Median CD3+ lymphocytes infused: 1×10^7 kg in 71% of DLI. Median interval transplant-first DLI: 8 months (1–60). Others therapies for relapse: imatinib-dazatinib 10/chemotherapy 9/second alloHT 6/others 3. Thirty-seven patients developed GVHD. Patients alive: 31 (22 complete remission/9 disease); main cause of death: disease progression. There was remission in 16 of therapeutic DLI (12/19CML;2/2AL;1/5MM;1/2others), 5 months.

This form of adoptive cell therapy is a therapeutic option in selected patients after alloHT, with better results in CML. Overall survival after 1st DLI was 50.4% (±8.2%) at 8 years. GVHD was the most frequent complication, otherwise it was well tolerated.

(OP 86) Downregulation of Osteogenic BMP and Cartilage Matrix Degrading MMP Gene Expression in Delayed Compared to Standard Bone Healing

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The aim of this study was to compare the temporal expression pattern of factors related to cartilage and bone formation and endochondral ossification during standard and delayed bone healing for a more in-depth understanding of the molecular basis of disturbed bone healing and to elucidate timely adapted substitution of factors to stimulate the healing process.

A tibial osteotomy was performed in two groups of sheep ($n = 30$ each) and stabilized with either a rigid external fixator leading to standard healing or with a mechanically critical one leading to delayed healing. Hematoma/callus tissue was harvested

4, 7, 14, 21 and 42 days postop. qPCR was employed to determine the expression patterns of BMPs and other molecules.

Gene expressions of BMP2, BMP4, BMP7, Nog, MMP9 and MMP13 were distinctly lower in the delayed compared to the standard healing group at several time points from day 14, whilst no differential gene expression of Coll II and Coll X was found between both groups. Among the BMPs, BMP7 showed the most markedly differential expression. The first evident difference in BMP7 expression between both groups was found at day 14 suggesting that exogen substitution in the context of a therapeutic approach should be postponed. The differential expression pattern of both MMP9 and MMP13 suggests that there might be a failure in endochondral ossification in delayed bone healing.

Downregulation in gene expression of osteogenic BMPs and cartilage matrix degrading MMPs may account for a considerable delay of bone healing.

(OP 87) Drug Releasing Hybrid Scaffold: New Avenue in Cardiovascular Tissue Engineering

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Clinical application of tissue engineered vascular graft (TEVG) still needs to overcome several limitations. Our aim was to evaluate a novel hybrid device able to simultaneously provide a specific microenviroment to allow cell differentiation and to function as a drug delivery system.

We developed a multilayer hybrid construct with a middle pivotal fibronectin-collagen network and two functionalized Polylactic acid (PLLA) layers on its inner and outer sides. The inner side layer was realized as a drug delivery system releasing Heparin (HEP) useful in the immediate management of the scaffold engraftment, and Erythropoietin (EPO), important in differentiation and recruitment of endogenous stem cell populations.The outer one was engrafted with TGF- for muscular differentiation.

PLLA/HEP-EPO nanocomposite scaffolds were prepared by electrospinning. Polymer solutions containing 13% w/w PLLA alone or 13% w/w PLLA with HEP and EPO solution were electrospun (15 kV) forming a non-woven cloth. Membranes microstructure was evaluated by Scanning Electron Microscopy. After sterilization scaffolds were seeded with human mesenchymal stem cells and cell engraftment, viability, proliferation and differentiation were evaluated by light and confocal microscopy.

Mean fiber diameter was $1.5 \pm 0.9\,\mu m$ with a porosity favorable to cell attachment and culture. Both HEP and EPO release showed an initial burst within the first 24 hrs and a further smoother pattern. Microscopy was consistent with a good cell attachment and viability with changes in cell morphology resembling to endothelial phenotype.Detection of ki67+ cells confirmed cell proliferation and suggested the presence of a favourable mircorenviroment. A shift towards CD31 positivity could be observed in the functionalized scaffold.

(OP 88) Dynamic Mesenchymal Stem Cell Culturing for Bone Tissue Engineering

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Mesenchymal stem cells (MSCs) are a widely used cell source in tissue engineering and regenerative medicine because they can be expanded and differentiated easily *in vitro* as well as *in vivo*. MSCs can be obtained from a variety of tissues including adipose tissue. Static cell culture with the aim of tissue engineering applications has been proven to be disadvantageous since constructs grown in static cell culture lack the mechanical stability native tissue exhibits. Mechanical stimulation has therefore become a substantial tool in tissue engineering to accustom cells to their future physically active environment. Moreover, differentiation of stem and progenitor cells has been proven to be supported by mechanical strain. In this work, the effect of mechanical strain on the differentiation of a preosteoblastic cell line and adipose tissue derived mesenchymal stem cells was analysed. Nutrient supply especially on 3D scaffolds is heterogeneous in static cell culture leading to reduced growth rates or even death of the tissue on the scaffold. Thus, a specifically customised bioreactor systems have been developed to control tissue growth under dynamic culture conditions. In this work cultivation of adMSC on 3D scaffolds under controlled and reproducible conditions in a rotating bed system bioreactor is presented.

(OP 89) Dynamic Studies of Biomimetic Coated Polycaprolactone Nanofiber Meshes as Bone Extracellular Matrix Analogues

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This work aimed at studying the effects of dynamic culture conditions and biomimetic coating on bone cells grown on nanofiber meshes. In our previous work, biomimetic calcium phosphate coated polycaprolactone nanofibre meshes (BCP-NM) proved to be more efficient for supporting cell attachment and proliferation under static conditions, when compared to polycaprolactone nanofibre meshe (PCL-NM). However, no studies on the influence of bioreactors on the behaviour of cells cultivated on these materials were developed so far. In fact, *in vitro* cultivation of constructs using bioreactors which support efficient nutrition of cells has appeared as an important step toward the development of functional grafts. In the current work, osteoblast-like cells were seeded on both BCP-NM and PCL-NM. The formed constructs were cultured in a rotating bioreactor (Synthecon, RCSS-1, USA), for different time periods. Cell morphology and viability were assessed by confocal

microscopy. DNA and total protein quantifications were performed accordingly. No significant difference in cell proliferation was observed in BCP-NM and PCL-NM constructs kept under dynamic conditions when compared to the results obtained from static studies. However, under dynamic conditions, total protein contents were higher on BCP-NM constructs than in the uncoated ones. Moreover, PCL-NM presented a higher number of dead cells than BCP-NM. Obtained results point out that BCP-NM can support cell growth under dynamic conditions. The vast potential of using BCP-NM in applications related to bone tissue engineering was also confirmed.

Marie Curie Actions ''Alea Jacta Est Training Fellowship'' and the European NoE EXPERTISSUES (NMP3-CT-2004-500283) supported this work.

(OP 90) Dynamics and Interactions of Hemoglobin in Red Blood Cells

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We studied molecular dynamics and interactions of hemoglobin in whole human red blood cells with Incoherent Elastic Neutron Scattering and Small Angle Neutron Scattering. Thermal neutron wavelengths and energies match atomic distances and atomic thermal motions and allow their study in biological material without damage. Protein dynamics were measured on time scales of around 100ps which reflects protein side chain motions and long time self diffusion of the whole protein.

We could show that there occurs a transition of hemoglobin dynamics at human body temperature of 37°C in cells and solution. The measurements show that there is a break in the long term self diffusion coefficient at human body temperature. Complementary from our interaction studies, we found that hemoglobin start forming aggregated clusters at human body temperature.

(OP 91) Educational Aspects of Tissue Engineering: an Industrial Perspective

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Tissue engineering is a branch of regenerative medicine that attempts to convert insights from biomedical research into novel therapies composed of cells, growth factors and/or biocompatible structural materials. It aims to (i) repair, rather than replace damaged tissues; (ii) postpone radical surgery; and (iii) stimulate endogenous healing responses.

Society's need for these new clinical approaches, (e.g. biological therapies for cartilage repair), is indisputable and growing in line with the aging population. Unfortunately, the commercial and clinical success of tissue engineering has been poor to date because the early innovators failed to appreciate the need to resolve all the scientific, clinical, regulatory, commercial and logistical issues involved in bringing a complex and unfamiliar therapy from 'bench to bedside'.

Educators should therefore emphasise the multidisciplinary nature of regenerative medicine to students. They should stress that in order to be successful a new product must simultaneously resolve all the problems associated with its introduction (e.g. (i) definition of clinical need; (ii) cell/molecular biology of the product; (iii) structural materials and biomechanics; (iv) scale-up and production; (v) patents and IPR; (vi) regulatory approval and quality control; (vii) distribution, inventory control and logistics; (viii) immunology and graft integration; (ix) patient selection and adoption into clinical practice; (x) proof of cost-effectiveness and re-imbursement; and (xi) time to market and return on investors capital). Curricula, should encompass these topics and the management approaches (e.g. open innovation) required to build and maintain the multidisciplinary teams needed to develop regenerative medicine.

(OP 92) Educational Aspects of Tissue Engineering: the Academic Perspective

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Everyone recognises that tissue engineering is a rapidly emerging, multidisciplinary subject that, so far, is not grounded in traditional academic courses. A multitude of departments, institutes and centres concerned with tissue engineering have emerged in Universities throughout the world, but almost wholly being research or clinically oriented. This has been inevitable and, as with any emerging subject, is has been very sensible to identify its boundaries before constructing educational courses. The time is now coming, however, where there is a role for new courses, in order to provide for this education.

The subject of biomaterials provides an example of how this could be done since 40 years ago this subject was similarly in its infancy. We have witnessed the introduction of courses at undergraduate and postgraduate levels, some based in medical schools and others in engineering schools. We cannot pretend that we have always got this right and it is no means obvious that either the industrial or clinical end-users of the student output have fully benefited. The most obvious question has been whether it is possible or necessary to train 'biomaterials scientists' or for students to follow a major relevant discipline, for example materials science, cell biology or mechanical engineering, perhaps to PhD level, and then to develop the skills and knowledge for the transition into biomaterials science.

It is essential that the field of tissue engineering learns these lessons; this presentation will explore the options.

(OP 93) Educational Aspects of Tissue Engineering: the Perspective of Students and Young Investigators

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The Students and Young Investigators Section (SYIS) of TERMIS-EU aims to bring together an international community of students and young investigators engaged or interested in different aspects of science and technology of tissue engineering and regenerative medicine.

An essential aspect to accomplish this purpose is to promote education improving informed and current discussion on the scientific challenges and therapeutic developed technologies. A platform for the ''next generation'' of scientists in the field of tissue engineering and regenerative medicine can only be generated if their opinion, career expectations, motivations and needs are considered.

Several issues related with the multidisciplinary nature of regenerative medicine field were assessed within the S & YI section. This survey will give an outlook on different issues including: (i) opinion on Master/PhD/Post-Doc programmes; (ii) most relevant subjects/skills to include in their respective programmes; (iii) best format to deliver this information and enhance professional development; (iv) awareness of the students for activities, such as short courses, currently being organized; (v) understanding of the development of the area and the value of their contribution; and (vi) how S & YI see their future in a tissue engineering and regenerative medicine related area.

These issues will be explored in this presentation having in consideration the perspectives and opinions of Students and Young Investigators of different nationalities and from different backgrounds.

(OP 94) Effect of Hypoxia on Vascular Endothelial Growth Factor Expression in an Engineered 3D Cell Model

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Tissue hypoxia results in rapid angiogenesis *in vivo*, triggered by angiogenic proteins, including vascular endothelial growth factor (VEGF). We have developed an *in vitro* model using dense cell-seeded collagen constructs to engineer hypoxic gradients which can be quantified using a sensitive O_2 probe in real-time.

We have used 3 cell types: human dermal fibroblasts (HDF), bone-marrow derived stromal cells (BMSC) and pulmonary artery-derived smooth muscle cells (PASMC) seeded in collagen constructs at 2×10^6 cells, constructs were compressed, spiralled and statically cultured for up to 10 days. Levels of O_2 in the core fell from 145 mmHg at 20 mmHg for HDF's, 10 mmHg for PASMC's and 8 mmHg for BMSC's after 24 hours of static culture. Levels of O_2 in surface layers were 145 mmHg for all constructs. Following O_2 monitoring over 10 days, mRNA was extracted from spatially distinct zones to reflect O_2 gradients from core to surface of constructs. VEGF was up-regulated 10-fold in the core of HDF constructs after 7 days in culture, correlating low O_2 levels to increased angiogenic signalling. Further work is underway to establish signalling in other cell types. There was no evidence of decreased cell viability for HDF's within all zones of the construct, however BMSC's and PASMC's had lower viability in the core, and therefore are more sensitive to low O_2 levels.

This study raises the possibility of engineering naturally occurring angiogenic signals for induction of vascularisation in such 3D tissue engineered constructs post-implantation by manipulating cell density (hence O_2 consumption) to control cell responsive signalling.

(OP 95) Effect of Matrix Stiffness on Cellular Responses

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Several cell types respond to mechanical forces, key to *in-vitro* remodeling. We have tested the differential response of human dermal fibroblasts (HDFs) and human bone marrow stem cells (HBMSCs) using increasingly stiffer collagen matrices with the mechano-molecular regulation of specific extracellular matrix genes, following ramp load regimes.

Strain was applied using a computer driven tensional-Culture Force Monitor (t-CFM). Cell seeded collagen constructs were subjected to pre-strain (0% and 5%) and kept under tension. Ramp load applied was: a) 10% ramp over 1h, b) 10% ramp over 12 h pre-strain level (i.e 0% and 5%). Molecular outputs from constructs were also investigated. Both HBMSCs and HDFs showed no significant increase on force generation following ramp load stimulation, with either 10 and/or 20% FCS. ECM regulatory genes TIMP-2 and COL-3 showed up regulation, with 10% FCS, at 5% pre strain and ramp load over 1h. In contrast, genes MMP-2 and COL-1 showed down regulation at the same groups. At 20% FCS genes showed no regulation over 1h ramp loads, instead the slow ramp load 12h led to, again, MMP-2 down regulation and TIMP-2 up regulation, but up regulation of COL-1. HBMSCs showed no significant gene regulation at 10% FCS, and only minimal though significant down regulation of COL-1 with 20% FCS and slow ramp load regimes. ECM remodeling gene-regulation and turnover is important when stiff constructs, ramp rate and FCS levels are combined in bioreactors. This has major implications in TE, specifically in tissues where load bearing is a major function.

(OP 96) Effect of Membrane Surface on Hippocampal Neuronal Cell Differentiation

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Biomaterials such as membranes have become of great interest, since they offer the advantage of developing neuronal tissue that may be used for *in vitro* simulation of brain function. In an attempt to develop a membrane biohybrid system constituted of membranes and neurons the behaviour of neurons isolated from the hippocampus of the hamster Mesocricetus auratus were studied on membranes with different morphological properties.

Polymeric membranes in polyester (PE), modified polyetheretherketone (PEEK-WC), fluorocarbon (FC) and polyethersulfone (PES) coated with poly-L-lysine with different morphological surface properties (e.g., pore size, porosity and roughness) were used as substrates for cell adhesion.

Confocal and SEM analyses demonstrated that in response to varying the roughness of the membrane surface, hippocampal neurons exhibited a different morphology. Indeed cells grown on smoother membranes differentiated with a large number of neuritis with consequent formation of bundles. As a consequence while a very complex network was formed on FC membrane, cells tend to, instead, form aggregates and most of the processes are developed inside the pores of the membranes when rougher PEEK-WC surfaces were used. Metabolic results in terms of glucose consumption, lactate production and BDNF secretion confirmed the effect of roughness on the cell behaviour: neurons exhibited BDNF secretion at high levels on FC membranes with respect to the other membranes. Taken together these results suggest the pivotal role played by membrane roughness in the adhesion and differentiation of the hippocampal neurons and may thus constitute a valuable approach for future neurobiological studies.

(OP 97) Effect of Surface Modification on Morphology, Mechanism and Function of Human Adipose-Derived Stem Cells

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The effect of surface modification by using three materials, chitosan, gelatin and poly-ornithine on the morphology, mechanism and cell adhesion function of human adipose-derived stem cells (hADSCs) were observed under atomic force microscope (AFM), and the chemical actions between surface coatings and hADSCs were further investigated by using Raman spectra. The mechanism and morphology of hADSCs were observed under AFM, the existence on the glass slides modified with above three materials with different concentrations of 2%, 1%, 0.5% and 0.25% (w/w) respectively were also qualitatively assayed. Then, the growth condition and distribution of hADSCs on different coated surfaces were surveyed under inverted microscope and by hochest/PI double stainings. The mechanism of hADSCs and the effect of chemical actions between these materials and cells on cellular structure and performance were analyzed at molecular level. The results showed that the existence of surfaces modified with different concentrtions presented tremendous difference, the mean sizes of surface modificaitons were decreased with the degrade of concentration. The surface modified by protein groups showed rugged and rough with high-low protuberance, resulting in the comparative large rugosity. But to chitosan, the surface appeared very smooth and lower rugosity. The cell quantity and conditions on the surface modified by protein groups were clearly better than that of chitosan. The cellular structure and characteristic functional group of hADSCs were analyzed and dertemined by Raman spectra. It is indicated that the surface modification and its morphology play important roles to the cellular structure and function of stem cells.

(OP 98) Effective Nutrient Supply through Multi-Layer Scaffolds

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Introduction and Method: In tissue engineering scaffolds, the delivery of nutrients to cells and removal of waste products and cell metabolites is of outmost importance.

In previous work [1] we presented the preparation of highly porous (porosity $> 80\%$) micropatterned poly(l-lactic acid) (PLLA) sheets and showed the potential of multi-layer staking these sheets in 3-D constructs. The porous micropatterned sheets are prepared by one-step fabrication method Phase Separation Micromolding (PSμM) which is based on immersion precipitation on a micropatterned mold replicating the micropattern into the polymer sheet.

Nutrient transport can take place through the inner-porosity of the sheets as well as via perfusion of the micropatterned channels.

This work focuses on nutrient supply through these layers. Glucose and culture medium transport through the sheets with and without cells is studied to investigate the effect of the cell sheet layer on nutrient transport.

Results and Discussion: Modeling shows that nutrient diffusion is sufficient up to a few sheet layers for static culturing and low cell density. However, for clinical relevant constructs ($\geq$1 mm thickness) more sheets are needed and nutrient transport through the sheets in static culture is not sufficient anymore. Preliminary experimental results confirm these predictions.

The preliminary experiments indicate as well that the cell sheet layer might in fact be the limiting step in nutrient transport through the porous sheet layers, and perfusion of the micropattern channels is required to ensure sufficient nutrient transport through the complete multi-layer scaffold.

Reference:

[1]Papenburg, B.J, *et al*., Biomaterials 28(2007), 1998

(OP 99) Electrospun Polymeric Meshes for Application in Bone and Cartilage Regeneration

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A successful approach in tissue engineering practice is related to the development of bioactive scaffolds with proper biocompatibility, morphology and mechanical strength. In this work, the evaluation and processing of raw polymeric materials, the characterization of the manufactured systems and their culturing in dynamic environment are performed. Three-arm star poly(epsilon-caprolactone) (PCL*), 88% hydrolyzed polyvinylalcohol (PVA) and poly(lactic-co-glycolic acid) (PLGA), were processed by means of a custom-

ized electrospinning apparatus allowing control over applied voltage, solution flow rate, aeration and collection shape and size. Processing conditions were optimized and correlated to morphology and mechanical properties of the collected meshes. Morphology and mechanical properties of PCL* meshes were significantly affected by flow rate (e.g. fiber diameter of 1,5 micron at 1 ml/h and 2,75 micron at 16 ml/h). Cytocompatibility *in vitro* tests on these meshes showed cell viability at 12th day. PVA meshes displayed fiber size ranging between 100–500 nm and strength and strain at break comparable to those of human articular cartilage. Mesh stabilization in aqueous environment was accomplished by glutaraldehyde crosslinking, without affecting fibers morphology. Regarding PLGA fibrous meshes, the fiber size depended mostly on voltage and flow rate, varying within 1 and 5 micron. The loading of active agents was also approached by preparing albumin-loaded PVA meshes and retinoic acid-loaded PLGA meshes. Preliminary testing showed encouraging results in terms of effective loading and controlled release. Finally, in order to perform dynamic cell culture onto the optimized meshes, a bioreactor system allowing for the employment of either a perfusion or a rotating wall chamber was designed.

(OP 100) Electrostatically Spun Scaffolds with Controlled Topographies Induce Changes in Stage-Specific Schwann Cell Development, Myelination and Functioning

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A tissue engineered solution to peripheral nervous system damage or disease is increasingly sought. The Schwann cell is vital in maintaining, regenerating and protecting these peripheral nerve structures and cells, and in its ability to develop lineage-specific functionality, particularly myelination. The aim of this work was to investigate the potential of electrostatically spun scaffolds, with controlled topographies, to control the cellular development and specific functioning.

A range of polyurethane scaffolds (Tecoflex® SG-80A) was electrostatically spun using different spinning parameters. Scaffolds were characterised using SEM, AFM, image analysis and FTIR, for inter-fibre separation, fibre diameter, surface roughness, void fraction, fibre orientation and surface chemistry. UV-Ozone sterilised scaffolds were cell-seeded with human Schwann cells (5×10^4 cells per $1cm^2$ of sample), for culture periods of 1, 2, 4, 7, 14 and 28 days. Immunohistochemistry for a range of extracellular matrix, adhesion, function and developmental stage-specific markers was analysed. SEM and image analysis examined cell coverage, number, spreading, orientation and cellular interactions.

All scaffold forms were determined through the fabrication conditions. All aspects of underlying topographical features directly induced changes in cellular behaviour, with increased topography producing a ''lag'' in the cellular responses. The scaffold properties were also significantly linked to differences in the adhesion mechanisms, with high upregulation of extracellular matrix molecules correlating with increased topographical features. Topography also correlated with cell development, with individual scaffolds directly inducing stage-specific changes in myelin production and cell functionality.

Electrostatically spun PU scaffolds showed significant potential as controllable scaffolds for application-specific peripheral nervous system tissue engineering.

(OP 101) Enabling Technologies for Tissue Engineering and Regenerative Medicine

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Tissue engineering and regenerative medicine has emerged as an innovative scientific field that focuses on development of new approaches to repairing cells, tissues and organs for clinical applications. Recent advances in this field have offered new therapeutic opportunities that facilitate the restoration and maintenance of normal tissue function. Consistent with the goals of tissue engineering and regenerative medicine, we have followed a strategy that has shown to be effective clinically. This approach involves the use of biocompatible matrices either with or without cells. The matrices are either used as cell delivery vehicles or as scaffolds to promote and enhance tissue regeneration. When cells are used, donor tissue is dissociated into individual cells, which are expanded in culture, attached to a support matrix, and re-implanted into recipient for recovery of tissue function.

Over the past several years, research investigations have been actively pursued in various tissue systems using a paradigm for tissue engineering and regenerative medicine that utilizes combinations of biomaterial scaffolds and cells. Although these engineered tissues have shown their potential applicability experimentally, only a limited number have successfully translated to clinical trial. This is due to various obstacles encountered in the tissue building process. Recent advances in novel biomaterials, new sources of cells, and scaffold fabrication strategies are being applied to these challenges. Significant progress has already been made in the development of the next generation of enabling technologies.

(OP 102) Endothelial Adhesion Molecule Expression on Electrospun Small Diameter Vascular Grafts

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The introduction of new vascular substitutes requires extensive preclinical characterization e.g. assessment of phenotypical changes of endothelial cells (EC) occurring after attachment to artificial scaffolds. In this study spontaneous attachment, proliferation, and adhesion molecule expression of EC seeded onto electrospun, small diameter polyurethane grafts was studied *in vitro*.

Human EC were seeded onto the luminal surface of the prostheses without additional pre-coating. Cell attachment and expression patterns of the endothelial adhesion molecules E-Selectin, ICAM-1, and VCAM-1 were studied using an immunogold labeling technique, scanning electron microscopy, and energy dispersive X-ray analysis (EDX). Mechanical properties of the prostheses were characterized by suture force measurement.

Vascular prostheses with an inner diameter of 2 mm, a uniform wall thickness of 0.1 mm and fiber diameters ranging from 500 to 2500 nm were fabricated. Electrospun polyurethane grafts showed a two-fold higher resistance to suture forces compared to native arteries. EC attachment was easily achieved without pre-coating the fiber matrix. Stimulation of EC with interleukin-1beta led to a significant upregulation of the adhesion molecules investigated. EDX quantitation showed no differences in the stimulatory responses of EC cultured on electrospun polyurethane in comparison to cells grown on tissue culture-treated coverslips.

We have shown that proper adjustment of the electrospinning parameters allows for the production of small diameter polyurethane grafts which are suitable for spontaneous EC attachment. The underlying synthetic graft surface did not impair the endothelial response toward IL-1 stimulation or adversely affect the regulation of adhesion molecules known to be crucially involved in endothelial-leukocyte interactions.

(OP 103) Endothelial Progenitor Cell Dysfunction in Patients with Progressive Chronic Renal Failure

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Patients suffering from end-stage renal failure are plagued by vascular complications such as thrombus formation of the hemodialysis shunt. Regenerative medicine aims to relieve these vascular symptoms by amongst others tissue engineering of autologous vascular shunts, combining endothelial cells and biodegradable materials. Autologous endothelial cells can be isolated from progenitor cells in the circulation from two distinct cell populations; the rare CD34-positive endothelial progenitor cell (EPC) and the more common CD14-positive EPC. However, vascular diseases often appear to associate with impaired EPC function. We hypothesize that patients with chronic renal failure (CRF) also have impaired EPC function. We therefore assessed the EPC numbers in the circulation of CRF patients during disease progression and dialysis treatment. Furthermore, we assessed angiogenic differentiation and endothelial cell function of these patient-derived EPC after culture on biodegradable diureidopyrimidinone polycaprolactone (PCLdiUPy). The frequency of circulating CD14-positive EPC in the circulation of CRF patients did not differ from healthy controls. In contract, CD34-positive EPC numbers decreased in all patient groups. *In vitro*, EPC of CRF patients showed a strongly reduced adherence to PCLdiUPy and a strongly reduced capacity for angiogenic differentiation. This was observed for all stages of CRF. Noticeably, the antithrombogenic behavior from EPC-derived endothelial cells did not differ between CRF-patients and healthy controls. In conclusion, autologous EPC from CRF patients are inappropriate for tissue engineering of hemodialysis shunts. Further research should reveal the causes of reduced adherence and aim at the development of 'smart' biomaterials that augment adhesion and differentiation.

(OP 104) Engineered Cartilage Generated by Nasal Chondrocytes is Responsive to Physical Forces Resembling Joint Loading

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We tested whether Engineered Cartilage generated by Nasal chondrocytes (ECN) is responsive to different regimes of loading, associated with joint kinematics and previously shown to be stimulatory of Engineered Cartilage generated by Articular chondrocytes (ECA).

Human nasal and articular chondrocytes, harvested from 5 individuals, were expanded and cultured for 2 weeks into porous polymeric scaffolds. The resulting ECN and ECA were then maintained under static conditions or exposed to the following loading regimes. Regime1: Single application of cyclic deformation for 30 minutes. Regime2: Intermittent application of cyclic deformation for a total of 10 days, followed by static culture for 2 weeks. Regime3: Application of surface motion for a total of 10 days.

Prior to loading, ECN constructs contained significantly higher amounts of glycosaminoglycans (GAG) (1.7-fold) and type-II collagen (1.7-fold) than ECA. ECN responded to Regime 1 by increasing collagen (1.4-fold) and proteoglycan synthesis (1.4-fold), to Regime 2 by increasing the accumulation of GAG (1.5-fold) and type-II collagen (1.6-fold), as well as the dynamic modulus (1.3-fold), and to Regime 3 by increasing the expression of superficial zone protein, at the mRNA (32.9-fold) and protein level (1.4-fold), as well as the release of hyaluronan (1.4-fold). ECA constructs were overall less responsive to all loading regimes, likely due to the lower extracellular matrix content.

Human ECN is responsive to physical forces resembling joint loading and can upregulate molecules typically involved in joint lubrication. The findings prompt for *in vivo* studies exploring the possibility of using nasal chondrocytes as a cell source for articular cartilage repair.

(OP 105) Engineered Heart Tissue for Target Validation and Cardiac Repair

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Over the past years methods have been developed to generate 3-dimensional, spontaneously and coherently beating engineered heart tissues (EHT) from embryonic chick, neonatal rat and mouse cardiac myocytes. Circular EHT may be useful as an *in vitro* model for target validation and drug screening for several reasons. (i) The system provides cardiac cells with a more physiological, 3D environment than classical 2D cultures, (ii) allows measurement of force of contraction, contraction kinetics and beating rate under controlled, isometric conditions, (iii) is stable for weeks, and (iv) allows efficient genetic manipulation with adeno- and lentivirus (overexpression, gene silencing). On the other hand, EHT have been tested as graft material for cardiac repair and improved the function of infarcted rat hearts. To translate the EHT approach to patients and to develop a human heart muscle system, recent efforts concentrate on optimizing experimental conditions for generating EHTs from human embryonic (hESC) and adult stem cells. To this end, cells from hESC-derivedspontaneouslybeatingembryoidbodiescanbedispersed and reassembled to generate human EHTs that contract synchronously, develop force of contraction and react to pharmacological stimulation qualitatively similar to normal human heart muscle.

(OP 106) Engineering Cartilage Like Tissue Using Polymeric Systems Derived from 2-Ethyl-2-Pyrrolidone-Methacrylate Combined with Hyaluronic Acid

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Hydrogels are potential candidates for Tissue engineering (TE), because of their water content, transport properties, and tissue like physical and chemical behaviour. This project was based on the production and characterization of implantable stimuli responsive scaffolds made of hyaluronic acid (HA) that presents a high water retention character, in combination with 2-ethyl (2-pyrrolidone) methacrylate (EPM), possessing temperature-dependent solubility in water, by bulk polymerization.

Semi-interpenetrated networks were produced with different EPM and HA concentrations using K2S2O8 as an initiator and triethylene glycol dimethacrylate or N,N-methylene-bisacrylamide as crosslinkers. PEPM-HA hydrogels were washed and freeze dried. Characterization of the systems was assessed by NMR, ATR-FTIR and SEM techniques. The swelling degree and degradation behaviour studies were conducted in a phosphate buffer solution. Biocompatibility studies were performed by culturing bovine chondrocytes (BC), L929, ROS and MES in the PEPM-HA hydrogels. After evaluating the cells' viability, BC were cultured under agitation for a period of 6 weeks. Distribution, morphology and extracellular matrix components deposition during the experiment were analysed. The different combinations of EPM-HA and crosslinkers led to hydrogels with different porous structures that could be intimately related to their swelling character. When BC where incubated for 72 h in different PEPM-HA hydrogels, cells remained viable and therefore were cultured for a longer period of time to predict their suitability for a cartilage TE approach. PEPM-HA hydrogels may constitute a valid alternative for pursuing future cartilage tissue engineering strategies.

(OP 107) Engineering of Hypertrophic Cartilage for Bone Repair

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Introduction: Using a hypertrophic cartilage graft could be an alternative approach to bone repair. Cartilage can survive in a relatively hypoxic environment, giving time for vascularization of the engineered graft to develop post-implantation. The aim of this research was to utilize different culture conditions to investigate whether nasal chondrocytes may be used to generate a cartilage construct with characteristics of hypertrophic tissue.

Methods: Rat nasal chondrocytes (P2) were cultured as pellets and on PGA scaffolds under conditions that could promote hypertrophic differentiation and matrix mineralisation (chondrogenic and osteogenic medium, addition of BMP-2, -7, leptin, b-glycerophosphate). Chondrocyte differentiation was evaluated by immunolocalisation of collagens (types I, II and X) and histochemical detection of proteoglycans, calcium deposition and alkaline phosphatase. Further investigation of phenotype (e.g. MMP13, VEGF, annexin, Runx2 gene expression) and potential of human nasal chondrocytes in hypetrophic cartilage formation is ongoing.

Results and Conclusion: Expanded chondrocytes successfully redifferentiated in pellets and on PGA scaffolds. Alkaline phosphatase activity and collagen type X expression was detected mainly in PGA constructs, and suggested that chondrocytes were entering the hypertrophic stage (however no matrix mineralisation was detected even with b-glycerophosphate). In conclusion, nasal chondrocytes may express a hypertrophic-like phenotype and therefore show some potential in bone tissue engineering strategies.

Acknowledgements: This work is sponsored by the EC under the Marie Curie EST programme (MEST-CT-2004-008104) and is part of the activity of the EXPERTISSUES Network of Excellence (NMP-2002-3.4.4.2).

(OP 108) Engineering Pre-Vascularized Tissue Substitutes for Clinical Use

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Initial take, development, and function of transplanted engineered epithelial substitutes are crucially dependent on the vascular supply by the adjacent mesenchyme. Therefore, the development of rapidly and efficiently vascularized (epithelio-mesenchymal) tissue substitutes is vital for tissue engineering and regenerative medicine. Here we report on the construction of highly organotypic capillaries in engineered mesenchymal (dermal) substitutes. We employed a 3D culture system consisting of human microvascular endothelial cells (HuMECs) isolated at high purity and seeded into a novel biodegradable fibrin-based hydrogel. The process of capillary formation *in vitro* was initiated by endothelial cells arranging into solid cords. Subsequently, these cells developed intracellular vacuoles that fused with the plasma membrane to form an extended intercellular lumen. Transplanted vascular structures were stabilized by mural cells recruited from the recipient animal. An autologous tissue substitute that can connect to existing vessels via inosculation and differentiate its engineered vasculature according to the tissue type it regenerates, may critically impact the emerging field of tissue engineering.

(OP 109) Enhancement of Chondrogenesis from Mesenchymal Stem Cells by Modulation of Signal Transduction Pathways

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MSCs isolated from human bone marrow have pluripotential capacity to differentiate into a variety of connective tissue lineages. However the very multipotentialities of MSCs pose drawbacks as a source of cells for cartilage repair because MSCs may differentiate into any tissue type depending on the milieu in which they are cultured. The objective of this study is to test the hypothesis that the modulation of signal transduction pathway promotes chondrogenesis and suppresses hypertrophic changes in the differentiation process of MSCs. We employed inhibitors of ERK (PD98059;100 nM) and p38(SB203580;100 nM) in MAPK pathway, and PTHrP (100 nM) which maintains chondrocyte phenotypes in growth plate. To investigate cellular proliferation, amount of DNA was measured after administration of each drug. DNA content increased by 42%, 21%,and 75% using PD98059, SB203580 and PTHrP treatment respectively. GAG contents per ug of DNA also increased by 67%, 16%, 46% with PD98059, SB203580 and PTHrP treatment respectively. Safranin-O staining showed that metachromatic staining and chondrogenic differentaion of cells increased by the treatment with PD98059 or PTHrP while SB203580 did not notably change the profile. Immunohistochemical staining for type II collagen demonstrated that PD98059 or PTHrP enhanced type II collagen expression in cell and matrix while SB203580 did not affected the expression. PTHrP reduced the expression of type X collagen compared with the control whereas SB203580 rather enhanced type X collagen. The results from this study show that modulators of signal transduction pathway, especially PTHrP, may be used to enhance chondrogenesis and suppress hypertrophic changes in the chondrogenic differentiation.

(OP 110) Enrichment of Adipose-Derived Mesenchymal Stem Cells Using Resveratrol

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Adipose tissue has been shown to contain a supply of mesenchymal stem cells (MSCs), however improved enrichment techniques are required before these cells can be used effectively. Resveratrol inhibits cyclooxygenase and activates Sirt1, which inhibit adipogenesis and induce apoptosis in adipocytes. This suggested that resveratrol could reduce the number of adipocyte progenitor cells and enrich the MSC population with osteogenic progenitors.

Adherent cells were isolated from the inguinal fat pads of Sprague-Dawley rats, plated at 5,000 cells/cm^2, and cultured in MSC growth media (GM) or osteogenic media (OM) (Lonza) containing 0, 12.5, or 25 μM resveratrol for 7 or 14 days. Flow cytometry was used to assess expression of MSC or osteoblast markers in the original population as well as following growth in GM or OM. MSCs were defined as CD73+, CD271+, and CD45−. Osteoprogenitors (OPC) were defined as osteocalcin positive.

Resveratrol caused a dose and time-dependent increase in cell number and of the percentage of both MSCs and OPCs. In GM containing 25 μM resveratrol, there was a 577-fold increase in MSCs at 7 days (5% of population), and a 106-fold increase in OPCs (21% of population) at 14 days. In OM, 25 μM resveratrol increased MSCs 27.8-fold (18% of population) at 7 days, and OPCs 29.9-fold (34% of population) at 14 days. These results show that MSCs and OPCs were present in the original adherent cell population and that resveratrol treatment enriched both cell populations. Moreover, effectiveness of the treatment was reduced when the adherent cells were cultured in OM.

(OP 111) Enrichment of CD271+ Cells from Culture-Expanded Mesenchymal Stromal Cells (MSCs) Selects Cells with *In Vivo* Bone Forming Capacity

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MSCs are postulated as an attractive stem cell source for bone tissue engineering (TE), but so far the amount of bone that can be produced by cultured human MSCs appears to be limited. CD271 defines a subpopulation in bone marrow containing CFU-F with a high proliferation capacity. We investigated the *in vitro* growth kinetics as well as the *in vivo* bone forming capacity of the culture-expanded CD271+ MSCs subpopulation.

Adherent cells from BM aspirates were passaged in standard culture medium until passage 8 (P8) and monitored for CFU-F and CD271 frequency. During culture, frequencies of CFU-F gradually decreased from 10% at passage 3 (P3) to 0% at P8 and of the NGFR+ fraction from 3% to 1%.

P3 CD271+ MSCs were sorted and expanded parallel to unsorted cells until P5. At P5, sorting resulted in a 7-fold increase in

frequency of CD271+ and 2-fold of CFU-F. After seeding of 200,000 P5 cells on Biphasic-Calcium-Phosphate scaffolds, hybrid constructs were implanted subcutaneously in nude mice, explanted after 6 weeks and analyzed for bone formation using histomorphometry. Sorted CD271+ cells showed a 9-fold improved bone formation compared to unsorted cells. No bone was formed in scaffolds without cells or P8 cells.

We show that *in vivo* bone formation of cultured MSCs resides in the CD271+ fraction and by selecting this population a better bone formation was achieved. This finding has implications for preparation of hybrid constructs in TE and for MSC therapy as a treatment for bone related diseases.

(OP 112) Establishment of Continuously Growing Human Adult Stem Cells from Adipose Tissue and Amnion for Cell-Based Therapies

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Recently, stem cell sources including adipose tissue or amniotic membrane have evolved as interesting targets for tissue regeneration research. However, due to lack of telomerase activity, mesenchymal stem cells cease growth after a limited number of population doublings which limits long-term analysis and expansion before use in cell-based therapies. Additionally, telomerase deficiency might impair the cells' differentiation potential as has been reported for bone-marrow mesenchymal stem cells. In order to get more insights into the role of telomerase in the general development of stem cells from adipose tissue and amnion, the catalytic subunit of human telomerase (hTERT) was overexpressed in these cells. Subsequently, the cellular phenotype, growth characteristics, surface marker profile, differentiation potential, immunogenic and immunomodulatory properties of the resulting stem cell lines were monitored. Indeed, while all of these analysed stem cell characteristics remained unaltered, the replicative life span was largely extended and growth characteristics were improved. Interestingly, among the generated hTERT lines, stem cell lines with reduced and improved differentiation and immunomodulatory potency were identified.This study therefore emphasizes that ectopic expression of hTERT maintains the parental phenotype of adipose and amnion derived mesenchymal stem cells and thus will be a useful tool for studying cell differentiation and tissue engineering approaches.

Acknowledgments: HIPPOCRATES (NMP3-CT-2003-505758), EXPERTISSUES (NMP3-CT-2004-500283).

(OP 113) Ethical Issues in Soft Tissue Engineering for Congenital Birth Defects in Children–What Do Experts in the Field Say?

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Paediatric applications of tissue engineering are expected to raise some unique moral questions. For instance, how should we deal with the unpredictability of the (long-term) effects of the materials used? And, is it morally acceptable to subject young children to highly experimental interventions? The use of soft tissue engineering for the treatment of congenital defects in children is the focus of the EuroSTEC project, which takes a translational route through *in vitro* and animal experiments to clinical trials and (eventually) clinical practice. To be able to respond adequately and timely to possible future moral issues, a prospective and anticipatory ethical analysis is essential.

Review of medical and ethical literature yielded an initial idea of the issues that might arise. To investigate what moral issues, if any, experts in the field expect to occur during development of tissue engineering applications for structural defects, a Delphi study was started, targeting professionals involved in EuroSTEC. Through a questionnaire consisting of open-ended questions, participants were invited to express their opinion about possible (future) ethical issues.

The first round of the Delphi study saw a response rate of 60%. The different nationalities, institutions, educational backgrounds and roles in the project were well represented among respondents. A first analysis showed that content of the answers was highly variable; ranging from no moral issues listed to a multitude for each phase. At the conference, a thorough analysis of the results of the first Delphi round and their conversion into topics for future rounds will be presented.

(OP 114) European Human Embryonic Stem Cell Registry—hESCreg

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The European Human Embryonic Stem Cell Registry (hESCreg) is a publicly accessible internet site for researchers to submit and obtain information on hESC lines, their availability, characteristics and utilisation. The primary objective of hESCreg is to pool comprehensive information on cell lines, starting with those that have been derived in Europe and also non-European lines, which are available to the scientific community at large.

Defined eligibility criteria guarantee a common standard for registration of each cell line in the registry. Scientific annotations on the cell lines include specific features, culture conditions, results obtained with the cells, information about differentiation potential, gene expression and application. Provided information will be regularly updated and completed.

Furthermore, information on the regulatory framework in the countries involved in hESC research will be provided as well as communication and feedback mechanisms. The registry closely co-operates with other European hESC research projects, as well as national and international registries and aims to contribute to the establishment of standardized evaluation parameters for human ESC lines.

hESCreg is co-ordinated by BCRT at Charité Universitätsmedizin Berlin and the Stem Cell Bank of Barcelona at the CRMB. It is supported by an international Scientific Advisory Board and an Ethics Advisory Board to guarantee quality, reproducibility, comparability and transparency of the registry. The UK StemCell Bank is a lead partner and responsible for quality assessment, code of practice and information on listed cell lines.

hESCreg is funded by the European Commission as a Specific Support Action within FP6.

(OP 115) Evaluating Cell Sources for Osteochondral Tissue Engineering

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Finding a promising cell source to achieve a viable tissue engineering construct for osteochondral transplantation was one of the tasks in Hippocrates. Hence, we established and characterized primary cell cultures from different origins including amniotic membrane, Wharton's Jelly from umbilical cord, articular cartilage, adipose tissue and bone marrow. For all cell sources, isolation, expansion and differentiation protocols were established and optimized.

Human cell populations were additionally characterized regarding their immunomodulatory potential demonstrating contact- and dose-dependent immunosuppression of mesenchymal and epithelial amniotic SC populations as well as adipose-derived SC.

For testing a tissue engineered osteochondral construct in a larger animal model cells from this species have to be applied to avoid host-versus-graft reactions. Hence, we established also protocols for harvesting, isolation, expansion, differentiation and scaffold seeding for porcine bone marrow mesenchymal stem cells and porcine adipose-derived stem cells for use in autologous *in vivo* models.

For small animal experiments cells from different sources were labelled by reporter genes and assessed *in vivo* by bioluminescence and *in vitro* by fluorescence microscopy. Cultures of human mesenchymal stem cells transiently transfected with a reporter gene were able to express the trans-gene for up to 3 weeks. The introduction of a reporter gene in the cells allows cell tracking over several days also in an *in vivo* experiment.

Acknowledgments: HIPPOCRATES (NMP3-CT-2003-505758), Lorenz Boehler Fonds, EXPERTISSUES (NMP3-CT-2004-500283).

(OP 116) Evaluation of Biomaterials-Based Tissue Engineering Technology for Regeneration Therapy

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To induce the natural regeneration and repairing of tissues and organs based on the self-healing potentials of body, there are two approaches; cell transplantation and tissue engineering. Tissue engineering is a biomedical technology or methodology to artificially create the environment which enables cells to enhance their proliferation and differentiation for tissue regeneration by making use of biomaterials. When applied to a certain tissue defect, a 3-dimensional scaffold of biomaterials with a good compatibility to cells will function as a substrate for cell-induced tissue regeneration. However, there are some cases when the mechanical property of scaffold is too poor to expect successful regeneration of bulky tissue. As one trial to tackle the problem, polymer fibers and ceramic granules are incorporated to mechanically reinforce the scaffold. In the body tissue, bio-signaling molecules (growth factors and genes) enable cells to enhance the proliferation and differentiation for regeneration and repairing of tissues. To enhance the *in vivo* efficacy of growth factor, the combination with drug delivery system (DDS) is practically promising. Substantial combination with DDS-modified growth factor is integrated to provide the scaffold with a biological function. For example, a scaffold of gelatin sponge capable for the controlled release of osteogenic BMP-2 could facilitate bone formation more homogeneously in the sponge at a larger volume. In this paper, some results on tissue regeneration by the cell scaffold and controlled release technologies are presented to emphasize significance of biomaterials-based tissue engineering in regeneration therapy.

(OP 117) Evaluation of Different Culture Conditions for Human Mesenchymal Stem Cells Derived from Second Trimester Amniotic Fluid

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Objective: To examine growth, phenotypic characteristics and differentiation potential of isolated mesenchymal stem cells (MSC) from second trimester amniotic fluid (AF) under different culture conditions.

Study Design: MSC were co-cultured on fibronectin, gelatin and polystyrene using three different culture media. Medium 1, 2 and 3 were earlier proposed growing human AF-derived MSC (in't Anker 2004), amnion mesenchymal cell (Ochsenbein-Kölble 2003) and bone marrow-derived mesodermal progenitor cell (Reyes 2001). MSC were characterized after at least 15 cell doublings by morphology and FACS-analysis and differentiation potential by immuno- and cytochemical staining. Karyotyping was performed prior and after > 15 cell doublings in order to show cytogenetic stability.

Results: Human MSC were isolated from second-trimester AF. Cell doubling time ranged between 49–104 h depending on culture conditions. Cytogenetic analysis showed normal karyotype. Medium 2 containing fetal bovine serum, epithelial growth factor, insulin, transferrin and tri-iodothyronine, stimulated cell growth better than the other two media and resulted in over 36 cell doublings.

Flow cytometry of cells showed typical MSC phenotype, positive for CD166, CD105, CD73, CD44, CD29 & HLA-ABC, but negative for CD45, CD34, CD14 & HLA-DR. The cells showed potential of differentiation into osteoblasts.

Conclusion: We were able to identify the most potent medium and culture conditions for AF-derived MSC. This protocol is further being used to explore the potential of AF-MSC in perinatal tissue engineering. Work supported by a grant of the European Commission (6th Framework Programme www.eurostec.eu, LSHC-CT-2006-037409).

(OP 118) Evaluation of Sterilization Procedures for Collagen-Based Matrices

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Introduction: A challenge in the use of collagen scaffolds as biomedical implants is the sterilization procedure, since this biological material collagen damages/denatures easily upon sterilization. The objective of this work is to evaluate the current available sterilization processes gamma-irradiation, beta-irradiation and ethylene oxide (ETO) to find the most appropriate method for sterilization of lyophilized collagen scaffolds. These sterilization processes are compared with non-sterilized scaffolds.

Materials and Methods: Preparation of type I collagen scaffolds:

Type I collagen scaffolds were prepared by means of freezing and subsequent lyophilization. Scaffolds were chemically crosslinked with/without heparin.

Applied sterilization procedures:
- 5 kGy Gamma-irradiation
- 10 kGy Gamma-irradiation
- 20 kGy Gamma-irradiation
- Beta-irradiation
- ETO

Characterization of scaffolds after sterilization: Scanning Electron Microscopy (SEM) was used to study the ultrastructure of the scaffolds; SDS-PAGE was used to determine degradation products. The extent of scaffold crosslinking and the heparin content were determined before and after sterilization.

Results: The gamma-sterilized scaffolds showed severe morphology changes, while sterilized scaffolds by ETO and beta-irradiation remained almost unchanged.

On a SDS-PAGE gel, Gamma-irradiated scaffolds showed collagen breakdown products, especially in non-crosslinked scaffolds.

The amount of amine groups showed differences after sterilization; Gamma-sterilization resulted in a dose-dependent decrease of amine groups. ETO sterilization also gave some decrease.

No differences were found in the amount of bound heparin (~17%) after various sterilization procedures.

Conclusion: This study shows that both beta-irradiation and ETO sterilization are promising methods to preserve the ultrastructure of collagen scaffolds.

(OP 119) Evaluation Technology of Tissue Engineered Constructs for Regenerative Medicine

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In order to realize an early introduction of regenerative medicine for practical use, it is necessary to ensure its safety and effectiveness. At the same time, development of related technologies, including devices to enable efficient measurement and evaluation of various characteristics of cells to be used, is needed. Many grants from Japanese government support the research in regenerative medicine. The New Energy and Industrial Technology Development Organization (NEDO) has organized one project to evaluate the process/products of regenerative bone, cartilage, heart and cornea. In this symposium I will focus on the technology, especially bone tissue engineering.

We have already treated the patients having osseous problems by using tissue engineered constructs. The construct consists of osteogenic differentiated cells (osteoblasta) derived from patient's bone marrow mesenchymal stem cells (MSCs) and bone matrix produced by the osteoblasts on various kinds of biomaterilas. To evaluate the constructs, we have developed *in situ* monitoring system to estimate the quantity of the bone matrix. We added calcein or tetracycline in the medium during the osteogenic culture of patient's mesenchymal stem cells and measured the fluorescence emission from the constructs by either commercial available or our original new equipment. The measurements well paralleled the bone matrix quantity determined by calcium contents. These data indicate usefulness of the monitoring system for bone tissue engineering and to be used for validation of the constructs in clinical applications.

(OP 120) Expansion of Chondrocyte Numbers on a Thermo-responsive-Grafted Surface.

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Introduction: Using thermoresponsive polymer grafted surfaces it is possible to expand number of cells without the use of proteolytic enzymes. It has been shown that chondrocytes gradually de-differentiate and lose their phenotype in monolayer. The aim was to investigate the potential use of a thermoresponsive surface for expansion of cell numbers to generate chondrocytes for tissue engineering.

Materials & Methods: Articular chondrocytes were cultured on commercially available thermoresponsive culture plates (RepCell®) and tissue culture plastic (TCPS) in monolayer. Cells were passaged either by decrease of temperature below lower critical solution temperature of the thermoresponsive surface, or by enzymatic treatment using a trypsin/EDTA solution. After passages 2 (P2) and 5 (P5), cells were seeded on PGA-scaffolds. Differential expression along with cell viability, proliferation rate, cell morphology, and surface markers were evaluated in monolayer cultures. 3D constructs were analysed by histochemistry and immunolocalisation of cartilage specific molecules.

Results & Conclusion: Chondrocytes de-differentiated on both surfaces, but cells expanded on RepCell® were metabolically more active. Constructs engineered from P2 expanded on RepCell®, appeared to have more extensive staining for collagen-II and proteoglycans, higher content of glycosaminoglycans, and weaker staining for collagen-I compared to constructs engineered from enzymatic-treated chondrocytes. The results indicated there was a difference in chondrocytic phenotype, suggesting chondrocytes not repeatedly exposed to trypsin/EDTA may be re-differentiated more successfully to produce a hyaline cartilage-like tissue.

Acknowledgements: This work is sponsored by the EC under the Marie Curie EST programme (MEST-CT-2004-008104) and is part of the activity of the NoE EXPERTISSUES (NMP-2002-3.4.4.2).

(OP 121) Expansion of Human Adipose-Derived Stem Cells in Spinner Flasks

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Human adipose-derived stem cells (hADSCs) are an emerging source of stem cells, which can be easily harvested without detriment to donors. Few studies have been published to date about the efficient expansion of hADSCs *in vitro* to provide enough cells for tissue construction, which play a key role in tissue engineering applications. In this study, the hADSCs was isolated from human adipose tissue by liposuction. The expanded conditions of hADSCs were investigated *in vitro*, especially in spinner flasks where the cells were cultured on the microcarriers. The expanded fold and the metabolism of glucose and lactic acid were assayed. During the whole culture process, the protein markers (CD29, CD44, CD105, CD106, CD34, CD13, CD45, HLA-DR) of hADSCs were detected by flow cytometry (FCM), and the multi-lineage differentiation of the expanded hADSCs was also tested. Besides, the cell viability, glucose and lactic acid concentration of hADSCs in spinner flasks and culture plate were detected and compared. The results showed that hADSCs could express specified stem cell protein markers and differentiate into adipocytes, osteoblasts and chondroblasts. The growth curves suggested that the viability of hADSCs cultured in spinner flasks was stronger than that in culture plate, and the expanded fold was also much higher than that in it. Moreover, hADSCs could still remain stem cell characteristics after being expanded in spinner flasks. In conclusion, these data suggest that the spinner flask is an available and alternative tool to expand a great number of hADSCs for tissue construction of tissue engineering.

(OP 122) EXPERTISSUES—a Network of Excellence for Tissue Engineering in the Bone, Cartilage and Osteochondral Field

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The main goal of EXPERTISSUES is to overcome fragmentation of European Research in the field of tissue engineering of bone, cartilage and osteochondral defects by applying an interdisciplinary and translational approach. EXPERTISSUES joins together academic centers and industry (* currently 22 coordinated by Rui Reis, Portugal) with expertise in biomaterials/processing, including bioplotting–soft/hardware, growth factor application/delayed release, (stem) cell biology including GMP production facilities, bioreactors and multiple preclinical models plus imaging techniques as well as clinical experience (maxillofacial, trauma and orthopedic surgery).

To achieve the objectives of the NoE, integrating activities are established, e.g. by joint internal project grants (influence of biomaterial surfaces, biomaterial inner structure on cell mobility, gene therapy approaches for bone regeneration, multi-center preclinical trials and TERM knowledge networks); by mobility of personnel including integrated PhD students, a joint training programme of hybrid researchers in different labs of the NoE; by establishment of a Common Quality Assurance System; by organization of workshops, seminars, courses and conferences; by launching the TERM (Journal of Tissue Engineering and Regenerative Medicine) journal and edition of scientific books as well as a shared electronic network on TERM; by ethical and technical inputs for the development of European Standards; by jointly submission of grants on the European level.

The final goal is the creation of the EXPERTISSUES European Institute of Excellence for Tissue Engineering and Regenerative Medicine (as an EEIG).

NoE EXPERTISSUES (NMP3-CT-2004-500283).

(OP 123) Exploiting Lymphatic Transport and Complement Activation in Nanoparticle Vaccines

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The development of vaccine technologies has emerged as a forefront healthcare initiative, especially technologies for use in developing countries, posing severe economic and logistic constraints. One must develop antigen targeting and adjuvant schemes that respectively facilitate delivery of antigen to dendritic cells (DCs) and elicit their activation. Here we engineered antigen-bearing nanoparticle vaccines with two novel features: lymph node-targeting and *in situ* complement activation. Following intradermal injection, interstitial flow transported our ultra-small nanoparticles (25 nm) highly efficiently into lymphatic capillaries and their draining lymph nodes, targeting half of the DCs there, whereas nanoparticles even 100 nm large were only 10% as efficient. Furthermore, the surface chemistry of our nanoparticles activated the complement cascade, which spontaneously generated a danger signal *in situ* and potently activated DCs. With the model antigen ovalbumin conjugated to the nanoparticles, we demonstrated humoral and cellular immunity in mice in a highly size-dependent and complement-dependent manner. We will also present data where we exploit nanoparticles as synthetic pathogens in order to elucidate molecular mechanisms of complement activation with DCs. Finally we characterized the adaptive immune response that complement induces.

(OP 124) Expression of Cyclooxygenases and Prostaglandin Receptors in the Growth Plate and Articular Cartilage of the Rat–Its Possible Role for Tissue Engineering of Cartilage

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Background: Tissue Engineering of articular cartilage remains an ongoing challenge. Since tissue regeneration recapitulates ontogenetic processes we use the growth plate as a model to target suitable signalling molecules and growth factors for the tissue engineering of cartilage. In previous investigations we demonstrated that in the growth plate cyclooxygenases (COX-1 and COX-2) are involved in the proliferation of chondrocytes. In the present study we analysed the expression of cyclooxygenases and prostaglandin receptors in articular cartilage and growth plates of young rats.

Methods Frozen sections from epiphyseal plates including the articular cartilage of 4 weeks old Sprague Dawley rats were fixed in paraformaldehyde (4 per cent) and stained by the alkaline phosphatase–anti-alkaline phosphatase (APAAP) method using polyclonal antibodies against COX-1, COX-2 and the prostaglandin receptors EP-1, EP-2, EP-3, EP-4. The tissues were counterstained with haemalaun according to laboratory standards.

Results: Growth plate chondrocytes and chondrocytes of the articular cartilage showed the expression of COX-1, COX-2 EP-1, EP-2, EP-3 and EP-4. The reserve zone of the growth plate showed only weak expression of COX-2 with numerous negative cells. The superficial layer of articular cartilage is negative for COX and EP-receptors.

Conclusions: Our findings indicate a functional role of cyclooxygenases and prostaglandin receptors not only in the growth plate but also in the maturation of articular chondrocytes. This opens interesting perspectives to improve the proliferation and maturation of chondrocytes in scaffold especially since prostaglandin receptor ligands are available in pharmaceutical quality.

(OP 125) Extracellular Matrix as a Biologic Scaffold for Urinary Bladder Reconstruction: the Role of Mechanical Loading in the Remodeling Process

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Extracellular matrix (ECM) scaffolds have been widely studied for their potential role in the functional reconstruction of several tissues and organs including the lower urinary tract, the gastrointestinal tract, skin, and cardiovascular structures among others. ECM derived from two layers of the porcine urinary bladder, the basement membrane and subjacent tunica propria (termed urinary bladder matrix–UBM) was used to replace a 30% resection of the dome of the urinary bladder in eight adult female dogs. Four dogs were catheterized for 24 hours following surgery at which time spontaneous filling and emptying of the bladder was allowed to occur for the remainder of the study. The catheter remained in place for 30 days in the following four dogs. Two dogs in each group were sacrificed after 30 days and the other two after 90 days. Dogs that remained catheterized showed 90% contraction of the scaffold material, incomplete urothelial coverage at 30 days, and a chronic inflammatory response within the underlying UBM scaffold and newly deposited host ECM. The dogs that were allowed spontaneous filling of the bladder showed 30% contraction of the scaffold area, complete urothelialization at 30 days with islands of smooth muscle in the subjacent remodeling tissue. By 90 days, these non-catheterized dogs showed sheets of functional smooth muscle arranged in patterns that resembled the normal muscularis externa with an adjacent submucosal layer and near normal bladder histomorphology. These findings indicate that mechanical signals play an important role in the remodeling process of this hollow organ.

(OP 126) Fabricating and Testing of Flexible Microelectrode Arrays for Neural Recordings

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In this study, flexible polymer-based microelectrode arrays (MEA) were designed, fabricated and characterized. The functionality of these arrays was tested in different neuronal recording cases *in vivo* and *in vitro*. Several designs of MEAs were realized varying the layout of array and material of insulation layer. Two different layouts were 8-channel MEA with 200 μm (circle-shaped, diameter) electrode sizes and 16-channel MEA with 100 μm electrode sizes. As insulation materials were tested polyimide Pyralin PI-2771 and epoxy-resist SU-8. The substrate material in all MEAs was 25 μm Kapton film coated with thin layer of polyimide PI-2525. Metallization layer is formed by structuring UV-resist (ma-N 1420), sputter-coating of biocompatible Ti and Pt thin films

(200 nm) and via lift-off procedure. A 3 µm thick insulation layer (PI-2771 or SU-8) was structured by UV-exposure to form electrode openings and connector pads. Thin film pads were designed to fit into 16-channel ZIF-type connector. Fabricated MEAs were characterized by impedance spectroscopy in saline. Electrode impedances of 100 µm and 200 µm electrodes measured at 1 kHz were 98k Ω (SD: 18k Ω) and 34k Ω (SD: 4k Ω), respectively. The preliminary biological studies showed promising results. Evoked potentials were recorded *in vivo* from the rat brain when electrical stimulus was conducted to the rat paw. We managed to record different responses in every 8 channels. 16-channels MEAs also gave encouraging results evoked potential recordings in rat hippocampal slices. In future, this biocompatible fabrication protocol is possible to adapt for development of different neural interfaces with higher spatial resolution.

(OP 127) Factor Xa and Thrombin Evoke Additive Calcium and Proinflammatory Responses in Endothelial Cells Subjected to Coagulation

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Endothelial cells react to factor Xa and thrombin by proinflammatory responses. It is unclear how these cells respond under physiological conditions, where the serine proteases factor VIIa, factor Xa and thrombin are all simultaneously generated, as in tissue factor-driven blood coagulation. We studied the Ca(2+) signaling and downstream release of interleukins (ILs), induced by these proteases in monolayers of human umbilical vein endothelial cells. In single cells, factor Xa, but not factor VIIa, complexed with tissue factor, evoked a greatly delayed, oscillatory Ca(2+) response, which relied on its catalytic activity and resembled that of SLIGRL, a peptide specifically activating the protease-activated receptor 2 (PAR2). Thrombin even at low concentrations evoked a rapid, mostly non-oscillating Ca(2+) response through activation of PAR1, which reinforced the factor Xa response. The additive Ca(2+) signals persisted, when factor X and prothrombin were activated *in situ*, or in the presence of plasma that was triggered to coagulate with tissue factor. Further, thrombin reinforced the factor Xa-induced production of IL-8, but not of IL-6. Both interleukins were produced in the presence of coagulating plasma. In conclusion, under coagulant conditions, factor Xa and thrombin appear to contribute in different and additive ways to the Ca(2+)-mobilizing and proinflammatory reactions of endothelial cells. These data provide first evidence that these serine proteases trigger distinct signaling modules in endothelium that is activated by plasma coagulation. In conclusion these results suggest the protease inducing clot formation might induce different biological events in downstream in platelet rich plasma clinical procedure.

(OP 128) Fetal Chorionic Villi and Amniotic Fluid Derived Progenitors As Autologous Cell Sources for Pediatric Cardiovascular Tissue Engineering

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Due to a lack of viable autologous replacement materials with growth and regeneration capacities current surgical treatment of cardiovascular malformations requires non-autologous, non-living replacements associated with disadvantages including obstructive tissue ingrowths and calcification. This typically causes re-operations over the patient's lifetime. Therefore, pediatric cardiovascular tissue engineering aims at the fabrication of autologous living replacements, ideally, already being available at or shortly after birth. Thus, the tissue engineering process has to be initiated prenatally as soon as the cardiovascular defect has been detected. Here, a novel concept using routinely prenatally obtained human fetal progenitors as autologous cell source for cardiovascular tissue engineering is presented.

Prenatal progenitor cells were isolated from chorionic villi and amniotic fluid. After expansion and characterization, cells were differentiated into the cell types required for heart valve tissue engineering: myofibroblast-like cells and endothelial-like cells. Thereby, cell phenotypes were analyzed by immunohistochemistry and flowcytometry. For fabrication of heart valves PGA/P4HB-based biodegradable scaffolds were seeded with mesenchymal-like cells derived from chorionic villi or amniotic fluid. Afterwards, constructs were cultured in a pulse duplicator system and subsequently coated with endothelial-like cells. After maturation, engineered heart valves demonstrated endothelialized layered tissue formation. Analysis of extracellular matrix elements and cell number revealed production of gycoaminoglycans and collagen (GAG 80%, HYP 5%) and cell numbers up to native tissue values.

In conclusions, the use of fetal progenitor cells as autologous cell source is a promising strategy enabling the fabrication of heart valves ready to use at birth for the early repair of congenital malformations.

(OP 129) Fibrin Delivery Systems for Elastic Tissue Regeneration

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Fibrinogen (Fgn), a soluble plasma protein found in all vertebrates, is a covalent dimer composed of pairs of three polypeptide chains called Aa-, Bb- and gamma-chains. Fgn is converted to an insoluble fibrin network following activation by thrombin and crosslinking by factor XIIIa following vascular injury and serves the primary role in haemostasis. Our interest in developing fibrin-based biomaterial technology is based on fibrin's central role in tissue binding and in the initiation of tissue repair and defence. Technologies

have been developed with some success for incorporation of growth factors into fibrin, ranging from simple admixing to covalent incorporation using the coagulation transglutaminase factor XIIIa. We are developing a novel platform technology where the growth factor is incorporated into Fgn's Aa-chain at the genetic level. We have chosen this region since evidence suggests that the C-terminus of Fgn's Aa-chain is not required for bioassembly or polymerization. The release profile and functional significance of this novel fibrin-delivery system will be compared to current fibrin delivery schemes. For comparison to our current fibrin delivery system using factor XIII coupling, hIGF1 cDNA was generated by PCR for incorporation into the expression vector where the transglutaminase substrate sequence from alpha2PI (Tg) for crosslinking into fibrin was added at the N-terminus.

(OP 130) Fibrous Scaffold for Encapsulation of Pancreatic Islets

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Conventional microencapsulation methods for cell-based therapies are limited by inadequate nutritional transport and surrounding fibrotic overgrowth, thus rendering long-term graft survival difficult to achieve. A fibrous scaffold is presented as an alternative encapsulation strategy of pancreatic islets. Fibers containing RINm5F cells are formed by interfacial polyelectrolyte complexation between alginate and water-soluble chitin. Additional polyelectrolyte coatings are used to reduce scaffold porosity and increase stringency of immunoisolation. The multicomponent fibers, measuring approximately 100 um in diameter, offer a higher surface-to-volume ratio and better diffusion dynamics than conventional alginate beads.The fabrication process well preserves the formation of viable cell aggregates as revealed by calcein staining, especially as aggregation is desirable for islet survival and insulin secretion. Efficiency of coating is demonstrated by changes in surface zeta potential with each layering at physiological pH. Functionality of encapsulated islets was validated by rat C-peptide ELISA. A sustainable release of C-peptide is observed with 20 mM of glucose challenge, with a total of 3 ng for every 5×10^5 cells after 100 minutes. The efficiency of immunoisolation is further assessed by gel permeation chromatography. Protein markers ranging from 5 kDa to 300 kDa are encapsulated in the fibers and allowed to diffuse out, revealing a marked decrease in the concentration of bigger molecules in the supernatant. Potential crosslinkers are currently being evaluated to improve the scaffold porosity and islet survival.

(OP 131) Frictional Properties of Tissue Engineered Cartilage Generated Using Standard or Bioreactor Culture

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Introduction: Tissue engineered (TE) cartilage may, in the future be used to repair articular cartilage in joints and restore the low frictional properties of the surfaces. Research to date suggested that the properties of constructs cultured using standard culture conditions (orbital shaker) were inferior to those of native articular cartilage. Advanced culture conditions in bioreactors might therefore be essential to engineer a functional tissue with low friction properties.

Objective: This study compared the fictional properties of TE cartilage cultured under standard culture conditions and a rotating wall vessel (RWV).

Methods: TE cartilage constructs were cultured under standard conditions and in a RWV. Indentation and start-up friction tests were performed with PBS as a lubricant. The presence of GAGs, type I and II collagen were investigated.

Results: All the constructs exhibited a time-dependent increase in deformation and friction during the tests. The friction values were however, lower for the constructs cultured in the RWV ($\mu 20\,\text{min} = 0.20 \pm 0.02$) than those under the semi static culture conditions ($\mu 20\,\text{min} = 0.25 \pm 0.06$), and closer to native tissue ($\mu 20\,\text{min} = 0.20 \pm 0.05$). The composition of the extracellular matrix of the constructs cultured in the RWV was also superior.

Conclusion: TE constructs from the bioreactor showed functional properties including low friction in common with native tissue. By using these more advanced culture conditions, it was possible to improve the biological quality and frictional properties of TE cartilage.

Acknowledgement: The authors are grateful to the White Rose University Consortium for funding, and the work was performed as part of the EXPERTISSUES Network.

(OP 132) Functional Control of Myoblasts in a Strained Fibrin Gel

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In vitro engineering of biomimetic skeletal muscle tissue is one of the candidates for the future treatment of the skeletal muscle disease. To control cell functions including patterning, proliferation, and differentiation in three-dimensional (3D) matrices might be crucial for this purpose. To address this goal, we used fibrin gel as a 3D cell culture matrix and tried to control cell functions in this 3D gel.

When continuous tensile strain was applied to a cylindrical shaped fibrin gel to the opposite uniaxial direction, the bundle-like structures which aligned parallel to the strain direction were formed in the gel. Myoblasts cultured in this fibrin gel demonstrated a specific alignment in the gel, proliferated to the specific one direction, and finally formed aligned cell sets in this gel. When we applied different degrees of strain to the cells in the gel, cell proliferation was the highest in the high-strained gels and was attenuated in the low-strained gels. In contrast, myoblast differentiation was the highest in the low-strained gel, but was inhibited in the high-strained gel. These results indicate that this 3D fibrin gel culture system enables one to control patterning, proliferation, and differentiation of myoblasts.

(OP 133) Gene Expression Profiling of HYAFF-11 Cartilage Transplants Generated by Chondrocytes from OA Donors

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Osteoarthritis (OA) is the most abundant disorder of the musculoskeletal system. It is characterized by the degeneration of articular cartilage due to mechanical wear. Currently Tissue Engineering (TE) technologies based on scaffold-supported chondrocytes are used to treat traumatic cartilage defects but not joint lesions due to OA as *in vitro* and clinical studies are lacking. Therefore, in this study the feasibility of chondrocytes from OA donors for cartilage TE was determined by investigating the differentiation capacity of chondrocytes from OA donors in comparison to healthy donors on gene expression level.

OA cartilage biopsies (Mankin Score 3-7) were taken from the femoral condyle of patients undergoing total knee replacement. Chondrocytes were isolated enzymatically and expanded in monolayer cultures. Chondrocytes from normal donors (ND) were obtained from excess samples of cells originally used for autologous chondrocyte transplantation. ND and OA chondrocytes were seeded onto HYAFF-11 scaffolds and were maintained up to 4 weeks under chondrogenic conditions (serum free, TGFbeta-1). Re-differentiation associated collagen type II formation was assessed by fluorescence immunohistochemistry. Furthermore, gene expression of monolayer as well as of scaffold cultured chondrocytes were analyzed applying Affymetrix HG-U133plus2.0 GeneChip microarrays and. A set of genes, that were differentially expressed between normal donor HYAFF-11 culture and monolayer cultures was generated and used to assess differentiation of OA chondrocytes. ND and OA chondrocytes showed a comparable formation of cartilage-associated collagen type II and a comparable expression of a relevant set of chondrogenic markers, which indicates the feasibility of OA chondrocytes for cartilage TE.

(OP 134) Generation of a Bioartificial Liver Using a Decellularized Whole Liver Bioscaffold

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Our laboratory recently developed a decellularization method able to generate an entire organ bioscaffold from a whole liver, preserving its functional vascular network. Preliminary studies showed the possibility to efficiently re-cellularize the bioscaffold using a perfusion cell seeding process in a bioreactor. Nonetheless, numerous technical issues need to be optimized to efficiently seed by perfusion primary hepatocytes to generate functional hepatic tissue. The purpose of this study was to investigate the feasibility of generating bioartificial liver by re-cellularizing the decellularized whole organ bioscaffold with primary hepatocytes and endothelial cells.

Mouse endothelial cells and freshly isolated rat hepatocytes were seeded through the portal vein of the bioscaffold. The seeded bioscaffolds remained in a bioreactor with constant culture medium perfusion up to one week. Microscopy was used to determine cell density and seeding efficiency. Immunohistochemistry was used to identify the engrafted cells and to detect hepatic tissue associated functions.

The perfused endothelial cells attached and formed a monolayer on the luminal side of the vascular channels. Rat primary hepatocytes showed homogeneous seeding and high cell density throughout the bioscaffold (with 1–1.5 cm thickness). Immunohistochemistry showed progressive tissue formation with expression of albumin, alpha 1-antitrypsin and cytochrome P450.

Our results demonstrate the efficient generation of a bioartificial liver with primary hepatocytes using a perfusion cell seeding process in a liver bioscaffold. Hepatic tissue associated functions were detected with three-dimensional tissue formation *in vitro*. This technology may provide a new approach for bioartificial liver engineering, critical for drug discovery and treatment of terminal liver diseases.

(OP 135) Generation of Functional Cardiomyocytes from Induced Pluripotent Stem (iPS) Cells for Myocardial Tissue Engineering

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So far, development of autologous bioartificial cardiac tissue (BCT) was hampered by the lack of a suitable cell source. The recent reprogramming of fibroblasts into induced pluripotent stem cells (iPS) for the first time offers an autologous pluripotent cell source with theoretically unlimited expansion potential. The combined overexpression of 4 transcription factors resulted in reprogramming of fibroblasts and generation of pluripotent cells that largely resemble ES cells.

It was now aim of our study to characterize the cardiac differentiation potential of iPS, to characterize the resulting iPS-derived cardiomyocytes on a molecular, structural and functional level, and to apply these cells to myocardial tissue engineering.

Differentiation of a murine iPS clone O9 resulted in spontaneously beating cell clusters comparable to those arising in differentiating mESC lines. Semi-quantitative RT-PCR analyses demonstrated expression of marker genes typical for mesoderm, cardiac mesoderm and cardiomyocytes. Immunohistological and ultrastructural studies showed expression of CM-typical proteins and cross-striated muscle fibrils. Electrophysiological studies by means of multi electrode arrays (MEAs) revealed evidence of functionality and ß-adrenergic signaling of the generated cardiomyocytes. Moreover, own iPS clones were generated and con-

firmed the cardiac differentiation potential of iPS cells. Finally, pilot studies aiming the generation of iPS-based bioartificial cardiac tissue (BCT) were performed.

This is the first study demonstrating the *in vitro* differentiation of iPS cells into functional cardiomyocytes. In contrast to ES cells, iPS cells allow for the establishment of autologous pluripotent customized stem cell lines, showing great promises for myocardial tissue engineering and other applications in regenerative therapies.

(OP 136) Genetically Engineered KSFrt Mesenchymal Progenitors Cells in Combination with Whole Body Optical Imaging Provide an Excellent Model to Study Effects on Cell Fate, Bone and Cartilage Formation and Biomaterials

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Mesenchymal Stem Cells have great potential for application in tissue engineering of bone and cartilage, especially in combination with biomaterials. Composition and structural properties of biomaterials can considerably influence MSC differentiation. At present, differentiation of MSCs *in vitro* or *in vivo* as well as osteogenic properties of biomaterials are poorly understood. Therefore, we created a model, which allows fast and reproducible production of stable cell lines, by introducing an FRT-site into the genome of the KS483 cell line. This site is used for the insertion of DNA to enable gene function studies by overexpression or knock down or by insertion of reporter genes to visualise and quantify specific biological processes. Insertion of a luciferase2 gene enabled us to follow cell fate *in vivo* by whole body bioluminescent imaging after subcutaneous implantation in nude mice for 20 weeks. Immunohistochemical analysis indicated absence of bone formation without further stimulation. However, when seeded onto BCP scaffolds, KSFrt-Luc2 cells have formed bone after 8 weeks, while KSFrt cells overexpressing Runx2 already generated bone after 3 weeks, indicating that proper stimulation significantly accelerated bone formation by KSFrt cells *in vivo*. Therefore, these cells provide an excellent model to investigate bone-inducing properties of biomaterials. To facilitate this, we generated an osteogenic reporter cell line by introducing an osteoblast-specific collagen I promoter driving expression of the luciferase2 gene. This cell line will be used to test drugs and biomaterials *in vitro*, followed by *in vivo* conformation of results using whole body optical imaging.

(OP 137) Genomic Guided ECM Biomaterials Development for Regenerative Medicine Applications

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Identification of appropriate ECM surrogates for cellular differentiation is a challenge for biomaterials development because little is known about most of the ECM proteome. To address the problem, effective methodologies to screen a wide range of biomaterials candidates for stem cell differentiation applications are needed. We have developed a novel high throughput ECM screening method which is guided by the genomic analysis of cellular differentiation.

Methods: Human amniotic fluid derived stem cells (hAFSCs) were used, and microarrays were performed on hAFSCs at various timepoints following myogenic, hepatogenic, vasculogenic, & osteogenic differentiations. RNA was isolated and hybridized to GeneChip. Highly up-regulated genes involved in ECM formation were identified. To demonstrate these ECM molecules could serve as potential biomaterials targets, several were selected for further study under osteogenic differentiation.

Results: Microarray analyses of differentiating hAFSCs provided over 40 ECM-related genes that were universally up-regulated as these cells reached their terminal fate. Some of the most significantly up-regulated genes are those involved in ECM production. 4 ECM-related genes were highly up-regulated upon osteogenic differentiation and selected for further study. The 4 selected ECM molecules (aggrecan, decorin, osteoblast specific factor, and thrombospondin) demonstrated significantly enhanced or inhibited effects on hAFSCs osteogenic differentiation in several ways, including concentration, combination, and temporal dependencies.

Conclusions: ECM biomaterials with highly up-regulated genes identified by micro-array profiling of differentiating stem cells demonstrated certain effects on efficiency and temporal patterning of cellular differentiation. This new genomic-guided ECM molecule screening represents a new approach for ECM mimicry in regenerative medicine applications.

(OP 138) Growth Factor Gene Therapy for Alveolar Bone Engineering

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Repair of craniofacial defects including alveolar bone lesions caused by trauma, congenital malformations or disease is a major goal of oral reconstructive therapy. Alveolar bone tissue engineering has been achieved with limited success by the utilization of bone replacement grafts, guiding tissue membranes and block grafting techniques. Over the past decade investigators have begun to utilize signaling molecules such as growth factors to restore lost bone support due to damage to the alveolar process. This presentation will review emerging therapies in the areas of materials science, growth factor biology and cell/gene therapy. Results from preclinical and clinical trials will be reviewed. The presentation will conclude with a future perspective on the use of novel biomimetic approaches such as gene delivery of signaling molecules and scaffolding matrices with the potential of accelerating dental implant osseointegration. Funded by NIH DE 13397, AO Foundation Switzerland and the ITI Foundation.

(OP 139) Growth Factor Releasing Biohybrid Silk-PLGA Scaffolds for Tendon and Ligament Tissue Engineering

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One of the major challenges facing tendon and ligament tissue engineering is the availability of suitable scaffolds. Hybrid scaffolds fabricated by combining knitted scaffolds with nanofibre coatings have been shown to be effective for tissue engineering of tendons and ligaments.

In this study, biocompatible knitted silk scaffolds were fabricated and coated with FGF-releasing PLGA nanofibres, using silk solution as intervening glue. Such scaffolds were optimized with terms of the method of silk processing, cell seeding, growth factor incorporation, and characterized subsequently for their efficacy in engineering tendons and ligaments by a variety of assays. Silk scaffolds degummed using an improved degumming method, employing ultrasonic agitation in the presence of a detergent, proved better by better retaining the mechanical properties of silk (38% stronger).

Scaffolds seeded with bone marrow stem cells on both surfaces allowed better cell proliferation (14–17% higher), as compared to scaffolds seeded with the same total number of cells on just one surface. Rolling up the scaffolds and continuing *in vitro* culture yielded ligament analogs. When the scaffolds were coated with FGF-releasing nanofibres, the constructs demonstrated increased cell proliferation (24% higher), collagen production (39% higher), upregulation of tendon/ligament specific extracellular matrix proteins (collagen I, collagen III, fibronectin and biglycan), as well as improved mechanical properties (21% stronger and 26% stiffer). Thus, the biohybrid scaffolds developed in the study proved suitable for tendon/ligament tissue engineering applications.

(OP 140) Hemocompatible Membranes for Blood Oxygenators

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Artificial blood oxygenation is required during surgical interventions such as coronary artery bypass grafting, in which both lung and heart functions are replaced by a heart-lung machine. In these equipments the membrane placed between the blood and the gas phase is object of new developments in terms of the characteristics of O_2 and CO_2 permeability and hemocompatibility. Anderson JM *et al.* (Academic Press, 1999) emphasized that the literature is contradictory with respect to the role of interactions between artificial material surfaces and the blood components that may lead to platelet adhesion/activation, activation of coagulation and complement, resulting in thrombus formation and systemic inflammation.

This work addresses:

- The preparation of polyurethane membranes with two soft segments: a polypropylene oxide based one with isocyanate terminal groups and another polyester or polyether based one with hydroxyl terminal groups (1).
- Surface characterization through spectroscopic and microscopic techniques: X-Ray Photoelectron Spectroscopy (XPS), Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM) and Field Emission Scanning Electron Microscopy (FESEM).

The correlation between bulk and/or surface characteristics of the membranes and their gas permeation and hemocompatibility characteristics is carried out in order to understand the mechanisms of membrane surface/blood components interactions. The knowledge of that and the versatility in casting bi-soft segments membranes with a wide variety of bulk and surface morphologies allows the tailoring of membranes with optimal gas permeation and hemocompatibility properties.

Reference:

[1]D. P. Queiroz, M. C. Gonçalves, M. N. de Pinho, J. Appl. Polym. Sci., 103, 315–320 (2007).

(OP 141) Home, Sweet Home: Recreating the Native Bony Environment *In vitro* Using a Novel Dynamic Cell-Culture System for Bone-Biology Research

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Introduction: Traditional cell-culture methods are ill-suited to study the intricacies of bone biology because they ignore the three-dimensionality of meaningful cellular networks and the lacuno-canalicular system. Reliance on nutrient diffusion limits scaffolds to 2–3 mm thickness. Static culture also ignores the importance of mechanical loading in regulating bone metabolism. A novel flow perfusion system was developed to overcome these limitations. Both bioreactor and osteoconductive/osteoinductive scaffolds resulted from evolutionary outgrowth of the native structure/function of bone and the design requirements crucial to successful experimentation.

Methods: Eight adaptable chambers house cylindrical scaffolds, up to 24 mm diameter $\times$10 mm thickness. A multi-channel peristaltic pump draws medium from parallel reservoirs and perfuses it through each scaffold (0.3–30 mL/min). Hermetically sealed valves permit sampling of medium. A gas-permeable membrane permit gas exchange. Tubing was selected to withstand continuous perfusion for $>$2 months without leakage. Porous scaffolds were manufactured using a novel 3D bioprinting process. These scaffolds were composed of hydroxyapatite/tricalcium phosphate with variable thicknesses, strut sizes, and pore sizes.

Results: Fluid shear stress ranged from 0.04–4 dyn/cm^2 at the cellular level and are described by finite element modeling. Scaffold architecture is characterized by micro-CT and scanning electron microscopy. Osteoblastic networks and canalicular channels are visualized by fluorescent confocal microscopy.

Conclusions: Unlike spinner flasks and FlexCell devices, this novel system simulates the natural 3D lacuno-canalicular system of native bone, ensuring chemotransportation throughout thick 3D scaffolds and allowing for the study of bone biological and biomechanical processes *in vitro*.

(OP 142) Host Responses in Tissue Engineering

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Therapies utilising cell delivery or accumulation to provide a medical treatment that is functional by cellular activity requires rapid integration and guided immediate communications with host tissues. The many and varied tissue engineering processes to achieve these objectives bring to the fore the critical host defence mechanisms that require to be at least anticipated if not controlled and utilised in providing long lived, functional cell therapies. Historically most implanted materials have lacked an inherent potential to trigger pathways in the specific immune response, but have always initiated a non-specific immune response. In regenerative medicine by taking a biological approach to treatments and regeneration the potential activation pathways of both the specific and non-specific immune pathways begin to at least in theory become significant areas that require further consideration and experimentation. How far does the clear potential from the theory for increased immune activity extend to being a real functional issue or risk for tissue engineering therapies and the patient?

A biological approach to therapies provides an abundance of the right type (proteins) of fundamental molecules for directed cell communication. The drive is towards providing autologous sources or protein free solutions, should be met by the fundamental understanding of what protein interactions are important, their kinetics, dynamics and thresholds. Do immunity thresholds exist for cell based therapies? An objective well characterised approach to determining for implanted organic materials the immune threshold potential is clearly required for both scientific and clinical success but is also fundamentally required for regulatory and safety issues.

(OP 143) How will We Repair Nerves and Bridge Nerve Gaps in the Future?

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Peripheral nerve injures are common and often result in impaired sensory and motor function. This is true especially when there is a nerve-defect that requies bridging. The present conventional technique of using nerve grafts is still the only clinical applicable one, but extensive research is ongoing to find other techniques to avoid autologous nervous transplantation and still achieve an improved functional recovery. The concept of tubulisation using biodegradable polymeric materials, either with or without luminal fillers, is suggested as an alternative. The naturally occurring biodegradable polymer Poly[(R)-3-Hydroxybuturate] (PHB), metabolised to the endogenous monomer D-(-)-3-hydroxybutyric acid, is one of the tubulisation materials that has been experimentally tested as an alternative for peripheral nerve grafts together with both Schwann cells and mesenchymal stem cells with good results.

In several previous experimental studies we have demonstrated that PHB is a suitable material to be used in combination with transplanted Schwann cells to bridge nerve gaps and to improve regeneration. The limitation is however that transplanted Schwann cells need to be autologous to avoid immunorejection. In some recent studies we have, however, been able to demonstrate that mesenchymal stem cells have the ability to differentiate to Schwann cells morphologically but also to achieve their nerve stimulating properties. The lecture will summarise the experimental and clinical results on using PHB as a synthetic nerve implant and its combination with Schwann and stem cells to enable bridging of nerve gaps and to enhance nerve regeneration.

(OP 144) Human Adipose Stem Cells–From Laboratory to Clinical Practice

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Currently, the most reliable method of reconstructing large bony defects involves painful harvest of bone. This causes donor site morbidity and a higher risk of infection. The use of other biological materials carries the risk of virus transmission.

Tissue engineered custom-made products will replace harvested flaps in the future. This new technology will be based on stem cells capable of self-renewal and differentiation into several cell types *in vitro*. The cells can be transplanted with a biomaterial scaffold without immunosuppression.

Adipose tissue is an appealing donor tissue for cell replacement, since it is redundant and can easily be harvested. Furthermore, adipose stem cells combined with osteostimulative biomaterials provide an attractive alternative for bone tissue engineering.

Adipose tissues were subjected to cell extraction techniques and the cells were expanded. For flow cytometry, cells were stained with antibodies and cell surface marker expression was analysed. Biocompatibility of biomaterials was tested by measuring cell proliferation and osteogenic differentiation *in vitro*.

Our surface marker expression data is in accordance with the results reported earlier. Furthermore, our data shows that adipose stem cells have the capacity to undergo osteogenic differentiation. At the moment, we have treated three patients with bony defects using adipose stem cells. These cases show that the use of autologous adipose stem cells combined with osteostimulative biomaterial results in faster generation of new viable bone. Moreover, our results show that adipose tissue stem cells can be utilized in fabrication of custom-made bone flaps. This will begin a new era of reconstructive surgery.

(OP 145) Human Cultured Epithelial Allografts: an Adapted Approach Introducing More Efficient Production Schemes and Resulting in Extra Patient Safety

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Introduction: Since 1987 keratinocytes are grown and used in our burn unit (> 800 patients were treated). During those two decades, our culture system has evolved considerably with an emphasis on safety assurance for the acceptor patients, increased quality and overall performance, and cost-reduction.

Methods: The major improvements consist in the introduction of neonatal foreskin keratinocytes (NFK), a feeder layer- and animal component free culture system, and a Quality Management System (QMS).

Results: In contrast to the irregular and unpredictable growth rates and end stages of normal adult human epidermal keratinocytes (HEKa), NFKs show a very regular and consistent growth, comparable to that of established cell lines, which lasts for up to 50 population doublings (theoretical expansion of a 1 cm^2 biopsy up to 100 km^2 of cultured epithelium, compared to 50 m^2 for adult keratinocytes). This tremendous proliferation potential allows the use of more strict and predictable subculturing schemes. Today, all culturing and banking procedures are performed taking into account the cGMP guidelines and ISO 9001:2000 QMS requirements. Recently, our lab tested of a new totally defined and animal free culture medium. This medium supported a regular and extended growth (up to 25 population doublings). The use of a totally defined and animal free medium is avoiding possible transmittance of potential animal viral agents. The support for the cells is given by a coating of recombinant human type-1 collagen instead of the traditional bovine collagen. We also tested (safe) membranes as a support for human epithelial grafts.

(OP 146) Human Dermal Fibroblasts, Keratinocytes and Adipose Stem Cells Behaviour on Patterned Electrospun Nanofiber Meshes

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Natural extracellular Matrix (ECM) creates a unique cellular microenvironment. It acts as a support to organize cells in tissues, maintains their structure and works also as a reservoir for cytokines, thus controlling cell growth and differentiation. A well-defined biomaterial surface topography is believed to be adequate to mimic native ECM for guiding cell growth or tissue regeneration. This structure can be achieved by using an electrospinning technique, which allows producing a non-woven nanofibrous structure with topographic features mimicking the natural ECM.

This study evaluates the influence of micro-topography of patterned Polycaprolactone (PCL) nanofiber meshes, aimed at being used in skin regeneration approaches. The morphology, adhesion and proliferation of primary cultures of human keratinocytes (hKC), dermal fibroblasts (hDFs), and adipose-derived stem cells (hASCs), isolated from the abdominal region, was evaluated after seeding in those structures.

In vitro studies showed that the characteristic morphology of each cell type and respective phenotype was maintained on the patterned electrospun nanofiber meshes during the culture period. SEM micrographs demonstrated that these cells adhered better on the randomly distributed areas of the nanofibers than in the aligned ones. Furthermore, DNA quantification and metabolic activity analysis confirmed the enhanced performance of the cells adhered on the random structures. Additionally, the patterned areas were able to induce cell alignment along the nanofibers.

The combination of the organized and random structures into patterned nanofiber meshes, being able to control cell distribution and proliferation, showed promising characteristics for upcoming studies regarding skin tissue engineering applications.

(OP 147) Human Mesenchymal Stem Cells (hMSC) Increase the Stiffness of Collagen Meshes: Dependence of the Crosslink Percentage

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Introduction: The use of graft materials in uroginecology surgery has been promoted to compensate for the inherent weakness in autologous tissues. The ideal graft should be inert, noncarcinogenic, strong, nonallergenic, noninflammatory, convenient, and affordable[1]. The advantages of collagen matrix laminates made from porcine dermis as implantable xenograft products are well known. Collagen-based meshes possess good mechanical properties, strong resistance and poorly antigenicity. Moreover, they allow a secondary cellular colonization and can become less degradable if modified.

Material and Methods: Porcine collagen meshes (Pelvicol, BARD®) with different crosslink degrees were tested to determine their mechanical properties. Monotonic and cyclic uniaxial tensile tests were carried out along the two principal directions of the mesh. hMSC were seeded and cultured for one week at 37°C in a CO_2 incubator.

Results: Significant differences in mechanical properties between samples with different crosslink percentages were observed. The partial crosslink mesh showed higher anisotropy. The hMSC colonized the collagen mesh and also increased the young modulus of the collagen meshes depending on the crosslink percentage. Damage accumulation due to cyclic load depends also on the crosslink percentage.

Conclusions: Increasing the crosslink percentage produced a stiffness gain with a decrease in the damage accumulation in the collagen meshes. MSC could increase the stiffness of the collagen meshes but not of the full crosslinked ones. This increase is inversely proportional to the crosslink degree.

Reference:

[1]W.A. Silva and M.M. Karram, Scientific basis for use of grafts during vaginal reconstructive procedures, Curr Opin Obstet Gynecol 17 (2005), pp. 519–529.

(OP 148) Human Wharton's Jelly Stem Cells can be Differentiated *In Vitro* into Endothelial Cell Lineage with the Appropiated Culture Mediums

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Introduction: Mesenchymal stem cells have the ability to differentiate into several lineages, and they have high levels of self-renewal. The objective of this study was to verify this potential of differentiation in Human Wharton's Jelly Stem Cells (HWJSC).

Material and Methods: HWJSC were isolated from fresh umbilical cords by mechanical and enzymatic methods. They were separately cultured in Amniomax (Gibco) and M199 (Gibco) supplemented with 1% EGF (Sigma) until reach the 4th passage. Cells fixed in 10% formalin were used to analyze the specific endothelial line markers vWF and CD31. Proliferation studies were realized by trypan blue techniques.

Results: HWJSC showed high proliferation rate in both culture mediums, but Amniomax cultures had a faster cellular proliferation than M199. Cells cultured in M199 did not express vWF until the 4th passage and the marker appeared to be very light at this time. No positive vWF cells were found in Amniomax cultures at any passage. M199 cultures were positive to CD31 since the 1st passage and the positive average percentage was increasing with the passages (from 9% in the 1st to 40% in 4th). This marker was found in 2nd to 4th passages of Amniomax cultures in less than 1%.

Conclusion: If we are able to differentiate *in vitro* HWJSC into endothelial cell lineage using M199 medium supplemented with 1% EGF in the first four passages, we could use this cells in tissue engineering as a substitute of endothelial cells.

This work was supported by a grant from: FIS (PI-061781)

(OP 149) Imaging Techniques to Improve and Control Tissue Engineering Strategies

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With recent advances in tissue engineering there is a strong need for quantitative image processing of three-dimensional natural and engineered biomaterials models to improve and control tissue engineering strategies. A number of new microstructural imaging modalities have been put forward recently allowing quantification with high precision and accuracy. Although biomedical imaging technology is now readily available, few attempts have been made to expand the capabilities of these systems. Nevertheless, quantitative endpoints have become an important factor for success in basic research and the development of novel therapeutic strategies in tissue engineering. Microtomographic imaging is key to these developments and is expected to shed light on the relationship between cell in-growth and viability and structural features of the host material. As part of the presentation, new strategies for advanced imaging techniques to improve and control tissue engineering strategies in skeletal applications will be presented. Results will include the use of imaging for tissue engineering specifically in studies of bone and cartilage repair. The focus will be on hierarchical micro- and nano-imaging as well as image-guided biomechanics. In conclusion, microstructural bone imaging is a nondestructive, non-invasive, and precise procedure that allows the measurement of natural and engineered biomaterials as well as the repetitive 3D assessment and computation of microstructural and micromechanical properties in laboratory, animal and patient studies. The procedure can help improve predictions of material failure, clarify the pathophysiology of skeletal diseases, and define the response to therapy.

Acknowledgment: Partial funding EU/NoE EXPERTISSUES (NMP3-CT-2004-500283).

(OP 150) Improve Tissue-Engineered Bone Regeneration by Introducing Endothelial Cells-Initiating Vascularization

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Natural bone growth greatly depends on the precedent vascular network to supply the oxygen and essential nutrients, while remove metabolites. Likewise it is crucial for tissue engineered bone to establish a vascular network which temporally precedes the new bone formation, and spatially originates from inside of graft. Recapitulating the archetype of skeletal development we developed a complex bone graft used to repair rat bone defects. Based on the cell morphology, quantification of specific antigen molecule, matrix mineralization or capillary-like growth, we demonstrated that the endothelial cells and osteoblast were able to be differentiated and expanded from rat bone marrow mononuclear cells population. Using methods of point-injection and low pressure, the seeded endothelial cells and osteoblast were capable of assembly into microvascular networks and form bony matrix in grafts. In sex-mismatch implantation, Y chromosome assay identified the exogenous origination of these cells and contribution to the vascularization and osteogenesis. By pre-seeding endothelial cells, endothelial cells-initiating vascularization was able to promote osteogenesis, prevent ischemic necrosis and improve the mechanical properties in engineered bone tissue. Taken together, the present study integrating complex cell populations and complex scaffold materials were an effective technique to improve osteogenesis in engineered bone graft and provided important perspective for therapy of mass bone defect clinically.

(OP 151) *In Situ* Generated Scaffolds for Tissue Regeneration

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Injectable polymeric systems play a fundamental role in the emerging "*in situ* generated implants" field, which aims at engineering biomedical structures at their site of performance. The water solutions of reverse thermo responsive display low viscosity at ambient temperature and exhibit a sharp viscosity increase as temperature rises within a narrow interval, producing semi-solid gels at body temperature.

This contribution describes various of the strategies pursued in our laboratory, aiming at tailoring the rheological, mechanical and biological properties of novel reverse thermo-responsive polymers, expanding, therefore, their clinical applicability. Particularly promising are: (i) The generation of high molecular weight thermo responsive polymers, and (ii) The use of functionalized building

blocks that combine thermo-responsiveness and *in situ* cross-linkability. Additionally, these thermo-responsive gels were also reinforced at their site of performance.

Of special significance is the development of scaffolds for Tissue Engineering based on reverse thermo responsive polymers, as an alternative to the currently used pre-formed constructs comprising biodegradable polymers.

The objective of the "*in situ* generated implants" area is not only to deploy materials, but primarily to engineer structures at a precise body site, having specific geometric and mechanical characteristics. This study presents, therefore, the *in vitro* production of macroscopic constructs (e.g. tubular conduits), capitalizing on the improved mechanical properties of the reverse thermo responsive polymers developed. Mono-layered structures as well as conduits comprising two and three layers were engineered *in vitro* and their mechanical response (burst strength, compliance) wil be presented.

(OP 152) *In Situ* Tissue Engineering - Multilineage Potential and Migratory Capacity of Human Periosteum Derived Cells

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Objective: Recently, a mesenchymal stem cell like character of human periosteum-derived cells (PCs) was shown. The aim of our study was to investigate their bone and cartilage formation on the molecular level applying microarrays and their chemokine-dependent migration potential, which is essential for *in situ* tissue engineering applications.

Methods and Results: PCs isolated from periosteum covering mastoid bone were expanded in medium containing human serum, embedded in fibrin-PLGA fleeces and induced towards osteogenesis (dexamethasone) and chondrogenesis (TGFbeta3). Matrix formation was verified by von Kossa (osteogenesis) and alcian blue and collagen type II staining (chondrogenesis) respectively. Gene expression profiling using Affymetrix microarrays was done on PC samples from native tissues, expansion cultures and 3D transplants (osteogenesis: day 7, 14, 28; chondrogenesis: day 14, 28, 42). Data were analyzed with software tools for cluster and pathway analysis. During differentiation culture osteogenic marker genes (e.g. Cbfa-1, osteopontin) and chondrogenic marker genes (e.g. aggrecan, COMP, collagen type II, Sox9) were upregulated. Hierarchical clustering of differentially expressed genes showed a molecular relationship between native tissue and tissue-engineered bone and cartilage transplants. PCs were also checked for their expression profile of chemokine receptors on the mRNA (PCR) and protein (antibodies) level. A chemotactic response to CCL2, CCL25, CXCL8, CXCL12, and CXCL13 was measured in a multiwell chemotaxis assay.

Conclusion: In conclusion, PCs cultured in fibrin-PLGA constructs can be induced to form bone and cartilage, and they migrate after chemokine stimulation. Therefore, they represent promising candidates for *in situ* tissue engineering applications of skeletal defects.

(OP 153) *In Vitro* Characterization on the Interactions between Carboxymethyl-Chitosan/Poly(Amidoamine) Dendrimer Nanoparticles and Neurons/Glial Cells

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Central nervous system associated disorders are a leading cause of disability worldwide. In recent years targeted drug delivery nanoparticle based systems for intracellular application have been put forward has a possible therapeutic route to follow. In this sense the objective of the present report was to characterize and evaluate the possible applicability of recently developed carboxymethylchitosan/poly(amidoamine) (CMC/PAMAM) dendrimer nanoparticles in central nervous system (CNS) cell populations. Atomic force and transmission electron microscopy observations revealed that these nanoparticles possessed a nanosphere-like shape and sizes between 2–123 nm. Moreover it was also possible to confirm by UV/VIS spectrophometry that these nanoparticles could be bound to FITC for tracing purposes. Experiments with post-natal hippocampal neurons and cortical glial cells revealed that both cell populations were able to internalize the CMC/PAMAM dendrimer nanoparticles. The internalization rates changed according to the cell populations, reaching a maximum peak after 48 hours of incubation. Overal astrocytes and microglial cells disclosed higher internalizaton rates (around 100% of the total cell sub-population) followed by neurons and oligodendrocytes (up to 80–90% of the observed cells). Further experiments for periods of up to 7 days revealed that these values were maintained or even increased if CMC/PAMAM dendrimer nanoparticles were periodically added to the culture medium. Finally it was also observed that cell viability and proliferation were not significantly affected by the presence of the above referred nanoparticles. Further studies will be focused on loading relevant drugs for future applications in CNS disorders.

(OP 154) *In Vitro* Comparison of Cardiac-ECM and Urinary Bladder Matrix for Cardiac Remodeling

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While cardiac surgery with synthetic materials improve heart function, they do not form contracting tissue or grow with the patient. One successful regenerative medicine approach for tissue restoration has been the development of naturally occurring extracellular matrix (ECM) scaffolds. Recently, porcine urinary

bladder matrix (UBM) was used to repair myocardial tissue that contributed to regional function in a canine and porcine models, but did not fully regenerate contractile tissue. Given the highly specific composition, organization, and function of individual organ ECM, a xenogeneic ECM scaffold derived from the organ that is targeted for repair (i.e., the heart) may provide the optimal biomechanical behavior and biological signals for site specific remodeling. Therefore, a Cardiac ECM (C-ECM) scaffold from a porcine heart has been developed.

C-ECM development included decellularization, chemical and physical characterization, and cell seeding with comparison to UBM. Decellularization of a whole porcine heart was performed using retrograde perfusion of trypsin, Triton X-100, deoxycholatic acid each for 2 hours and peracetic acid for 1 hour. Decellularization was confirmed with H&E, DAPI, and Pico Green for cellular remnants and DNA content. Movat's Pentachrome and GAG assays were used for chemical characterization. Physical characterization was performed with SEM and uniaxial testing in multiple orientations. Scaffolds were seeded with cardiomyocytes, endothelial, and progenitor cells. Seeded constructs were characterized with ATP biocompatibility assays and confocal microscopy.

C-ECM is ready to be compared to UBM and Dacron in a full thickness right ventricle defect in a GFP bone-marrow chimera rat model scheduled for March 2008.

(OP 155) *In Vitro* Culture of Human amniotic Cells: Phenotype and Differentiation Capacity

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Amniotic membrane is a promising cell source for tissue engineering. This abundantly available and uncontroversial tissue contains epithelial and mesenchymal cells which show stem cell and immunomodulatory characteristics. We have studied the biological variability and adipogenic and osteogenic differentiation capacity of purified amniotic cells in various differentiation media. We further investigated the consequences of *in vitro* expansion, comparing freshly isolated and cultivated human amniotic epithelial cells (hAEC) regarding stem cell characteristics including surface antigen expression profile and their differentiation capacity.

All isolations of epithelial and mesenchymal cells homogenously expressed typical mesenchymal and embryonic stem cell markers. Osteogenesis could be induced in all epithelial and 75% of mesenchymal amniotic, while adipogenesis was detected only in mesenchymal and not in epithelial amniotic cell cultures. Differentiation capacity was strongly dependent on cell type and differentiation protocol but only slightly on biological variation.

We demonstrate that expression of surface antigens changes dramatically during cultivation of hAEC. The mesenchymal markers CD13, CD44, CD49e, CD54, CD90 and CD105 are strongly up-regulated during *in vitro* propagation. In contrast, expression of the embryonic markers TRA-1-60 and TRA-1-81 but not SSEA-4 rapidly decreases upon cultivation. The phenotypic shift is associated with reduced osteogenic differentiation. This suggests that phenotypic alterations of hAEC during cultivation might be associated with a functional reduction of the differentiation potential, which should be considered for potential application of these cells in cell based therapies.

Acknowledgments: HIPPOCRATES (NMP3-CT-2003-505758); EXPERTISSUES (NMP3-CT-2004-500283).

(OP 156) *In Vivo* Behaviour of Injectable Polymeric/Calcium Phosphate Cement Composites: an Experimental Study in Rabbits.

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Calcium phosphate compounds are osteoconductive and can reach to mechanical strenghts that are comparable to bone. Especially calcium phosphate cement (CPC) is a good candidate for bone repair since it can be shaped to the defect site after injection and render an optimal contact between bone and material. However, *in vivo* resorption and tissue ingrowth is slow. To improve these properties, microspheres can be introduced, which will create macroporosity into the system.

The current study focussed on the *in vivo* behaviour of injectable composites consisting of CPC with PLGA and Gelatin microspheres. Osteoconductive and osteoinductive behaviour were examined respectively in the femoral condyle and subcutaneous tissue of a rabbit, with implantation periods of 4, 8 and 12 weeks. Histological examination of the CPC/PLGA composite showed new bone formation and almost complete degradation of the CPC cement after 12 weeks of implantation. On the other hand, solid CPC cement was completely surrounded by newly formed bone and no degradation could be observed. However, CPC/gelatine composite revealed a moderate degradation and a lower bone contact. Subcutaneous implantation showed that all CPC/microsphere composites exhibited microspheres degradation. Furthermore, all composites showed a similar tissue response with a decreasing capsule thickness and moderate inflammatory response over time. In conclusion, the *in vivo* degradation of the CPC/microsphere composites is dependent of the degradation pattern of the individual microspheres. Although no clear osteoinductivity was found, the injectable cement showed excellent biocompability as well as osteoconductivity.

(OP 157) *In Vivo* Effects of TGF-β1-Loaded Polymeric Microsphere Incorporation in Injectable Cap Cement

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Calcium phosphate cements (CPCs) represent feasible synthetic bone substitution and regeneration materials. To facilitate degradation, composites of CPC and degradable, polymeric microparticles have been developed. In addition to the creation of porosity within the implant after microparticle degradation, these microparticles can be loaded with appropriate biologicals (e.g. growth factors) to direct biological responses.

The current study focused on the *in vivo* effects of injectable composites consisting of CPC with microspheres, either or not loaded with transforming growth factor-β1 (TGF-β1). Bone augmentation properties of an injectable CPC/PLGA composite were evaluated using an *in vivo* rat model, in which the composite was injected onto the skull for implantation periods of 2, 4, and 8 weeks. Histological and histomorphometrical analyses showed that microsphere loading with TGF-β1 significantly increased initial bone-implant contact. Moreover, TGF-β1 loading significantly enhanced bone formation after prolonged implantation periods. Alternatively, an injectable CPC/gelatin composite was implanted in a cylindrical femoral condyle defect in rabbits for 4, 8, and 12 weeks. Irrespective of TGF-β1-loading, all implants showed an increase in mechanical strength with implantation time. Further, TGF-β1-loading significantly accelerated implant degradation, whereas no effects on bone-implant contact or bone formation were observed compared to composites containing non-loaded gelatin microspheres. These results demonstrate the applicability of CPC/microsphere composites for bone augmentation and substitution, and demonstrate that growth factor loading of the microspheres can further direct biological responses.

(OP 158) *In Vivo* Engraftment Potential of Human Bone Marrow and Amniotic Fluid Stem Cells Cultured Under Osteogenic Conditions

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The potential immune privilege presented by mesenchymal stem cells (MSCs) makes them a promising population in tissue engineering and regenerative medicine. In this study, we investigated the effect of culture medium (expansion versus osteogenic media) over the *in vivo* potential of adult stem cells derived from human bone marrow (hBMMSCs) and amniotic fluid (hAFSCs).To this end, we isolated hAFSCs from day 6 supernatant of the cultures of amniotic fluid obtained from amniocentesis, and hBMMSCs were a kind gift from Biopredic. Cells were maintained in culture until confluence either in expansion or in osteogenic media (7 days), and then transplanted into 58 to 62 day-old foetal sheep at a concentration of 1×10^6 cells/foetus. Pregnant ewes were fasted for 24 hours. General anaesthesia was induced with thiopental sodium and maintained by inhalation anaesthesia with isoflurane and oxygen. After general anaesthesia, the ewes were positioned in dorsal recumbency and prepared in a sterile surgical environment for a ventral midline celiotomy. The abdomen was exposed through a ventral midline incision and the gravid uterus located. After the identification of the foetus inside the uterus and their gentle handling contention against the inner epithelium layer of the uterus, cells were transplanted into the intraperitoneal foetus cavity by injection through the intact uterus walls. The animals were euthanized sixty days after transplant, and samples from various tissues were collected. The engraftment and phenotype of human-derived cells was evaluated by flow cytometry and immunocytochemistry analysis.

(OP 159) *In Vivo* Evaluation of Fibrin-Based Tissue Engineered Heart Valves in a Sheep Model

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Objective: Our group has previously demonstrated the synthesis of dynamically-conditioned tissue-engineered heart valves based on an autologous fibrin scaffold. The present study aims to evaluate the structure and mechanical stability of fibrin-based heart valves following implantation in a sheep model.

Methods: Autologous tissue-engineered heart valves were moulded using a fibrin scaffold, ovine carotid artery-derived myofibroblasts and endothelial cells, before subjection to 28 days of mechanical conditioning in a bioreactor. Following conditioning, tissue-engineered valves were implanted in the pulmonary trunk of the same animals ($n=4$) from which the cells had been harvested; identical valves conditioned in parallel served as controls. Valves were explanted after 1 and 2 months and analysed using routine histology, immunohistochemistry, electron microscopy (EM) and extracellular matrix (ECM) assay.

Results: Explanted valve conduits had excellent tissue consistency after 2 months *in vivo*. Routine histology showed excellent tissue development and cell distribution, functional blood vessel ingrowth in the conduit wall, with no evidence for inflammation. Immunohistochemistry and ECM assay demonstrated almost complete resorption of fibrin gel components and replacement with ECM proteins. A monolayer of vWf-positive endothelial cells lined the valve surface, and was shown to be completely confluent using scanning EM. The resident valve tissue cells were in excellent health, as evidenced by transmission EM.

Conclusions: Preliminary results of implanted fibrin-based tissue engineered heart valves are encouraging, with remarkable

tissue remodelling and mechanical stability after 2 months *in vivo*. The results suggest that it may be possible to construct truly ''autologous'' tissue-engineered heart valves on a patient-to-patient basis.

(OP 160) *In Vivo* Immune Response to Allogeneic Porcine Mesenchymal Stem Cell (MSC) Transplantation: a 12-Day Course of FK-506 Abbrogates Humoral Response

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Purpose: Several reports have highlighted the low immunogenic profile of MSC. Therefore, we decided to test *in vivo* the humoral response to allogeneic MSC transplantation and the effect of transient immunosuppression in a porcine model.

Methods/Material: MHC-controlled mini-swine SLAcd and SLAdd were used as donor and recipients, respectively. Two sites of transplantation were selected: subcutaneous and intracardiac. In our control group ($n = 5$), animals received no immunosuppression. In the study group ($n = 11$), 1×10^6 allogeneic MSC/kg were injected. Tacrolimus was given from day 0–12. Recipient sera were collected weekly after MSC transplantation. The presence of specific anti-donor IgM and IgG was tested by flow cytometry and by complement-mediated cytotoxicity assay.

Results: In the control group, all animals developed humoral responses in both IgM/G classes that persisted up to ten weeks. A single subcutaneous injection failed to elicit a complement-mediated cytotoxicity but subsequent re-challenge did.

In the study group, only 2 out of 11 animals developed a transient humoral response, both in IgM and IgG, whereas all others failed to develop donor-specific antibodies. However, none of the sera tested from those 11 animals could elicit a complement-mediated cytotoxic response.

Conclusion: Allogeneic MSC can elicit prolonged humoral responses despite their putative low immunogenic profile. As already shown for experimental allogeneic organ transplantation, a transient immunosuppressive regimen can overcome the initial B cell response. Our result suggests that *in vitro* and *in vivo* characteristics of MSC might differ and emphasizes the importance of pursuing research on allogeneic stem cell transplantation.

(OP 161) *In Vivo* Tissue Engineering of Vascular Grafts Using High Specific DNA-Aptamers as Capture Molecules for Circulating Endothelial Progenitor Cells

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Objective: Due to their insufficient biocompatibility and high thrombogenicity, small diameter artificial vascular prostheses still do not show a satisfactory patency rate. *In vitro* endothelialization of artificial grafts before implantation has been established experimentally years ago, but, has never been used for routine clinical applications. This study deals with the coating of graft surfaces with capture molecules for circulating endothelial progenitor cells (EPCs), mimicking a pro-homing substrate to fish out EPCs from the bloodstream after implantation.

Methods: Aptamers against EPCs were generated by systematic evolution of ligands by exponential enrichment (SELEX), a technique from combinatorial chemistry. They can be selected from a library of 1015 starting nucleotides. We have spotted a defined aptamer onto a hydrogel coated surface, installed in a flow chamber, to detect the attachment of EPCs from fresh human whole blood. Finally these cells were cultivated in growth factor enriched medium and fluorescence marked antibodies against CD34, CD 31, von Willebrand factor and VEGFR-2 were used to characterize the cell attachment.

Results: After eight SELEX rounds 36 aptamers in total were cloned, sequenced, synthesized and evaluated by flow cytometry. The best binding aptamers were immobilized in a flow chamber. The captured cells were positive for CD34, CD 31, von Willebrand factor and VEGFR-2 and able to differentiate into a confluent endothelial layer.

Conclusion: We hypothesize that *in vivo* self-endothelialization of blood contacting implants by homing factor mimetic capture molecules for EPCs may bring new perspectives towards future clinical applications of stem cell and tissue engineering strategies.

(OP 162) *In-Vitro* Investigation of Esophageal Cell Organization and Collagen Scaffold Interaction

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Esophageal tissue and organ demand is foreseen not only in the pediatric but also in the adult population. Since the intention of esophageal tissue engineering project is to replace the esophagus in the thoracic esophagus, two cell types-esophageal epithelial cells and smooth muscle cell have been identified to engineer esophageal tissue. The aim of this study was to isolate, culture and seed rat esophageal epithelial cells. Using the Sprague-Dawley-rat model, esophagus tissue was isolated and treated with enzymatic solutions to release the esophageal epithelial cells from the mucosal lining. These cells were cultured to investigate the organization of these cells under culture conditions and to evaluate there interaction with collagen scaffolds.

Optimizing of esophageal epithelial cell protocols were necessary to increase the cell yield. Microsurgical techniques were employed to slide through the esophageal epithelial lining to provide maximal exposure for cell extractions. Collagen coating of tissue culture plates was necessary and was found to increase cell attachment which was extremely low on non-coated surfaces. Medium with serum offered better cell viability, proliferation and differentiation when compared to serum free media.

Positive identification of esophageal epithelial cells were done using immunofluorescent and immunohistochemical CK-14 markers which as specific for esophageal epithelial cells. Collagen sponges were rolled into tube and sutured and viable esophageal epithelial cells were demonstrated 4 weeks after seeding on the collagen sponge tubes. The initial results from our investigations are promising steps in the tissue engineering of esophagus.

(OP 163) Influence of the EPC-BMSC Proportions Included in a PRP Gel on Cell Differentiation

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Bone formation highly depends on osteogenic cells presence at the implant sites, but vascularisation is also crucial. Our aim is to establish a precellularised bone graft containing both endothelial and bone cells. We previously showed a strong effect of Platelet Rich Plasma (PRP) on BMSC differentiation into osteoblast, and that direct contact between BMSC and HUVEC enhances osteoblastogenesis and vessels-like formation. Willing to develop an autologous bone grafts, we then studied the effect of endothelial progenitor cells (EPC) on BMSC differentiation, and the influence of the cellular proportion (EPC vs BMSC) on cell differentiation. BMSC were isolated by density-gradient centrifugation. EPC (CD133+) cells were isolated from mononuclear cell fraction of bone marrow using magnetic beads (Miltenyi Biotec). PRP was prepared from thrombocyte concentrates diluted in plasma (2×10^6 platelets/mL). PRP was activated using 0.5U thrombine. EPC and BMSC were mixed in different proportions: 100%EPC, 75%-25%, 50%-50%, 25%-75%, 100%BMSC. Cell proportions were mixed together within a PRP gel (3D). LDH analyses showed cell viability of both cell types in 3D, after 21 days of culture, for each proportion. In 3D, we observed an up-regulation of runx2 and dlx5 genes for BMSC co-cultured with EPC when compared to BMSC alone. The expression was found higher for the proportion 25%EPC-75%BMSC compared to higher EPC proportions. This work shows the high potential of EPC as an autologous source of endothelial cells, and their positive effect on BMSC differentiation.

(OP 164) Inhibition of Proteoglycan Production Influences Cartilage Collagen Network Formation

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Cartilage tissue engineering is hampered by slow production of collagen. In the present study we investigate the influence of proteoglycans on collagen network.

Bovine chondrocytes were cultured in alginate beads with(out) 0.25 mM para-nitrophenyl-beta-d-xyloside (PNPX). PNPX is an exogenous acceptor for galactosyl transferase I that prevents glycosaminoglycans (GAGs) being incorporated in the matrix. GAG and collagen deposition and distribution in matrix and medium, collagen crosslinks, aggrecan and collagen II gene expression, and mechanical properties were determined.

At day 21, 33.3 ± 4.0 µg GAG/bead was deposited in controls and 6.6 ± 1.4 µg GAG/bead with PNPX. Almost no GAG was detectable in culture medium of controls, this increased with PNPX. AGCN gene expression was slightly upregulated, not reaching statistical significance. 21.6 ± 4.4 µg collagen/bead was deposited in controls and 14.4 ± 4.0 µg collagen/bead with PNPX. Collagen II expression was not affected by addition of PNPX. PNPX addition resulted in a shift in GAG deposition from cell-associated to Further-Removed Matrix (FRM) (64% in FRM versus 55% in controls) and collagen (53% in FRM versus 29% in controls). The number of collagen cross-links decreased from 0.13 ± 0.02 HP/collagen in controls to 0.08 ± 0.03 HP/collagen in the presence of PNPX. Mechanical properties, measured by stiffness and ability to hold water, were decreased when PNPX was added.

The absence of a GAG network results in diffusion of collagen away from the cells and probably also excretion into the culture medium, leading to less collagen deposition. GAGs therefore seem to contribute in the formation of a functional collagen network.

(OP 165) Injectable Gellan Gum Hydrogels as Supports for Cartilage Tissue Engineering Applications

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Gellan gum is an extracellular microbial polysaccharide from Sphingomonas paucimobilis that forms a firm and transparent gel in the presence of metallic ions. This hydrogel presents some interesting features that allow its use as a cell encapsulating support, or as an *in vivo* injectable system. In this work, the usefulness of gellan gum hydrogels as supports for cartilage tissue engineering applications was shown. Processing versatility of gellan gum into different structures such as discs, fibers, membranes, particles, and 3D porous structures using temperature and pH based technologies was demonstrated. The characterization of these structures was conducted using rheological analysis, dynamic mechanical analysis, transmission electron microscopy and cytotoxicity assessment. *In vitro* tests with human articular chondrocytes were conducted during a 8 weeks period. The histological characterization of the tissue engineered constructs was performed using different stainings for cartilage extracellular matrix (ECM) components. At the molecular level, real time PCR was used to quantify the expression of cartilage ECM markers such as col I, col II, aggrecan and Sox9. The *in vivo* performance of the developed structures was investigated in mice. The properties of the materials revealed that gellan gum is adequate to be used as a cell encapsulation support or as an injectable system. The *in vitro* results showed that the typical cartilage ECM components, col II and aggrecan, were being expressed throughout the culturing periods. The *in vivo* results obtained so far are rather promising envisaging the use of gellan gum hydrogels in the cartilage tissue engineering field.

(OP 166) Interface Integration of Rapidly Engineered Multilayer Collagen Scaffolds

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The ability to engineer tissue interfaces, with the possibility of measuring interface integration would offer an invaluable tool in surgical tissue reconstruction and repair biology. Plastic compression (PC) of collagen hydrogels has been shown to rapidly produce dense, mechanically strong collagen sheets (100 μm thick) with high cell viability(Brown *et al*. 2005). In this study we investigated the interface integration of multilayer scaffolds based on such compressed collagen constructs.

Acellular or HDF-seeded collagen gels were cast on top of each other, in order to produce bilayer constructs. After setting, acellular constructs were compressed and fitted onto a mechanical testing system to measure the interface adhesive strength. Cell-seeded constructs were either compressed at D0 and cultured for 7 days(compressed culture) or left attached to the casting well during the culture period and compressed on D7(compliant compressed culture). Interface adhesive strength was then measured.

After 1 week culture strength of the integration between two collagen layers increased 6-fold ($p < 0.05$), compared to acellular constructs. For compliant compressed cultures, interface break force was higher ($2282 \pm 383 \times 10^{-5}$N) compared to compressed cultures ($1636 \pm 145 \times 10^{-5}$N). Cell migration across the interface i.e. cell translocation might play an important role in the integration process. We tracked cell migration in both models at 24 hours and found that cell migration was greater in the complaint-compressed culture.

Integration of tissue engineered structures with host tissue post implantation is critical for successful outcomes. We have developed an *in vitro* model which can define integration parameters and after some refinement should be capable of predicting *in vivo* integration outcomes.

(OP 167) Interleukin-18 System Plays an Important Role in Keloid Pathogenesis via Epithelial-Mesenchymal Interactions

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Keloid scarring is a dermal fibroproliferative disorder characterized by increased fibroblast proliferation and excessive production of collagen-extracellular matrix (ECM) components. To date, the role of cytokines in the keloid pathogenesis has not been unraveled. Interleukin-18 (IL-18) is a pro-inflammatory cytokine that plays important roles in wound healing, fibrogenesis and carcinogenesis, and our aim was to study the role of IL-18 sytem in keloid pathogenesis. Results revealed that IL-18, IL-18Rα and IL-18Rβ expression were elevated in keloid tissue compared with normal skin tissue. Studies on the expression of IL-18 and its antagonist, IL-18 binding protein (IL-18BP) using a coculture model demonstrated severe IL-18/IL-18BP imbalance in keloid keratinocyte/keloid fibroblast cocultures with significant elevation of bioactive IL-18 whereas IL-18BP levels which remained the same. Overproduction of bioactive IL-18 in keloid cocultures could be due to increased Caspase-1 and decreased Caspase-3 expression in keloid tissue, as well as decreased soluble IL-10 levels observed in keloid cocultures. The important inductive effects of IL-18 on keloid fibroblasts (KF) was further underscored by the observation that exposure of KF to IL-18 resulted in increased cellular proliferation, collagen-ECM component synthesis, increased secretion of profibrotic cytokines, as well as decreased production of matrix-degrading proteases and antifibrotic cytokines. The present study has proven that the IL-18 system plays an important role in keloid pathogenesis via epithelial-mesenchymal interactions. It also suggests a therapeutic potential of PI3K, MAPK, Sp1 and mTOR inhibitors in the treatment of keloid scarring.

(OP 168) Intervertebral Disc Regeneration Using Adipose Stem Cells; a Large Animal Model in Goats

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Introduction: Adipose stem cells (ASCs) can be obtained rapidly, in high yields, with minimal patient discomfort. This allows stem cell therapy concepts in which the stromal vascular fraction (SVF) containing ASCs is harvested, either or not stimulated differentiation-induced, and re-implanted within one single surgery. For preclinical feasibility assessment, we developed a mild disc degeneration model in goats.

Purpose: SVF still contains additional cell types apart from ASCs (endothelial cells, monocytes, erythrocytes (RBCs)). Since haemophilic arthropathy models showed that monocytes/RBCs may evoke radical formation and inflammatory responses, we evaluated whether RBC removal and/or other cell types from SVF is mandatory for inflammation-free IVD regeneration.

Methods: Degeneration was induced in lumbar IVDs of six goats with Chondroitinase ABC (cABC). After 12 weeks, these IVDs were randomly injected with PBS, SVF, SVF meticulously RBC-deprived using Optiprep®, or cultured ASCs (isolated during cABC degeneration induction surgery and cultured to homogeneity). After twelve weeks, goats were euthanized and analyzed using X-ray, MRI, and histology.

Results: PBS resulted in disc height loss, decrease in MRI Index and mild degenerative changes on histological examination. SVF resulted in severe inflammatory responses, resulting in severe degenerative changes in all parameters. Histologically, round cellular infiltration and osteoclastic activity were observed. This inflammatory response was observed in neither cultured nor Optiprep® ''treated'' discs. The disc height and MRI index were decreased comparable to PBS-injected discs.

Conclusion: Removal of RBCs from SVF prevented a negative, inflammatory response in degenerated IVDs, implying that RBC removal from SVF is crucial for IVD regeneration.

(OP 169) Intra-Individual Comparison of Human Ankle and Knee Chondrocytes *In Vitro*: Relevance for Talar cartilage Repair

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As compared to knee chondrocytes (KC), talar chondrocytes (TC) have superior synthetic activity and increased resistance to inflammatory stimuli. We thus investigated whether these properties are maintained when TC are isolated from their own environment and de-differentiated *in vitro*.

TC and KC ($n = 10$) were expanded in monolayer for 2 passages and then cultured in pellets for 3 and 14 days or in hyaluronan meshes (Hyaff®-11) for 14 and 28 days. The generated tissues were assessed biochemically [glycosamminoglycans (GAG), DNA, collagen I and II], histologically (Safranin-O) and by RT-PCR (collagen I and II). The proteoglycan and collagen synthesis of the pellets were measured following or not exposure to IL-1b.

Following 14 days of pellet culture, TC and KC expressed similar amount of collagen I and II mRNA and produced tissues with comparable amount of GAG and collagens. Proteoglycan and collagen synthesis increased between 3 and 14 days of culture to a similar extent for TC and KC. The drop in synthetic activity in response to IL-1b was similar among TC and KC.

Following 14 days of culture in Hyaff®-11, TC and KC generated tissue with similar amounts of GAG and collagens. The increase in the contents of these macromolecules from 2 to 4 weeks culture was larger (up to 2.2-fold) in tissues generated by KC.

The superior synthetic activity of TC as compared to KC is lost when chondrocytes, isolated from their original matrices, are de-differentiated and subsequently induced to re-differentiate, suggesting a critical role of the tissue environment in determining the properties of KC or TC.

(OP 170) Intracellular Fate Investigation of Bio-Eliminable Polymeric Nanoparticles by Confocal Laser Scanning Microscopy (CLSM)

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The present contribution is reporting on the assessment of *in vitro* cytotoxicity and intracellular fate of poly[(glycylglycine methacrylamide)x-co-N-(2-hydroxypropylmethacrylamidey)] bioeliminable polymer samples and relevant nanoparticles in Balb/c 3T3 cloned A31 mouse embryo fibroblasts cell line by using Confocal Laser Scanning Microscopy (CLSM). Nanoparticles were prepared by co-precipitating the polymers with fluorescein labeled human serum albumin (HSA-FITC) as the fluorescent probe and as the model protein drug. The toxicity of the polymer samples consisting of 25, 50 and 100%, respectively, of glycylglycinemethacrylamide monomeric units (GGMA), was investigated in terms of cytoskeleton morphology by exposing cell cultures to various concentrations of polymers for 24 h. Under normal culture conditions, fibroblast cells exhibit characteristic spreading and shape, however, when the cell cultures were subjected to chemical, metabolic or physical stress, their morphology changed in terms of cytoskeletal architecture and reducing their visibility. The co-polymer samples at 25% GGMA monomeric units showed a lower toxicity even at high concentrations [8.5 mg/mL]. The cellular uptake of polymeric nanoparticles was determined by comparing incubation of fibroblasts with HSA-FITC loaded particles to HSA-FITC alone at three different time endpoints. The results indicate that the nanoparticles were up-taken by the cells in a time dependent fashion. Computer assisted analysis of nanoparticles fluorescence emission suggests a possible lysosomal escape as intracellular fate.

(OP 171) Isolation and Characterisation of Stem Cells from Different Human Salivary Glands

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There is continuously growing interest in adult stem cells as a source for regenerative medicine and tissue engineering applications. The characterisation of novel stem cell sources is therefore of utmost concern.

Recently we reported for the first time the presence of adult stem cells with mesenchymal characteristics in human parotid gland tissue. In this study we isolated stem cells from human submandibular glands and compared these cells to cells from parotid origin and characterised both in more detail.

Cells were isolated from normal submandibular and parotid glands by enzymatic digestion. Following initial proliferation cells were characterized by flow cytometry. For differentiation specific induction media and growth conditions were used with the purpose to generate adipogenic, osteogenic and chondrogenic cells. Differentiation was assessed by histochemical and immunocytochemical stainings as well as by the demonstration of specific mRNA using RT-PCR.

Cells from both gland types had surface characteristics very similar to mesenchymal stem cells. They were e.g. positive for CD13, CD29, CD44, and CD90 and negative for CD34 and CD45. Cells could be induced into adipogenic, chondrogenic and osteogenic celltypes, demonstrating their differentiation capability.

In this study we demonstrated that stem cells with mesenchymal characteristics can be derived from both adult parotid and submandibular glands. Thus, human salivary gland tissue might be a novel promising tissue source for future applications of these stem cells in tissue engineering and regenerative medicine.

This work was supported by Novartis Foundation for Therapeutic Research and the Network of Excellence EXPERTISSUES

(OP 172) Isolation and Expansion of Human Umbilical Cord Perivascular Cells (HUCPV)

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Adult mesenchymal stem cells from bone marrow (BMSC) represent the most commonly used cell source for applications like cell-based therapies or bone marrow transplantations. However, due to the limited number of BMSCs available for autogenous use and additionally the possibility of donor site morbidity, there is a need to identify alternative cell sources. Recently, Wharton's Jelly (WJ) of the human umbilical cord has been identified as a potential new cell source. Cell populations derived from this tissue have been shown to be major histocompatibility complex (MHC) class I/II negative, making them a potential cell source for allogeneous therapies, without ethical constraints.

In this study an outgrowth protocol was compared with a collagenase digestion. The outgrowth of HUCPV ($n = 6$) started around day 10. The first harvesting time point was dependent on the amount and length of WJ surrounded vessels per flask. The time range was between 24 days for three vessels and 45 days for one vessel per flask. The harvests yielded an average of 5.6×10^6 cells. Thereafter, the outgrowth of cells was much faster, leading to an average harvesting time of 9 days for outgrowths 2–4. The maximum cell yield summed up to 24×10^6 cells, in average 19×10^6 cells were isolated during the four outgrowths. In comparison to the collagenase digestion the cell yield increases by a factor of 10. Cell expansion was dependent on the seeding density. The highest expansion (34-fold) was achieved at a cell density of 500 cells/cm^2.

(OP 173) Isolation of Adipose Stem Cells (ASCs) Subpopulations with Distinct Differentiation Potential

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ASCs are becoming the elected cells for TE applications because ASCs have been easily isolated and have shown good differentiation potential. The aim of this work was to isolate the ASCs using immunomagnetic beads coated with different antibodies (Ab) markers and to test the differentiation potential of the different subpopulations isolated. The Ab used were CD29, CD44, CD49d, CD73, CD105, STRO-1 and NGFr (p75). Once isolated, the cells were cultured with Basal Medium until the confluence, then the cells were trypsinized and divided in 3 groups. The first one was used to perform RT Real-Time PCR for CD44, CD105; CD73, CD90 and STRO-1. The second group was cultured with Osteogenic Medium for 3 weeks. The third one was used to set up a pellet culture with chondrogenic medium and cultured for 3 weeks. After 3 weeks of culture with osteogenic medium or chondrogenic medium the samples were retrieved. Alizarin Red Staining and RT Real-Time PCR for Osteocalcin and Osteopontin were used to establish the osteoblast differentiation potential. Alcian Blue, Toluine Blue and Safranin O staining and RT Real-Time PCR for Agrecan, Collagen I, Collagen II, Collagen X and Sox 9 assess the chondrogenic differentiation potential. The described method was effective to isolate distinct subpopulations which present different gene expression profile relative to the stem cell markers studied. The expression of osteogenic and chondrogenic markers showed that these subpopulations exhibit significantly different differentiation potentials.

Acknowledgements: Marie Curie Actions Alea Jacta Est, Project HIPPOCRATES, NoE EXPERTISSUES.

(OP 174) Lactide-PEG Cryostructures with Stem Cells as Tissue Engineering Scaffolds in Reconstruction of Cranial Bone Defects in Rat Model

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Poly(L-lactide)(PLLA)-poly(ethylene glycol)(PEG) copolymers were synthesized by ring opening polymerization of lactide dimer with PEGs with different molecular weights, and molar ratios. They were characterized with GPC, ^{1}H-NMR and DSC. Cryostructures were prepared from these copolymers in organic medium. Salt crystals in the size range of 100–200 μm were used to create macropores pores by a salt-extraction process in addition to the relatively smaller pores that are formed during cryogelation. Scaffolds with dimensions of 8 mm in diameter and 1 mm height were used in a critical size cranial bone defects in rats with or without using mesenchymal stem cells that were obtained from rat bone marrow. Animals were sacrificed on 1, 3 and 6th months after implantation, and the specimens were taken out from the implantation site with the surrounding tissues for histological analysis. Histological data confirmed ingrowth of connective tissue cells with vascularization within the scaffold. Group with mesenchymal stem cells resulted with much better scores and bone mineralization. It has also been reported that the scaffolds were almost fully degraded at 6th month after implantation.

(OP 175) Lessons from the Sea: Biomaterials Development Inspired by Marine Bioadhesives

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Biomedical engineers can easily find inspiration for exciting research directions by looking to the oceans. Barnacles, starfish, sea cucumbers, and mussels are examples of the many marine organisms that generate adhesive materials. Although the details of formation and bonding within these biological materials are not completely understood, barnacle and mussel adhesives are known to be protein-based materials. The animals apply proteins to surfaces and then cross-link these proteins to yield the final, cured adhesive matrix. These bioadhesives exhibit various properties appealing to the tissue engineer: a cross-linked protein environment, the formation of strong bonds to surfaces, low toxicity, and the ability to set in wet environments. Studies in our lab have begun by characterizing marine adhesive materials. We are working with live mussels, extracted adhesive protein, and synthetic peptide models. Our results indicate that mussels use iron for the key reagent to cross-link their adhesive proteins. With this knowledge in hand, we are testing mussel adhesive for biomaterial development. Although promising results are being obtained, using the natural material can be somewhat limiting. Consequently we are taking advantage of our characterization insights to design new synthetic biomaterials. We have combined mussel adhesive cross-linking chemistry with the accessibility and flexibility of bulk polymers. Early results show these materials to exhibit strong adhesive properties. Ongoing efforts are focused on developing surgical adhesives, dental cements, bone cements, and cell growth scaffolds for organ generation.

(OP 176) Linking the Physical Environment to Cell Behaviour–Applications for Tissue Engineering and Regenerative Medicine

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Physical cues such as the mechanical environment are well known to influence cell behaviour and growth of tissues. Using *in vivo* and *in vitro* models, we have been able to study the mechano-receptors which control these interactions in a variety of cell types. We have been studying the potential ways to activate or regulate these mechano-receptors for tissue engineering applications using three approaches. Firstly, we design growth environments which apply physiological stresses similar to the ones which the implant will experience in a patient. The stresses can vary according to the ultimate functional tissue design. Secondly, we have extended to create scaffolds which augment the mechanical receptors by providing targeted release agonists. In this way, we can allow mechanical cues to be enhanced with release strategies promoting tissue growth in areas subjected to mechanical loads. In both approaches, mechanical environments are applied to combinations of cell seeded biomaterials which ultimately relies on the initial mechanical integrity of the construct. For conditioning of brittle or soft scaffolds e.g. ceramics or injectables, we have developed a technology for direct activation of cell membrane mechanoreceptors using magnetic nanoparticles coated with specific antibodies or peptides, e.g. TREK1 or RGD. We then apply a varying magnetic field activating the cells remotely across a 3D construct. Our work has shown targeted differentiation and matrix production of MSCs in 2D and 3D tissue engineering configurations. In addition, the magnetic particle technology can be adapted for *in vivo* tissue engineering where cell seeded constructs mature inside the patient.

(OP 177) Living Biological Annuloplasty Rings with Potential to Growth for Reconstructive Mitral Valve Surgery

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Introduction: Growing materials for reconstruction of cardiac structures are of big advantage for pediatric surgery. In the present study, we investigated the integrative capacity of viable and non-viable allo- and xenogeneic tracheal cartilage rings, (allo-/xeno-vTCR; allo-/xeno-nTCR), respectively, implanted for mitral valve (MV) annuloplasty.

Methods: Ovine and porcine native vTCRs and deep-frozen nTCRs were implanted in MV position for 3 and 9 months ($n = 3$, each) in juvenile sheep. The size of each implanted TCR was adapted to the MV size. MV function and TCR position were analyzed by echocardiography. The TCR viability was analyzed by LIVE/DEAD assay. The TCR integration was evaluated by macroscopy, ring planimetry, H&E-, Movat-pentachrome-, von Kossa-stainings, SEM.

Results: No echocardiographic differences were detected after implantation. Before explantation in the xeno-vTCR and the xeno- and the allo-nTCR groups a significant TCR-shrinkage with development of MV stenoses were observed, whereas the size of all allo-vTCRs was distinctly increased that paralleled the somatic growth of the animals. No MV insufficiency was observed in all groups. The laboratory analyses showed the superior integration of allo-vTCRs with significantly lower rate of ring dehiscence, inflammation and calcification as compared to others. Consistently, viable cartilage cells were found only in allo-vTCRs and also a complete endothelialization was observed in this group only.

Conclusion: Since allo-vTCRs implanted in MV position show excellent intracardiac tissue integration paralleled with ring size increase according to the somatic growth, native viable TCR may represent a well suited living material for reconstructive cardiac surgery especially for pediatric patients.

(OP 178) Long Term *In Vivo* Performance of Starch-Based Scaffolds

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Natural-based biomaterials are mainly constituted by proteins or polysaccharides which, under certain circumstances, may be recognized as natural host invaders. Starch-based scaffolds, produced by different methodologies, have been shown to hold adequate properties regarding mechanical performance and *in vitro* biocompatibility. This study aimed at evaluating the *in vivo* inflammatory response to starch-based scaffolds. Wet-spinning produced starch-polycaprolactone (SPCL) scaffolds were subcutaneously implanted in rats for 8 and 12 weeks in order to assess their long-term *in vivo* behaviour. Macroscopically, the implantation area did not show signs of inflammation. At the different time points the implanted scaffolds and surrounding tissue were explanted, together with the neighbouring lymph nodes, and histological analysis was performed. The image analysis of the histological samples showed good integration of the scaffold in the subcutaneous tissue, with the presence of collagen fibres and newly formed blood vessels. A mild inflammatory infiltrate was observed, although some foreign-body giant cells were detected in the surroundings of the scaffold's fibres. The histological analysis of the lymph nodes showed no signs of activation. All together, these findings allow concluding that the wet-spinning produced starch-based scaffolds did not elicit neither inflammatory nor immune responses after long periods of implantation in rats and are suitable for long-term tissue engineering approaches.

Acknowledgements: This work was partially supported by the European Union funded STREP Project HIPPOCRATES (NMP3-CT-2003-505758) and was carried out under the scope of the European NoE EXPERTISSUES (NMP3-CT-2004-500283).

(OP 179) Marine Polysaccharide Multilayers: PH Responsive Systems for the Surface Modification of Tissue Engineering Scaffolds

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The success of some polymeric scaffolds for Tissue Engineering is hindered by its surface chemistry, which in many cases leads to a significant foreign body response. To overcome this, the present project intend to explore a strategy of surface modification through electrostatic self-assembly, first reported in the 1990s, by the construction of multilayered systems by assembling a polycation and a polyanion in an alternate fashion.

In the present work, polyelectrolyte multilayers constituted only by marine polysaccharides are studied by Quartz Crystal Microbalance with Dissipation monitoring, QCM-D, focusing on the effect of pH in their construction, properties and stability. The results show the effectiveness of layer-by-layer assembly with the consecutive deposition steps, with the mass of each layer being dependent on the solution pH. Moreover, the effect of pH is also present after the preparation of the multilayer, once the resonance frequency of the crystal changes when it is submitted to solutions with different pHs. This was attributed to an effect on the multilayer structure due to the ionization/deionization of different groups within the macromolecules. The variation in dissipation of energy with pH supports this, once dissipation is dependent on the viscoelastic properties of those multilayers. In this way, these multilayers may constitute a pH responsive smart material.

The knowledge acquired will be later transferred to 3D substrates–porous scaffolds–with the final goal of controlling their surface properties, maintaining the mechanical properties of the original scaffolds.

(OP 180) Measuring Ion/Element Changes in Cells in Culture to Monitor Cell Death

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To be successful, tissue engineering requires that cells are grown and differentiate outside of the body for a period of time and it is necessary to be able to determine the viability of these cultures before re-implantation occurs. Traditional methods used for the assessment of viability of cell culture lines such as exclusion of vital dyes and the MTT assay have the disadvantage that they reflect late changes in cell physiology affecting a large number of the cell population, and generally denote that the culture is moribund.

Cell death by either necrosis or apoptosis involves alteration in cell volume with a concomitant change in intracellular ion/element concentrations. The EM technique of X-ray microanalysis has a long tradition in the measurement of element changes in cells undergoing necrotic insult such as e.g. heavy metal poisoning, where cell swelling is accompanied by early increases in the intracellular concentrations of sodium and chloride with a decrease in potassium only in the later stages. More recently this technique has shown that apoptotic cell death is accompanied by a different profile with immediate decreases in the concentrations of potassium and chloride reflecting the early shrinkage that occurs in this pathway. In both necrosis and apoptosis these changes in element profile occur well before any of the recognised markers of cell death, and, since very few cells are required for EPXMA, this is a sensitive technique for monitoring cells in culture.

(OP 181) Mechanical Stimulation of Adipose-Derived Stem Cells Embedded in a 3-Dimensional Fibrin Construct

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The mechanical environment has the ability to alter the differentiation of mesenchymal stem cells (MSC). We developed a novel method for generating three-dimensional MSC-based constructs using fibrin gel casting as described for myoblasts[1]. Adipose-derived stem cells were seeded on top of a fibrin gel or mixed within the gel. Over the next 8 days, cell-mediated tension contracts the

gel around two artificial anchors, resulting in small tubular constructs 1–2 mm diameter and 10–12 mm length. Following formation, these constructs were connected to a stepper-motor and were uniaxially loaded to 110% of their resting length at 0.1 Hz over 14 days. Histological analysis of the constructs after the 14 days of stretch showed a high density of collagen between the anchors. Since MSC cells normally don't produce large amounts of collagen, this indicates that the stem cells within the fibrin gel constructs had differentiated down a tendon/ligament phenotype. These data indicate that MSC/fibrin-based gels provide a novel method to engineer three-dimensional functional constructs, load the cells either in tension or compression, and rapidly determine the phenotype of the MSCs. The fibrin MSC model may be used to drive MSC cells towards the tendon/ligament or cartilage phenotype *in vitro*.

Reference:
[1]Huang YC, Dennis RG, Larkin L, Baar K. Rapid formation of functional muscle *in vitro* using fibrin gels. J Appl Physiol. 2005;98:706–713.

(OP 182) Mechanism of Haemoglobin Sensing Body Temperature

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The talk works on answering the question whether hemoglobin (protein) can ''sense'' body temperature. When human red blood cells (RBCs) were aspirated into 1.3 μm pipettes ($DP = -2.3$ kPa), a transition from blocking the pipette below $Tc = 36.3 \pm 0.3^{\circ}C$ to passing it above Tc occurred (passage transition). With a 1.1 μm pipette, no passage was seen and RBC volume measurements were possible. With increasing temperature RBCs lost volume significantly faster below than above a $Tc = 36.4 \pm 0.7$ (RBC volume transition). Colloid osmotic pressure (COP) measurements of RBCs in plasma ($25^{\circ}C \leq T \leq 39.5^{\circ}C$) showed a turning point at $Tc = 37.1 \pm 0.2^{\circ}C$ above which the COP rapidly decreased (COP transition). In NMR T1 relaxation time measurements the T1 of RBCs in plasma changed from a linear ($r = 0.99$) increment of T1 below $Tc = 37 \pm 1^{\circ}C$ at a rate of 0.023 s/K, into a parallel to the temperature axis above this point (RBC T1 transition). In conclusion: during micropipette aspiration, an amorphous gel forms in the spherical trail of the aspirated RBC, consisting of mostly hemoglobin and water. At Tc the gel turns fluid and Van-der-Waals bonds brake due to thermal energy enabling cell passage. The passage, the volume, the COP, and the RBC T1 transitions all happen at Tcs close to body temperature suggesting a glass-like transition as mechanism. Tc may mark the set point of a species' core body temperature inscribed in the primary structure of a species' hemoglobin and possibly other proteins (cited from ''Bioengineering in cell and tissue research,'' GM. Artmann & Shu Chien (ed.), Springer 2008)

(OP 183) Mechanobiology: Computer Simulations for Tissue Engineering

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In this paper I will present research relating to how computer simulations relating mechanical stimulation to tissue phenotype can be performed. Issues to be addressed include: (A) stimuli controlling cell differentiation [1], in particular mesenchymal stem cell fate, (B) dispersal of cells within the scaffold due to crawling and convection in the fluid (C) proliferation and apoptosis of cells, and the relationship to mechanical stimuli in the cellular microenvironment [2,3], (D) angiogenesis: the budding, growth, and branching of capillaries in the granulation tissue and the differentiated tissue, and possible modulation of this process by mechanical forces. Also the local oxygen tension and therefore the differentiation of cartilage is affected by inhibitions on angiogenesis, (E) variability in outcome: this is due to either (i) environmental factors such as variation in loading or diffusion of nutrients and (ii) genetic differences between individuals and possibly inter-individual differences in cellular mechanosensitivity.

References:
[1]Prendergast P.J., Huiskes R., Soballe K. Biophysical stimuli during tissue differentiation. Journal of Biomechanics 30, 539–548, 1997
[2]Kelly D.J., Prendergast P.J., Prediction of optimal mechanical properties for a scaffold used in osteochondral defect repair, Tissue Engineering 12, 2509–2519, 2006
[3]Byrne D.P., Lacroix, D., Kelly, D.J., Planell J. & Prendergast P.J., Simulation of tissue differentiation in a scaffold as a function of porosity, Young's modulus, and dissolution rate of a scaffold: application of mechanobiological models in tissue engineering. Biomaterials 28, 5544–5554, 2007

(OP 184) Medtech Industry Requirements for the Cooperation with Spin-Off Companies

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European universities currently promote the foundation of spin-off companies to accelerate product realisation of innovative ideas. It is an open secret that such spin-offs frequently fail. What are the reasons for these failures which lead to a loss of investment and personal disappointments. As large companies are seeking cooperations with innovative SMEs, several conditions are necessary to be considered prior to starting a successful collaboration: 1. The performance of an innovative product should have been proven in *in vitro* or animal trials. 2. Logistics of applied biomaterials and their quality/reproducibility should have been established. 3. Ideas for approval and legal conditions have to be assessed before and intelectual properties (IP) in terms of patents been fixed. 4. At best a prototype of the innovative device should be available, whereby considering that manufacturing of such a device might need financial resources of up to 3–7 Mio €. Subsequent clinical trials in cooperation with large companies might then easily add to further 15–20 Mio €. 5. The competence of an SME approaching a large company might also be evaluated by a local audit and SMEs should not hesitate to undergo such investigations. 6. One detail should not be neglected: timing of contacts should be performed rather early. Establishing cooperative programms and budgets needs time. Large companies finish their budget-planning for subsequent years in late summer. Being late would postpone the whole process by a further year. These and other details will be discussed.

(OP 185) Membrane Biohybrid Systems for Liver Tissue Engineering

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The reconstruction of human organ functions has proved to be an important challenge besides for reducing the need for organ replacement also as model system for studying disease, drug, infection and molecular therapeutics. Polymeric membranes can be used in engineering of the liver tissue for the construction of *in vitro* liver functions and modulation of cell behaviour.

In this study we report on the synthesis of novel semipermeable membranes able to support the long-term maintenance and differentiation of human liver cells and on the strategies to optimise cell-biomaterial interactions in biohybrid systems.

We developed membrane biohybrid system constituted by membranes made from a polymeric blend of modified polyetheretherketone and polyurethane (PEEK-WC-PU) and human hepatocytes.

Human hepatocytes organize in a 3D structure in the membrane biohybrid system maintaining a polygonal shape, which would lead to better functional maintenance, so many of the features of the liver *in vivo* are reconstituted. Liver specific functions investigated in terms of urea synthesis, albumin production and total protein secretion are maintained at high levels. Hepatocytes are able to biotransform diazepam, which is an anti anxiety agent (benzodiazepines), through the formation of its typical metabolites including temazepam, N-desmethyl-diazepam and oxazepam. This engineered liver construct is able to promote adhesion and to provide a microenvironment able to elicit specific cellular responses.

Acknowledgments: The Authors acknowledge European Commission through the Livebiomat project, Contract No. NMP3-CT-2005-013653 and European Network of Excellence on Nanoscale-based membrane technologies (“NanoMemPro 500623-2-26-7-2004).

(OP 186) Membranes for Bioartificial Organs and Tissue Engineering

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Membrane technology is of major importance in medical applications. Membranes are used in drug delivery, artificial organs, tissue regeneration, diagnostic devices, as coatings for medical devices etc. In fiscal terms, the value of medical membrane products is far larger than all other membrane applications combined. Only in the US, the medical membrane market approaches 1.5 billion dollars per year and grows steadily.

In this keynote lecture, a description of European Network of Excellence: ‘‘NanoMemPro’’ and the research activities of the partners on ‘‘membranes for bio-rtificial organs and tissue engineering’’ will be initially presented. Furthermore, examples of application of membranes in bio-artificial organs will be discussed; including bio-artificial kidney, liver, pancreas etc [1]. In tissue engineering, membrane science can play an important role in the design and construction of better scaffolds for cell culture [1, 2]. The optimum design of cell culture bioreactors for delivery of nutrients and oxygen, using flat sheet and hollow fiber membranes is an important challenge and will also be discussed in detail.

References:

[1]D. F Stamatialis, B. J. Papenburg, M. Gironés, S. Saiful, N.M.S. Bettahalli, S. Schmitmeier and M. Wessling. J. Membr. Sci., 308 (2008) 1.
[2]B. J. Papenburg, L. Vogelaar, L. Bolhuis–Versteeg, R.G.H. Lammertink, D. F. Stamatialis and M. Wessling, Biomaterials 28 (2007) 1998.

(OP 187) Mesenchimal Stem Cell Improved Bone Regeneration Around a Hip Prosthetic Uncemented Stem

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Non cemented prosthetic stem is the gold standard for hip replacement. In presence of poor quality bone, cementation is necessary. To avoid the use of cement, we tested the ability of a cell based composite to enhance bone ingrowth around a hip prosthetic uncemented stem.

Twelve adult sheep were divided into 2 groups. Group I received unilateral uncemented hip emiarthroplasty. In Group II a composite material made of type I collagen, autologous platelet-rich plasma and mesenchymal stem cells, was injected into the femoral canal followed by a press-fit insertion of the stem. X-Ray, histology and histomorphometry were performed.

The roentgenograms in group I showed 2 cases of peri-prosthetic fracture and one case of incorrect positioning of the stem. In group II we had one case of dislocation and one case of subsidence of the prosthesis. These animals were not evaluated histologically.

Histomorphometry showed a higher percentage of newly formed bone inside the medullary canal in group II (18,72%) in comparison to group I (4,61%). Inside the cortex there were no differences between the 2 groups. The bone contact to the titanium femoral stem was higher in group II (7,3%) than in group I, where no bone contact was detected.

The cell-based substance is able to improve bone remodelling in a two months sheep model and the contact between newly formed bone and the titanium stem.

The addition of a composite material based on collagen, PRP and mesenchymal stem cells can improve the secondary fixation of a hip uncemented femoral stem.

(OP 188) Mesenchymal stem Cells Differentiate Towards Endothelial Cells in a Prevascularized Bone Tissue Engineering Setting

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Introduction: One of the current limitations of tissue engineering is the inability to provide sufficient blood supply in the initial phase after implantation. A strategy to overcome this limitation is prevascularization. We previously developed a spheroidal system to co-culture human mesenchymal stem cells (MSC) with human endothelial cells (HUVEC), resulting in the formation of a 3D prevascular network. Here we study the role of the different cell types in the network formation in this system using BrdU labeling combined with immunostaining.

Results and Discussion: After 10 days of co-culture, CD31 positive and vWF positive prevascular structures could be seen throughout the entire spheroid. BrdU staining revealed that these structures consisted of both HUVEC and MSC. Although MSC that stained positive for CD31 were already seen after 3 days, positive vWF staining of MSC was only seen after 5 days of co-culture.

The mechanism of endothelial differentiation of MSC in this system remains largely unknown, although experiments using anti-VEGF antibodies and indirect cocultures indicate that both secreted factors and direct cell contact are important for the endothelial differentiation and organization of MSC. Future research will focus on unraveling the mechanism and trying to replicate the results without the addition of endothelial cells.

Conclusions: This study indicates that human mesenchymal stem cells have the potential to differentiate towards endothelial cells and can be incorporated in prevascular structures in a bone tissue engineering setting. This is an important finding, since this might enable prevascularized bone tissue engineering from a single cell source.

(OP 189) Mesenchymal Stem Cells: a Novel Cell Therapy Based Approach for the Treatment of Autoimmune Diseases

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Mesenchymal stem cells (MSCs) represent a heterogeneous subset of stromal stem cells that can be isolated and expanded *ex vivo* from many adult tissues and can differentiate into mesodermal tissues such as fat, bone and cartilage. Under some experimental conditions, MSCs have been reported also to transdifferentiate into cells from the other two germinal lineages. Recent data suggest that MSCs can interact with cells of both the innate and the adaptive immunity and modulate their function. Based on these findings, we have shown that i.v. injected MSC can ameliorate both relapsing-remitting (PLP-induced) and chronic progressive (MOG-induced) experimental autoimmune encephalomyelitis (EAE) through the induction of peripheral tolerance. Upon i.v injection, GFP labeled MSCs home inside the lymph nodes and the inflamed CNS where they engraft without evidence of transdifferentiation into neural cells. However, MSCs may promote survival of damaged neural cells through paracrine mechanisms sustained by striking anti-inflammatory, trophic and anti-apoptotic activities. Due to the relative simplicity of growing them *in vitro*, MSCs currently represent the ideal source for adult stem cells amenable to therapeutic development in immune-mediated diseases.

(OP 190) Mimicking Interstitial Microenvironments for Lymphatic Capillary Tissue

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Lymphangiogenesis depends mainly on three factors: interstitial flow, the extracellular matrix, and the matrix environment (chemical and physical factors). We have previously shown *in vivo* as well as *in vitro* that the organization of lymphatic endothelial cells (LEC) is guided by slow interstitial flow. Additionally, it is known that vascular endothelial growth factor (VEGF)-C is important for lymphatic proliferation and migration via signaling through its receptor VEGFR-3. We have engineered a VEGF-C protein that can covalently bind to fibrin such that it can be liberated by cellular proteolytic activity. Using a novel 3D radial flow chamber developed in our lab, we studied the effects of subtle flow on cells cultured in this VEGF-C linked matrix. These studies showed that liberation of the bound VEGF-C protein from the fibrin gel synergizes with slow flow to promote the formation of small capillary networks, presumably by preferentially cleaving VEGF-C in the direction of flow thereby biasing the free morphogen gradient and inducing subsequent morphogenesis. We also show that fibroblasts co-cultured with LECs strongly promote lymphangiogenesis and give them more relevant morphologies and possibly stabilize them. These new models of the lymphatic microenvironment can thus be used both to better understand the biology governing lymphangiogenesis as well as provide engineering design criteria for creating functional lymphatic vessels *in vitro* and for regenerative medicine.

(OP 191) Molecular Mechanisms in Schwann Cells that Control Axon Growth and Nerve Repair

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In comparison to nerve fibre tracts in the central nervous system, peripheral nerves have a remarkable ability to re-grow following injury. This difference is largely due to the different reaction to injury shown by the glial cells in the two locations, and to the particular properties of Schwann cells, the glial cells of peripheral nerves. Normally, Schwann cells are highly dif-

ferentiated, tightly ensheathing all axons and forming insulating myelin sheaths around the larger ones. Nerve injury triggers a radical change in these cells: They start to proliferate, the myelin sheaths collapse and are phagocytosed and digested by Schwann cells and macrophages and Schwann cells start expressing a range of molecules that promote neuronal survival and axon growth. Overall this represents a process of de-differentiation, because this injury-response takes the differentiated cells of normal nerves back to a phenotype that is very similar to that of immature Schwann cells found in developing nerves prior to myelination. Remarkably, this de-differentiation process is the basis for the ability of axons to regenerate after injury, and when it is prevented axon re-growth is blocked or severely impaired. The molecular processes responsible for the de-differentiation are therefore fundamental for nerve repair. This talk will discuss some of the molecules that control the de-differentiation programme, in particular the transcription factor c-Jun and the signalling receptor Notch. It will describe how molecular failures within this programme result in failure of axon regeneration and functional nerve recovery.

(OP 192) Monitoring Cell Based Therapy by Magnetic Resonance Imaging

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Magnetic Resonance Imaging (MRI) is one of the most powerful tools for high-resolution, non-invasive imaging. Apart from monitoring anatomical changes and functional recovery, the location and potentially functional changes of cells can be followed by MRI. It has recently been demonstrated that the migration of low cell numbers can be monitored in rodents after cell labeling with MRI contrast agents and subsequent implantation.

We have focused on methodological aspects of cell labelling strategies for robust and sensitive visualisation of multipotent adult progenitor cells, mesenchymal stem cells and embryonic stem cells. The sensitivity, stability, toxicity and adverse effects on the cell biology by the labelling procedure was studied for ultrasmall, small and micron-sized iron oxide particles. *In vivo* imaging was performed in control animals and a stroke animal model.

Although, the actual labelling procedure can be straight forward, there are several issues that require careful consideration for (stem) cell labelling and their *in vivo* applications. Among these issues are:

(1) Generation of highly sensitive contrast for the visualization of small cell numbers

(2) Generation of unambiguous contrast that distinguishes cells from other sources of hypo- or hyperintensity in MR images

(3) Stable binding or incorporation to avoid contrast agent leakage or transfer to cells of the host

(4) Prevention of toxicity or harmful alterations of cellular processes by the contrast agent

(5) Minimal influence on the physiological behavior of the cells in the host (for example migration of differentiation potential)

(6) Generation of contrast even after continued cell proliferation (dilution)

(OP 193) Morphometric and Mechanical Characterization, Insulin Loading and *In-Vivo* Biocompatibility of Chitosan Particles Aggregated Scaffolds for Tissue Engineering

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In tissue engineering, scaffolds development presents, among others, 3 key requirements: adequate morphological characteristics, mechanical performance and *in-vivo* biocompatibility. The aim of the present study was to evaluate chitosan-based scaffolds produced by particle aggregation in these key issues. Furthermore, chitosan scaffolds were loaded with insulin to promote chondrogenic differentiation. Micro-Computed Tomography (μ-CT) was carried out for accurate morphometric characterization quantifying porosity, interconnectivity, particles and pores size that shown to be adequate. Dynamical Mechanical Analysis (DMA) showed that scaffolds are mechanically stable in wet state with a storage modulus of 4.21 ± 1.04 MPa at 1 Hz frequency. Insulin-loaded scaffolds were characterized and studied with a pre-chondrogenic cell line (ATDC-5). The *in-vitro* release was carried out mimicking cell culture conditions quantified by micro-BCA. When seeded with ATDC-5, insulin-loaded scaffolds promoted the chondrogenic differentiation as assessed by SEM, DNA and GAG content, histology and real time-PCR. Furthermore, chitosan scaffolds were evaluated *in-vivo* using a rat muscle-pockets defect model for different implantation periods (1, 2 and 12 weeks). The histological and immunohistochemistry results have demonstrated that chitosan scaffolds are biocompatible. In addition, scaffolds interconnectivity shown to be favourable to the connective tissues ingrowth into the scaffolds and to promote the neo-vascularization even in early stages of implantation. It is concluded that chitosan scaffolds produced by particle aggregation could serve as alternative, biocompatible, and safe biodegradable scaffolds for tissue engineering applications.

Acknowledgements: FCT (SFRH/BD/11155/2002) and EU funded projects HIPPOCRATES and EXPERTISSUES.

(OP 194) Mouse Embryonic Stem Cell Expansion in a Microcarrier-Based Stirred Culture System

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The aim of this study was the scaling-up of mouse embryonic stem (mES) cell expansion in a microcarrier-based stirred culture system while maintaining their pluripotency and neural commitment potential. The 46C mES cell line was used as a model system. The

scale-up of 46C mES cell expansion was studied in spinner flasks (30–50 mL), in serum-containing (DMEM/FBS) or serum-free (SF) media, both supplemented with LIF. In order to immobilize mES cells, two different microcarriers were evaluated: a microporous gelatin-covered microcarrier (Cytodex 3) and a porous gelatin microcarrier (Cultispher S). The expansion of mES cells (5×10^4 cells/ml) was firstly performed using DMEM/FBS medium on Cytodex 3 microcarriers (0.5 mg/ml) for 8 days. The maximum cell concentration achieved was $(1.9\pm0.1)\times10^6$ cells/ml on day 6, which represented a 38 ± 2-fold increase. In the next step, the expansion of mES cells was performed in the spinner flask on the Cultispher S microcarriers (1 mg/ml) both in DMEM/FBS and SF medium. Maximal cell densities achieved during 8 days in culture were $(2.6\pm0.7)\times10^6$ and 3.5×10^6 cells/ml, which corresponds to fold increases of 50 ± 15 and 70 for DMEM/FBS and SF, respectively. After 8 days of culture under stirred conditions in SF medium, approximately 90% of the expanded cells were viable. More importantly, the cells maintained their ability to differentiate into neural progenitors with more than 90% of Sox1-GFP+ cells obtained upon the neural commitment protocol. In addition, after expansion, cells stained positively for alkaline phosphatase, indicating that a high percentage of cells remained pluripotent.

(OP 195) MRI Evaluation of Deformation Fields and Glycosaminoglycan Concentrations in Tissue-Engineered Articular Cartilage

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Noninvasive magnetic resonance imaging (MRI) is a technology that enables the characterization multiple physical phenomena in biological tissues. Mechanical function of engineered tissue is a primary endpoint for the successful regeneration of many biological tissues such as articular cartilage, spine, and heart. Here, we demonstrate the application of MRI to characterize mechanical function of tissue-engineered articular cartilage (agarose) constructs. The mechanical function of an articular cartilage defect model was evaluated in terms of displacement and glycosaminoglycan (GAG) concentration fields computed throughout the tissue construct. Two techniques were demonstrated using the articular cartilage defect model: (1) phase contrast-based methods (i.e. displacement encoding by stimulated echoes with a fast spin echo readout) to characterize deformation fields of the tissue under compressive loading and (2) gadolinium-enhanced imaging to determine GAG concentrations. Using these two techniques, displacement and GAG concentrations fields were found to vary nonuniformly depending on spatial position. Displacements were highest at the articular surface and in the tissue constructs compared to surrounding cartilage. Tissue surface geometry corresponded to observed displacement fields. GAG concentrations were lowest in the tissue constructs compared to the surrounding cartilage. GAG content was increased in agarose constructs but not restored to that of surrounding cartilage following treatment with serum-free, or BMP-7 or 10% FBS supplemented media. The use of multiple MRI techniques to assess tissue mechanical function suggest that deformation is related to tissue geometry and underlying biomolecular tissue constituents, and highlight the need for successful tissue integration following repair.

(OP 196) Multi-Tiered Sequential Processing and Analysis of 3D Tissue-Engineered Constructs

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Introduction: Many analytical tools are relevant to the study of tissue-engineered constructs, but some are destructive and preclude further testing of the same sample. Here, we describe a streamlined process that allows multiple analytical modalities to be applied, sequentially, to the same sample.

Methods: A flow-perfusion bioreactor was used for dynamic cell culture of various cell types (MC3T3-E1 pre-osteoblastic cells, primary murine osteoblasts, NHOst cells, and human osteoprogenitor and mesenchymal stem cells) seeded in porous hydroxyapatite/tricalciumphosphate scaffolds. Following dynamic cell culture, samples were sectioned along the median plane. One half was imaged by environmental scanning electron microscopy (SEM) for cellular morphometry along the top, bottom, and median cut surfaces. Following SEM, mechanical testing was performed. The second half of the sample was fixed in glutaraldehyde, dehydrated in ethanol, progressed through graded acetone-polymethylmethacrylate cycles under vacuum, and cured at 47°C in the dark. Cured blocks were sequentially sectioned and ground/polished to desired thicknesses (50 nm-25 μm) on semi-automated processing equipment. Cells labeled with fluorescent tags prior to experimentation were viewable by laser confocal microscopy immediately, without any additional processing. SEM or transmission electron microscopy (TEM) of non-sequential sections allowed detailed imaging at various depths within the scaffold. Slides were also processed by standard histological (e.g., trichrome) and immunohistological (e.g., osteocalcin) staining for light or laser confocal microscopy.

Conclusions: With advances in tissue engineering, a uniform method of processing is needed. This processing algorithm is capable of extracting a large amount of data from the same samples by non-destructive sequential analysis.

(OP 197) Multilineage Progenitors of the Enteric Nervous System: Interesting and Useful?

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The enteric nervous system (ENS) is composed of a complex network of interconnected ganglia distributed throughout the length of the gastrointestinal tract. Most enteric neurons and glia are derived from a small population of progenitors which originate in the vagal neural crest and invade the foregut from where they migrate to colonise the entire organ. Mutations in genes encoding extracellular signals and their intracellular mediators often lead to partial colonization of the gut by ENS progenitors and failure of gangliogenesis in the distal colon (congenital colonic aganglionosis = Hirschsprung's disease-HSCR). We have generated mice expressing only one of the two Ret receptor ty-

rosine kinase isoforms (Ret51) and they are characterized by a ''HSCR-like'' phenotype. In parallel studies, we have developed protocols to culture ENS Progenitor Cells (EPCs) from the fetal and postnatal mouse gut. Clonal analysis of such EPCs has established that they represent self-renewing multilineage progenitors capable of generating both neurons and glial cells. Interestingly, EPCs can also be generated from Ret51 mutant mice, raising the possibility that they can be used in an autotransplantation assay to replenish the agagnlionic gut segments with ENS progenitors. To examine these possibilities we have been transplanting EPCs into the wall of gut maintained in organotypic cultures or *in vivo*. I will describe our efforts to maximize the efficiency with which we obtain EPCs from embryonic and postnatal gut and report on our preliminary studies on the transplantation of EPCs into the gut wall with the ultimate aim of restoring a functional ENS.

(OP 198) Myocardial Tissue Engineering Using ES Cell-Derived Cardiomyocytes and Elastomeric Polymers

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Heart failure (HF) results from heart tissue having limited regenerative capacity following a heart attack. At end stage of HF, heart transplantation is the only option. However, donor shortages leave cell therapy, including tissue engineering solutions, as alternative strategies. In our approach preformed patches of myocardial cells are attached to a biodegradable engineered biomaterial support using embryonic stem cell (ESC)-derived cardiomyocytes (ESC-CM). We report on the characterisation of a series of biodegradable elastomeric materials describing also tests carried out to demonstrate their biocompatibility with cells, including ESC-CM. Multiblock copolymers containing 30 wt% hard segments of poly(ethylene terephthalate) (PET) or poly(butylene terephthalate) and 70 wt% soft segments composed of dilinoleic acid (DLA) or poly(ethylene glycol) were selected. Additionally, PET/DLA copolymers were used for preparation of composites containing 0.2 wt% of titania nanopowder with the aim to induce nanotopographic features. D3 mouse ESC line (from ATCC) and human ESC H7 (from Geron Corp) that were induced to differentiate via embryoid body formation started to beat spontaneously from day 7 and day 9–12 onwards, respectively. All materials tested were biocompatible with differentiating cardiac cells, which did not show any signs of cytotoxicity or adverse effects. This was evidenced by the continuous beating of the cardiomyocyte clusters for more than 35 days on these materials. It was noted that isolated cells adhered and spread well on all materials tested even in the absence of gelatin, and the presence of TiO_2 nanoparticles was not toxic to the cells but encouraged their adhesion, spread and proliferation.

(OP 199) Nano/Microparticle Incorporated Chitosan Fibers as Tissue Engineering Scaffolds

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The aim of this study was to develop a bone tissue engineering scaffold with an inherent bone morphogenetic proteins BMP-2 and BMP-7 sequential delivery system. BMPs were encapsulated in poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and poly(lactic acid-co-glycolic acid) (PLGA) nano/microparticules which are then introduced to a chitosan matrix by two methods: embedding in the chitosan fibers and then forming the scaffold or by forming the chitosan scaffold and then introducing the nano/microparticules.

Nano/microparticles loaded with BSA (model protein) or BMPs were prepared by double emulsion/solvent evaporation technique. The structure, encapsulation efficiency and BSA release were studied. Chitosan-based fiber mesh scaffolds were prepared by wet spinning. Incorporation of nano/microparticles into fiber mesh scaffolds was achieved by two methods: incorporation within the fibers and by post-seeding. For incorporation within the fibers, particles were mixed with chitosan solution and wet spun as presented above. Post-seeding was obtained by adding a particle suspension in dH_2O onto the scaffold followed by the application of vacuum-pressure cycle.

Among the particles prepared, 20% PLGA nano/micro spheres and 20% PHBV nano/micro capsules were chosen as the rapid and slow release components of the sequential delivery system, respectively. Wet spun chitosan scaffolds produced from 4% chitosan (w/v) revealed smooth surfaces and used for further studies. Presence of particles within the fibers was shown by SEM analysis for the first incorporation method. Post-seeding did not influence the final release pattern, but suppressed the burst release. Incorporation of BMP-2 and BMP-7 carrying particles and the effect of GF release on MSCs are being studied.

(OP 200) Nanoscale Organization of Biomimetic Peptide Motifs on Polymer Surfaces: a Molecular Printing Approach

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The cells in tissues receive key solid-phase signals from extracellular matrix (ECM) through interactions of their transmembrane recognition proteins with specific domains on ECM molecules. Therefore, biomaterials to be used as scaffolds for guided tissue regeneration should exhibit corresponding recognizable structures, either as whole ECM molecules or their fragments, typically peptides, on their surfaces. A number of evidence shows that not only the presence but also, and most importantly, the molecular organization of peptide ligands on the surface

represents a key factor in creation of functional biomimetic cell/biomaterial interfaces. The importance of spacing of fibronectin-peptide mimics on a tens-of-nanometers scale to produce functional integrin clustering into focal adhesions has been demonstrated on planar model substrates. The presented work focuses on creation of nanoscale organized surfaces of biodegradable polymer materials, such as polyesters (e.g., PLA, PLGA), applicable to real 3D scaffolds. To achieve distribution of peptide motifs on a scale comparable with the size of individual transmembrane macromolecules, e.g., integrins, a "molecular printing" technique, based on deposition of a molecular layer formed by well-defined functional block copolymers was developed. The molecular characteristics of copolymer films and the surface topology of biomimetic groups obtained through molecular assemblies of copolymers were characterized. The applicability of the technique to polyester surfaces is discussed. The positive effect of clustering of fibronectin-derived peptides through association of functionalized copolymer molecules on the cell adhesion and phenotype differentiation in cell cultures was demonstrated.

Acknowledgment: The support by Academy of Science of CR (Grant No.: 1QS500110564) is acknowledged.

(OP 201) Neural Driven Angiogenesis through Local Secretion of Neurotrophic Factors

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Nerves and blood vessels are closely associated. Moreover, sensory nerves were shown to determine the branching pattern of the vascular network in skin, and to promote the arterial differentiation of blood vessels. We developed a unique *in vitro* model featuring a pre-formed three-dimensional neurites network on which a capillary-like network was allowed to organize and mature. Sensory neurons and glial cells were cultured with fibroblasts in a collagen-chitosan sponge to reconstruct the neural network for 14 days and human endothelial cells were then seeded on the tissue to build a capillary-like network for 17 additional days. Neural cells induced a 27% increase in the number of capillary-like tubes (CLT) formed in the tissue. This effect was abolished when K252a, an inhibitor of the TrkA, B and C receptors for the NGF, BDNF and NT-3 neurotrophins respectively was added to the culture medium. Moreover, we demonstrated that when 10 ng/ml of NGF, 0.1 ng/ml of BDNF, 15 ng/ml of NT3 and 50 ng/ml of GDNF were added to our endothelialized reconstructed connective tissue model, a major increase from 40 to 80% in the number of CLT was observed. This is the first *in vitro* demonstration of a direct angiogenic effect of peripheral neural cells on human endothelial cells through the release of neurotrophic factors and of the angiogenic potential of NT-3 and GDNF, the later belonging to an other family of neurotrophic factors.

(OP 202) Neurogeneic and Osteogeneic Potential of Placental-Derived Cells: Why Should We Use Placenta?

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Objective: To determine the osteogeneic and neurogeneic differentiation potential of placental-derived mesenchymal stem cells (MSC)

Study Design: Fetal MSCs from first trimester placental chorionic villi and term gestation chorion were isolated and grown in the presence of EGF and FGF-2 as floating spheric clusters. After plating on collagen, neural differentiation was initiated with retinoic acid and growth factors. Differentiation into neurons, oligodendrocytes and astrocytes and their progenitors was monitored immunohistochemically and by RT-PCR of neural genes. 2D-PAGE followed by high performance liquid chromatography (HPLC) with subsequent tandem mass spectrometry (MS/MS) was used for protein identification.

Results: Placental-derived MSC's differentiated into osteogeneic, chondrogeneic and adipogeneic cell lines. After 5–7 days in the neurogeneic medium, placental MSC formed rapidly proliferating neurosphere-like structures which stained strongly positive for nestin. 60–80% of the cells outgrowing from stimulated neuropheres were positive for Tuj-1 and TUC-4, both markers specific for immediately postmitotic neurons (untreated controls: 4%), also confirmed in the Proteomics analyses.10% of the neurons expressed markers for more mature postmitotic neurons (NeuN; MAP1B; NF-M; NSE). Mature (MAP2+/TAU1+/NF200+) neurons were rarely found (1%). A part of the neurons had dopaminergic, another serotonergic or glutamatergic (but not GAGAergic) character. A fraction of 5–10% of the neurally differentiated cells had oligodendrocytic character:

Conclusion: Placental MSCs can differentiate into early neural progenitors and might be an ideal source for autologous stem cell graft for peripartum neuroregeneration

(OP 203) New Biosynthetic Bacillus-Derived Hyaluronic Acid as an Attractive Potential Biomaterial for Tissue Engineering Applications

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Hyaluronic acid (also referred to as HA or hyaluronan) is a natural linear and unbranched polysaccharide. It is a major constituent of the extracellular matrix of soft connective tissues and the synovial fluid of articular joints. HA is ubiquitous in human and animal tissues, where it exhibits significant structural, rheological, physiological, and biological functions. Its distinctive moisturizing and visco-elastic properties, coupled with its lack of immunogenicity and toxicity, have led to a wide range of proven and marketed applications within the cosmetic, biomedical and pharmaceutical industries. These include skin moisturizers, osteoarthritis treatment, ophthalmic surgery, adhesion prevention, dermal fillers, and wound healing. Recent studies also report the use of HA-based materials for tissue engineering.

Hyaluronic acid is currently obtained commercially from rooster combs and certain strains of Streptococcus. However, the rooster comb extraction process is faced by growing concern over the use of animal-derived products and requires extensive purification to re-

move antigenic avian proteins. In addition, Streptococci are fastidious microorganisms to grow and can potentially produce exotoxins. Finally, in both industrial processes, large amounts of organic solvents are used during the purification of hyaluronic acid. To avoid these complications, an alternative and safe fermentation strain, namely Bacillus subtilis, has recently been used to produce HA. This improved process, during which no animal-derived materials are employed, relies on defined and aqueous-based recovery steps.

In this work, we introduce Bacillus-derived hyaluronic acid as a new biosynthetic material. We also highlight its advantageous properties which make this biopolymer particularly attractive for tissue engineering applications.

(OP 204) New Developments in Neural Tissue Engineering

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During phases of active neurodegeneration, factors seem to be transiently elaborated to which neural stem cells (NSCs)–a prototype for most stem cells—may respond by migrating (even long distances) to degenerating regions where they attempt to restore homeostasis by a variety of mechanisms. This may include, (but is not limited to) differentiating, towards the replacement of degenerating neural cells of multiple types, not only neurons but also requisite non-neuronal ''chaperone'' cells, all of which are essential for the proper development and reconstitution of function. NSC's are drawn to inflammatory niches, where they then exert anti-inflammatory actions. These ''repair mechanism'' may also reflect the re-expression of basic developmental programs (particularly during temporal ''windows'' following injury). There is an enormous amount of ''programmed'' cross-talk between stem cells and the milieu that add complexity but also enrich therapeutic promise to the system. In addition, NSCs in their native state (as well as following genetic-engineering) may serve as vehicles for protein delivery allowing for the possibility of simultaneous cell replacement & gene therapy (e.g., with factors that might enhance differentiation, neurite outgrowth, connectivity, neuroprotection, anti-inflammation, anti-scarring, and angiogenesis). Cell-cell contact with communication through gap junctions appears to represent another mode of cross-talk. Multi-model approaches to most neurological conditions are likely required. The stem cell may serve as the ''glue'' for these. When combined with certain synthetic biomaterials, NSCs may be even more effective in ''engineering'' the damaged CNS towards reconstitution.

(OP 205) New Materials for 3D-Scaffolds for Tissue Engineering Applications

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Stereolithography has become a very appealing method for the production of three-dimensional scaffolds for several tissue engineering applications, e.g. for bone and blood vessel replacement. This layer-by-layer technique is based on the photopolymerization of commonly acrylate-containing resin formulations and allows the fabrication of cellular structures with defined pore sizes and attainable wall thicknesses down to 100 μm. Nevertheless, residual, unreacted acrylate groups have shown to be highly cytotoxic toward endothelial and osteoblast-like cells and a high local concentration of poly(acrylic acid), formed by *in vivo* degradation, may impair cell function. Therefore, a new class of vinyl ester-based monomers, forming harmless poly(vinyl alcohol) upon *in-vivo* degradation, has been synthesized. *In-vitro* biocompatibility assays revealed that the concentrations leading to cytotoxic cell damage are about two orders of magnitude higher compared to similar acrylate systems. This finding is corroborated by excellent cell adhesion and proliferation observed on the surface of vinyl ester-based polymer specimens. High reactivity for photopolymerization and good mechanical properties of the polymers further indicate the suitability of these monomers for rapid prototyping of 3D-scaffolds in the field of tissue engineering.

(OP 206) New Monitoring Parameters and Increased Efficiency in Bone Tissue Engineering Using Longitudinal Micro-Computed Tomography in a Novel Bioreactor

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Mechanical stimulation of *in vitro* engineered bone tissue constructs is believed to have strong osteogenic effects, if applied appropriately. Nevertheless, because of its inherent complexity, further advancement is needed to improve efficiency and control of this approach. In order to shed light onto the dynamics of mineralization under cyclic loading, we recently introduced a concept combining direct mechanical compression of 3-dimensional tissue engineered constructs and longitudinal monitoring by micro-computed tomography (μCT). Here we demonstrate the application of this approach by studying the osteogenicity of cyclic mechanical loading. Bone marrow-derived mesenchymal stem cells were seeded onto porous silk scaffolds and cultured under osteogenic conditions. Mechanical loading of the scaffolds using different loading regimens was applied five times per week and non-destructive imaging by μCT once a week. Differences in tissue quality were monitored longitudinally by using novel tissue quality parameters (such as ''mineralization rate'' and ''surface growth activity'' but also ''tissue stiffness''). Efficiency of tissue culture was optimized by individualized termination of each culture, either when reaching a previously defined target bone volume density of 2%, or when the mineralization performance of a specimen was too low to reach this target within a reasonable period of time. We expect that the introduced monitoring parameters offer new insights into the mineralization dynamics under different mechanical loading regimens. Furthermore, we envisage this approach not only to rationally allow the termination of the culture of mal-performing specimens but also

to make use of variable mechanical loading regimens to bring such specimens back on track.

(OP 207) NGF Release from Functionalised Collagen-Thermosensitive Polymer Networks Supports Neuronal Cell Growth and Differentiation

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Smart polymeric gels constitute a new generation of biomaterials that are being developed for their use as biomedical devices, scaffolds for tissue engineering and biosensors. The combination of biodegradable materials as collagen type I, with stimuli thermosensitive polymers (such as poly-(N-isopropylacrylamide), PNIPAam, or poly (ethylpyrrolidone methacrylate), PEPM) ushers new possibilities to develop hydrogels that exhibit properties of both components. For the purpose of demonstrating this concept, semi-interpenetrated networks (semi-IPNs) of collagen and crosslinked PNIPAam or PEPM, and IPNs of collagen crosslinked with PAMAM dendrimers and crosslinked PNIPAam or PEPM were prepared. NIPAam or EPM, and a trifunctional acrylic crosslinker, were polymerized in the presence of non- and crosslinked collagen. Scaffolds were characterized by FTIR spectroscopy, DSC, swelling and rheological studies, and by collagenase assay. Formulations were loaded with neural growth factor (NGF) and subsequently neural cellular behaviour and drug release studies at different temperatures was investigated. The prepared semi-IPNs and IPNs exhibited temperature sensitivity in the swelling studies from unswollen (37°C) to swollen (R.T.) with swelling degrees that increase with the collagen content. Transition temperature modulation was detected by DSC increasing from 27°C to 32°C when increasing the collagen content. In addition, the rheological studies performed from 45° to 5°C at constant frequency and oscillating stress also showed a clear transition in both storage and loss moduli that were also dependent on the network composition. The release of NGF from NGF pre-loaded collagen-thermosensitive polymer scaffolds supported the differentiation of neuronal cells on the scaffold.

(OP 208) Nonmelanoma Skin Cancer *In Vitro* Reconstructed

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The identification of genetic changes has led to an increased understanding of tumorigenesis, identifying the involvement of putative oncogenes and tumor-suppressor genes. These genes and their protein products have further potential as targets for disease management and, ultimately, therapy. Such approaches are equally applicable to nonmelanoma skin cancer (NMSC), such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Up to now, many studies on tumor cells have been performed under standard *in vitro* cultures. In standard culture conditions, neoplastic cells, as expected, can grow only in a two-dimensional array that differs from their native three-dimensional organization in nodules or masses and although interesting results have been obtained, three-dimensional scaffolds would better allow the tumor cells to organize into nodules or masses. With the present work we cultured keratinocytes isolated from skin cancer in 3D conditions by using scaffolds made with hyaluronic acid derivatives. (benzyl ester of hyaluronic acid- Hyaff materials) These three-dimensional tumor cell cultures were be used to study genetic changes and in particular to define genetic profile, cell spatial organization, cell/cell and cell/extracellular matrix interactions of skin cancer keratinocytes.

(OP 209) Not All Fibrin Matrices are Equally Suitable for Tissue Regeneration

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Fibrin is both an attractive matrix for drug or cell delivery because it consists of biologic components of the natural clotting cascade, which is the basis for tissue regeneration. There are different commercial fibrin products, which all have a different composition regarding salts and excipients. There is also a clear difference in biochemical properties between the commercial fibrin preparations and autologous fibrin sealants.

Fibrinogen is a molecule that is very sensitive to small changes in the solvent composition, resulting in different fibrin clot. There are two different kinds of fibrin clots: white, non transparent "coarse clots" formed at an ionic strength and pH value within the physiological range, and transparent "fine clots" produced at a higher ionic strength and/or pH value, which are less cell friendly. There are frozen and lyophilized versions of commercial fibrin products available on the market as well as those with fast or slow clotting properties. The stability of fibrin clots is an important quality criterion for fibrin sealants since premature clot lysis could cause post-surgical bleedings or dissociation of glued tissues. To a variable extent, fibrin persistence *in vivo* can be controlled by adding antifibrinolytic agents, of which some synthetic can have adverse effects.

Such due to differences in composition, different fibrin products have different mechanical and cellular properties, which can dramatically change the applicability for tissue engineering and regenerative medicine.

(OP 210) Novel Biodegradable Polymeric Microparticles for the Localized Delivery of Differentiation Agents in Bone Tissue Engineering Applications

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Growth and differentiation factors can be used to guide the biology in tissue engineering, ranging from promoting cell proliferation, to morphogenic activities initiating a cascade of events leading to tissue formation *in vivo*. Synthetic glucocorticoids (e.g. dexamethasone, DEX) and bone morphogenetic proteins (BMPs) are particularly relevant in bone tissue engineering, as they are able to induce osteoblastic differentiation.

In this work, starch-poly-ε-caprolactone (SPCL) microparticles were developed as a matrix for the controlled release of DEX and BMP-2. The developed system was characterized in terms of morphology and size distribution. DEX and BMP-2 were entrapped into SPCL microparticles at different percentages. The loading and encapsulation efficiency, as well as their release profile, were evaluated by HPLC and ELISA quantification.

Particles with a spherical morphology and size distribution between 10 and 150 μm were obtained, which indicates their potential to be used as an injectable system for the localized delivery of differentiation factors *in vivo*. The release behaviour of the entrapped molecules seems to be governed by diffusion and degradation of the polymeric matrix allowing their sustained release for controlled stem cell differentiation.

To investigate the potential of the developed carrier system for delivering factors for stem cell differentiation, adipose stem cells (ADSC) will be cultured in contact with the carrier systems. The release of entrapped molecules on the differentiation of ADSC towards the osteoblastic lineage will be studied by assaying the expression of phenotypic markers, such as high ALP activity, and genes (osteocalcin, osteopontin, Runx2) using real-time PCR.

(OP 211) Novel Genipin Cross-linked Chitosan-Silk Based Sponges for the Regeneration and Repair of Cartilage Using a Tissue Engineering Approach

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Development of materials that can interact positively with tissues is important to regenerative medicine strategies success. Cartilage tissue engineering (TE) scaffolding is a field of continuous evolution, and sponges derived from the combination of polysaccharides and proteins are expected to mimic the naturally occurring environment in the articular cartilage matrix, providing an optimum environment for tissue growth and regeneration. Chitosan (Cht) and Bombyx mori silk fibroin (SF) are excellent candidates for sponges design due to their intrinsic characteristics. The present work aimed to improve the chitosan biocompatibility through blending with Cht-SF and genipin-cross-linking. Hydrogels, produced by cross-linking of Cht-SF, were freeze-dryed to obtain the cross-linked chitosan/silk (CSG) sponges. Rheological and mechanical properties, structural aspects and morphological features of CSG sponges were evaluated. CSG sponges possess stable and ordered structures due to protein conformation changes from alpha-helix/random coil to beta-sheet structure, porous and globular-like surface morphologies, and pH/swelling dependence at pH 3, 7.4 and 9. To evaluate sponges' suitability for cell studies, ATDC5 chondrocyte-like cells were seeded onto CSG sponges and ATDC5 viability (MTS assay), proliferation (DNA test), morphology (SEM analysis) and matrix production (GAGs quantification) were assessed after 14, 21 and 28 days of culture. ATDC5-sponge constructs showed a significant higher adhesion, proliferation and matrix production with the time of culture when compared to Cht, suggesting CSG sponges as potential candidates for cartilage TE strategies. Acknowledgements. Thanks to Portuguese Foundation for Science and Technology, STREP Project HIPPOCRATES (NMP3-CT-2003-505758) and European NoE EXPERTISSUES (NMP3-CT-2004-500283)

(OP 212) Novel Injectable Gel Encapsulating Human Articular Chondrocytes for Cartilage Tissue Repair and Regeneration

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Cartilage tissue loss, as a result of trauma, congenital disorders and diseases of joints, involving structural damage of articular cartilage surface, is a substantial clinical problem representing a major challenge for cartilage tissue engineering. The aim of our study was to evaluate the *in vitro* and *in vivo* behavior of human articular chondrocytes encapsulated within a novel carrageenan *in situ* injectable hydrogel for cartilage tissue engineering and regeneration. Human articular chondrocytes (Hac) were expanded using a well defined serum free medium able to support cell proliferation and differentiation with high cell chondrogenicity. Specifically, Hac were encapsulated within the hydrogels and cultured *in vitro* for 28 days. Results showed that there was a statistically significant increase of cellular viability, with deposition of extracellular matrix (ECM) and subsequent maintenance of chondrocyte differentiated phenotype, as revealed by expression of chondrogenic markers and reinforced by histological analysis. Optical sections acquired by APOTOME (Zeiss) of Hac hydrogel labelled with Hoechst (viable cell dye) allowed the construction of 3D models images demonstrating an homogenous viable cell dispersion as well as a increasing cell number over the time. Bovine full-thickness articular cartilage defects were injected with the biodegradable hydrogel loaded with Hac and implanted subcutaneously in nude mice. Histological results documented the formation of a new human origin cartilaginous repair tissue, clearly indicating the potential of this novel cell delivery system for cartilage tissue engineering.

(OP 213) Novel Micro-Nanofibrous Multilayer Scaffold for Bone Tissue Engineering Applications

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Fibrous structures mimicking the natural extracellular matrix (ECM) morphology are considered promising scaffolds for Tissue Engineering (TE). Scaffold architecture determines its structural integrity, mechanical strength, transport properties and the microenvironment for cell adhesion and proliferation. Therefore, several scaffold processing techniques were developed to control the scaffold architecture. Rapid prototyping (RP) allows the production of scaffolds with precise control of porosity and internal pore architecture; while electrospinning (ES) technique has become very popular in the TE community due to the production of submicron ECM-like fiber meshes.

This work aims to evaluate a novel starch-based scaffold obtained by the combination of starch-polycaprolactone micro- and polycaprolactone nano-motifs, produced by RP and ES, respectively. Scanning Electron Microscopy (SEM) and micro-Computed Tomography micrographs showed a multilayer scaffold composed by parallel aligned microfibers, in a grid-like arrangement, intercalated by randomly distributed nanofibers forming a mesh-like structure.

Human osteoblast-like cells (Saos-2) were dynamically seeded on the multilayer scaffolds using spinner flasks to improve cell seeding efficiency within the scaffold, and the constructs were subsequently cultured under static conditions for 1 and 7 days. SEM micrographs and Hematoxylin-Eosin staining showed the predominant cell attachment and spreading on the nanofiber meshes, which enhanced cell retention at the bulk of the RP scaffold. Cell viability (MTS assay) and proliferation (DNA quantification) results demonstrated the advantageous effect of the combined micro-nano architecture, as compared to the micro structure on cellular performance. These results demonstrated the high potential of the innovative multilayer scaffolds for improving cellular response when pursuing bone TE strategies.

(OP 214) Novel Nano-Composite Biomaterial for Osteochondral Tissue Engineering:Pilot Clinical Study

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Introduction: Osteochondral articular defects represent a key concern in orthopedic surgery. The objective of this pilot clinical study was to test safety and performance of a newly developed type-I collagen-hydroxyapatite (HA) nanostructural bio-mimetic osteochondral (O.C.) scaffold which reproduces cartilage-subchondral bone morphology.

Methods: A gradient composite O.C. scaffold, based on type-I collagen-HA, was obtained by nucleating collagen fibrils with hydroxyapatite nanoparticles at physiological conditions.

30 cases (9F, 21M, mean age 29,3 years) with knee osteochondral lesions (8 medial femoral condyle, 5 lateral condyle, 12 patella, 8 femoral troclea) were treated with scaffold implantation from January 2007 to July 2007. The lesions size went from 2 cm^2 to 6 cm^2. All patients achieved minimum 1 year follow up and were clinically evaluated using the International Repair Cartilage Society score.

Results: IKDC objective score improved after 1 year showing a normal or nearly normal knee in 80% of patients at 1 year of follow up. Similar results were obtained with the IKDC subjective score and with Tegner score. Cases with previous surgery had the worst results, while associated surgery doesn't influenced significantly the clinical outcome. 1 case failed and were reoperated.

MRI evaluation demonstrated good bone and cartilage formation and only in the failed case no integration of the graft was found.

Conclusions: This open one-step surgery was used for treatment of big osteochondral defects. The results of this technique at short follow-up are encouraging. Better results can be obtained in young patients with no previous surgery.

(OP 215) Novel Smart Cryogels for Drug Delivery

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Biodegradable thermoresponsive cryogels were prepared by free radical polymerization of N-isopropylacrylamide monomer and a macromer composed of hydrolytically degradable oligolactate-2-hydroxyethyl methacrylate and dextran by cryogelation protocol. They were characterized by several techniques, i.e., FTIR, ^{1}H-NMR and SEM. Thermoresponsive behaviour of the cryogels with different chemical compositions was determined by swelling experiments and imaging NMR. These novel smart cryogels exhibited "volumetric phase change temperatures, VPCT" between 20°–37°C depending on the composition. The composition of cryogels and the medium temperature were found key parameters affecting *in vitro* degradation rate and profile. Two forms of simvastatin, hydrophobic and hydrophilic, were loaded into the cryogels following two different protocols: (i) embedding the drug after cryogelation, and (ii) incorporating simvastatin during cryogelation. FTIR and ^{1}H-NMR confirmed the drug loading. SEM micrographs demonstrated that hydrophobic simvastatin molecules were deposited on the walls of the pores of the cryogels, while hydrophilic simvastatin molecules were distributed within the solid polymer phase. *In vitro* release of simvastatin from the cryogels was investigated for possible application as Simvastatin delivering scaffolds for regeneration of bone defects. Different release profiles were observed in the *in vitro* release experiments, which were varied significantly depending on the

type of simvastatin, the composition of the cryogel and the medium temperature.

(OP 216) Organized Kidney Tissue Structures for the Treatment of End Stage Renal Disease

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Introduction: While dialysis can prolong survival for many patients with end stage renal disease (ESRD), only renal transplantation can restore normal function, and renal transplantation is severely limited by a critical donor shortage. Previous work showed that cultured renal cells seeded on artificial renal devices can form functional renal structures producing urine-like fluid. In this study we investigated the feasibility of creating 3D renal structures for *in situ* implantation within the native kidney tissue.

Methods: Primary renal cells from 4 week old mice were grown and expanded in culture. Cells were placed in a collagen-based 3D culture environment. Cells were labeled with fluorescent markers and injected into mouse kidneys for *in vivo* formation of renal tissues. Collagen injection without cells and sham operated animals served as controls.

Results: Culture expanded single renal cells formed tubular structures after 4 days in the collagen-based 3D culture conditions. The tubules, lined with renal epithelial cells, progressively increased in length and divided to form branching structures. Implanted renal cells formed tubular and glomerular structures within the kidney tissue, as confirmed by the fluorescent markers. There was no evidence of renal tissue formation in control and sham operated groups.

Conclusion: These results demonstrate that cultured renal cells can reconstitute into organized kidney structures in a collagen-based 3D culture system. The implanted cells self assemble into tubular and glomerular structures within the kidney tissue. The data suggest that this may be the preferred approach to engineer functional kidney tissues for the treatment of ESRD.

(OP 217) Osteoblast and Osteoclast Differentiation in an *In Vitro* Three-Dimensional Model of Bone Turnover

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There is an increasing interest in developing innovative *in vitro* tissue models using typical tissue-engineering approaches. The expansion of models based on integration between cells and biomaterials could help to better understand basic mechanism of human diseases and could be also used for the screening of new drugs, by testing their efficacy and safety before clinical trials. This study was designed to develop a novel 3D *in vitro* model of bone turnover using Skelite (67% Si-TCP and 33% HA/B-TCP) scaffolds in order to investigate osteoblast-osteoclast interactions in a 3D environment and to compare it to traditional two-dimensional Petri-dish cultures. Murine primary osteoblasts from C57Bl6/J mice and osteoclast precursors from GFP transgenic mice (C57Bl/6-Tg(ACTB-EGFP)1Osb/J) were co-cultured on 3D Skelite® scaffolds and on standard plastic culture dishes. We compared the growth and the differentiation of these cells in both culture conditions by proliferation assay (3H-Thymidine incorporation) and histology. Osteoblast and osteoclast differentiation were then monitored by Real Time PCR for Runx2, Osterix, Osteocalcin, Cathepsin K, TRAP after co-culture in normal medium and following an osteogenic stimulation. The GFP expression was examined to monitor the cell type ratio during the cultures. The expression of each gene was normalized to GAPDH and calibrated to the percentage of GFP+ or GFP- cells if expressed by osteoclasts precursors or osteoblasts respectively. In conclusion, our data show that the three-dimensional mineralized environment induces osteoblast and osteoclast differentiation and that the osteoclastogenic inductive properties of murine osteoblasts are strongly dependent on their stage of maturation.

(OP 218) Osteoconductive Scaffolds Obtained by Means of *In Situ* Surface Functionalization of Wet-Spun Fibre Meshes for Bone Regeneration Applications

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The success of bone tissue engineering (TE) strategies is strongly dependent on the development of new synthetic materials combining osteoconductive, osteoinductive and osteogenic properties. Recent studies suggest that biomaterials incorporating silanol groups promote and maintain osteogenesis with or without biological stimuli. This study aims to evaluate the osteoconductivity and osteogenic properties of novel wet-spun fibre mesh scaffolds of SPCL (blend of starch with polycaprolactone) with or without superficial functionalized silanol (Si-OH) groups by seeding/culturing them with goat marrow stromal cells (GBMCs). A calcium silicate solution was used as a non-solvent to precipitate the SPCL solution by wet-spinning and to develop an *in situ* functionalization methodology of the SPCL fiber mesh with silanol groups. GBMCs were seeded onto SPCL scaffolds with or without Si-OH groups (control) for 7 or 14 days in different culture medium; shifting osteogenic growth (beta-glycerophosphate, ascorbic acid) or differentiation (dexamethasone) factors. The fiber meshes-GBMCs constructs were characterized analyzing cellular viability, proliferation, early differentiation as well as matrix mineralization. The bioactive 3D fibre meshes with superficial Si-OH functional groups produced by a one-step wet-spinning technique not only sustained cell proliferation and viability, which increased with the culturing time, but also the higher ALP activity levels and matrix production in the Si-OH fiber meshes indicated that Si-OH groups improve cellular functionality towards the osteoblastic phenotype, which we believe to have an enormous potential for bone regeneration applications.

(OP 219) Osteogenic Differentiation of Human Bone Marrow Cells Cultivated in Single Cell 3D Microenvironments

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Cell shape and regulation of biological processes such as proliferation and differentiation are to a large degree connected. Changes in cell shape correlate with changes in gene expression such as up- or down-regulation of proteins involved in proliferation, ECM production, cellular signalling or differentiation. This possible relationship is therefore of great interest for tissue engineering strategies as well as for the development of cell based sensors. For such studies we use a technology where single cells can be studied in engineered quasi three-dimensional (3D) microenvironments.

Osteogenic differentiation encompasses a well organized series of events including expression of various regulatory factors. This gradual process can be followed by different marker proteins being expressed at various time points, comprising early and late genes. The aim of our project is to live monitor osteogenic differentiation of single cells in quasi 3D microenvironments.

Gene constructs reporting for osteogenesis were created by fusing fluorescent proteins to promoters of specific marker proteins for nucleofection of human cells, which allows to live monitor osteogenesis on a single cell level. In addition cells were immunohistochemically stained against bone-specific proteins to detect differentiation. The 3D microwells were produced by standard microfabrication techniques in silicon followed by thin film replica molding in PDMS. Passivation of the surface was followed by functionalization of the wells with fibronectin.

Cells adhere and grow inside the functionalized microwells, avoiding the passivated surface. Immunohistochemical staining as well as the GFP reporter strategy allow single cell monitoring of osteogenic differentiation in 3D microenvironments.

(OP 220) Osteogenic Differentiation of Human Dental Neural Crest-Derived Progenitor Cells: a Model for Developing Bone

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Human tooth contains a distinct population of neural crest-derived progenitor cells (dNC-PCs) which give rise to specialized daughter cells like neurogenic, chondrogenic and osteogenic lineages (Degistirici *et al*., 2008). Here, the ability of dNC-PCs to develop engineered bone in a self-propagating culture system was studied. For preparation of monolayers, a defined number of dNC-PCs in growth medium was seeded in plastic dishes. After some days of osteogenic stimulation, dNC-PCs had formed a multilayer. Flat cells covering the surface (outer cells) as well as cuboidal cells trapped within the layer (inner cells) were found. With prolonged culture time, an organic matrix that is not mineralized initially was secreted by the inner cells, which showed an osteoblast-like phenotype. When further cultured, the multilayer detached from the substrate and started to contract. As an active process, a three-dimensional structure was formed which subsequently calcified. For example, collagen fibrils which are produced by the inner cells formed ordered arrays and small and large calcified/calcifying matrix vesicles containing amorphous deposits were present. The Ca/P ratio was examined within this tissue. Indeed, electron dispersive X-ray analysis showed that the collagen fibrils were mineralized to a certain extent. The experiments so far show that long-term cultivation of dNC-PCs in osteogenic medium leads to formation of a hard tissue that can serve as an *in vitro* model for developing bone. These results suggest that dNC-PCs could also be used for tissue engineering for the bone defects of the craniofacial region.

(OP 221) Particularities of Tissue Engineering in Pediatric Applications: EuroSTEC

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EuroSTEC: European program on Soft Tissue Engineering for Children. The aim of this project is to use modern tissue engineering approaches to treat children with structural disorders present at birth, such as spina bifida, urogenital defects, gastroschisis, diaphragmatic hernia and esophageal atresia. The project strives to take a translational route through *in vitro* and animal experiments to early clinical trials. Tailor-made ''smart'' biomatrices (scaffolds) will be prepared using natural scaffold molecules (collagen, elastin) and/or manmade polymers (poly lactic/glycolic acid), and will be substituted with regulatory molecules such as growth factors and glycosaminoglycans. A variety of cells, including stem cells, fibroblasts, muscle cells and urothelial/epithelial cells will be cultured *in vitro* and seeded into biomatrices. Biomatrices thus prepared, will be implanted using novel animal models for major congenital birth defects, and evaluated for their capacity to regenerate the correct tissues. Clinical trials for diaphragmatic hernias will form the start of the patient registry and protocol development for future clinical studies. Ethical and regulatory issues will be fully addressed before final clinical application, and parents and children will have to be able to understand these new treatment options. A dialogue with society, including patient's associations, will be sought. Demonstration activities will be undertaken to increase the awareness of new treatment modalities based on new soft tissue engineering techniques.

(OP 222) Perfusion and Tissue Engineering Scaffolds: Micro versus Nano Pores

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A bedrock concept in tissue engineering (TE) holds that synthetic cell-support scaffolds (e.g. polymer or ceramic) should be highly porous; range 20–200 μm diameter. Pore walls, consequently, tend to be dense for mechanical strength. This is partly (i) for rapid nutrient exchange and (ii) to give space for cell growth. However,

recent characterisation, of real time (RT) O_2/glucose gradients in biomimetic collagen scaffolds indicates a major flaw, as these factors eventually oppose each other. In contrast to such traditional synthetic scaffolds, biomimetic matrices (eg collagen, fibrin) commonly comprise cell-seeded nano-fibrillar meshes with corresponding nano-porosity. Precise RT core O_2/glucose monitoring in controlled, graded-density collagen constructs has shown, surprisingly, that cell density-dependent consumption, not matrix density, dominates.[1] The present analysis compares the traditional polymer TE scaffold model with biomimetic, nano-fibrillar matrices. The critical factor is that small nutrients diffuse so effectively across these nano-matrices, that multi-μm pores are unlikely to improve exchange. However, since cell consumption/density dictates deep perfusion, the very cell in-growth needed for successful culture generates the steepest, most damaging gradients for core cells. This will be exaggerated by the minimal matrix accumulation in early cell aggregates and the dense polymer pore walls. We suggest, then, that biomimetic nano-fibril scaffolds may hold the key to effective deep-cell perfusion, since the logic of traditional multi-μm pore design is eroded by its own success: rapid, 3D cell in-growth.

Reference:
[1]Cheema U., Rong Z., MacRobert AJ., Vadgama P. Brown RA. 2008. Oxygen diffusion through plastic compressed collagen constructs. In Prep.

(OP 223) Phenotypic Comparison of Intervertebral Disc Cells with Articular and Nasal Chondrocytes: Towards a Phenotypic Signature for Annulus Fibrosus and Nucleus Pulposus Cells.

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There is a surge of interest in applying tissue engineering principles to treat spinal disorders associated with the intervertebral disc (IVD). However, the cellular and molecular characteristics of IVD, an essential prerequisite for the development of tissue engineering, are poorly defined. We aimed at characterizing intervertebral disc by comparing the phenotype of Annulus fibrosus (AF) and Nucleus pulposus (NP) cells with articular (AC) and nasal chondrocytes (NC). Rabbit IVD were immunohistologically characterized and the level of transcripts from the freshly isolated cells was assessed by real-time PCR. Alcian Blue staining reveals an increasing content of sulfated glycosaminoglycans from the outer to the inner part of the AF. Conversely, Masson's Trichrome staining evidences an inverse distribution for total collagen that was abundant in the outer AF and decreased towards the inner AF and NP. Finally, whereas the immunostaining for type II collagen was negative in the outer AF, it appeared strongly positive in the inner AF and NP. Differential expressions of the transcripts were observed. Type II collagen (COL2A1)/Type I collagen (COL1A1) and COL2A1/aggrecan (AGC1) ratios in IVD cells were significantly different from those in AC and NC. Interestingly, type V collagen, COL1A1 and Matrix Gla Protein (MGP) were strongly expressed in AF but barely detectable in NP cells. As expected AC and NC expressed COL2A1, AGC1 and MGP. In view of a stem cell-based tissue engineering approach for IVD regeneration, the differential expression of these markers could become instrumental in monitoring and, eventually, triggering stem cell differentiation towards IVD cells.

(OP 224) Poly(Ether Imide) Membranes as a Matrix for Cell-Polymer Interactions

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Aim of this work are polymeric membranes prepared from poly (ether imide) (PEI)to meet the demands of Regenerative Medicine concerning (selective) separation, bio/hemocompatibility, and sufficient stability of the matrix. Poly(ether imide) was chosen because it can be steam-sterilized, its membrane forming properties, and because it can be modified covalently with nucleophiles in order to bind biofunctional ligands. The talk gives an overview on scaffolds prepared from poly(ether imide) for biomedical applications. The ease of surface functionalization of PEI can be used to create tailor-made surfaces for specific medical applications or for further modification of the pore structure. The promising results concerning cell interactions (e.g. fibroblasts, endothelial cells, platelets, keratinocytes) make PEI scaffolds a promising matrix for many applications such as biohybrid organs, apheresis, wound healing, or implants.

Support by the EU(NoE NanoMemPro) and the Helmholtz Gemeinschaft and Mecklenburg-Vorpommern (Nachwuchsgruppe 0402710) is gratefully acknowledged.

(OP 225) Polyelectrolyte Complex Fibrous Scaffold for Liver Tissue Engineering

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Liver transplantation as a therapy for liver failure is often hampered by a shortage of donor tissue. The delivery of liver-differentiated human mesenchymal stem cells (hMSCs) is a potential therapy to aid in liver regeneration. In this study, a polyelectrolyte complex (PEC) fibrous non-woven scaffold is employed to deliver differentiated hMSCs *in vivo*. The PEC fibres are synthesized by a process of interfacial polyelectrolyte complexation, which has its benefits in being a room-temperature, aqueous-based reaction. The fibres used in this experiment are mainly composed of natural biopolymers, chitosan and alginate, and are crosslinked by silica. The alginate component of the fibre has been chemically modified to present an integrin-binding peptide, RGD, which enhances attachment of the cells to be delivered. Bone marrow-derived hMSCs are differentiated *in vitro* by a combination of extracellular matrix and conditioned medium. The differentiated cells are seeded onto the RGD-modified chitosan/alginate fibrous scaffolds and allowed to attach overnight. The cell/scaffold construct is then implanted into the livers of Wistar rats, from which 70% of the liver has been removed. Immunolabeling of histological sections reveals minimal capsule formation and hMSC expression of a subset of hepatocyte

markers after two weeks. Hence, we have demonstrated that the RGD-modified chitosan/alginate fibrous scaffold is useful for delivering pre-differentiated hMSCs *in vivo*.

(OP 226) Polyethylene–Mediated Gene Delivery into Rat Bone Marrow Stem Cells

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Stem cells possess the inherent capability of transforming and differentiating into many cell types and specific cell type. Based on this potential, stem cell-based gene therapy is promising for the reconstruction of injured tissues or organs. Thus, the aim of this study is to evaluate transfection efficiency in rat bone marrow stem cells (rBMSCs) using DNA/polyethyleneimine (PEI) complexes.

The formation of complexes from DNA and PEI was performed by the adding of the PEI solution to the DNA solution. Gel retardation assay showed the optimal N/P charge ratio where PEI completely binds DNA. The particle size of DNA/PEI complexes measured by dynamic light scattering (DLS) showed diameters of up to 500 nm. From AFM measurement, DNA/PEI complexes showed round-shape. These results indicated that DNA was sufficiently condensed by PEI to give DNA/PEI complexes. The viability of rBMSCs in the presence of DNA/PEI complexes was evaluated by the MTT assay. As the relative amount of PEI was increased, its cytotoxicity to rBMSCs increased. The DNA/PEI complexes were transfected into rBMSCs. After gene transfection, enhanced green fluorescence protein (EGFP) was monitored by flow cytometry. Naked DNA showed no EGFP expression, whereas delivery of DNA/PEI complexes to rBMSCs showed EGFP expression and resulted in 2–10% transfection. Transfection efficiencies of DNA/PEI complexes increased with increasing N/P charge ratio.

In conclusion, we have shown that PEI acts as an effective gene carrier for rBMSCs.

(OP 227) Polymer–ECM Hybrids Combining Engineered Shape with Cell Produced Matrix Components

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Synthetic biodegradable polymers are a current class of safe biomaterials and they combine mechanical strength with processability and reproducibility. Their biodegradability and tuneable degradation rate allow them to offer temporary mechanical support for cells. However, they suffer from poor acceptance by cells and almost complete absence of biological activity. Conversely, scaffolds made from extracellular matrix components like collagen often exhibit excellent adhesion, adequate cell growth and can be remodelled *in-vivo*. However, they suffer from limited processability, poor strength, source variability and low biological activity.

The objective of the 3G-SCAFF project is to develop the ''third generation'' bioresorbable intelligent polymer-ECM composite materials that have a bioactive information built into them using engineered cells and thus can activate specific tissue regeneration.

This approach for developing materials for Tissue Engineering and Regenerative Medicine ranges from synthesis and processing of synthetic polymers over cell engineering and culture systems to *in-vivo* tests in relevant animal models. The partners involved in this project with complimentarily competences and expertise in polymer synthesis (Uppsala University), biomedical textiles (RWTH Aachen University), cell- and tissue engineering (Swiss Federal Institute Lausanne), mechanotransduction and bioreactor (University College London) and clinical applications in urology (Lausanne Research Hospital, CHUV) and skin (Karocell AB, Sweden) will present the way from molecules to engineered cell constructs.

(OP 228) Polymer/ECM Composite Biomatrix for Regeneration of Soft Tissues

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A three dimensional scaffold is a common but challenging tool in modern soft tissue engineering. On one hand it needs be porous to allow cell penetration and resorbable. On the other hand, the scaffold has to have mechanical properties allowing for suturing and giving compliance similar to the surrounding tissue.

Collagen is the most abundant natural polymer in the body and possesses important characteristics needed for attachment and proliferation of cells, which makes it an interesting candidate for preparing scaffolds. However, such biomatrices lack mechanical strength, making them fragile and difficult to use for more mechanically demanding applications.

To overcome this problem we designed a construct that consists of a biodegradable synthetic polymer knitting, incorporated in a collagen sponge. The polymer fabric acts as a mechanical reinforcement, while the collagen provides the necessary three dimensional cues for cell integration and proliferation. Several preparation methods are being explored in order to control the orientation and the porosity of the collagen.

This composite system will be evaluated for soft-tissue regeneration e.g. for repairing large congenital defects in children within the frames of the European program on Soft Tissue Engineering for Children (EuroSTEC).

(OP 229) Polyureyhane/BMP-4 for Periodontal Tissue Engineering in an Osteoporotic Rabbit Model

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Introduction: Osteoporosis is a global problem and is the underlying cause of more than 1.5 million fractures annually. Presumed correlations between age, systemic osteoporosis and periodontal disease, tooth loss and changes in the quantity and quality of jaw bone have been reported. Biomaterials for tissue engineering are continuously developing for enhancement of tissue regeneration and as delivery systems for BMP gene therapy. The present work evaluated the regenerative potential of polyurethane/BMP-4 gene encoded scaffold for periodontal tissue engineering in an osteoporotic rabbit model.

Materials and Methods: The first stage included synthesis of biodegradable polyurethane matrix containing plasmid DNA vectors encoding Bone Morphogenetic Protein–4 (BMP-4) as a therapeutic transfectant. In the second phase, an osteoporotic rabbit model was established by ovarectomizing 6 female New Zealand white rabbits aged 5–7 months old. Animals were sacrificed 2 and 4 months post-ovarectomy and histological and histomorphometric analyses were performed. The third phase involved the use of 6 normal and 6 osteoporotic rabbits to induce periodontal defects on the distal surface of the 2 lower central incisors. For all the animals, the left defects were left empty and the right defects received the polyurethane/BMP-4 scaffolds. Animals were sacrificed 2, 4, and 6 weeks after the periodontal surgery.

Results & Conclusions: The New Zealand white rabbit was a suitable model for osteoporosis as confirmed by histological analyses of bone status. The implanted polyurethane/BMP-4 matrix was capable of regeneration of periodontal structures; alveolar bone, highly organized periodontal ligament attachment in defects in normal and osteoporotic rabbits.

(OP 230) Preferential Cellular Behaviour is Seen with a Collagen-Elastin-Like Polypeptide Scaffold

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Collagen scaffolds cross-linked with microbial transglutaminase have shown to enhance angiogenesis and resist wound contraction in *in-vivo* models [1]. Using this scaffold as the structural foundation; it is hypothesised that the introduction of a functionalised elastin-like polymer can be fully integrated, providing enhanced mechanical and programmed cell viability. In addition, this elastic component, designed using recombinant methodology, will provide inherent proteolytic resistance to the scaffolds as well as increased functionality by carrying specific sequences for endothelial cell adhesion and enzyme substrate. The objective of this study was to study physico-chemical properties and preferential cellular behaviour of a tethered ELP-collagen scaffold. The effect of ELP introduction on collagen fibrillogenesis, thermal behaviour, porosity and mechanical properties was evaluated using atomic force microscopy, differential scanning calorimetry and rheology respectively. In addition, cell viability and morphology were assessed using different cells lines (fibroblasts, human umbilical vein endothelial cells and smooth muscle cells) using AlamarBlue™ reduction assay in conjunction with confocal and bright filed microscopy. The analysis of these parameters allowed us to conclude, that the introduction of ELP in enzymatically cross-linked collagen scaffolds significantly enhances mechanical properties without significantly affecting other physico-chemical properties. In addition, enzymatically cross-linked collagen-ELP scaffolds favoured endothelial and smooth muscle cells over fibroblasts. This preferential attribute could be used to modulate cellular responses not only in wound healing models but in cardiovascular and reconstructive tissue engineering fields.

(OP 231) Preparation of Macroporous 3D Bonelike® Structures Through 3D Machining Techniques

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Bioactive and bioresorbable materials can be used in bone regenerative surgery, avoiding bone grafts.Using these biomaterials it is possible to construct three-dimensional (3D) structures, which can be used in regenerative bone surgery, as well as scaffolds, for tissue engineering applications. This work reports a 3D machining technique that allows the preparation of scaffolds with a reproducible pore size, pore distribution and pore interconnections in opposite to some conventional techniques used to prepare porous materials. This technique also allows the preparation of scaffolds with a defined architecture with the right shape to a specific implant site. This work reports the use of 3D machining techniques in the preparation of 3D macroporous Bonelike®, a glass reinforced hydroxyapatite. A virtual 3D structure model was created and a CNC milling device machined the Bonelike® dense structure. *In vitro* testing using human bone marrow showed that cells were able to adhere and proliferate on 3D structures surface and migrate into all macropores channels. Moreover, these cells were able to differentiate, since mineralized globular structures associated with the cell layer were identified. The results obtained showed that 3D structures of Bonelike® allow with success cell migration into all macropores, as well as the human bone marrow stroma cells proliferation and differentiation.

Acknowledgments: The authors would like to thanks to the FCT for their support in this work through the project POCI/CTM/59091/2004.

(OP 232) Preparing Bone Using an Injectable Hydrogel Scaffold

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Autologous bone grafts are routinely used to heal large bone defects but has the disadvantages limited graft quantity, donor site morbidity, pain for the patient, and cost of harvesting. A promising alternative to this autografting is the delivery of osteoinductive

growth factors, such as members of the bone morphogenetic protein (BMP) family. It is, however, estimated that less than 20% of the more than 1 million patients in US and Europe who develop non-healing bone fractures can today be treated by bone growth stimulation to heal. One reason for this is that the clinical efficiency of BMP is critically dependent on the delivery strategy: when delivered in solution, BMPs will be rapidly cleared, resulting in suboptimal healing. When employing carriers that can retain and sequester BMPs greatly enhances efficacy and reduces protein dose by localizing the morphogenetic stimulus.

To address this problem we have developed a new hydrogel carrier that has demonstrated the desired features of an injectable replacement for bone grafts: with BMP-2 it induces bone formation in animals, it induces vascularisation, it is sterilizable, forms a gel upon injection, shows little or no inflammatory reaction and is based on naturally occurring hyaluronic acid which has a firmly established biocompatibility.

(OP 233) Processing Biodegradable Thermoplastic Elastomers for Applications in Soft Tissue Replacement and Augmentation

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A poly(ester urethane urea) (PEUU) with hydrolytically labile soft segments has been shown to act as a thermoplastic elastomer and has been processed into a variety of formats to meet clinical needs in the treatment of cardiovascular disease and soft tissue replacement. Early pre-clinical testing has demonstrated the promise of this platform in several settings. For pediatric cardiac surgery, a full-wall thickness right ventricular outflow tract reconstruction was performed in rats with a porous PEUU patch and lead to complete functional scaffold replacement without complications. For adult disease, placement of an epicardial patch onto a subacute myocardial infarct in rats resulted in positively altered ventricle wall remodeling and improved cardiac function. A tissue engineered blood vessel generated by a combination of thermally induced phase separation and electrospinning, followed by seeding with muscle-derived stem cells, was successfully implanted in rat (aortic) and porcine (carotid) models. Abdominal wall replacement was achieved in an infected, trauma model with controlled antibiotic release. Processing methods could yield structural and mechanical anisotropy by electrospinning or thermally induced phase separation with thermal gradients and composites could be made by blending with collagen or extracellular matrix digests. For controlled release, growth factors (e.g. bFGF, VEGF) could be blended into the polymers as could a small molecule ligand for a rheostatic ligand/gene promoter system. Electrostatic coprocessing of polymer and cells allowed formation of tissue constructs with microintegrated cells and fibers. This platform offers attractive features for numerous applications in regenerative medicine.

(OP 234) Production of Bone Substitutes Within Bioreactor-Based Systems: Modeling and Culture of Preosteoblastic Cells

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Aim: Our project aims at developing a bioreactor-based device dedicated to the production of bone substitutes *ex vivo*, by combining bone marrow stem cells and biomaterials. The goal of this study is to better characterize the flow and the mechanical stimuli to which the cells are exposed within such systems by means of computational fluid dynamics software, and to identify the appropriate configuration for culturing bone cells on microparticles.

Methods: Modeling software (Gambit, TGrid and Fluent) was used to determine fluid velocity, static pressure and shear stresses, parameters that may greatly impact cell proliferation and differentiation. In parallel, preosteoblastic cells (MC3T3-E1) were seeded and cultured dynamically on glass microbeads. Three bioreactor configurations were investigated: vertically and horizontally positioned packed beds, fluidized beds as well as monolayers of beads within plane-parallel chambers. Cell survival and proliferation rates were determined by cell counting. Cell distribution and morphology were evaluated using ESEM and methylene blue staining. The experimental data were subsequently correlated to the numerical simulations.

Results: The fluidic environment could be investigated. A patent effect of bioreactor configuration was observed on both survival and proliferation of preosteoblastic cells. Horizontally positioned packed beds and monolayers of beads lead to the best results in terms of cell survival and proliferation.

Conclusion: Computational fluid dynamics is a valuable tool to better comprehend the effect of dynamic culture on cells and to determine the optimal experimental conditions required to produce bone substitutes. Monolayers seem to represent an appropriate configuration for culturing bone cells on micro-particles.

(OP 235) Production of Cytokines and Response to IL-1b by Human Articular Chondrocytes at Different Stages of Tissue Maturation

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Implantation of engineered cartilaginous tissues represents a promising strategy for the repair of cartilage defects. In the injured joints, proinflammatory/catabolic mediators can impair the implanted engineered tissue. We aimed at investigating whether an increased extent of tissue maturation *in vitro* would reduce the production of pro-inflammatory chemokines and the IL-1b-mediated catabolic effects by human articular chondrocytes (HAC).

HAC isolated from knee cartilage biopsies of 6 individuals were expanded in monolayer for 2 passages and then cultured in pellets for 3 or 15 days in chondrogenic medium. Tissue maturation was assessed biochemically [glycosaminoglycans (GAG), DNA] and histologically (Safranin-O). MCP-1, IL-8 and TGFb-1 were quantified in culture surnatants or in tissues by real time RT-PCR.

At 3 or 15 days of culture some tissues were exposed to IL-1b and specific metalloproteases (MMPs) were quantified in the culture media.

By increasing culture time, HAC accumulated more GAG (4.3-fold), released lower amounts of IL-8 (12.0-fold) and MCP-1 (10.0-fold) and higher amount of TGFb-1 (5.4-fold). PCR analysis confirmed respectively lower and higher expressions of IL-8 and TGFb-1 mRNA in more mature tissues. As compared to HAC cultured for 3 days, those cultured for 15 days responded to IL-1b releasing lower MMP-1 (12.6-fold) and MMP-13 (1.8-fold) amounts. Quantification of cytokines in response to IL-1b is in progress.

We showed that chondrocytes embedded within a more aboundant cartilaginous matrix release lower levels of pro-inflammatory chemokines and exhibite less IL-1b-mediated catabolic effects. The implantation of more mature cartilaginous tissues could therefore guarantee superior graft survival and functional outcome.

(OP 236) Production of PDLA and PLGA Scaffolds by Microfabrication and Electrospinning Techniques

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Properties, structure and morphology of scaffolds have a key role in determining seeded cells behavior and scaffold fate once implanted. Among the various methods of scaffold production used for polymer based materials are microfabrication and electrospinning techniques which can produce scaffolds with different geometry and order. Microfabrication permits the production of 3-D scaffolds with tailored regular porosity and micro-resolution, while non-ordered or less-ordered nanostructures are obtained by the electrospinning process.

In this study we compare properties and *in vitro* behavior of scaffolds produced by electrospinning and by a home-made microfabrication apparatus.

The microfabrication apparatus consists of three computer controlled slides and an automatic pumping device connected to a syringe. Slides can move independently with micrometric resolution in three orthogonal directions while the syringe extrudes a thin filament of polymer solution through a 60 μm stainless steel capillary needle at constant flow rate. The electrospinning system makes use of a high voltage between the needle and a target to form a mat of fine fibers. By changing the electrospinning process parameters such as voltage, distance and flow rate nets with different morphology can be obtained.

In this study, poly(D,L-lactic acid) (PDLA) and poly(D,L-lactide-co-glycolide) (PLGA) (50:50) scaffolds were prepared by the microfabrication and electrospinning techniques. Rheological tests were performed to characterize polymer solutions. The properties of the prepared scaffolds were investigated by DSC, ATR-FTIR, SEM. Cell culture tests were performed with MG63 osteoblast and MRC5 fibroblast cell lines on the scaffolds.

(OP 237) Programming Cells *In Situ*

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There are hundreds of clinical trials of cell therapy currently underway, with the goal of curing a variety of diseases. Simple cell infusions lead to large-scale cell death (>90%), and little control over cell fate. Typical tissue engineering approaches do not regenerate host tissues, but instead lead to the formation of a new tissue mass that replaces the original tissue and must be integrated with the host. We propose a new approach, in which material systems are first used either as cell carriers or attractors of host cell populations, and in either case the material then programs the cells *in vivo* and ultimately disperses the cells to surrounding host tissues or organs to participate in tissue regeneration or destruction. We have first addressed this possibility in the context of skeletal muscle regeneration and vascularization, as materials were designed to transplant progenitor cells, promote their proliferation, and ultimately release the cells in a controlled manner over time to repopulate host tissue. Further, this concept has also been examined in the context of cancer vaccines, in which antigen-presenting cells were recruited to the material, matured, and then dispersed to lymph nodes to activate an immune response. These examples demonstrate the broad potential for this approach to cell therapies, and suggest one may ultimately be able to bypass most or all *ex vivo* manipulations in cell therapies.

(OP 238) Prostaglandin E2 as an Innovative Signalling Molecule for the Tissue Engineering of Cartilage

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Background: Prostaglandin E2 (PGE2) is involved in physiological homeostasis and plays a role in cartilage metabolism. Thus, PGE2 could serve as a candidate for tissue engineering strategies. We investigated the incorporation of PGE2 into biodegradable poly-L-lactide-co-glycolide (PLGA) microspheres and its release over 154 h. Furthermore, we analysed the effect of PGE2 on human articular chondrocytes seeded in a gelatine-based, oriented scaffold.

Methods: PGE2-modified microspheres were produced by a co-solvent emulsification method. Upon incubation in 1.0 ml serum-free medium at 37°C, the supernatants were removed at defined time points up to 154 hours. PGE2 concentrations were analyzed by gas chromatography tandem mass spectrometry (GC/MS/MS). The effect of this PGE2-release system was analysed in freshly isolated primary human chondrocytes cultivated on oriented, collagen I-coated gelatin scaffolds with and without PGE2-containing microspheres. Cell morphology was visualized after

2 weeks by Calcein-AM staining and laser-scanning confocal microscopy.

Results: Microspheres showed a prolonged release of PGE2 with a linear concentration increase over the first 12 hours, followed by a plateau phase and a slow decrease until 154 h. Cell culture and Calcein-AM staining of chondrocytes revealed that cells cultured on the collagen scaffolds with PGE2 microspheres preserved a rounded form resembling native articular chondrocytes, whereas those cultured in the absence of PGE2 attained an elongated, fibroblast-like morphology.

Conclusions: We demonstrated the prolonged release of PGE2 from PLGA-microspheres. Furthermore, our results from chondrocyte cultures suggest that PGE2 enhances the preservation of the chondrocyte phenotype. Thus, PGE2 is an interesting candidate for tissue engineering of cartilage.

(OP 239) Rapid Engineering of a Biomimetic Skin Substitute

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Skin substitutes should promote recovery of function by reproducing the anatomy, physiology and biologic stability of uninjured skin. Plastic compression(PC) of collagen hydrogels is promising as it rapidly produces dense, mechanically strong and biomimetic scaffolds. We studied the possibility of further developing this into a skin substitute.

Collagen constructs were prepared as two layer mimics of native skin. A dense 'dermal' layer was developed by PC of collagen gels, seeded with human dermal fibroblasts. Human keratinocytes were seeded onto the surface and constructs were submerged in media for 2 days before being raised to the air-liquid interface and cultured for an additional 12 days. One set of constructs was embossed with 3D micro-ridges into the dermal-epidermal interface during the PC process, by embossing the putative interface of the initial collagen gel with a template pattern of 40um diameter glass fibres, parallel and 100um apart.

The skin-like ECM construct formed rapidly, taking a max of 1 hour to fabricate and up to 14 days to culture keratinocytes to a multilayer. Histological analysis and immunofluorescence staining(anti-cytokeratin) indicated that a stratified and differentiated epidermis was formed. SEM showed a pattern of deep ridges on the collagen surface which induced the formation of invaginations of the epidermal layer as shown by histological analysis. Epidermal thickness was 14 ± 6 μm for ridged interfaces compared to 40 ± 14 μm for smooth interfaces, indicating that the topography of the microtextured interface influenced the stratification rate of keratinocytes.

These findings support ongoing research for developing compressed collagen scaffolds into biomimetic skin substitutes.

(OP 240) Recent Advances of Term in Korea: Commercialization and Academic Research

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In Korea, basic researches on tissue engineering and regenerative medicine (TERM) have grown rapidly last decade in the academic and industry fields. A lot of basic researches have been supported by national research grants. Based on these basic researches, commercialization of TERM products has continued. Now, about 10 products are being launched in market. These products include 8 cell therapy products and 1 scaffold product and 1 tissue engineered product. Also, some products are under investigational new drug (IND) application. By recent outstanding results from the stem cell research, a major cell source of the above products is mesenchymal stem cell derived from bone marrow or umbilical cord blood for the treatment of ischemic stroke etc. Some combined products such as bioartificial liver system are under preclinical study. Despite of this breakthrough in commercialization, it is strongly suggested to secure a lot of the original and core technology for TERM in Korea now.

(OP 241) Redifferentiation Mechanisms of Human Articular Chondrocytes in Three-Dimensional Scaffolds

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In order to obtain a sufficient number of cells for matrix-associated autologous chondrocytes transplantation (MACT), human articular chondrocytes are expanded in monolayer culture. During this cultivation time, the chondrocytes undergo a dedifferentiation process, which is partially reversed, when the cells are seeded on three-dimensional scaffolds. The aim of this study was to enlighten some molecular aspects of the redifferentiation process on the basis of two different scaffolds, which are already used for MACT. Human articular chondrocytes were seeded on a colllagen I/III membrane (Biogide®) and a hyalruonic acid fleece (Hyaff®). Expression levels of collagens, BMPs, BMPRIb, Noggin, Connexin43 and CD44s were determined by real-time PCR at different time points. Furthermore the samples were analysed histochemically and immunhistochemically. Gene expression of Col1, Gdf5, CD44s and Connexin 43 was upregulated during monolayer culture in comparison with native cartilage and was partially downregulated in the scaffolds. BmprIb expression was increased in monolayer culture, but in Hyaff and Biogide the expression completely returned to levels of native cartilage. Col2 and Noggin expression was dramatically diminished in monolayer culture. After 5 weeks three-dimensional culture we found Col2 and Noggin expression slightly increased in comparison with monolayer culture. Expression of Bmp6 was decreased in monolayer culture and in the scaffolds. Bmp7 expression was not detected in any sample. Although the two scaffolds highly differed in composition, they showed a very similar influence on chondrocyte redifferentiation. Our data indicate that major changes in the expression of differentiation/proliferation factors caused by three-dimensional scaffolds may induce redifferentiation.

(OP 242) Regeneration of Muscular Defects by a Composite Graft Made of Mesenchymal Stem Cells and Human Acellular Collagen Matrix

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Purpose: This work investigates, in 2 animal models, the reconstruction of muscular defect by a composite graft made of "Mesenchymal Stem cells (MSc) and human acellular collagen matrix."

Methods: MSc cells were isolated from transgenic "Green Fluorescent Protein" pigs and seeded on human acellular collagen matrix (incubation time: 2–3 weeks). Two experimental models of muscular defects were tested on nude rats ($n = 16$): (i) abdominal wall defect and (ii) muscular necrosis induced by electrocoagulation (4 necrosis areas of 16mm^2/animal). Three experimental groups were designed to cure muscular defect: 1) composite "MSc/collagen matrix," 2) Collagen matrix alone and 3) No repair. Histomorphometry were performed, at 2, 4 and 8 weeks post-implantation, to assess pig MSc cells detection (anti-GFP antibody), matrix remodelling (Masson's Trichrom/dystrophin) and revascularization (von Willebrandt factor).

Results: No significant difference of muscular defect remodelling was observed at 2 weeks for each experimental group. In both animal models, human acellular collagen matrix remained non-recolonized up to 8 weeks post-transplantation. In contrast, a significant higher area of composite implants was recovered by muscular cells and vascular structures between 4 and 8 weeks for composite grafts in comparison to collagen matrix alone ($p < 0.05$ for Group 1 vs. Group 2).

In both models, pig MSc remained undifferentiated and tissue recolonization was performed by native nude rat endothelial and muscular cells up to 8 weeks post-implantation.

Conclusion: A composite graft of MSc/human acellular collagen matrix can promote the remodelling of muscular cell defect by paracrine effect of MSc cells on the native environment.

(OP 243) Regional Expression of Articular Cartilage Surface Lubricants following Mechanical Loading: Implications for Tissue Engineering

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Mechanical signals are critical for restoration strategies in tissue engineering and regenerative medicine. The objective of this study was to investigate the role of mechanical signals in the expression of superficial zone protein (SZP), a lubricating biomolecule in articular cartilage. Regional expression of SZP was first quantified in cartilage obtained from the femoral condyles of immature bovines using immunoblotting and visualized by immunohistochemistry. Contact pressure mapping in whole joints was accomplished using pressure sensitive film and a load application system for joint testing. Friction measurements on cartilage plugs were acquired in using a pin-on-disk tribometer modified for reciprocating sliding. Direct mechanical stimulation by shear loading of articular cartilage explants was performed with and without inhibition of TGF-beta signaling, and SZP content in media was quantified by enzyme-linked immunosorbent assay. SZP localization in femoral cartilage was initially identified, with anterior regions exhibiting high SZP expression. Regional SZP patterns were regulated by mechanical signals and correlated to tribological behavior. Direct relationships were demonstrated between highest SZP expression, maximum contact pressures, and lowest friction coefficient. SZP expression and accumulation was increased by applying a shear stress, depending upon location within the knee, and was decreased to control levels with the use of a specific inhibitor of TGF-beta type I receptor kinase and subsequent phospho-Smad2/3 activity. The regional localization of SZP may be a critical design endpoint for tissue engineering and regenerative medicine strategies aimed at functional restoration of the articular cartilage surface with optimal tribological properties.

(OP 244) Rheological Characterization of Pathological Blood Alterations and Possible Effects on EC Blood Treatment Processes Based on TE Constructs

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TE constructs are often used in bioreactors coupled to artificial organs to treat blood, or plasma continuously pheresed from it, of patients with metabolic disorders that, also through administered drugs, alter blood composition and rheological properties. Nevertheless, these devices are designed as if blood were Newtonian with standard viscosity. In this work, we report on a technique to characterize both viscous and elastic blood behavior based on deformation measurements under low constant stress (creep), and blood rheological characterization of nephropatic and transplant patients.

Blood collected with EDTA from healthy individuals, nephropatic and renal transplant patients of both genders was tested in a Rheometrics DSR200 with parallel plates ($D = 40$ mm, gap $= 1.5$ mm) at 0.09 Pa. From deformation-to-stress ratio (compliance) vs. time curves it was estimated the shear viscosity, as the reciprocal slope at long times, blood elasticity, as the hysteresis area between Newtonian and experimental compliance, and a specific blood elasticity, as the differential-to-total hysteresis area ratio.

The technique yielded reproducible sound parameter values with viscosity consistent with literature values at the given shear rate, blood elasticity decreasing with hematocrit, and specific blood elasticity independent of hematocrit and gender. Viscosity and specific elasticity of nephropatic and transplant patients was lower than the controls, decreasing with progression of renal damage of the former, and were correlated with altered blood structure. During EC treatment lower viscosity may cause lower pressure drop and transmembrane pressure for given permeate flux. Reduced cell deformability may favor clotting in narrow channels, and in membrane manifolds in the device headers.

(OP 245) Role of Austrian Cluster for Tissue Regeneration in the Hippocrates Project

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The Cluster was mainly responsible for three areas, growth factors and its monitoring, (stem) cells (see the Linz Branch) and *in vivo* models including imaging. BMPs play an impotant role in bone of critical size defects, therefore different Bmps were expressed and purified in different expression systems in addition to FGFs or derivates thereof.

Non viral gene therapy is another focus within our growth factor research. For this reason, cDNAs of the most important BMPs (BMP-2, BMP-4 and BMP-7) were cloned by RT-PCR and subcloned into an eukaryotic expression plasmid and used, partly also in bicistronic vector systems.

To establish a fluorescent based system to evaluate osteogenic differentiation of human ASC, human CollagenIα1 as well as osteocalcin promoter-reporter vector systems were used. The promoter activity was monitored by fluorescence microscopy and semi-quantitative RT-PCR.

Different cell/growth factor combinations in different scaffolds were tested in different spezialised osteochondral models (mice, rats, rabbits, pigs).

The process of degradation and the removal of scaffold material was shown using fluorescently labelled hydrogel in combination with a real time *in vivo* imaging system (Maestro)

Transfection with a luciferase reporter gene allowed both detection and quantification of implanted cells via a highly sensitive CCD camera for at least 20 days in nude mice.

The mobility of the cells can further be influenced by choosing different modes of application. The real time tracking of implanted cells provides a valuable tool to detect processes of integration,homing or healing in defect model experiments

(OP 246) Self-Assembling Peptide Amphiphiles for Articular Cartilage Regeneration

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Despite significant surgical advances and other therapies, treatment of damaged articular cartilage due to injury or degenerative disease remains a major medical challenge. This is largely due to the limited capacity for self-regeneration of mature cartilaginous tissues. The goal of these studies was to develop injectable peptide amphiphile (PA) gels to enhance articular cartilage regeneration. These aqueous gels consist of small peptide-based molecules that self-assemble to form nanofibers of similar dimension and architecture to collagen fibrils.

In vitro experiments were completed to determine the viability and chondrogenic differentiation of human mesenchymal stem cells (hMSCs) within PA scaffolds. Cells were cultured within PA gels with or without the incorporation of varying amounts of the chondrogenic-inducing transforming growth factor (TGF)-β1 for up to 4 weeks. Live/dead assays and gene expression of aggrecan and type II collagen demonstrated good cell viability and successful chondrogenic differentiation within the PA scaffolds with the addition of TGF-β1.

PA gels were then evaluated for their ability to enhance tissue formation in an *in vivo* rabbit model. Full thickness articular cartilage defects were created in the trochlea of rabbit knees followed by microfracture. TGF-β1 alone or PA gel containing TGF-β1 were subsequently injected within the defects. Four weeks post-op, histological evaluation revealed a 2-fold increase in the amount of tissue fill within defects containing the PA gels and TGF-β1 compared to defects with TGF-β1 alone. These results demonstrate the capability of using less invasive injectable PA gels to augment current clinical practice for regenerating cartilage tissue.

(OP 247) Self-Assembling Peptides for Scaffolds in Regenerative Medicine: Production Using Recombinant DNA Technology

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Peptides have been rationally designed which spontaneously self-assemble into hierarchical structures in solution in response to specific physico-chemical triggers to provide a new generation of well-defined biopolymers. An 11 mer peptide, $P_{11\text{-}4}$ (n-QQRFEWEFEQQ-c) that forms a self-supporting isotropic hydrogel at concentrations of 10–30 mg/ml in physiological medium has utility in regenerative medicine applications. Production of the peptide using solid-state chemical synthesis is not cost effective for biomedical applications and so our aim is to generate the peptide at high yield and purity using a recombinant DNA approach.

The pET31b(+) E. coli expression system was used for high level expression of $P_{11\text{-}4}$, generating approximately 0.6 g peptide/L. The coding sequence for $P_{11\text{-}4}$ was fused in tandem repeats to the fusion protein ketosteroid isomerase (KSI) to allow high yield, insoluble expression. Plasmids containing the KSI-His-$(P_{11\text{-}4})_3$ construct carrying three $P_{11\text{-}4}$ tandem repeats were transformed into BL21 (DE3) and expressed for 42 hours in an autoinduction system. Subsequent inclusion bodies that formed were harvested by centrifugation after cell lysis. A hexahistidine tag on the construct was used to purify the protein by nickel affinity chromatography. Recombinant $P_{11\text{-}4}$ was obtained by cyanogen bromide cleavage of internal methionine residues at the end of the KSI and between each individual peptide.

Recombinant $P_{11\text{-}4}$ was shown to spontaneously self assemble into hydrogels in physiological tissue culture medium. Recombinant $P_{11\text{-}4}$ was not toxic to cells and was shown to support three-dimensional proliferation of L929 cells and primary human dermal fibroblasts over 28 days of culture.

(OP 248) Senescence-Related Myocardial Differentiation of Human Bone Marrow Mesenchymal Stem Cells Induced by Polystyrene Niche and its Reversal/Prevention by Extra Cellular Matrix Culture

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In the present study, it was revealed that human bone marrow mesenchymal stem cells (MSCs) undergo senescence-related differentiation into myocardial lineage during *in vitro* culture without any induction treatment. This phenomenon occurred over the whole population of MCSs, much different from conventional differentiation with limited frequency of occurrence, and was accompanied by change of morphology into large, flat cells with impeded proliferation which are the representative indications of MSC senescence. By culturing MSCs under several culture conditions, it was determined that medium type used and induction treatment with 5-azacytidine were not associated with the phenomenon, but serum starvation condition, under which proliferation is most severely hampered, caused senescence progression and up-regulation of cardiac markers. Nevertheless, MSCs gradually developed into myocardial phenotype under normal culture conditions over prolonged culture period and heterogeneous populations were formed. In perspectives of research and clinical applications, this must be prevented for fair and consistent outcomes. Hence, a more biomimetic niche environment was constituted for hBM-MSCs by culturing on a conventionally available ECM, as this phenomenon seemed to have derived from the diverse nature of polystyrene niche from that of *in vivo* environment. Consequently, cells cultured on ECM regained spindle-shape morphology, increased in their proliferation rate by two folds, and showed decreased expression of cardiac markers at both mRNA and protein levels. In conclusion, these results indicate that progression of MSC senescence may occur via myocardial differentiation during *in vitro* polystyrene culture and this can be overcome by employing appropriate ECM culture techniques.

(OP 249) Silicon—Hydroxyapatite Bioactive Coatings (Si-HA) from Diatomaceous Earth and SiO2. Study of Adhesion and Proliferation of Osteoblast-Like Cells

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The aim of this study consisted of investigating the influence of bioactive ceramic coatings of silicon substituted hydroxyapatite (Si-HA) over the behaviour of the human osteoblast-like cell (SaOS-2) line. Pulsed laser deposition (PLD) was the selected technique to deposit the coatings over titanium substrates. Diatomaceous earth and SiO_2, together with commercial hydroxyapatite were respectively the silicon and HA sources used to produce the Si-HA coatings. HA coatings with 0% of silicon were used as control of the experiment. The Si-HA thin films were characterized by Fourier Transform Infrared Spectroscopy (FTIR) demonstrating the efficient transfer of Si to the HA structure in the form of SiO_4^{4-} groups. The *in vitro* cell culture was established with three-dimensional titanium discs covered with Si-HA bioactive ceramic coatings. Cell attachment, proliferation and their osteoblastic activity was followed up to 7 days respectively by, Scanning Electron Microscopy (SEM), DNA and alkaline phosphatase (ALP) quantification. The SEM analysis demonstrated the similar adhesion behaviour of the cells on the tested materials and the maintenance of the typical osteoblastic morphology along the time of culture. The Si-HA coatings did not evidence any type of cytotoxic behaviour when compared with commercial HA coatings, although the proliferation rate was enhanced on the commercial HA from day 3 to day 7 of culture. In what concerns the osteoblastic activity no significant changes were observed for the tested culture times.

This work was supported by the UE-Interreg IIIA (SP1.P151/03) Proteus Project and Ministerio de Educación y Ciencia (MAT 2004-0291).

(OP 250) Silk Fibroin Based Strategies for Bone and Cartilage Repair

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Silk fibroin is receiving large attention for possible application in medicine and namely in tissue engineering. Different processes can lead to the fabrication of gels, sponges or nets that have been already tested *in vitro* and sometimes confirmed in animal tests the attractive properties of this protein. Looking at bone and cartilage repair, fibroin can provide injectable gels, able to promote fast repair of bone defects, sponges that could be implanted or inserted in the damaged cartilage site, nets that can be used in hybrid formulae for more complex needs such as osteochondral defects.

For instance, fibroin water solution can gel after acidic, thermal treatments or other treatments, these resulting in gels with different rheological properties; sponges can be prepared by freeze-drying and salt leaching with the formation of materials with various architecture and mechanical behavior; nets can be formed by solvent induced fibres bonding or electrospinning, providing materials able to induce "in volume" or "on the surface" tissue engineering processes. In all these formulations, fibroin preserves its ability to induce cell adhesion and proliferation, and to guide tissue formation when implanted.

The lecture will overview some of the results achieved by this research group also together with Expertissues partners as part of the strategies of the NoE Expertissues addressed to the development of natural polymers based solution for bone and cartilage repair.

(OP 251) Silk Fibroin Processing and Thrombogenic Responses

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Silkworm fibroin, the core of the silk filament, is an attractive protein-polymer for biomedical applications due to its biocompatibility, ability to promote tissue regeneration and slow biodegradation. Fibroin can also be processed into a variety of 2-D and 3-D formats to match morphological and structural features to specific applications. Depending on the method of preparation and the source of the silk (e.g, Bombyx mori race) with different amino acid chemistries, the polymer can assume different structures, which lead to different mechanical and biological properties. Of particular interest in the field of biomaterials and associated biocompatibility is the interaction of material surfaces with biological defense systems, such as blood coagulation and inflammation. The complement interactions are mediated by a combination of biomaterial structure and chemistry, as well as serum protein modifications of surfaces. The focus of the present research was to correlate the structure of the silk fibroin-derived biomaterials with plasma protein adsorption, platelet activation and inflammatory cell (THP-1 cell line) adhesion and activation.

The chemistry of the two types of silk studied influenced the crystallinity of the films, hydrophobicity, surface roughness and biological interactions. Protein adsorption was lower on samples with the higher crystallinity and hydrophobicity, mainly the chemotactic factors (C3a, C5a, C3b), while other protein adsorption such as fibrinogen were comparable. As a consequence, platelets and immune cells used in the present work responded to the different chemical and physical patterns among the various films in terms of their adherence, activation, and the secretion of inflammatory mediators by monocytes.

(OP 252) Silk Fibroin-Based 3-D Porous Scaffolds for Cartilage Tissue Engineering

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Tissue-engineering scaffolds require chemical, structural and mechanical properties to provide templates that enable cells to adhere and produce their extracellular environment. Previously, we showed that chondrocytes prefer PLGA-scaffolds with mechanical properties similar to native cartilage, but cell-loading problems limit their use for cartilage production *in-vitro*. Here we investigated chondrocyte growth on scaffolds produced using a natural extracellular-matrix (ECM) protein, silkworm derived fibroin as the model biopolymer.

Scaffolds were prepared from 7% fibroin/water (w/v) using the salt leaching method and were characterized in terms of morphology, amino acid composition, molecular weight, conformation, thermal behaviour, porosity size, water absorption capability and mechanical properties. Sponges with a 500–1180 μm pore size were used to assess chondrocyte growth. Confluent cultures of rat costochondral chondrocytes were released from their native ECM by trypsin-digestion, suspended in medium, and loaded onto 3 mm×6 mm cylindrical sponges (10^4 cells/mm^3) by static gravity-dependent filtration and by dynamic perfusion via an orbital shaker, resulting in 87.1% and 81.8% loading efficiency, respectively. Cell/scaffold constructs were cultured in DMEM containing 10%FBS, ascorbate and antibiotics for 28-days.

At harvest, live/dead staining showed >90% cell viability regardless of loading technique. Cell number, determined as a function of DNA content, increased 3.3-fold in the static-loading group but decreased 50% for the dynamic-loading group. The chondrocytes on the scaffold retained their capacity to synthesis cartilage-ECM, producing 2 μg sulfated-glycosaminoglycan/μgDNA, based on DMMB-staining. Thus, natural silk fibroin scaffolds permit efficient gravity-dependent cell loading and support cell proliferation and ECM production *in-vitro*, suggesting they are good alternatives to synthetic polymeric scaffolds *in-vivo*.

(OP 253) Silk Nanoparticles for Delivery of Human BMP-2 in Bone Regenerative Medicine Applications

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A tissue engineering approach combines the use of scaffold biomaterials, stem cells and growth factors. Bone morphogenetic proteins (BMPs) are growth factors that have sparked a great interest in tissue engineering due to their strong ability to promote new bone formation. Herein, we report the use of silk derived nanoparticles as carriers for delivery of human BMP-2. Silks are attractive biomaterials for tissue engineering due to its biocompatibility, slow biodegradability and excellent mechanical properties. Recombinant human BMP-2 was expressed in Escherichia coli and purified by affinity chromatography, showing bioactivity in human adipose stem cells. BMP2-containing silk particles were then prepared by a water-in-oil emulsion method. Scanning electron microscopy (SEM) revealed that silk formed nanoparticles of 100–200 nm in absence of BMP-2 and of 300–350 nm when BMP-2 was incorporated. Western-blot using an antibody against human BMP-2 allowed to detect that the growth factor loaded into the particles, and that it could be slowly released, maintaining the original BMP-2 conformation. In human adult adipose stem cells, the particles showed no significant cytotoxicity and an increase in alkaline phosphate activity. Ongoing research includes the study of particle size by Dynamic Light Scattering (DLS), release assays using FITC-labeled BMP-2 and FTIR analysis. Silk nanoparticles showed to be a promising option as biomaterials for the delivery

of bone growth factors such as BMPs for regenerative medicine purposes.

(OP 254) Single Integrin Detection on the Surface of Osteosarcoma Derived Cells by means of Functionalized AFM Probes

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Integrins are transmembrane glycoproteins made of non-covalently bonded alfa- and beta-chains. They are involved in cell-extracellular matrix and cell-cell interaction and then in fundamental processes such as cell adhesion, growth, and differentiation [1]. During tissues development, the presence of environmental conditions permissive to cell differentiation is associated with the expression of proper cell surface receptors [2]. To this regards, the possibility to investigate cell membrane at the single molecule level is of high potential in the field of tissue engineering. In this work AFM probes have been successfully functionalised and tested for the detection of integrins expressed on the surface of a line of osteosarcoma-derived osteoblasts named MG-63. To this purpose, monoclonal antibodies specific to the β1 subunit of the integrins expressed by this cell line have been covalently immobilized on the tip of silicon nitride AFM cantilevers by means of a bifunctional linker (PEG) [3]. Force curves have been collected in culture medium over the surface of both fixed and living cells obtaining maps of the interaction between the immobilized antibodies and the respective cell membrane receptors. The obtained results demonstrate the possibility to detect specific receptors on the surface of living cells and then the high potential of the AFM technique in the field of tissue engineering to investigate cell response to external environmental conditions.

References:
[1]Hynes R. O. (1987), Cell, 48:549–554.
[2]Rosso F. *et al*. (2004), J. Cell. Phys., 199:174–180.
[3]Hinterdorfer P. *et al*. (1996), Proc. Natl. Acad. Sci. U.S.A., 93:3477–3481.

(OP 255) Site-Specific Patterned Differentiation of Embryonic Stem Cells to Vascular Cells

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Due to oxygen diffusion limitations, engineering of cardiac tissues of physiologically relevant thicknesses (>1 cm) requires engineering blood vessels. Blood vessels are primarily comprised of endothelial cells (ECs) and smooth muscle cells (SMCs). ECs line the lumen whereas SMCs surround the ECs and provide vascular integrity. We and others previously demonstrated that primitive mesodermal progenitors derived from embryonic stem cells (ESCs) express the receptor, Flk1, for vascular endothelial growth factor (VEGF). When grown on collagen IV (ColIV), Flk1+ cells preferentially differentiate further into ECs or SMCs in the presence or absence of soluble VEGF-A, respectively. Previously, we demonstrated that azidobenzoic acid-modified chitosan (Az-chitosan), a cell-repellent cationic hydrogel, can be used to pattern cells. We hypothesize that blood vessels can be engineered by differentiating ESCs on micropatterned ColIV with or without VEGF-A. We immobilized VEGF-A onto negatively-charged ColIV and micropatterned this solution onto Az-chitosan coated coverslips. Flk1+ ESCs differentiated in embryoid bodies for 3 days were FACS sorted and seeded onto patterned surfaces to induce site-specific differentiation into vascular cells. Immunostaining analyses demonstrated that approximately 70% of sorted cells differentiated into vascular cells. Using immobilized VEGF, $52 \pm 14\%$ of cells differentiated to ECs and 19% to SMCs. While in the absence of immobilized or soluble VEGF, ECs and SMCs accounted for $19 \pm 14\%$ and 49% of the differentiated cells, respectively. Therefore, ESCs are capable of differentiating into vascular cells in a site-specific manner. Future work will examine the signalling pathways of the immobilized VEGF and extend this system to 3D structures.

(OP 256) Six Month Control of Diabetes by Transplantation of Encapsulated Pig Islets in Diabetic Primates Without Immunosupppression.

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Objective: This study assessed the capacity of alginate encapsulated islets to reverse diabetes in "pig to primate" model.

Methods: Adult pig islets were encapsulated in microcapsules and implanted under the kidney capsula of 4 Streptozotocin-treated primates or in subcutaneous macrodevice in 5 additional diabetic animals. As controls, primates received non-encapsulated pig islets ($n = 2$) or empty capsules ($n = 2$). Body weight, fasting blood glucose (FBG), insulin, porcine C-peptide, HbA1C (glycosylated haemoglobin) and anti-pig antibodies were evaluated in sera. Immunostaining for CD3,CD68,C3,C9 and insulin were performed on explanted grafts.

Results: Non-encapsulated pig islets were rejected within 7 days. Although a significant reduction of FBG and a transient increase of insulin/porcine C-peptide levels were observed during 2 weeks after microencapsulated pig islets implantation, a gradual decline of function was observed after 6 weeks. After subcutaneous transplantation of a macrodevice, diabetes was corrected up to 6 months: normal FBG (52–107 mg/dl) and HbA1C (after 16 weeks) reached $8 \pm 1.4\%$. Two animals were retransplanted with a new macrodevice between 25 and 35 weeks after the first graft whereas the latter clearly dysfunctioned. Diabetes was completely controlled again ($\text{HbA1C} = 7.4 - 9.8\%$) ten weeks after retransplantation. Although anti-pig IgG antibodies were evidenced in primates sera, immunohistology demonstrated insulin positive cells inside the graft and no sign of graft rejection. Permeability testing by lectin-FITC revealed no permeablity to molecules over 150 KDa for alginate used in macrodevice.

Conclusions: Adult pig islets encapsulated in a subcutaneous Macrodevice can completely reverse STZ-induced diabetes up to 6 months without immunosuppression.

(OP 257) Skeletal Myogenic Differentiation of Mesenchymal Stem Cells Isolated from Human Umbilical Cord for Skeletal Muscle Tissue Engineering

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We have investigated the phenotype of mesenchymal stem cells (MSCs) from human umbilical cord (UC) and their potential to differentiate into a skeletal muscle phenotype as a new approach to skeletal muscle tissue engineering. Furthermore, we investigated whether UC MSCs were capable of differentiation into multiple lineages, such as osteoblasts, adipocytes and neural cells.

Human UCs were harvested from full term deliveries. Each UC was cut into $1mm^2$ pieces and then cultured in DMEM-LG containing 15% FCS for 7–10 days. The pieces were removed and the phenotype of the adherent cells investigated using FACS. During cell population expansion, UC MSCs gave rise to adherent layers of fibroblast-like cells expressing MSC-related antigens SH2, SH3, CD90, CD29 and CD117 and negative for CD34, CD45, CD133, CD106, HLA-ABC and HLA-DR. More importantly, when these UC-derived MSCs were incubated in myogenic conditions for up to 6 weeks, they expressed myogenic markers in accordance with a typical myogenic differentiation pattern. Both RT-PCR analyses and immunohistological staining showed that two early myogenic markers, MyoD and myogenin were expressed after 3 days of incubation but not after 2 weeks. At week 6, most of the UC MSCs expressed myosin heavy chain, a late myogenic marker. Our results demonstrated that UC MSCs possess a potential of skeletal myogenic differentiation and also imply that these cells could be a suitable source for skeletal muscle repair and a useful tool of muscle-related tissue engineering. Finally, osteoblastic, adipocytic and neural differentiation was demonstrated when cultured in typical differentiation media.

(OP 258) Smart "Temperature Responsive" Self-Assembled Monolayers

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Self-assembled monolayers (SAMs) are a class of molecular assemblies that are prepared by spontaneous adsorption of molecules from solution onto a solid substrate. The process of SAMs and more recently, self-assembly of multilayer systems have attracted a great deal of interest. SAMs technology provides a powerful tool for generating monolayers of biological molecules on various solid substrates. The orientation of monolayers offers great versatility in terms of the complex bio-recognition, which might provide a method for the *in vitro* development of biosurfaces that are able to mimic naturally occurring molecular recognition process. Recently we were able to form smart (i.e., temperature responsive) poly(N-isopropylacrylamide) (Poly(NIPA)) based SAMs on model substrate surfaces. We have investigated the effects of dipping time and solution concentration on the formation of SAMs with 3-aminopropyltrimethoxysilane (APTS) molecules on the Si(001) surfaces. Poly(NIPA) was synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization to contain two different functional groups at two ends (i.e., -SH and -COOH), then was covalently attached to the surfaces via surface APTS molecules via the–COOH terminal groups. 5'-thiolated oligodeoxynucleotides (ODNs) were immobilized onto these thiol-terminated poly(NIPA) molecules on the surface by disulfide bond formation. It was possible to control the hybridizations of these immobilized ODNs with the target "complementary" ODNs within the medium by changing the temperatures (from 25°C to 45°C) which was monitored by an ellipsometer

(OP 259) Smooth Muscle Cell Seeding of Decellularized Scaffolds: Further development Toward a More Native Architecture for Tissue Engineered Blood Vessels

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Vascular Smooth muscle cells (VSMCs) impart important functional characteristics in the native artery, and therefore, should logically be incorporated in the development of tissue engineered blood vessels designed for vascular repair or replacement. However, the native architecture and low porosity of naturally derived biomaterials (i.e., decellularized vessels) has impeded efforts to incorporate VSMCs into the wall of tissue engineered blood vessels. To this end, the goal of this study was to develop improved methods for seeding VSMCs onto decellularized porcine carotid arteries. Decellularized vessels were prepared in the absence and presence of the adventitial layer, and statically seeded with a pipette containing a suspension of rat aortic VSMCs. Following cell seeding, re-cellularized engineered vessels were place in a custom bioreactor system for 1–2 weeks to enhance cellular proliferation, alignment and maturation. Seeding of SMCs was dramatically enhanced by removing the adventitial layer of the decellularized porcine artery. Moreover, cyclic bioreactor conditioning (i.e., flow and pressure) augmented SMC proliferation and accelerated formation of a muscularized medial layer. Fura-2 based digital imaging microscopy revealed marked and reproducible depolarization-induced calcium mobilization following bioreactor preconditioning in the absence, but not in the presence of the adventitia. The major finding of this investigation is that following 2 weeks of bioreactor preconditioning, re-cellularized, adventitia denuded, native scaffolds possessed a relatively thick layer of VSMCs capable of mobilizing calcium in response to depolarization. These findings represent an important first step toward the development of tissue engineered vascular grafts that more closely mimic native vasculature.

(OP 260) Starch/Ethylene-Co-Vinyl Alcohol Fiber Mesh Scaffolds: Production, Characterization and Surface Modification

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Tissue engineering scaffolds must provide cell anchorage sites, mechanical stability and structural guidance. Fiber mesh structures have been considered as adequate scaffolds for tissue engineering due their high surface area available for the adhesion and proliferation of different cell types. In addition, they present a good degree of interconnectivity between pores, which is essential for the migration of cells and diffusion of nutrients.

Herein, we propose a new route to produce fiber mesh scaffolds from a starch/poly(ethylene-co-vinyl alcohol) (50/50-%wt) blend. Scaffolds with porosity up to 80% were obtained by a simple wet spinning technique based on solution/precipitation of a polymeric blend and subsequent fiber sintering to stabilize the produced 3D structures. The proposed wet spinning procedure avoids thermal degradation, typical for the conventional melt spinning techniques, as shown by thermal gravimetric analysis.

The high surface area and the presence of –OH groups in the blend components caused relatively high values of water uptake (about 110%). On the other hand, the samples presented very low percentages of weight loss in buffer solution, which reveals their stability in aqueous media.

To enhance cell attachment and proliferation, plasma treatment was applied to the produced scaffolds. The untreated and treated scaffolds were examined using SaOs-2 human osteoblastic cell line. The results showed that SaOs-2 were able to attach and proliferate on the studied materials. However, double concentration of DNA was measured for the modified scaffolds after 2 weeks of culture. Moreover, the cells seeded onto the treated samples showed more spread morphology with extended filopodia.

(OP 261) Starch/Gellan Gum Hybrid 3D Guidance Systems for Spinal Cord Injury Regeneration: Scaffolds Processing, Characterization and Biological Evaluation

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Spinal cord injury (SCI) represents a significant health and social problem and therefore it is urgent to find strategies that can specifically target this problem. In this sense the objective of the present work was to develop a new range of 3D tubular structures aimed at inducing the regeneration within SCI sites. Up to six different 3D tubular structures were initially developed by rapid prototyping-3D bioplotting–based on a biodegradable blend of starch. The mechanical properties of these structures were assessed by DMA, in both dry and wet conditions, and their morphologies/porosities analysed by micro-CT and SEM. Afterwards, gellan gum hydrogel was injected in the central area of structures. Biological evaluation was then carried out by determining their cytotoxicity, using MEM extraction and MTS test, as well as by encapsulation of oligodendrocytes (M0III cell line) within the hydrogel phase. The histomorphometric analysis showed a fully interconnected network of pores with porosity ranging from 70%–85%. Scaffolds presented compressive modulus ranging from 17.4 to 62.0 MPa and 4.42 to 27.4 MPa in dry conditions and wet conditions respectively. Cytotoxicity assays revealed that the hybrid SPCL/Gellan Gum scaffolds were non cytotoxic as they did not cause major alterations on cell morphology, proliferation and metabolic activity. Finally, preliminary direct contact assays showed that the hybrid scaffolds could support the *in vitro* culture of oligodendrocyte like cells. Further work will focus on the behaviour of these scaffolds when implanted in SCI animal models.

(OP 262) Stem Cell Sheet Engineering on Polyelectrolyte Multilayer Films

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Cell sheet engineering has emerged as a novel method for creation of three dimensional tissue models without the need for biodegradable scaffolds. We aim to develop a new methodology that builds on the use of electric charge to accomplish non-enzymatic harvest of stem cell sheets. We arranged charge-sensitive polyelectrolyte multilayer films (PEMs) by stacking of 9 alternating layer pairs of poly-L-lysine and hyaluronic acid. These ''soft'' stacks were either functionalized with fibronectin or additionally stiffed by crosslinking with [EDC/NHS]. We have established a new protocol for isolation of Placenta-derived mesenchymal stem cells (PD-MSC), that we tested here for creation of cell sheets. PD-MSC were cultured on experimental stacks and examined for morphology (phase microscopy), viability (life/death stain) and vitality (WST-1 assay) 48 hours post-seeding. The principal findings are: (1) Native ''soft'' PEM films do not support stem cell adhesion even after functionalization with fibronectin. (2) Only ''tuned'' PEM films stiffed by crosslinking support stem cell adhesion and outgrowth of dense stem cell sheets. (3) PD-MSCs expressed mesenchymal stem cell and adult cell markers. (4) On tissue plastic polystyrene, PD-MSCs could be successfully differentiated towards three mesodermal lineages. (5) PD-MSCs maintained as dense cell sheets during differentiation. Crosslinked charge-sensitive PEM films are functional substrates for sheets of human mesenchymal stem cells. PD-MSC can successfully adhere on such PEM films and form viable dense sheets. Ongoing steps of research concern whether stem cell sheets grown on PEMs can be successfully differentiated and harvested by applying of electricity.

(OP 263) Stem Cells and Biomaterials for the Treatment of Spinal Cord Injury

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Mesenchymal stem cells (MSCs) have the capacity to migrate towards lesions and induce regeneration. MSCs labeled with

iron-oxide nanoparticles were transplanted into rats with a spinal cord compression lesion (SCI). *In vivo* MRI, used to track their migration and fate, proved that MSCs migrated into the SCI. To bridge the cavities we implanted blocks of hydrogel or nanofiber scaffolds, either alone or seeded with MSCs. We compared the effects of implanted rat MSCs with the implantation of a freshly prepared mononuclear fraction of bone marrow cells (BMCs) or the injection of granulocyte colony-stimulating factor (G-CSF). Seven days after SCI, rats received either MSCs, BMCs or G-CSF intravenously. Animals grafted with MSCs, BMCs or treated with G-CSF had significantly smaller lesions and higher BBB scores than did control rats. Autologous BMC implantation was therefore used in a Phase I/II clinical trial in patients with acute and chronic SCI ($n = 34$). In other studies, hydrogels (degradable and nondegradable) or nanofiber scaffolds were implanted into rats with a SCI. In both acute and chronic SCI, the implants reduced scar formation and bridged the lesion, providing a scaffold to reform the tissue structure. Furthermore, hydrogel or nanofiber scaffolds seeded with stem cells can bridge a lesion site in acute as well as in chronic SCI and increase functional recovery. We conclude that scaffolds in combination with stem cells can improve regeneration by bridging gaps after SCI, by mechanically supporting ingrowing cells and axons and by the rescue and replacement of local neural cells.

(OP 264) Stem Cells Derived from Placenta: Where do We Stand?

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The immunological characteristics and early embryological origin of human placenta suggest that it could be a source of cells for allo-transplantation. Additionally, cell recovery from placenta is non-invasive and its use is ethically sound.

Human amniotic epithelial cells (hAEC) express embryonic stem cell markers, and differentiate *in vitro* toward lineages of all three germ layers. Meanwhile, human amniotic and chorionic mesenchymal stromal cells (hAMSC and hCMSC) display phenotypic and functional characteristics similar to mesenchymal stromal cells of other origins, particularly, differentiation toward mesodermal, but also ectodermal and endodermal lineages.

In vitro studies show that amnion- and chorion-derived cells are non-immunogenic and can induce immunomodulation. Interestingly, it has recently been shown that amnion-derived cells harbour T-cell suppressive or stimulatory subpopulations. Long-term engraftment was observed after xenotransplantation of human amniochorionic derived cells into different animal models.

Preclinical studies show that amnion-derived cells show promise for restoring various tissue functions. hAEC transplanted into immunodeficient mice gave rise to cells with hepatocyte morphology expressing human albumin or α-1 antitrypsin, with circulating human α-1 antitrypsin detected. hAEC xeno-transplanted into lesioned areas of spinal cord-injured animal models survived for up to 120 days, and improved performance in locomotor tests was observed. Meanwhile, hAMSC transplanted into rat myocardial infarcts survived for 2 months and differentiated into cardiomyocyte-like cells, reducing infarct size and improving infarct border capillary density and left ventricular function.

The *in vitro* differentiation potential, immunomodulatory characteristics and promising preclinical data suggest that placenta cells hold great promise for application in regenerative medicine.

(OP 265) Stereological Methods in the Assessment of Functionalised Scaffolds *In Vivo*

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Animal wound healing models provide an important platform to evaluate tissue engineering strategies that aim to reverse chronic healing. However, there is no standard method of analysis to assess the healing response in a functionalised scaffold; aspects such as trangene expression, cellular and tissue response need to be quantified. Stereology is an engineering tool used to make quantitative estimates of the 'amount' of a geometrical feature within an object (1), based upon observations made on two-dimensional (2D) sections. The aim of this study was to develop stereological methods to quantify the tissue response in a standardised rabbit ear ulcer model. Four 6 mm punch biopsy wounds were created on the rabbit ear and the following treatments administered randomly: scaffold containing therapeutic gene, scaffold alone, therapeutic gene alone and no treatment. At 7 days, tissue was harvested, cut across the midline of the wound, and prepared for histology. Six random fields of view were captured from each section at 400× magnification. A 192 point grid was used to quantify volume fraction of cells. A cycloidal grid of known radius was used to assess surface density, length and radial diffusion distance of blood vessels. Epithelial gap was measured at low magnification. Results showed that the methods of stereology used were successful in describing differences in total volume of cells involved in inflammation, proliferation and angiogenesis, providing unbiased information on the stage of healing.

Acknowledgements: Baxter Healthcare, Vienna, Health Research Board, Faculty of Engineering

(1) Unbiased Stereology 2nd Edition - Howard and Reed

(OP 266) Stimuli-Responsive Biomineralization onto Biodegradable Substrates

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Bioactive composites that enable the formation of an apatite layer onto the surface are important in field of tissue engineering and regenerative medicine, namely in the development of osteo-

conductive biomaterials for orthopaedic applications. The aim of this work was to control the biomineralization event by triggering external stimuli, namely, temperature and pH. Poly (L-lactic acid) (PLLA) reinforced with Bioglass®, was modified by grafting either poly(N-isopropylacrylamide) (PNIPAAm) or chitosan to the surface, by using plasma activation methodologies.

Regarding the PNIPAAM-grafted surfaces, it was found that temperature could trigger the formation of apatite upon immersion in SBF above the LCST of PNIPAAm, but no apatite could be formed at 25°C.

For the chitosan-grafted substrates the apatite formation upon immersion in SBF was analysed. It was found that the formation of apatite could be blocked when the pH was 5.4. On the other hand, a dense apatite layer was formed at pH 7.4.

These results suggest that the formation of apatite or possibly other kinds of minerals could be controlled by such "smart," in these case pH- and thermo-responsive, surfaces. For the smart surfaces analysed in this work, the apatite formation was always blocked when the grafted chains adopted an extended conformational state.

(OP 267) Stromal Cells from the Infrapatellar Fat Pad Allow One-Step Surgical Procedure for Regeneration of Cartilage Tissue *In Vitro*

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Current therapies for osteoarthritis lack regenerative capacity. Therefore stem cell therapies are being developed as a promising alternative. Adipose tissue is an attractive source of stem cells for these therapies since they provide a rich source of adipose tissue-derived mesenchymal stem cells (ASCs). If sufficient ASCs can be harvested, a one-step surgical procedure could be devised in which harvesting of adipose tissue and the treatment of the osteoarthritic defect with these ASCs are performed within a single surgical procedure. Therefore we investigated whether ASC quantity and quality in the stroma of the infrapatellar fat pad allows for application in a one-step surgical procedure for the regeneration of osteoarthritic cartilage.

Infrapatellar fat pads were harvested from 20 patients undergoing knee arthroplasty. The frequency of Colony-Forming Units in the stromal vascular fraction (SVF) of the infrapatellar fat pad was $3.4 \pm 0.8\%$. These units showed bilineage differentiation along the osteogenic and adipogenic pathway. When cultured (P3), a homogeneous cell population was obtained with a population doubling time of approximately two days and a surface marker expression profile matching that of ASC. When cultured in a poly (D,L-lactide-co-caprolactone) scaffold, both cultured ASCs and freshly isolated stromal cells showed chondrogenic differentiation potential *in vitro* using RT-PCR analysis and (immuno) histochemistry.

In conclusion, due to the high ASC-frequency in the stroma of the infrapatellar fat pad, the favorable proliferation rate and the chondrogenic differentiation potential, this stroma is a suitable candidate for a one-step surgical procedure for the regeneration of cartilage tissue.

(OP 268) Study of Growth and Differentiation of BMSC on Synthetic Polymeric Matrices.

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Bone tissue has the ability to repair minor injuries through remodeling. However, when the host source of osteoprogenitors is compromised at the defect site, one effective treatment may be cell-based therapy. These cells may be supported in a synthetic biodegradable scaffold to regenerate bone lesion. In this work we study poly-ester- and polyfumarate-scaffolds and their biocompatibility with bone marrow mesenchymal stem cells (BMSC). Surface properties and matrix porosity were evaluated by scanning electron microscopy (SEM), and hidrophilicity (water contact angle) of poly-e-caprolactone (PCL) and poly-diisopropyl-fumarate (PFIP) scaffolds obtained by solvent casting and lyophilization. PFIP showed a smooth surface while PCL presented some rugosities on its surface. Scaffolds obtained by lyophilization presented well distributed and homogeneous porosity compatible with bone microarchitechture. PCL presented higher hydrophylicity than PFIP. However, neither PCL nor PFIP were degraded after 21 days of incubation at 37°C in phosphate saline buffer. Biocompatibility was evaluated by culturing BMSC on plastic (control) or polymeric matrices and incubated at 37°C for different periods of time. Cell morphology, adhesion (1 h), cell proliferation (24 h) or osteoblastic differentiation (15 days) was evaluated. BMSC growing on the matrices developed a well defined stress fibers network without cytotoxicity signs. Cells attached and grew well on the scaffolds and expressed the osteoblastic marker alkaline phosphatase after 15 days in culture, at a similar rate that the cells growing in the control dishes.

In conclusion, our results suggest that these polymeric scaffolds are suitable for bone tissue engineering purpose.

(OP 269) Study of Protein Adsorption onto Biodegradable Marine Origin Polyelectrolyte Multilayer Films Followed *In-Situ* with QCM-D

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The development of new coatings and surface modification strategies for biomaterial is of prime importance for understanding and controlling cell-material interactions. In this context, protein adhesion plays a major role in determining the biocompatibility of materials and scaffolds. Here, it is described the use of a Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) to follow *in-situ* the adsorption of Bovine Serum Albumin (BSA) onto a polyelectrolyte multilayer film composed by chitosan (CHI) and alginate (ALG), previously prepared by electrostatically driven

Layer-by-Layer (LbL) assembly. The interaction between BSA and CHI/ALG multilayers was assessed, manipulating multiple variables like terminating charge layer, pH, number of polyelectrolyte layers and chemical crosslinking, to study the rate and amount of protein adsorbed. Furthermore, the use of a QCM-D allows us to understand the viscoelastic properties and the hydration state for the multilayer build-up. The results evidence the influence of the outermost layer and the pH conditions in the attachment of the protein to the CHI/ALG system. This study highlights the ability to incorporate biomolecules into complex multilayer films as being potentially valuable for biomedical applications, including tissue engineering and regenerative medicine.

(OP 270) Substrate Surface Chemistry Effects on Human Mesenchymal Stem Cell Differentiation in 3-D Culture

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Different functional surface chemistries control not only initial protein adsorption and production, but also the differentiation potential of human mesenchymal stem cells (hMSCs) in 2-D culture [1,2]. In this study, the different functional chemical groups of methyl (-CH_3), amino (-NH_2), thiol (-SH), hydroxyl (-OH) and carboxyl (-COOH) have been grafted on to the surface of 3-D porous ceramic materials. hMSCs were cultured *in vitro* in contact with unmodified and modified 3-D scaffolds with -CH_3, -NH_2, -SH, -OH and -COOH groups in basal, chondrogenic and osteogenic media for time periods up to 28 days *in vitro*. The 3-D scaffolds were characterized by scanning electron microscopy (SEM), mercury intrusion porosimetry (MIP), attenuated total reflection FTIR (ATR-FTIR) and X-ray photoelectron spectroscopy (XPS). Differentiation of viable cells at the mRNA level was determined by targeting beta-actin, ornithine decarboxylase, collagen I, collagen II, sox-9, osteopontin, osteocalcin, osteonectin and CBFA using real time PCR. Fluorescent immunohistochemistry was used to evaluate the production of collagen I and II, osteocalcin and CBFA1. The results indicated that the functional chemical groups had been successfully grafted on to 3-D scaffolds with no distinct changes in morphology. Both the 3-D structure and chemical properties of these materials had effects on controlling hMSC differentiation with/without additional biological stimulation.

References:

[1]Curran J. M., Chen R. and Hunt J. A., Biomaterials, 2006, 27,4783–V4793.

[2]Curran J. M., Chen R. and Hunt J. A., Biomaterials, 2006, 26,7057–V7067.

(OP 271) Supercritical Fluids: an Emerging Technology for the Preparation of Scaffolds for Tissue Engineering

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The field of tissue engineering has evolved greatly since the idea of combining active compounds and scaffolds to create artificial tissues. Small molecular weight drugs that control proliferation differentiation of cells can be incorporated into biodegradable scaffolds to induce cellular differentiation and tissue remodeling. The scaffold plays, therefore an important role not only as a physical support but also in the cell proliferation and differentiation.

One of the most important stages of tissue engineering is the design and processing of a porous 3D structure, with high porosity, high interconnectivity between the porous and uniform distribution. A variety of processing techniques have been developed and include solvent casting and particles leaching, compression moulding and particle leaching, thermally induced phase separation, gas-foaming processes, among others. The main disadvantages of these methods are the use of organic solvents and the high temperatures required. The presence of residual organic solvents is being rigorously controlled by international safety regulations, it is necessary to warrant the complete removal and absence of these substances, without exposing bioactive compounds to high temperatures, which may degrade them. Supercritical fluid technology appears, therefore as an interesting alternative to the traditional processing methods.

The unique properties of supercritical fluids have been explored and have led to the development of a number of polymer processing techniques. Regarding tissue engineering and scaffold preparation the most interesting techniques available are impregnation, foaming, phase inversion and foam injection moulding. In this contribution theses techniques will be presented and discussed along with some examples.

(OP 272) Supra-Molecular Nanodevices for Gene and Drug Delivery

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Polymeric micelle, the self-assembly of block copolymers with core-shell architecture, is a promising nanodevice for drug and gene delivery. There are several relevant properties in polymeric micelle as nanodevice systems, such as longevity in blood circulation, tissue-penetrating ability, spatial and temporal controlled drug release, and reduced inherent toxicity. Also, engineering of the block copolymer structure allows the preparation of polymeric micelles with integrated smart functions, such as targetability as well as stimuli-sensitivity. This presentation overviews the recent achievements of polymeric micelles as smart nanodevices for drug and nucleic acid delivery to solid tumors. Then, the focus will be placed to the application of gene-loaded polymeric micelles as non-viral vectors in the field of regenerative medicine. Further, the future perspective will be addressed in the last part of this presentation, directing to the new medical paradigm of smart nanotheranostic systems controlled by external physical stimuli.

(OP 273) Supramolecular Peptide Nanofibers for Tissue Engineering Applications

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Conventional polymers are extensively studied as biomaterials for tissue engineering. Recently, we have shown an innovation in biomaterials design by means of directed non-covalent interactions based on four-fold hydrogen-bonding 2-ureido-4[1H]-pyrimidinone (UPy) dimers. Both passive and active scaffolds could be produced by introducing a modular approach using these dynamic UPy-UPy interactions. This approach has shown great benefits in processability, mechanical tunability and most importantly biofunctionalization. The creation of a toolbox with different UPy-modified polymers, bioactive molecules and imaging probes allowed for the off-the-shelf assembly of bioactive biomaterials by easy mixing without the need for additional elaborated synthesis steps. Investigation of this UPy-polymer system in great detail revealed that besides UPy-UPy dimerization also additional hydrogen-bonding takes places when prepolymers are end-functionalized with UPy-moieties via a urethane or urea group; resulting in formation of nanofibers. UPy-modified peptides were easily extracted from UPy-polymers lacking the lateral interactions. However, when prepolymers were UPy-functionalized via a urethane or urea group, UPy-peptides could be incorporated into the nanofiber stacks. The nanofiber containing UPy-polymers were mixed with several UPy-modified extracellular matrix (ECM)-peptides, derived from laminin, fibronectin and different collagens, to mimic the natural ECM. *In-vitro* studies showed that renal epithelial cells behave differently on the various supramolecular peptide nanofiber films, with respect to their phenotype and ECM production. The association constants of the bioactives with the material can be regulated by using these different supramolecular interactions which allows for the design of biomedical materials that can be tuned on the axis between non-covalent and covalent modification.

(OP 274) Surface Interactions Between Materials and Biology

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The role of surfaces of matrices for regenerative medicine is to induce specific cellular responses that will guide tissue regeneration. For this reason, strategies for tissue engineering materials are also directed to surface modification methods able to overcome surface limitations or to improve the surface crosstalk between implant and biological environment. Surface designed scaffolds could specifically control the biological events occurring at the material-tissue interface. Adsorption and desorption of proteins from biological fluids will form on the scaffold dynamic surfaces with proteins at different conformations, eventually exposing specific sites able to dialogue with cells membrane receptors. Cell response to scaffolds surfaces will be determined by a signalling cascade as a consequence of ligand-receptor interactions.

In the past, surface modification strategies have been mainly aimed at decreasing almost randomly protein adsorption; nowadays, strategies are aimed at minimizing non specific interactions while controlling the protein adsorption process, to promote specific cellular activation and tissue regeneration. Surface modification can be morphological or/and chemical, designed at nano- or micro-level. Model surfaces can be used for *in vitro* evaluations, for a biological tuning of surface properties and adsorbed protein types and distribution, as well as adsorption kinetics. For instance, proteins can be visualized by using functionalized immunogold nano-particles or quantum dots marked with fluorescence, and by using Atomic Force Microscopy with specifically functionalized tips. Adsorbed protein characterization, i.e. type, quantity but also distribution on the surface, can help to understand the following cell response events. Examples will be illustrated.

(OP 275) Survival and Remodelling of Endothelialized Collagen Modules in Animal Models

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Modular tissue engineering is intended to result in vascularised constructs. Functional cells are embedded in sub-millimetre sized collagen rods (modules), and the outside surface is covered with endothelial cells (EC). Upon implantation, the transplanted EC are expected to connect with the host vasculature, enabling blood flow through the assemblage of modules. However the inflammatory response encountered upon implantation drives a remodelling process that limits the survival of the transplanted EC. Here, we evaluate the viability of transplanted EC in different animal models with a view to understanding the optimum implantation strategy for maximizing EC viability. Collagen modules (without embedded cells) were seeded with human umbilical vein endothelial cells (HUVEC) or rat aortic endothelial cells (RAEC). HUVEC survived for 7 days and formed primitive vascular structures in an omental pouch in nude rats treated with clodronate liposomes to temporarily deplete peritoneal macrophages. Alternatively, in SCID mice, some HUVEC on modules injected minimally invasively in subcutaneous sites were found to form vessel-like structures for up to 21 days. Allogeneic RAEC covered modules did not survive when implanted in an omental pouch of rats without immunesuppressive treatment. However treating animals with tacrolimus (Prograf) and atorvastatin (Lipitor, to support EC survival) extended survival to 7 days (longest time point checked) and provided some preliminary evidence for new vessel formation. Further analysis is parsing the relative roles of apoptosis and innate immune responses in EC survival.

(OP 276) Synthesis and Characterization of Matrix Metalloprotease-Sensitive Elastin-Like Based Bioactive Hydrogels

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One of the main challenges in tissue engineering is the obtaining of advanced materials acting as artificial extracellular matrices (aECM). Alternative candidates to actual employed synthetic

materials are represented by biopolymers with a rich complexity of structure and function as advanced scaffolds for tissue engineering. Among the different families of protein-based polymers (PBPs) one has proven an enormous potential, the Elastin-like polymers (ELPs), which are based on repeating sequences, "building blocks," found in the natural elastin. These polymers show an unmatched biocompatibility joint with a remarkable combination of excellent properties that are difficulty found together in other polymer family: a strong tendency to self-assemble into elastic fibrillar structures; the formation different topological structures in the nanometer scale; an acute smart behavior depending to polymer architecture and environment conditions. Additionally, specific functionalities as cell adhesion sequences and bioactive functions can be included in their structure adding especially peptide by the use of genetic engineering tools in developing recombinant protein-based polymers. This communication shows the achievement of recombinant elastin-like protein polymers that have been designed and bioproduced in E. coli. They can be easily cross-linked to obtain elastomeric hydrogels. All of them contain, cell adhesion domains for extracellular ligand binding. Moreover specific protease target domains are employed to improve the biopolymers bio-processability. The insertion of proteases recognition site of human elastases allows us to achieve definite degradation of the biopolymer in the same natural way of elastin during the ECM rearrangement. The mechanical, physical-chemical and *in vitro* analysis confirm their excellent behaviour.

(OP 277) Synthesis of Extracellular Matrix Components By Tissue-Engineered Human Oral Mucosa Subtitutes

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Introduction: The extracellular matrix is one of the main components of all kinds of connective tissues. However, *in vitro* reproduction of all proteins that are normally present in the native extracellular matrix is very difficult. In this work, we have carried out a gene expression analysis of the major components of the extracellular matrix in both human native controls and bioengineered oral mucosa samples.

Materials and Methods: Human oral mucosa substitutes were generated by using fibrin-agarose scaffolds with fibroblasts entrapped within and keratinocytes seeded on top. To analyze the gene expression profile of controls and artificial oral mucosa samples, total RNA was analyzed by using Affymetrix U133-plus 2.0 microarray chips. Genes with a role in extracellular matrix were selected, and genes with at least 2-fold increased expression in the group of controls or in the group of bioengineered samples were considered overexpressed.

Results and Discussion: Oral mucosa substitutes showed overexpression of genes encoding for several metallopeptidases, biglycan, several collagens, decorin, elastin, fibronectin, laminins beta1 and gamma1, for example. In contrast, native oral mucosa expressed high amounts of RNA of ADAMTS18, ADAMTS20, ADAMTS6, ADAMTSL1, collagen 14, fibrillin 3, laminin beta4 and matrilin. Expression of more than 100 genes was similar for native and artificial tissues. These results suggest that our artificial stroma is able to induce the cells to initiate an active process of remodeling by synthesizing several extracellular products that normally exist in native oral mucosa controls.

Supported by CTS-06-2191 and CM2005/011 from Junta de Andalucia.

(OP 278) Synthetic Fibrin-Like Matrix for Bone Tissue Engineering

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Materials from natural sources were successfully used as cell delivery or tissue regeneration devices in medical applications due to their innate biological properties. Synthetic extracellular matrices (ECM) represent interesting alternatives for biopolymers as they can be specifically designed with respect to their mechanical properties, susceptibility to proteolytic activity and presentation of cell adhesion ligands or morphogens. Here we present modularly designed poly(ethylene glycol) (PEG)-based hydrogels that are formed by Factor XIII (FXIII) catalysis. The new class of biomaterials enables the formation of matrices containing multiple tethered bioactive molecules and cell-responsive enzymatic substrates in a simple one step reaction. In the context of bone tissue engineering we have designed the system to contain building blocks that include enzymatically coupled cell adhesion ligands, the osteogenic growth factor BMP-2 and slow released VEGF. In this growth factor combination vascular and bone formation should be improved. In contrast to materials from natural sources these novel artificial ECMs allow the nearly independent control of properties including matrix stiffness, protease susceptibility and presentation of biological cues. The tailoring of these properties in a wide range enables us to rationally control cell behavior in both *in vitro* and *in vivo* contexts. These matrices could be useful tools for experimental cell biology as well as for *in vivo* applications such as bone tissue regeneration.

We thank for support by SNF Switzerland, Inion OY, Tampere, Finland, and microcomputed tomography by Prof. R. Müller, Institute for Biomedical Engineering, ETH Zürich

(OP 279) Tendon Regeneration with Fat Derived Stem Cells

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This study investigates the treatment of tendon defects with the help of autologous fat derived stem cells. In an *in vivo* rabbit model the central third patella tendon defect was created. A single step procedure, harvesting, preparation and implantation of stem

cells, was chosen. Fatty tissue was separated from the surrounding tissue and collagenase digestion was performed to isolate stem cells. There were three treatment groups. The defect was filled with a blood clot in group A, fibrin in group B and fibrin and adipose derived stem cells in group C ($n = 6$ per group, per time point). A three to seven millimeter defect in the central third of the rabbit patella tendon was filled with one of the three solutions. After three, five and eight weeks the experiment was terminated. Gene expression profiling was performed with reversed transcriptase PCR for collagen type I, aP2, Tenascin X and GAPDH. Probes were taken in the surrounding tissue as well as in the defect.

Our results showed significant increase in gene expression of collagen type I, aP2 and Tenascin X after five weeks in group C in comparison to group A and B.

We conclude that implanted fat stem cells lead to high cell activity in the tendon defect. Fat related marker PCR shows also a significant increase. In further studies stem cells should get some tendon specific predifferentiation. Grants: Expertissues, Lorenz Boehler Funds.

(OP 280) The Biocompatibility and Immunogenicity of Self-Assembling Peptides for Use in Tissue Engineering and Regenerative Applications

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Introduction: Previous work has established criteria for the formation of hydro gels from chemically synthesised self-assembling beta-sheet peptides under physiological conditions. This enabled the design of peptides that spontaneously self-assemble into scaffolds of potential utility in tissue engineering and regenerative applications.

The aim of this study was to determine biocompatibility and immunogenicity of two rationally designed self-assembling peptides.

Methods: Biocompatibility was determined using primary human fibroblasts and L929 cells *in vitro*. Immunogenicity of P11-4 or P11-8 was determined in a) naive BALBC mice b) mice immunised with peptide c) mice immunised with keyhole limpet hemocyanin (KLH) -conjugated peptide. Antibodies levels against the peptides were determined by ELISA. Lymphocyte responsiveness was determined in lymphocyte proliferation assays. Serum antibody titre to the peptides and peripheral blood lymphocyte proliferation in response to the peptides was determined in six human volunteers.

Results and Conclusions: Following culture with human and murine cells P11-4 or P11-8 demonstrated no cytotoxicity. Naive mice possessed no antibodies against P11-4 or P11-8 and no lymphocyte response was demonstrated. Mice immunised with peptide alone did not demonstrate a lymphocyte response and antibodies against P11-4 or P11-8 could not be detected in serum. Mice immunised with KLH conjugated peptide did not demonstrate a lymphocyte response but an antibody titre of 1/64 was measured against P11-4 and P11-8. Human volunteers demonstrated no prior sensitisation to the peptides.

(OP 281) The Development of Biomimicking Silk Scaffold for Ligament Tissue Engineering

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Although most *in vitro* studies indicate that silk is a suitable biomaterial for ligament tissue engineering, *in vivo* studies of implanted silk scaffolds for ligament reconstruction are still lacking. The objective of this study is to investigate anterior cruciate ligament (ACL) regeneration using mesenchymal stem cells (MSCs) and silk scaffold. The scaffold was fabricated by incorporating microporous silk sponges into knitted silk mesh, which mimicked the structures of ligament extracellular matrix (ECM). *In vitro* culture demonstrated that MSCs on scaffolds proliferated vigorously and produced abundant collagen. The transcription levels of ligament-specific genes also increased with time. Then MSCs/scaffolds were implanted to regenerate ACL *in vivo*. After 24 weeks, histology observation showed that MSCs were distributed throughout the regenerated ligament and exhibited fibroblast morphology. The key ligament ECM components including collagen I, collagen III, and tenascin-C were produced prominently. Furthermore, direct ligament-bone insertion with typical four zones (bone, mineralized fibrocartilage, fibrocartilage, ligament) was reconstructed, which resembled the native structures of ACL-bone insertion. The tensile strength of regenerated ligament also met the mechanical requirements. Moreover, its histological grading score was significantly higher than that of control. In conclusion, the results imply that silk scaffold has great potentials in future clinical applications.

(OP 282) The Development of Urinary Bladder Matrix-Fibrin Hybrid Scaffolds and the Effects of Cyclical Hydrostatic Pressure.

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Designing an artificial scaffold that can successfully control three dimensional cellular remodelling and tissue growth is still a predominant challenge in tissue engineering. We have previously shown that hybrid scaffolds prepared from porcine urinary bladder matrix (UBM) and fibrin has potential as vascular scaffolds. However, it is unclear if our novel scaffold will withstand the mechanical forces in the circulatory system. Here we examined the effects of cyclical hydrostatic pressure on endothelial cells co-cultured on hybrid UBM-fibrin scaffolds and attachment of fibrin on UBM scaffolds.

Human Umbilical Vein Endothelial Cells (HUVECs) were grown on hybrid UBM and fibrin scaffolds. Seeded hybrid co-constructs were exposed to either atmospheric or pulsatile pressure (50/110 mmHg) inside bioreactor for 24 hours. Various aspects of

cellular growth were analyzed using immuno-florescence and MTT based cell count kit 8. Attachment tests were carried out using a tensile testing machine.

Experiments show that cell survival in this system is comparable with that seen in the controls. After application of cyclical physiological pressure, cell morphology appeared to change, with cells appearing more organized. There was no significant difference in the growth of cells in the two groups of experiments. The attachment test showed that the failure load of 0.08 N was required to dislodge the fibrin material. This is below the levels of wall shear stress present in an arterial wall.

The successful development of UBM- fibrin hybrid scaffold cultured with endothelial cells under physiological pressures establishes a stable platform for *in-vivo* investigations of this novel hybrid scaffold.

(OP 283) The Effect of Donor Antigen-Pulsed Dendritic Cells on Survival of Skin Allograft in a Rat Model

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Prevention of acute rejection in skin allografts without continuous immunosuppression lacks reports in worldwide literature. Need for chronic immunosuppression precludes the use of tissue allograft as a routine surgical reconstructive option. Recently dendritic cells(DC) gained considerble attention as antigen presenting cells that are also capable of immunologic tolerance induction. This study assesses the effect of alloantigen-pulsed dendritic cells in induction of survival increase in a rat skin allograft model.

Recipient derived dendritic cells were harvested from rat whole blood and cultured with GM-CSF and IL-4 for 2 weeks. Then donor-specific alloantigen pulsed dendritic cells were reinjected into tail vein before skin graft. Rat dorsal skin allografts were transplanted in 5 subgroups. Groups: I) untreated, II) anti-lymphocyte serum (ALS, 0.5 ml), III) FK-506(2 mg/Kg), IV) DCp, VI) DCp and FK-506. Graft appearance challenges were assessed postoperatively. The group V(DC and FK-506 treated) showed longest graft survival rate(23.5 days) than other groups; untreated(5.8 days), ALS(7.2 days), FK-506(17.5 days), DCp(12.2 days). Donor antigen pulsed host dendritic cell combined with short-term immunosuppression prolong skin allograft survival and has potential therapeutic application for induction of donor antigen specific tolerance.

(OP 284) The Effect of Surface Mechanics on Cell Function and Dynamics

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Surface mechanics can play a pivotal role in cell function, proliferation, and migration. In order to understand this role, we performed a comprehensive study on the effects of substrate mechanics on dermal fibroblasts cultured on functionalized HA hydrogels, where the mechanics are controlled by cross linking density. We studied both flat surfaces, as well as electropsun hydrogel fibers. Using Scanning Force Modulation Microscopy we measured the relative modulii of live cells on these substrates and found that they scale with surface rigidity. Confocal microscopy indicated that the cells on the harder substrates were far better extended with large actin fibrils and surface area, while those on softer substrates were rounded with thinner actin fibrils and smaller surface area. Cell migration studies showed that while individual cell velocities were larger, mass migration was severely hindered on softer surfaces. These results can be correlated with finite element analysis coupled with digital image scanning correlation microscopy which was able to measure the traction forces exerted by the cells on the different substrates. The results showed that while cells on the softer substrates exerted the smallest traction forces, the force gradients on the surfaces with intermediate mechanical response produced the optimal conditions for single cell migration. Based on these findings, we were able to model the migration velocity as the sum of two opposing terms; one is a function of the substrate mechanics, while the second is an inverse function of the distance between adjacent cells. Implications to oriented fibers will be presented.

(OP 285) The Effects of Human Adipose-Derived Stem Cells on T-Cell Mediated Immune Response

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Mesenchymal stem cells derived from adult bone marrow or adipose tissue are known to be less immunogenic and immunosuppressive. But the detail of immunologic effect of adipose-derived stem cells (ADSCs) is not revealed yet. This study is aimed to investigate the effect of ADSCs on lymphocytes proliferation and the production of cytokines *in vitro*. The ADSCs extracted from omental or subcutaneous fat were expanded and the phenotype of the ADSCs was identified by cell surface markers using flow cytometry: positive for CD29 and CD44, but negative for CD34, CD45, CD117 and HLA-DR. The ADSCs were able to differentiate into osteoblast or adipocytes with induction media. Third-party ADSCs added to mixed lymphocyte reactions(MLR) induced overall suppressive effect on lymphocytes proliferation, but in part stimulated, whereas the suppressive effect of ADSCs on the non-specific lymphocyte proliferation provoked by PHA-A was totally profound. The Stimulated Index was 11.69% to 156.5% in MLR and 0.99% to 14.44% in PHA-A assay. The changes of cytokines production were analyzed from the supernatant collected during the lymphocyte proliferation reactions using ELISA. The production of TNF-α, IL2, IFN-γ was increased during the MLR and PHA-A stimulation. Adding ADSCs, only the level of TNF-α was decreased in MLR, whereas all of three cytokines was decreased in PHA-A stimulation. The level of Th1 cytokines such as IL-2 and IFN-γ in MLR was significantly increased during co-culture with ADSCs regardless of suppressive or stimulated response.The effects of ADSCs on immunologic or nonspecific lymphocyte stimulation different in extent and cytokine regulation.

(OP 286) The Impact of Tissue Engineering on Laboratory Animals–an Ethical Assessment

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Animal experiments play an essential role in the development of the field of tissue engineering. Laboratory animals are used to study the fundamental processes involved in tissue engineering and they function as models of human disease and injury for testing new products. Furthermore, some of these products contain xenogeneic cells or materials. Significant from an animal-ethical point of view, however, is that these uses further the instrumentalisation of animals and can cause them considerable discomfort. Moreover, doubts can be and are raised about the adequacy of the current animal models and therefore about the usefulness of the experiments.

On the other hand, human cells and human engineered tissue like artificial skin are already applied as alternative methods to animal use. If the promises of tissue engineering were to come to fruition, the field might yield ever more refined products, maybe even whole organs, that potentially could replace laboratory animals and thereby would drastically decrease the need for them.

In my paper, I will try to assess both types of impacts of tissue engineering on laboratory animals and discuss whether the latter might be used as an argument to justify the former.

(OP 287) The Influence of RGD-Containing Peptides on Bone Marrow Stromal Cell Phenotype

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It has been previously suggested that the cell culture substrate can influence the maintenance of BMSC characteristics. We hypothesized that human BMSC (hBMSC) attachment, cell surface marker and integrin expression could be influenced by culture on a RGD-bound cell culture substrate. Two RGD peptides (cyclic and linear), as well as vitronectin (VN) and denatured collagen 1 (dC1) were tested. For covalent bonding, two types of tissue culture plastic were activated, and the peptides then attached at varying concentrations. To assess cell attachment, hBMSCs were cultured on the surfaces for 4 hours and then their adhesion plaques stained using an anti-human vinculin antibody. A peptide dose was then selected based upon vinculin plaque-forming ability and surfaces were treated at this concentration. After 15 days of hBMSC culture, cells were then stained for integrin subunit β1, integrins αVβ3 and αVβ5, as well as the hBMSC surface markers CD73, CD105, and CD164. Marker expression was analysed using flow cytometry. CB surfaces covalently bound to either of the two peptides significantly improved vinculin plaque formation ($p \leq 0.05$). Covalently attached peptides performed better than when adsorbed ($p = 0.046$ for the linear peptide, and $p = 0.035$ for the cyclic peptide). There was no significant difference between attachment methods in the VN and dC1 groups. At 1000 and 1×10^4 ng of peptide the highest levels of vinculin plaques were observed. This dose of linear peptide increased the expression of β1, integrins αVβ3 and αVβ5. Both the peptide and dC1 reduced the the expression of CD73, CD105, and CD164.

(OP 288) The Interaction Between Demineralized Bone Matrix Scaffolds with Human Umbilical Cord Mesenchymal Stem Cells for Bone Tissue Engineering

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Mesenchymal stem cells are multipotential cells capable of differentiating into osteoblasts, chondrocytes, adipocytes, tenocytes, and myoblasts. Human umbilical cord contains stem cells that are a rich source of primitive multipotent mesenchymal cells. Demineralized bone matrix (DBM) has been widely used in orthopedic, periodontal, and maxillofacial applications and extensively investigated as a material to induce new bone formation. The purposes of this study were to characterize human umbilical cord mesenchymal stem cells (UCMS) and to examine the interaction between DBM scaffolds with UCMS. The altered gene expression during osteogenesis of UCMS was also investigated. UCMS were studied using *in vitro* functional mesenchymal stem cell assay and were determined their cell surface antigen expression. Osteoblast differentiation of UCMS was determined using alkaline phosphatase assay, osteocalcin, and Von Kossa staining assay. Total RNA was isolated from UCMS in the absence or presence of DBM scaffolds and analyzed using osteogenesis cDNA gene expression array. The selected genes were verified using reversed transcriptase-polymerase chain reaction (RT-PCR). Analysis by flow cytometry demonstrated that UCMS express cell surface antigens used to define MSCs isolated from umbilical cord such as CD29, CD44, CD90 and CD105. DBM-treated UCMS were differentiated into osteoblasts that stained positive for alkaline phosphatase, mineralization (calcification) and expressed osteocalcin. When analyzed by cDNA array and RT-PCR analyses, we found that the highly upregulated genes were runx2, vdr, tgfb2, flt1 and smad2 whereas the highly downregulated genes were smad7. These results indicated that UCMS with DBM scaffolds could provide an alternative approach for bone tissue engineering.

(OP 289) The Millefeuille Principle: a New Approach to Tissue-engineered Skin Reconstruction.

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Introduction: We previously presented our Millefeuille skin construct, as a possible future tool for replacing lost or severely damaged skin. We believe that focusing on constructs that come with a potential for vascularisation, and are made up entirely of the host's own expanded cells, will diminish infection and rejection rates in skin substitutes.

We tested the Millefeuilles more extensively in our porcine model, and gathered some more insight in the construct's ultrastructure.

Materials & Methods: Porcine fibroblasts and keratinocytes were isolated from small skin fragments, expanded *in vitro* and grown into sheets. In the meantime, blood outgrowth endothelial cells (BOECs), a subset of endothelial progenitor cells (EPCs) were harvested from peripheral blood samples, isolated and cultured.

In the next step, the Millefeuilles were prepared: multi-layered fibroblast and keratinocyte sheets were superimposed, with BOECs sandwiched between them.

The Millefeuille skin constructs were applied to full thickness skin wounds in a pig model, and compared to various conditions, with and without fibroblast sheets and/or EPCs.

Results: Reepithelialization rates in our Millefeuille-treated wounds reached 100%, versus 41% in control wounds, in addition to a lower contraction rate (19% vs 68%). Our Millefeuilles furthermore increased the total lectin content in the wounds 3-fold, compared to salines. Fibronectin content (and therefore a more pronounced proangiogenic extracellular matrix) was even 4 times higher than controls. Wounds treated with the laminated sheets showed MT1-MMP levels up to 2.6 times higher than saline. The EPC treated wounds displayed more regularly arranged vessel-like structures.

(OP 290) The Role of Integrin Alpha5beta1 as Mechanotransducer in Chondrocytes Embedded in Agarose

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Recent studies have shown that integrins act as mechanoreceptors in articular cartilage. In this study, we examined the role of integrin $\alpha5\beta1$ in the mechanoregulation of both ECM gene expression in the short term and ECM protein synthesis in the long term.

Chondrocytes were isolated from full-depth porcine cartilage and seeded in 3% agarose constructs. These constructs were loaded in semi-confined compression with 15% strain at 0.33 Hz for 12 hours, in the presence or absence of GRGDSP, which blocks integrin $\alpha5\beta1$ function, or GRADSP oligopeptide, as control. The levels of mRNA for aggrecan, collagen-2 and MMP-3 were determined by semi-quantitative PCR at several time points up to 24 hours post-stimulation. Cell viability, dsDNA and sGAG content were determined at several time points up to 28 days post-stimulation.

The applied loading induced different temporal responses on gene expression and ECM protein biosynthesis. The mRNA levels for aggrecan and MMP-3 were increased immediately and 1 hour after loading and GRGDSP counteracted these effects. In contrary, the loaded groups demonstrated a significantly lower amount of GAG over the entire culture duration, compared to the unstrained control. Blocking the $\alpha5\beta1$ integrin had no effect on the long-term biosynthetic response.

The combined results confirmed the role of integrin $\alpha5\beta1$ as mechanotransducer in the regulation of GAG mRNA expression for chondrocytes seeded in agarose under the applied loading regime. However, this did not translate into more GAG rich ECM matrix in the mechanically stimulated tissue engineered constructs.

(OP 291) The Role of the Primary Cilia in Mediating Mechanically Induced Increases in Matrix Production During Bone Tissue Engineering.

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Dynamic mechanical loading is used to enhance matrix formation in bioreactor culture of tissue engineered bone. When a cell seeded porous scaffold is compressed in a bioreactor, substrate strain and fluid flows are generated. Previously we have shown that dynamic cyclic compression applied for 2 hours every 5 days induces matrix formation by MLO-A5 osteoblastic cells. Primary cilia are sensory organelles which translate extracellular mechanical cues into a biochemical response, recently shown to be active in bone cells (Malone, PNAS, 2007). We investigated the effects of fluid shear stress on bone matrix production by osteoblasts with intact and defective primary cilia. MLO-A5s were seeded on fibronectin coated slides with 5×10^4 cells per slide and subjected to dynamic fluid flow at 2 Pa for 2 hours on day 3 of culture. Cilia function was inhibited by knockdown of polaris, which is required for cilia biogenesis and maintenance. Collagen content, assessed by Sirius red on day 8, increased in flow groups compared with nonflow groups, but the difference was eliminated in the absence of primary cilia. Gene expression of osteopontin and type 1 collagen was upregulated 2 fold by cells exposed to flow but there was no effect of flow in the knockdown group. In conclusion, fluid flow induced shear stress mediates bone matrix production by osteoblasts and the primary cilium is involved in mechanotransduction of the flow signal. Therefore, primary cilia may play an important role in mechanically induced matrix formation during bioreactor culture of tissue engineered bone.

(OP 292) The Synthetic Growth Surface Topography of Ultra-Web™ Enhances Cell Culture Performance

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The extracellular matrix (ECM) is involved in modulating cellular growth, development, and function through a complex array of biochemical and physical cues.

The novel cell culture surfaces from Corning, Ultra-Web™ Synthetic Surfaces are composed of randomly orientated electrospun polyamide nanofibers, and functionally mimic the architecture of the ECM. In size Ultra-Web nanofibres (~280 nm) are similar to collagen and other ECM fibers, this functional characteristic provides nanotopography for cell attachment and growth, which promotes *in vivo* -like cell responses. Ultra-Web Synthetic

Surface is available with two surface chemistries:

1. Untreated electrospun polyamide nanofibers with an uncharged slightly hydrophilic surface,
2. Polyamine treated electrospun polyamide nanofibers with a positively charged surface for enhanced cell attachment, and covalently linking biomolecules.

In this study, we examined neurite outgrowth of neurons, dome formation of kidney epithelial cells, spheroid formation of hepatocytes, and, maintenance and differentiation of primary human mesenchymal stem cells (hMSCs) on Ultra-Web surfaces. Specifically, we compared the performance of synthetic nanofibrillar surfaces to several other types of synthetic and biological surfaces. Each system demonstrated significant morphological or functional improvements over traditional two-dimensional plastic and coated alternatives. Additionally, the nanofibrillar surface was compatible with several assay formats, including luminescence, fluorescence-based calcium flux and cellular imaging. Together, our data suggest that this new synthetic surface may provide a unique means to achieve more physiologically relevant cell culture results.

(OP 293) Therapeutic and Research Potential of Human Stem Cells

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Stem cells offer great therapeutic potential for human disease conditions where the loss of specific cell types is the major pathophysiological feature of individual disorders. Embryonic stem (ES) cells derived from 6–8 day old human embryos offer the most therapeutic potential as these cells are capable of generating every cell and tissue type in the human body. If we can control the differentiation of ES cells, then cell replacement for profound human disorders such as Parkinson's disease, insulin-dependent diabetes, heart disease, stroke, multiple sclerosis, rheumatoid arthritis, spinal cord damage, and macular degeneration could become standard new therapies. This presentation will examine the state of the art in the development of stem cell therapies and outline some of the technical and ethical considerations as we progress toward clinical and research application of human ES cells.

(OP 294) Time-Course Study of Cell-Cell Junctions Development in Bioengineered Human Corneas

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Introduction: One of the main challenges of corneal tissue engineering is the development of an efficient substitute of the human cornea. In this work, we have carried out a sequential study of development and differentiation of intercellular junctions in partial substitutes of the human cornea generated by tissue engineering.

Materials and Methods: Cultured human cornea epithelial cells were cultured on top of artificial corneal stromas created by culturing human keratocytes in fibrin-agarose hydrogels. Immunofluorescence and scanning electron microscopy analyses of the artificial corneal epithelium were carried out *ex vivo* at different stages of development.

Results and Discussion: Sequential analysis of desmosome formation revealed that immature corneas with one or two epithelial cell layers did not show any cell-cell junctions. In contrast, mature artificial corneas displayed a multilayered epithelium with a high number of desmosomal junctions and a high expression of the desmosomal proteins plakoglobin 3, desmoglein 3 and desmoplakin. Nevertheless, samples that had been incubated fully submerged in culture medium preferentially expressed desmosomal proteins in the most apical cell layers, whereas corneas cultured using air-liquid culture techniques showed higher desmosomal protein expression in intermediate cell layers, similar to normal human control corneas. These results suggest that desmosomal formation and maturation was very similar for normal human control corneas and partial corneal substitutes submitted to air-liquid culture techniques. Consequently, corneal substitutes developed in this work could possibly be used as efficient partial human cornea substitutes.

Supported by CTS-06-2191 from Junta de Andalucia.

(OP 295) Tissue Engineered Lung Using Somatic Lung Progenitor Cells: a Potential Regenerative Lung Therapy

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The theory behind regenerative therapy is to obtain the ideal cell or mixture of cells that will integrate itself within the existing lung structure. One method of identifying the ideal cell is to create an *ex vivo* system capable of recreating the native organ on a specialized matrix. Within the lung a specific cell type that is capable of regenerating the lung has not been identified. Our research shows that murine or human engineered bronchiole-alveolar tissue developed from a mixture of somatic lung progenitor cells (SLPCs) is self-sustaining in culture within a rotary bioreactor for 8 weeks. Engineered tissues were examined using quantitative flow cytometry to determine cell phenotypes and qRTPCR to show expression of TTF-1, SPC, PECAM-1. Expression of protein products was evaluated by immunocytochemistry and western blotting and included surfactant proteins A, B, C and D, Clara Cell Protein 10 (CC10), alpha-actin and CD31. Cell types included but were not restricted to Type I and Type II pneumocytes, Clara cells, endothelial cells, neuroendocrine cells, smooth muscle and mucin secreting cells cells. Engineered tissue constructs derived from human SLPCs when engrafted onto the backs of nude mice produced type I and type II pneumocytes as well as endothelial cells. SLPC derived tissue constructs produced from strain matched (C57Bl6) mice that were implanted into the pleural space survived and retained the presence of type I and II pneumocytes after 10 days. Autologous SLPC derived sheep tissue constructs after implantation also developed into cell types found in the distal lung.

(OP 296) Tissue Engineered Vascular Prostheses

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Objectives: Patency of shelf-ready small calibre vascular prostheses is still sub-optimal. Vascular tissue engineering may be a new approach for improving vascular prostheses, however, biodegradable scaffolds are needed. The purpose of our study was to evaluate biocompatibility and thrombogenicity of the following electro-spun nanofibre polymer grafts: poly-dioxanone(PDS) co-polymer with polylactic-acid(PDS-PLA) or co-polymer with polycaprolactone(PDS-PCL) and to assess their *in vivo* vascular remodelling.

Methods: These medical grade polymers were selected because of biomechanical strength, surgeon's handling, degradation, cellular ingrowth and tissue reaction in subcutaneous and vascular implantation models. In 27 Sprague Dawley rats, 2 mm-ePTFE grafts (n=9 controls), 2 mm-PDS-PLA grafts ($n = 9$) and 2 mm-PDS-PCL grafts ($n = 9$) were interposed in the infrarenal abdominal aorta for 3, 6 and 12 weeks. Digital substraction angiography was performed for patency assessment before euthanasia and grafts were harvested for morphologic and scanning electron microscopic examination.

Results: Patency rates were excellent for all types of grafts (100%). Angiography at follow-up showed aneurysmal formation in one of three PDS-PLA grafts and none in PDS-PCL grafts. The neoendothelialization measured by morphometry was significantly increased in PDS-PLA and PDS-PCL compared to ePTFE grafts at 3-weeks ($p < 0.01$).

Conclusions: Patency of small calibre electrospun PDS-PLA and PDS-PCL grafts is excellent. Despite timely cellular ingrowth and neo-matrix formation, aneurysm formation still represents a major problem for degradable synthetic vascular grafts. Therefore, combinations or wrapping of the grafts with slower degrading polymers or new co-polymer combination, such as PDS-PCL are showing promising results. In conclusion, biodegradable, electrospun PDS-based polymer prostheses may represent a good alternative for future tissue-engineered vascular grafts.

(OP 297) Tissue Engineering of Peripheral Nerves: Guidance of Axons on Pre-Aligned Schwann Cells on Polymer Filaments

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The peripheral nervous system is capable to regenerate lesioned nerves because most cells of the nerve tissue remain viable. Deteriorated axons can regenerate if the continuity of the nerve tissue is preserved. Within the nerve axonal regrowth occurs along thousands of aligned Schwann cell (SC), so called *bands of Buengner*. To improve the therapeutic performance of synthetic implants used to bridge nerve gaps we have developed biohybrid bands of Büngner inside nerve guides.

30 µm thick micro-structured poly-caprolactone (PCL) filaments were produced by a melt extrusion process. Each endless filament had six longitudinal micro grooves. To overcome the non-permissiveness of the polymer filament surface, different plasma treatments and subsequent coatings with a synthetic polyamine and an extracellular matrix protein were investigated. Seeding of pre-treated PCL filaments with SCs resulted in longitudinal cell alignment. A metabolic labeling techniques revealed that SCs retained their proliferation potential. When dorsal root ganglions were explanted onto SC-seeded filaments, a highly oriented axonal outgrowth could be induced.

Implantation of cell-free-PCL filaments into lesioned sciatic rat nerves resulted in complete coverage of the implant by invading cells. In order to foster SC immigration we analyzed SCs *in vitro* and found that recombinant neuregulin-1 (NRG-1) accelerated cell migration. Therefore, the future addition of NRG-1 to nerve guide tubes with PCL filaments might accelerate SC recruitment from both neighboring nerve endings and in turn aid axonal regeneration.

Funded by BMBF 0313728 A,B

Biotech. Bioeng. (2006) **93**, 99–109; Biomaterials (2007) 27, 1425–36; Neurosurgery (2006) 59, 740–748.

(OP 298) Tissue Engineering Strategies for Vascularization

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One of the major challenges in the field of tissue engineering remains 'size'. How to engineer sufficiently large tissue systems *in vitro* for utility *in vivo*, where scale and integration *in vivo* are meaningful. This challenge is due to metabolic oxygen demands by the cells used in the process coupled with diffusion limitations of oxygen and nutrients within tissue constructs. With advances in biomaterials designs and control, cell biology, cell signaling and bioreactor features, we have been probing these issues with a variety of approaches to improve the scaling and size of tissues grown *in vitro*. Further, an Improved understanding of matrix-cell interactions has provided new direction to the design and study of biomaterial scaffolds to direct cell and tissue outcomes *in vitro* and *in vivo*. The ability to deliver appropriate topography, structure and chemistry to cells to direct their fate is emerging as a useful strategy relevant to functional tissue formation. Toward the goal of tissue vascularization *in vitro*, we utilize fibrous protein scaffold systems (e.g., collagens, silks) and control material processing to generate options for tissues. Our specific areas of interest include novel biodegradable microfluidic systems to house vascular networks, new modes to form mechanically robust vascular tubing for co-culture studies, and new modes to use co-culture to generate vasculature networks. Some of these examples will be described to highlight the importance of matrix design, cell interactions and bioreactor considerations in the context of functional tissue engineering.

(OP 299) Tissue Engineering with Primary Cultures and Cell Lines on Different Scaffold and Implant Materials

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Most implant materials can often also be used as scaffolds for tissue engineering. The present work considers Hydroxyapatite (HA) for bone substitutes and bone engineering scaffolds activated by cold plasma treatment for further drug/bioactive molecule loading.

In this study, HAs with specific internal pore sizes and porosities were elaborated by varying the sinter temperature and/or adding graphite and PMMA as porogenous agents. The micro-, meso- and macro-porous HA samples were obtained with internal pore size of 1–10 μm, 10–50 μm and 400 μm respectively. The biological tests, concerning cell viability, proliferation and morphology (SEM), and the cytochemical staining of actin and vinculin, were conducted with MC3T3-E1 osteoblasts. An applying example of above material was given by the study on the different antibiotics (ATBs) loading directly or after cold plasma treatment under different conditions. ATB loading amount and their kinetic antibacterial effectiveness have been assessed by UV spectrophotometry and bacteria inhibition tests.

The biological tests confirmed the excellent cyto-compatibility of HA fabricated by our technique. All samples, in particular micro-porous HA, showed a higher proliferation than the TCPS controls. Cytochemical staining revealed a well-developed cytoskeleton and organized focal adhesion contacts. By direct impreganating with ATBs (vancomycine, ciprofloxacine or gentamycine), micro-porous HA showed significantly higher ATB adsorption capacity, and prolonged ATB release time with anti-bacterial effects under physiological conditions, comparing with dense and meso-porous HA. Moreover, these efficiencies of bioactive molecules loading could be further improved by physical or chemical treatments such as cold plasma under $50\%O_2$–$50\%He/NH_3$ atmosphere prior to impregnation.

(OP 300) Towards an Intraoperative Engineering of Osteogenic and Vasculogenic Grafts with Freshly Isolated Human Adipose Tissue-Derived Cells

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We recently demonstrated that perfusion of human adipose-derived cells (ADCs) for 5 days through ceramic scaffolds establishes a three-dimensional culture system of osteoprogenitor and endothelial cells and generates osteogenic-vasculogenic constructs. We here investigated the principle of an intraoperative approach, with cell harvesting, their seeding in scaffolds and subsequent grafting of constructs within the same surgical procedure.

ADCs were enzymatically isolated from adipose samples of 7 healthy donors, yielding $2\times10^5 \pm 1.5\times10^5$ nucleated cells per ml of tissue, of which $8\% \pm 6.5\%$ were clonogenic as determined by CFU-f assays. $27 \pm 4\%$ of freshly isolated ADCs expressed mesenchymal (CD105 and CD90) and $43 \pm 34\%$ endothelial (CD31 and CD34) markers, as determined by cytofluorimetry. Freshly isolated ADC were embedded in fibrin (Baxter, Vienna, Austria) and wrapped around tricalciumphosphate (ChronOs®, Synthes) or hydroxyapatite (Engipore®, Finceramica, Faenza, Italy) cylinders. ADC-fibrin-scaffold constructs were then immediately implanted subcutaneously in nude mice for eight weeks. *In vivo*, human CD34+ endothelial progenitors formed functional, irrigated blood vessels inside the construct. The human mesenchymal progenitors formed a dense matrix positive for BSP with some morphological similarity to osteoid tissue in areas initially loaded with human clonogenic cells. However, obvious bone formation was not observed in hematoxylin/eosin staining of tissue sections.

Here we provide evidences that intraoperative engineering of autologous cell-based vasculogenic bone graft substitutes could be achieved by wrapping an ADC-fibrin gel around ceramic-based scaffolds. Further studies are necessary to determine whether the engineered constructs, implanted in a relevant orthotopic model, would be able to induce de novo formation of bone tissue.

(OP 301) Towards Prosthetic Replacement of Bruch's Membrane: Comparison of Polyester and Electrospun Nanofiber Substrates.

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Purpose: Replacement of the Retinal Pigment Epithelium (RPE) could be beneficial in Age-related macular degeneration (AMD), but may not be effective if Bruch's membrane (BM) is damaged. We explore two biocompatible substrates that simulate properties of BM.

Methods: Polyester culture inserts (PET) are 10 μm thick and permeable (Corning). Commercial electrospun polyamide nanofiber (EPN) inserts are adherent to impermeable plastic (Donaldson). The EPN can be detached from the plastic with ethanol to obtain free and thus permeable EPN. Scanning electron microscopy (SEM) studies were performed on all growth substrates. Fetal and aged adult human RPE cells were cultured on above substrates. RPE marker proteins were studied using immunofluorescence and immunoblotting.

Results: PET is a smooth surface with widely-spaced small pores: in contrast, EPN is a fibrillar meshwork with potential fluid transit everywhere. The EPN surface can show either flattened or rounded nanofibers, depending on whether that side had been attached to plastic or free. On PET, fetal cells remelanized and maintained a hexagonal monolayer over 3 months. Fetal cells grown on commercial (impermeable) EPN also showed typical RPE characteristics. In preliminary experiments, aged RPE showed better differentiation on commercial EPN than PET. Free (permeable) EPN was less supportive. Confluent fetal RPE monolayers broke up into a lattice pattern at days 5–7, but showed somewhat better attachment on flattened (as opposed to rounded) fibers

Conclusion: Permeable PET and impermeable EPN support differentiation of RPE cells. An increased flat surface for cellular attachment improves maintenance of an RPE layer on a permeable substrate.

(OP 302) Transcriptional Regulation of Regenerative Gene Expression by Biomechanical Activation of Chondrocytes-PCL Constructs

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Biomechanical activation of tissue engineered grafts is important for musculoskeletal tissue that are dynamically loaded *in vivo*. However, the intracellular mechanisms that are activated by biomechanical signals are little understood. We hypothesized that biomechanical forces act on cell-scaffolds in two ways, (i) induce expression of regenerative genes associated with cell proliferation and differentiation, and (ii) suppress transcriptional activation of proinflammatory genes expressed at the site of injury. To prove this hypothesis, chondrocytes were cultured on 3-D microfiber polycaprolactone scaffolds for 6 days, and subjected to dynamic compression (15% strain, 0.5 Hz). To depict the injured tissue environment, constructs were exposed to interleukin-1beta (IL-1). Examination of gene expression demonstrated that compressive forces significantly upregulate SOX-9, aggrecan, collagen type II, and BMP-2 mRNA. Simultaneously, compressive forces suppress IL-1β-induced expression of cyclooxygenase, IL-1, MMP-1 and MMP-13, suggesting that these signals are also antiinflammatory. More importantly, compressive forces upregulated reparative genes in the presence of IL-1β. Further examination of the intracellular signaling showed that compressive forces inhibit IL-1β-induced nuclear translocation of NF-kappaB by suppressing I-kappaB kinase phosphorylation by its upstream kinases. Simultaneously, these signals activate RAS GTPase activity, to activate C-myc and ELK-1 transcriptional activity via phosphorylation of ERK 1/2 to upregulate cell proliferation and aggrecan expression. Thus, our findings provide the evidence that of biomechanical signals are critical in upregulating regenerative potential of chondrocyte in PCL scaffolds despite the inflammatory environment surrounding the implant in the injured tissue. Acknowledgements: Grants AR04878, DE15399, HD40939.

(OP 303) Translating Novel Scientific Findings into Regenerative Therapies

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The Berlin-Brandenburg Center for Regenerative Therapies—BCRT—is located at the Charité—Universitätsmedizin Berlin and is a joint initiative with Germany's largest research organisation, the Helmholtz association. This Center for Translational medicine is funded by the Federal Ministry of Science and Education and is supported by over 15 Regional research organisations.

The research program of the BCRT comprises work in five medical research fields linked to overlapping platforms on basic research, bio-engineering, and translational research. Initially, the Center will put particular emphasis on research and clinical programs related to musculoskeletal and immunological applications, since aspects of regenerative medicine are most advanced in these areas at the Charité and are closest to clinical translation. In addition, the Center will also explore additional opportunities for regenerative therapies in the cardiovascular system, nervous system, and liver-related applications.

The BCRT is located in a newly reconstructed building at the Charité Campus Virchow Clinic and will host 23 newly implemented research groups (16 professorships), supplemented with established groups from the respective research fields.

To advance novel therapeutic concepts from the lab bench into the clinic, BCRT offers selected industry partners options for interacting at different levels:

- EARLY ACCESS: Sponsors will receive early access to research results and product candidates employing various communication instruments.
- JOINT PROJECTS: Will be conducted as fee for service as well as codevelopment projects with outcome sharing.
- STRATEGIC ALLIANCES:

Long-term exclusive co-developments in selected research fields.

(OP 304) Transplanted Human Amniotic Stem Cells Survive in the Rat Vascular Wall Following Endothelial Injury

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Human amniotic epithelial stem cells (HAESCs) are newly discovered sources of multipotent implantable cells which may be harvested without ethical concerns. We hypothesized that these epithelial cells can survive and inhibit neointima formation following acute endothelial injury. Adult male Wistar rats were anesthesized with Halothane and a balloon catheter was inserted into the common carotid artery through the external carotid. The balloon was inflated and deflated three times in order to induce endothelial injury. Brome-deoxy uridine (BrDU) labeled HAESCs were injected into the arterial lumen immediately after the removal of the balloon. Forty eight hours after implantation HAESCs were located at the luminal side of the damaged but not the intact artery.

Three weeks after the injury constrictive neointima formation was observed at a comparable level in transplanted and sham animals. BrDU positive cells were found in the intact arterial wall but not at the injured side. Moreover, acetilcholine-induced vasodilation was preserved in the sham-transplanted injured arteries, but it was reduced in the transplanted vessels. We conclude that amniotic stem cells are capable of surviving in another species without any immunosuppression, however, functional improvement is not related to the presence of grafted cells.

Supported by OTKA D45933, T049621, TET A4/04, Bolyai and Oveges Fellowships.

(OP 305) Ulvan: a New Natural Derived Biomaterial Obtained from Renewable Marine Resources

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It is not hard to understand the commitment set in tissue engineering research and the effort to find better performing materials. The ultimate goal: an ideal scaffold with cell and tissue compliant mechanical properties that supports cellular attachment, growth and differentiation. In this study, a new natural derived polysaccharide–ulvan–found at the cell wall of green algae Ulva, the common sea lettuce, was investigated. The aim of this research work is to study and develop novel biomedical applications based on this polysaccharide, with special emphasis to tissue engineering. The polysaccharide ulvan, composed of sulphated rhamnose, xylose, glucoronic and iduronic acids, was isolated from Ulva batch by step extraction using hot water and precipitation with organic solvents. The obtained polysaccharide was then used to produce polymeric films by solvent casting. As ulvan is soluble in water, a crosslinking step was necessary to render the membrane insoluble in water and more chemically stable at physiological conditions. Membranes produced were characterized by SEM and FTIR-ATR; swelling behavior and bioactivity was investigated and the mechanical performance assessed by both quasi-static tensile testing and dynamical mechanical analysis. The properties of ulvan structures studied in this work suggest a great potential of this natural sulphated polysaccharide for the herein envisaged applications. The knowledge generated during this work about the properties of this readily available polymer is a necessary and important step to uncover innovative biomedical applications.

(OP 306) Upregulation of Mesenchymal Stem Cells Differentiation and Proliferation in Coculture with Endothelial Cells

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Human mesenchymal stem cells (hMSCs) have been widely used in bone tissue engineering due to their high proliferative potential, their default osteogenic differentiation pathway, and their major role in endochondral and intramembranous bone formation *in vivo*. Studies with MSCs have demonstrated that these cells, growing in a monolayer culture, differentiate into osteogenic pathway under chemical stimulation. Also hMSCs osteogenic potential has shown to be conserved through several subcultures and even after cryogenic preservation. The induction of MSC osteogenesis is a highly programmed process that involves soluble factors as well as cell-cell and cell-matrix contacts. This work aims to study the influence of endothelial cells in the proliferation and differentiation of MSCs. For this purpose hMSCs (Cambrex) were culture in direct contact with human umbilical vein endothelial cells (HUVEC, ScienCell). The cells were cocultured in 24 well plates during 4 weeks and at several time points the hMSCs proliferation and differentiation were evaluated. hMSCs number in the coculture was assessed by FACS. Their differentiation was evaluated by alkaline phosphatase (ALP) activity quantification and by ALP staining, as well by the expression of osteogenic gene markers. It was possible to observe a higher proliferation in hMSCs in coculture than in monoculture. In coculture the ALP activity values were also higher and accompanied by the increase of intensity in ALP staining. The gene expression of the transcriptional factor Cbfa1 and of ALP was also upregulated in coculture. These data seems to indicate that HUVECs stimulate proliferation and enhance differentiation of hMSCs.

(OP 307) Using Native and Denatured Collagen Nanosurfaces to Control the Attachment and Subsequent Mineralization of Primary Cells and Cell Lines

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In bone tissue, collagen I is the major constituent of the extracellular matrix and serves as an organic scaffold for the osteocytes, mineral plates and non-collageneous proteins. It has further been shown that the *ex vivo* expansion of bone marrow mesenchymal stem cells on denatured collagen (gelatin) appears to preserve the potential for osteogenic differentiation.

The present study characterizes the effect of two substrates, namely collagen type I and gelatin, on the *in vitro* culture and osteogenic differentiation of mouse calvaria pre-osteoblasts and human adipose derived precursor cells. The main factors examined are cell attachment, cell morphology and the extent of mineralization by the cells.

The influence of the nano-structured substrates on the mineralization of the cultures after 28 days of osteogenic induction was also studied. Similar levels of mineralization are observed of pre-osteoblasts that are plated on collagen type I and tissue culture plastic, which is used as a control. In comparison, there is a greater extent of mineralization of both cell types that are plated on gelatin.

These findings demonstrate that although the cells display a larger spread area upon plating on collagen I, gelatin results in a stronger

adhesion at very short contact times and enhance the mineralization of the pre-osteoblasts after of osteogenic induction *in vitro*. Therefore, it is suggested that the mechanisms of cellular adhesion to collagen and gelatin are inherently different and consequently, influence the attachment, morphology and mineralization of the cells to varying extents.

(OP 308) Validation of Transplantable Epithelial Cell Sheets for Regeneretive Medicine

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As we reported (Nishida *et al*. NEJM 2004), we have clinically applied transplantable epithelial cell sheets fabricated on temperature-responsive culture surfaces to treat limbal stem cell deficiency. In order to achieve the safe and reliable treatment, we have developed a number of validations of fabricated cell sheets performed one day prior to the transplantation including the maintenance of epithelial stem/progenitor cells, differentiation exhibiting barrier functions as well as mucosal properties, stratification and so on. Now, we are going to propose the established validation protocols as domestic and international standards to evaluate transplantable epithelial cell constructs for the treatment. In the presentation, we'd like to show the details of the validation protocols and discuss the utility and expected roles in regenerative medicine.

(OP 309) Vascular Tissue Engineering: a Clinician-Scientist's Perspective

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Atherosclerotic and congenital vascular disease has made it necessary for surgeons to replace/bypass segments of diseased or dysfunctional vessels. Whilst the gold standard for bypass remains autologous vein, increasingly this is not always available due to either previous use or the vein being unsuitable due to disease eg varicose veins. Hence, there has been a concerted effort by the scientific community to develop a tissue engineered alternative with properties similar to autologous vein. The first tissue engineered blood vessel was produced over 20 years ago and since then, several groups, including ours, have been working in this area using a number of different techniques.

Recently, with the emergence of sophisticated tools such as bioreactors, engineered scaffolds, cell sourcing techniques and imaging methods, novel vascular products have been developed.

This talk will cover the different approaches to vascular tissue engineering with emphasis on the integration of vascular biology into the design of these novel products. A clinical perspective of the current status and future directions in this field will also be given.

(OP 310) Vascularization Strategies in the Context of Tissue Engineering

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One of the essential determining factors for the success of tissue engineering approaches is the ability to provide a rapid and fully integrated vascular system to supply oxygen and nutrients to the regenerating cells. Various solutions include the use of pre-vascularized tissue flaps, incorporation of pro-angiogenic drug- or gene-delivery systems into the biomaterial, pre-seeding with autologous endothelial cells (EC) and, increasingly, adult stem cell strategies. In all of these knowledge of the nature and control mechanisms of the regenerative niche in the tissue of interest is essential. Our research is focussed on employing *in vitro* techniques with human cells to characterize the biology of endothelial progenitor cells (EPC) and how circulating EPC could be recruited to the regenerative niche in bone. It is still unclear what contribution to the vasculature comes from EPC and from the *in situ* microcirculation. Our recent data indicate that both EPC and microvascular EC can self-assemble to form vessel-like structures in interaction with osteoblasts, also in the presence of porous biomaterials of various classes (polymers, metals, ceramics) (1,2). As yet unpublished work concerns *in vivo* studies in the nude rat and mouse to investigate the survival capacity of pre-seeded EPC on biomaterial scaffolds (silk fibroin) and hydrogel matrices (Matrigel™) and their possible integration into the pre-existing microvasculature. Future work will study the possible role played by circulating EPC.

The authors acknowledge EU support via the NoE EXPERTISSUES.

References:

[1]Fuchs S *et al*. Tissue Engineering 2007; 13: 2577
[2]Unger RE *et al*. Biomaterials 2007; 28: 3965

(OP 311) Versatile Polyurethane Scaffold Material for Tissue Engineering

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A set of 2K-polyurethane-systems as scaffolds for regenerative medicine has been developed. Its properties can be customized in a broad range to address different tissues. For the regeneration of soft tissues (ranging from fat to cartilage) formulations have been chosen that can be cast to an open-pore foam without a skin. The polymers are made from aliphatic diisocyanates and different polyester-polyols. The porosity and the surface properties can be adjusted by biocompatible additives. If hard tissues like bone shall be reconstructed, the material can be filled with up to 75% of ceramic particles (preferably HAP or TCP). Scaffolds are built up by dis-

pensor techniques. The mechanical properties of these scaffolds can be varied by means of the ceramic content. The Young's modulus is between 900 and 1600 MPa, the flexural strength between 5 and 10 MPa. These values are comparable with those of trabecular bone.

Steam sterilization of both materials is possible.

The degradation time of the polyurethanes can be adjusted from several months up to two years by variation of the polyol composition, the diisocyanate and fillers. A key factor is the hydrophilicity of the building blocks. The pH during the degradation is within a narrow range, even if the medium is not changed. No toxic degradation products are observed.

The compatibility with differents cells (cartilage, bone, fat, nucleus pulposus) and tissues has been proven. The constructs are well integrated into the surrounding tissues at different implantations sites and induced the ingrowth of bloodvessels.

(OP 312) Visualization of Structural Evolution in Tissue Engineering Tendon

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Introduction: Birefringence, the main polarization property in some biological tissues, is caused by a difference in index of refraction, and is often exhibited by fibrous structures. We use the level of birefringence as a mark to assess the structural evolution of tissue engineering tendon during a prolonged culture period in response to external cues by polarization optical coherence tomography.

Materials and Methods: Primary rat tenocytes were extracted from young rats. A Flexcell Tissue Train Culture system was used to mechanically stimulate tissue engineered tendon with the aim of improving the production and alignment of collagen within the tendon. The tenocytes (passage 2) were suspended throughout the collagen solution prior to gelation. Four different cell seeding density from 1×10^6 cells/ml to 10^5 cells/ml were examined. The tendon constructs were loaded cyclically for one hour per day for 11 days or 14 days at 1% or 1.5% strain level and 1 Hz.

Results and Discussion: A progressive change in the ordered matrix structure of the tendon constructs has been visualized by PSOCT. Birefringence was detected when the tendon constructs were subjected to the stretching where the loading was in the direction of the collagen alignment. The level of birefringence increased over time and with higher cell seeding density. It was also noticeable that the constructs became thinner over time and that there was a relationship between seeding density and structure of the engineered tendons. PSOCT demonstrates a powerful on-line tool for monitoring tissue growth and structural development in tissue engineering.

(OP 313) "Smart" and Stimulus Responsive Chitosan-Based Scaffolds/Cells for Bone Tissue Engineering: Influence of Lysozyme Upon Scaffold Degradation and Osteogenic Differentiation of Cultured Marrow Stromal Cells Induced by Cap Coatings

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The present study reports the use of non-porous, "smart" and stimulus responsive chitosan-based scaffolds with the capability of gradual *in situ* pore formation for bone tissue engineering applications. Biomimetic calcium phosphate (CaP) coatings were used as a strategy to incorporate lysozyme at the surface of chitosan-based materials the main objective of controlling their degradation profile as a function of immersion time. In order to confirm the concept, degradation tests with concentration similar to those incorporated into CaP chitosan-based scaffolds were used to study the degradation of the scaffolds and the formation of pores as function of immersion time. Degradation studies with lysozyme (1.5 g/L) showed the formation of pores, indicating an increase of porosity (~5% - 55% up to 21 days) resulting in porous 3-D structures with interconnected pores. Additional studies investigated the influence of CaP biomimetic coating on osteogenic differentiation of rat marrow stromal cells (MSCs) and showed enhanced proliferation and differentiation of rat MSCs seeded on the CaP coated chitosan-based scaffolds with lysozyme incorporated with bone matrix production and mineralization as demonstrated by calcium deposition measurements. The ability of these CaP coated chitosan-based scaffolds with incorporated lysozyme to create an interconnected pore network *in situ* coupled with demonstrated positive effect of these scaffolds upon osteoblastic differentiation of MSCs and mineralized matrix production illustrate the strong potential of these scaffolds for application in bone tissue engineering strategies.

(OP 314) Adipose Tissue as a Reservoir of Regenerative Cells

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Beside the classic role of adipose tissue in plastic and reconstructive surgery, the importance and the role of adipose tissues in energy balance have been greatly expanded to endocrine tissue. Some years ago, by analogy with mesenchymal stromal cells from bone marrow (MSC), the discovery that various phenotypes can be obtained from stroma cells of adipose tissue raises great hope in cell therapy and regenerative medicine. Indeed and as well known, such tissue can be easily harvested in adults and could represent an abundant reservoir of regenerative cells. Although MSC and ASC share common features, genomic, proteomic and functional studies revealed clear differences and that both cells are distinct. ASC can differentiate towards osteoblast, chondrocytes, skeletal muscles but are also strongly angiogenic via paracrine effects and a true endothelial potential. To go further in this way, we set-up GMP conditions for ASC culture and gathered many pre-clinical data in order to perform a clinical trial. However, many studies including ours reveal the existence of rare cells among ASC with features similar to true stem cells. One of the next challenges will be to be able to sort these cells a priori. Altogether, these promising results are attracting increasing groups and investigators and in the future

could lead to consider adipose tissue as one of the referent tissues for regenerative medicine.

(OP 315) Materiomics: Dealing with Complexity in Tissue Engineering

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As the human body holds some 200 cell types that synthesize a multitude of both soluble and solid actives in addition to a variety of components that provide various means of mechanical support it will be clear that extremely complex interactions stand at the basis of the proper functioning of all tissues.With the increase of complexity, certainly when this is associated with a, at best, only partial understanding of the underlying mechanisms, special strategies need to be applied to unravel or direct processes that result from such complex interactions. Rather than striving for a full understanding of the underlying mechanisms upon which to base ones actions, it might be more productive to rapidly screen a multitude of approaches and select the one with the most optimal result. Surprisingly, in tissue engineering this approach is still largely unexplored.In this presentation, apart from a selective overview of the current state of high throughput in tissue engineering, we will discuss the production of large libraries of material geometries that will allow us to screen thousands to millions of substrates. We propose the name *MATERIOMICS* for the discipline of high throughput methods in biomaterials and tissue engineering science.

(OP 316) Tailoring "Smart" Polymeric Surfaces

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The interface generated between all biomedical systems and their physiological environment largely affects their biological performance. In the Tissue Engineering field, the surface properties of the scaffold play a crucial role in determining the outcome of the tissue regeneration process.The temperature-triggered reversible coiling and uncoiling of water-soluble polymeric chains grafted on the surface of a substrate, represents a promising strategy to engineer ''switchable'' surfaces. Following a concept initially set forward by Okano *et al* using poly(NiPAAm) chains, this study capitalizes on the temperature-triggered ability of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblocks, to produce surfaces that can cause cells to gently detach at unison, generating functional cell sheets to optimize tissue regeneration.A diversity of reactive moieties can be generated on polymeric surfaces, by exposing them to different plasma treatments and use them as anchoring sites for further derivatization of the substrate.The PEO-PPO-PEO triblocks were grafted onto the surface *via* diisocyanate spacers, after exposing the substrate to plasma of ammonia, whereby amine groups were generated. The present study describes the generation of these ''switchable'' surfaces, and investigates their composition and thermoresponsiveness using various analytical techniques, such as XPS, contact angle measurements and HR-SEM. Dendrimer-like surface structures were built by exposing the substrate to multi-stage processes comprising alternative plasma polymerization and derivatization steps. The ability of these surfaces to allow cell grow at 37 degrees, while triggering the fast and efficient detachment of organic cell sheets, was demonstrated.

(OP 317) Regenerative Medicine Perspectives and Possibilities in the Technological and Clinical Area

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Regenerative medicine has led to completely new approaches and possibilities in the treatment of illnesses and bodily damage. Regenerative medicine makes use of the recovery potential already available in the individual for example in the form of organ specific stem cells. There are various techniques and approaches for generating tissue. They enable an adaptation to the individual's particular needs. As a result we are on the way to developing a tailor-made therapy for some important symptoms. At the same time technical systems come into use such as bioreactors, hybrid systems (combination systems from two origins, e.g. for the liver outside the body; autologous liver cells in synthetic balls) as well as complete biological implants. Since autologous tissue is immunologically better tolerated than exogenous tissue, the main aim remains of regenerating tissue in the living organism. Our research area covers among other things the search for a biological bone or rather cartilage replacement with autologous stem cells and mineralising structures, for a biological liver replacement, for a complete biological implant for a damaged heart, e.g. heart valves, which will also grow in children as they grow. In addition medicines and new types of growth factors have provided new forms of therapies which can initiate regeneration in living tissue. Cultivating the tissue e.g. *ex vivo*, requires a sort of extracellular scaffold of synthetic or of animal material. Living cells of the patient are then cultivated in this so-called matrix. This cultivation is carried out in a bioreactor which we specially designed for this purpose. The restructure and replacement of the matrix in the bioreactor with autologous tissue is then continued after the implantation in the living organism.

(OP 318) Derivation of Chemically Defined, Serum-Free Culture Parameters for the Expansion and Phenotypic Maintenance of Clinically Important Lineage Committed and Pluripotent Cell Types

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Currently cell culture techniques rely on the presence of animal-derived products as media supplements for the expansion of cell

populations destined for implantation. This study replaced the requirement for fetal calf serum during *in vitro* cell culture using an array of chemically-derived or recombinantly synthesised products to generate a media suitable for the growth of a range of cells relevant for current clinical applications. This included bone marrow-derived mesenchymal stem cells (MSC), a proven multipotent cell.

Media combinations were synthesised de novo using raw components. Cells of primary human origin were seeded and passaged in these media. Cell phenotype was characterised using a combination of flow cytometry and immunohistochemistry, visualised by laser scanning confocal microscopy.

Defined media supported the expansion of cells including dermal fibroblasts, smooth muscle cells, osteoblasts and articular chondrocytes up to 50 passages with proliferation rates comparable to commercially available bespoke media. The defined media also supported the proliferation and undifferentiated phenotype maintenance of several multipotent cells including MSCs and dental pulp progenitor cells whose undifferentiated phenotype was characterised by the presence of the intracellular markers Nucleostemin and STRO1 and the putative extracellular marker subset CD29+, CD34−, CD44+, CD45−, CD90+ and CD105+.

The findings in this study have established the critical foundations for overcoming the major regulatory requirements for the progression of cellular therapies from flask to clinic by optimising chemically defined pharmacopeia grade media, entirely devoid of animal-derived components for the *ex vivo* expansion of cells destined for therapeutic intervention.

(OP 319) Test and Validation of a Bioreactor for the Stimulation of Engineered Cartilage Constructs with Combined Regimens of Hydrostatic Pressure and Interstitial Perfusion

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During joint physiological loading *in vivo*, the complex mechanical stimuli which act simultaneously on chondrocytes include hydrodynamic shear stress, cell deformation and increased interstitial pressure. Bioreactor systems represent valuable instruments to investigate the roles of several culture parameters, although single rather than combined stimuli have often been studied. The present study tested and validated a bioreactor developed for culturing tissue engineered cartilage in the presence of different levels of hydrodynamic shear stresses and hydrostatic pressures. Primary bovine chondrocytes were seeded on non-woven scaffolds. Constructs were cultured for 5 days in Petri dishes and then for 5 additional days as follows: Group-1 with interstitial perfusion (0.4 mL/min) combined with hydrostatic pressure (10 MPa, 1 Hz for 4 h/day), Group-2 with interstitial perfusion (0.4 mL/min) without hydrostatic pressure, and Group-3 in static Petri dishes. A total of 112 cartilage constructs were studied in four independent experiments. Cell viability, assessed as MTT/DNA, was significantly higher ($p < 0.01$) in Group-1 than all other groups. Sulfated glycosaminoglycans (sGAG) content was significantly higher in Group-1 than Group-3 ($p < 0.01$, $626 \pm 85\,\mu g$ vs. $326 \pm 46\,\mu g$); sGAG content in Group-2 ($425 \pm 59\,\mu g$) tended to be higher than Group-3, but with no statistical significance. Histological analyses were consistent with the performed biochemical analyses. After only 5 days of stimulation, application of diverse mechanical stimuli successfully elicited different responses and resulted in higher quality cartilaginous constructs than static culture. We successfully validated a bioreactor for controlled application of different mechanical stimuli, which can lead to a more accurate definition of tissue-forming environments and mechano-transdcution mechanisms.

(OP 320) Tisseel Fibrin Glue use in Spinal Surgery: Effects on Bone Morphogenetic Protein Diffusion, Bone Morphogenetic Protein-Stimulated Bone Growth, and Soft Tissue Swelling

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Fibrin glue is used as a carrier of osteoinductive materials, including recombinant human bone morphogenetic protein-2 (rhBMP-2), which has become an increasingly popular method for augmenting fusion in the lumbar and cervical spine. However, rhBMP-2 may "leak" from the placement site and stimulate undesirable bone growth in the spinal canal and neural foramina. In addition, there have been some reports of soft tissue swelling after rhBMP-2 use in cervical procedures. Thus, we conducted a series of experiments to evaluate whether fibrin glue can limit rhBMP-2 diffusion *in vitro* and protect spinal nerves from rhBMP-2 stimulated bone growth *in vivo*. Lastly, we also evaluated the edematous effects of rhBMP-2 and fibrin glue in rabbit muscle tissue. For the *in vitro* studies, rhBMP-2 soaked collagen sponges were encapsulated in Tisseel fibrin glue (Baxter Healthcare) and immediately incubated in saline. Subsequent measures of saline rhBMP-2 concentrations showed that fibrin glue significantly limits, but does not completely block, rhBMP-2 diffusion. To determine whether fibrin glue can protect against detrimental bone growth, rats were given laminectomies and fibrin glue was placed over the dura and transverse processes/intertransverse space prior to rhBMP-2 placement. The results of this study showed that fibrin glue can control rhBMP-2 stimulated bone growth and thus prevent undesirable bone formation. Lastly, in a preliminary study, MRI analyses of rabbit muscle tissue implanted with rhBMP-2 soaked collagen sponges and fibrin glue suggest that individually these materials are not associated with increased edema. These findings should be further investigated in human clinical studies.

Poster Presentations

(P 1) Exploration of Transglutaminase 2 As a Biological Tissue Glue

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Aim: The clinical caseload of surgical wounds is very high and physicians have long sought an efficient biological method of wound repair that requires little time and minimizes discomfort for their patients, yet produces a good cosmetic outcome. Our aim is to develop a biological tissue glue based on the extracellular matrix stabilizing enzyme transglutaminase to covalently bond tissue layers to promote suture-less closure of surgical wounds.

Methodology: Human skin samples were cryosectioned and assayed for native transglutaminase activity using standardized histochemical procedures. Availability of substrate sites in human skin, with different brands of exogenous transglutaminases 2, was assayed with a set of substrate peptides. Tissue adhesive properties of the enzyme were studied and optimized on human skin specimens *in vitro* by tissue bonding experiments.

Results: Histochemical studies proved that human skin naturally express localized transglutaminase activity. The assay of exogenous transglutaminase activity revealed that skin has abundant substrate sites for the enzyme in all the histological layers and an efficient commercial brand of the enzyme was identified. Results from tissue bonding experiments indicated efficient tissue adhesive property of transglutaminase without causing any surrounding histological tissue damage.

Conclusion: We have shown the strong potential of extracellular matrix cross linking enzyme—transglutaminase 2 as a biological tissue glue to achieve rapid and efficient tissue repair.

(P 2) 3d Durotaxis Within a Collagen Matrix

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While matrix stiffness has been implicated in cell adhesion and migration, most studies have focused on the effects of substrate stiffness in 2D. This work describes a novel continuous stiffness gradient model for studying such processes in 3D.

Wedge-shaped collagen scaffolds were compressed to produce sheets of a desired (0.25 mm) uniform thickness, but with increasing collagen density along the length of the sheet. Dynamic mechanical analysis, carried out on 1 mm wide strips obtained from the two ends and the middle of each sheet, showed that the elastic modulus increased from 1057 ± 487 KPa to 2305 ± 693 KPa at the soft and stiff end respectively and was 1835 ± 31 KPa in the middle. In constructs seeded with sepharose marker beads prior to compression, mean sepharose bead density rose from 10 ± 1 to 71 ± 12 at the soft and stiff end respectively and was 19 ± 5 in the middle, indicating successful engineering of a density gradient corresponding to the measured stiffness gradient. Growth-arrested human dermal fibroblasts cultured within such constructs for 3 and 6 days, accumulated preferentially towards the stiff part of the gradient. Durotactic migration was significant after 6 days. This model provides a new means for studying cellular mechanotaxis and patterning cells in three dimensions.

(P 3) A Bioactive Biodegradable Guided Bone Regeneration Membrane

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Medical devices derived from biomaterials of the 3rd generation are biocompatible, biodegradable and bioactive. Recently we developed and characterized a 3rd generation guided bone regeneration membrane and showed that the plasticizer N-methyl-pyrrolidone (NMP) is bioactive, since it acts synergistically with bone morphogenetic protein and enhances bone maturation and regeneration in several *in vitro* and *in vivo* models. In order to get a broader view on the bioactivity of NMP we performed micro array experiments which revealed that irrespective of BMP, NMP targets the transcription of several genes; two of those belonging to the natriuretic peptide hormone system.

The influence of the natriuretic peptide hormone system on bone growth is manifested in the human disease: Acromesomelic dysplasia Maroteaux type where mutations in the natriuretic peptide receptor 2 induce dwarfism. Over-expression and knock-out experiments in mice showed that also other elements of this system can affect the skeleton. The knock-down of npr-3 in mice, which serves as scavenger receptor and the over-expression of natriuretic peptide B lead to gigantism. Our results in the micro array experiments show that the plasticizer NMP decreases npr-3 expression and increases BNP expression in C2C12 cells, mimicking the knock-out and over-expression of those genes in mice. Therefore, the direct effect of NMP on the transcription of 2 elements of the natriuretic peptide hormone system in a pro osteogenic way could at least partially account for the accelerated bone healing seen under the influence of NMP in vivo.

Acknowledgment: SNF; AO-Biotechnology Fund, Inion OY.

(P 4) A Bioscaffold Enhances the Fibrillogenesis in Healing Medial Collateral Ligament

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Porcine small intestine submucosa (SIS) has been successfully used to improve the healing of ligaments and tendons. In the current study, our objective was to examine the organization of the extracellular matrix, measure the collagen fibril diameter, and analyze the gene expressions of the fibrillogenesis-related molecules, specifically, collagen types I, III, V and small leucine-rich proteoglycans including decorin, biglycan, lumican and fibromodulin, in the healing rabbit MCL treated with SIS at 6 weeks post-injury. Twenty rabbits were equally divided into two groups. In the SIS-treated group, a 6 mm gap was surgically created in the right MCL and a layer of SIS was sutured covering the gap. For the non-treated group, the gap-injured MCLs remained untreated. The results revealed that in the SIS-treated group the collagen fibers were more regularly aligned as well as the cell nuclei than that for the non-treated group. Additionally, in the SIS-treated group larger collagen fibrils (90–120 nm) appeared around the fibroblasts and the range of the fibril diameter distribution was from 24 to 120 nm while the collagen fibrils in the non-treated group were uniformly small and the distribution of fibril diameter was limited from 26 to 87 nm. Simultaneously, the gene expressions of collagen type V, decorin, biglycan and lumican were down-regulated by 41%, 58%, 43% and 51%, respectively ($p < 0.05$). The present results suggested that the significant reduction in the gene expressions of fibrillogenesis-related molecules in the healing MCLs following SIS treatment are closely related to the improved morphological characteristics.

(P 5) A Cartilage Ecm-Derived 3-D Porous Acellular Matrix Scaffold for *In Vivo* Cartilage Tissue Engineering with Pkh26-Labeled Chondrogenic Bone Marrow-Derived Mesenchymal Stem Cells

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We developed a natural, 3-D interconnected porous scaffold derived from cartilage extracellular matrix (ECM). Human cartilage was physically shattered, and cartilage matrix microfilaments were prepared after differential centrifugation, then decellularized sequentially with use of hypotonic buffer, TritonX-100, and a nuclease solution and made into a 3% (w/v) suspension. The scaffold was fabricated by simple freeze drying, then cross-linked by dehydrothermal treatment and immersed in a carbodiimide solution (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-hydroxysucinimide). On histology, scaffolds showed most of the ECM components after removal of the cell fragments, and scanning electron microscopy revealed a 3-D interconnected porous structure. Cellular viability assay revealed no cytotoxic effects. *In vitro* study showed that the novel scaffold could provide a suitable 3-D environment to support the proliferation and differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) to chondrocytes. BMSCs were induced along the chondrogenic lineages for 21 days in medium containing transforming growth factor beta-3 (TGF-β1), then labeled with fluorescent dye PKH26 and seeded onto scaffolds at 2×10^7 cells/ml. Cell-scaffold constructs were implanted subcutaneously into nude mice. Four weeks later, cartilage-like tissue formed, with positive staining for Safranin O, tuoluidine blue and collagen II. Cells in the samples seemed to confirm that they originated from the labeled BMSCs, as confirmed by *in vivo* fluorescent imaging and immunofluorescence examination. In conclusion, the cartilage ECM-derived porous scaffold shows potential as biomaterial for cartilage tissue engineering, and PKH26 fluorescent labeling and *in vivo* fluorescent imaging can be useful for cell tracking and analyzing cell-scaffold constructs *in vivo*.

(P 6) A Comparative Study on the Effects of Adipose Tissue Derived and Bone Marrow Mesenchymal Stem Cells on Neurons/Glial Cells Viability, Proliferation and Differentiation

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It is known that both Mesenchymal Stem Cells (MSCs) and Adipose-derived Stem Cells (ASCs) are able to ameliorate the CNS condition upon injury. However it is still not clear whether they have the similar or opposite effects on the different CNS derived cell populations. In this sense the objective of the present work was to understand if ASCs and MSCs preferentially act on different CNS derived cell populations. Hippocampal neurons and glial cells were exposed to MSCs and ASCs conditioned media (CM) (obtained 24, 48, 72 and 96 after 3 days of culture of HUCPVCs) for 1 week. Cell viability experiments (MTS test) revealed that CM obtained for both cell populations at all time points did not cause any deleterious effects on neurons and glial cells. Immunocytochemistry and total cell counts revealed that hippocampal cultures incubated with CM displayed higher numbers of neurons (MAP-2 positive cells) when compared to the control. However this effect was higher for ASCs CM (up to 6 fold) when compared to MSCs CM (up to 2 fold). Furthermore it was also observed in glial cell cultures that MSCs CM preferentially stimulated oligodendrocytes (O4) proliferation. Similar effects on hippocampal neurons populations were observed whenever direct contact co-culture systems were used. The work here in presented suggests that ASCs and MSCs release different growth factors. Moreover it was also observed that

ASCs secrete neuroregulary molecules that preferentially promote neuronal differentiation/survival, while MSCs mainly act upon glial cell populations, namely oligodendrocytes.

(P 7) A Fibroblast-Collagen Membrane Composite for Tendon Repair: an *In Vitro* Study

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Tendon repair is a current challenging clinical problem, as tendons have a poor intrinsic healing potential. The aim of this study was to identify an *in vitro* model of collagen membrane seeded with fibroblasts, as a potential tool for improving the biological and biomechanical properties of the repair tissue.

Achilles tendons' specimens were taken from young pigs. Specimens were cut in small pieces, cultured *in vitro*, in order to allow cells to leave the specimens and to reach the confluence. Fibroblasts were then enzymatically isolated, resuspended and expanded since confluence was reached again. The cells were seeded onto membranes of collagen type I and III of 6 mm of diameter at 3 different concentrations. Membranes were cultured *in vitro* at standard culture conditions for 2 and 5 additional weeks, then retrieved from culture for macroscopic, histological and SEM analysis.

Macroscopically, seeded membranes showed shrinkage and reduced biomechanical integrity compared to unseeded membranes. Histological examination demonstrated the presence of vital cells within the membranes with matrix production.

The results from this study demonstrate that swine fibroblasts can be seeded onto a collagen scaffold. These cells remain vital during *in vitro* culture. The shrinkage of the experimental samples was probably due to a enzyme produced by the fibroblasts. Further studies will demonstrate the survival of the cells and the reparative potential of fibroblast transplantation in an orthotopic *in vivo* model. We believe this model could be a valuable tool for tendon lesions, working either as a cell-carrier and a as patch augmentation.

(P 8) A Model for Studying the *In Situ* Lens Epithelial Cell Response To Artificial Lens Replacement

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Lens implantation is a routine procedure in elderly people. The opacified lens is replaced by a non-accommodating artificial lens. New approaches to replace the lens include the use of injectable gels that allow accommodation in presbyopic people. Surgical intervention in the lens capsule and application of gel materials invoke a strong response in lens epithelial cells (LEC). *In vivo*, they proliferate and trans-differentiate towards a cell type with fibrotic properties. This reduces accommodation amplitude and visual clarity.

Models exist *in vivo* and *in vitro* to study effects of treatment of the capsular bag to prevent the LEC response. The aim of this study was to develop a model in which the (prevention of) fibrosis can be studied in the intact lens ex vivo.

Pig eyes were operated upon. The natural lens material was removed, the capsular bag was treated and a silicone-based gel was injected as lens replacement. The entire lens was taken out and incubated in medium for 6 weeks. Then mechanical properties were measured relatively to freshly implanted lenses. The entire lens was fluorescently stained and visualized with CLSM.

Results indicate the formation of fibrotic tissue in untreated lenses. Fibrosis could be reduced by treatment of the capsular bag. Mechanical properties were influenced by the presence of fibrotic tissue. The model therewith serves as an ideal interpolation between *in vitro* and *in vivo* data. It can be used to evaluate new concepts of lens replacements that allow accommodation.

(P 9) A New Scaffold Based on Globular Proteins of Plasma

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Introduction: Several different materials have been employed as scaffolds in Tissue Engineering. In this work we describe the development, *in vitro* studies and preliminary results of grafting a new scaffold based on globular proteins of plasma.

Material and Methods: The scaffold was based on the mixture of plasma globular proteins (albumin) and a crosslinking agent (glutaraldehyde). Albumin was obtained from concentrates and directly from plasma or serum. The resulting mixture was frozen, lyophilised and hydrated. The behaviour of this new scaffold was studied *in vitro* by culturing several mesenchymal cell lines and *in vivo* by experimental grafting.

Results: The structure obtained after lyophilization and hydratation was porous and flexible with pores and septa of different thickness depending on albumin concentration. The scaffold was not toxic for the cells, which were able to take on the surface and express differentiation markers (type II collagen, oil red, Von Kossa ...). *In vitro*, the scaffold was not degraded by the cultured cells. After grafting the scaffold without cells, a progressive degradation without inflammatory reaction was observed. After 3–4 months, the scaffold was completely degraded. When the scaffold was grafted with differentiated chondrocytes, a complete cartilage structure was observed. When the scaffold was grafted with adipocytes, a fat tissue surrounding the scaffold was observed.

Conclusions: Albumin based scaffolds are non-toxic and well-tolerated. Cells are able to adhere and differentiate. This scaffold is easy to obtain, can be used to regenerate mesenchymal tissues and represent a new alternative to other materials for tissue engineering techniques.

(P 10) A Novel Approach for Combining Advanced Optical and Afm Nano Imaging—the Biomaterialsworkstation

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Optical microscopy and atomic force microscopy (AFM) make a powerful combination for the investigation of bio-materials, because the optical overview, fluorescence or spectroscopy information can be combined with the nano-imaging and nano-manipulation capabilities of the AFM. Previously this combination was mainly limited to transparent samples, where the AFM could measure from above simultaneously with an inverted optical microscope from below the sample.

JPK has developed a novel approach to allow the combination of upright microsocpy with AFM on the same sample region, so that non-transparent samples, including ceramic or metallic materials, can be used. A patented calibration procedure ensures that the region of interest can be found and re-imaged with micron precision, so now exactly the same area can be sequentially measured optically and with AFM. The JPK BioMaterialsWorkstation enables the full capabilities of advanced upright optical microscopy, including fluorescence techniques, immersion lenses and confocal laser scanning. This is combined with the AFM for high resolution nano-imaging, elasticity and adhesion measurements, as well as nano-manipulation and lithography. In particular, measurements with high resolution dipping lenses and AFM measurements in liquid become possible on the same sample area.

These capabilities open new possibilities for the investigation of bio-film formation, biocompatible materials, cell growth on scaffolds and many more biomaterials measurements.

(P 11) A Novel Bioreactor Design for Enhanced Stem Cells Proliferation and Differentiation in Tissue Engineered Constructs

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Recent studies have shown that culturing undifferentiated stem cells in appropriate biochemical environments and under mechanical stimulation could provide the correct signals for cellular proliferation, differentiation and subsequent extracellular matrix production. This triggered a growing interest about *in vitro* biomechanically-stimulating culture environments.

The main goal of this work was to evaluate the efficacy of a newly developed bidirectional perfusion bioreactor. This apparatus enables implementing complex culturing programmes. Both perfusion flow rate and direction can be varied along the culturing period. This compact and user-friendly system is made of autoclavable materials enabling to culture up to 20 samples simultaneously. This study aimed at determining the most effective programmes for enhancement of cellular proliferation and differentiation. Fiber-bonded starch polycaprolactone (SPCL) meshes were statically seeded with goat bone marrow cells (GBMCs). These meshes were cultured either statically or under several dynamic culturing programmes for 7 and 14 days. Collected samples were characterized by DNA and alkaline phosphatase (ALP) quantification and scanning electronic microscopy (SEM). DNA quantification results showed a tendency for greater cellular proliferation under static and unidirectional perfusion conditions. ALP activity results revealed an enhanced cellular differentiation rate under cyclic flow direction inversion. SEM results also showed greater cellular adherence and spreading in cell-scaffold hybrid constructs cultured under those conditions. In conclusion, this culture system can be used for enhancing cellular proliferation and differentiation by optimizing the combination of fluid flow rate and flow inversion frequency during culturing periods.

Acknowledgments: European NoE EXPERTISSUES (NMP3-CT-2004-500283), STREP HIPPOCRATES (NMP3-CT-2003-505758) and FCT CellStrain (POCI/V.5/A0059/2005).

(P 12) A Novel Plasma-Based Scaffold for Subcutaneous Islet Transplantation

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Several studies in the last years have shown the insulin independence achieved by intraportal islet transplantation in patients with type I diabetes. However, long-term results have indicated that the site and method of transplantation still need to be optimized to improve islet engraftment. The subcutaneous space has been widely assessed as a simple and safe alternative to the liver. Nevertheless, the sparse vascularization of this space is the main limitation for its use as an islet transplantation site. To overcome this drawback, new strategies have focused on the use of pro-angiogenic growth factors to enhance vascularization and on the use of different biomaterials as islet scaffolds. In this study we used plasma, obtained by directly centrifuging donor's blood, as scaffold, and fibroblasts, isolated from donor's skin biopsies, as the source of pro-angiogenic factors. Cultured fibroblasts were resuspended in plasma containing tranexamid acid. Next, fresh isolated islets were added to the mixture and coagulation was induced by $CaCl_2$, such that pro-coagulant factors such as thrombin were not necessary. Islets embedded in this "plasma-fibroblast gel" were transplanted subcutaneously in STZ-induced diabetic mice and compared with diabetic mice transplanted with free islets beneath the kidney capsule (control) and with normal mice. Our findings indicate that subcutaneous transplantation of pancreatic islets embedded in a plasma-fibroblast gel reverts type I diabetes in mice similarly to the standard procedure. This approach could be a useful and safe option to treat this disease in human clinical practice.

(P 13) A Novel Polysaccharide-Based Porous Scaffold for Cell Delivery Into the Infarcted Heart

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Introduction: Cell therapy is one therapeutic option for ischemic infarcts. However, direct injection into the heart leads to a major loss of cells. We hypothesized that an implantable porous scaffold could improve cell survival and enhance the cell engraftment into the target tissue.

Materials and Methods: Porous polysaccharide-based scaffolds were prepared according to a patented method by cross-linking pullulan and dextran followed by a salt-leaching technique. Bone marrow-derived fluorescent rat mesenchymal stem cells (rMSC) were seeded on 6 mm diameter-scaffolds (106 cells) the day before implantation. In 17 adult rats (Wistar or Lewis), myocardial infarction was induced by ligation of the left coronary artery. Seeded scaffolds were then grafted on top of the infarcted area. Animals were sacrificed at 1 week ($n = 3$) and at 1 month ($n = 14$). Cell distribution was evaluated on tissue sections with fluorescence microscopy.

Results and discussion: After seeding of fluorescently labeled rMSC on porous scaffolds, homogeneous cell distribution within the 3D structure was demonstrated by confocal microscopy. Seeded scaffolds were successfully applied locally onto rat infarcted tissues. Cell transfer from the scaffold into the injured tissue was clearly evidenced with numerous fluorescently labeled cells throughout the infarcted area in both rat autologous and syngenic models.

Conclusion: We described the feasibility of preparing porous polysaccharide-based scaffolds and of implanting the seeded scaffolds on a rat infarcted heart model. This technique of cell delivery resulted in an excellent engraftment of the cells. Future studies will evaluate the therapeutic effects for myocardial infarction of this type of cell therapy.

(P 14) A Novel Rat Mandibular Bone Regeneration Model using Hap/Gemosil® for Rhbmp-2 Delivery

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A new rat mandibular defect model was designed for bone regeneration. Preliminary studies were conducted to determine the effects on bone formation when using a poly(D,L-lactide-co-glycolide) (PLGA) and the hydroxyapatite carrier HAP/Gemosil® (HAPG) loaded with bone morphogenetic protein 2 (rhBMP-2) and biglycan (BGN), an important small leucine-rich repeat proteoglycan. The main goal was to analyze the usefulness of HAP/Gemosil® and these signaling cues in this model of bone regeneration.

Methods: A surgical defect was made in each rat hemimandible. Each defect ($n = 12$) was assigned to one of these groups containing: (1) water (Control); (2) rhBMP-2; (3) BGN; (4) rhBMP-2 and BGN; (5) PLGA; (6) PLGA loaded with rhBMP-2; (7) PLGA with BGN; (8) PLGA with rhBMP-2 and BGN; (9) HAPG; (10) HAPG loaded with rhBMP-2; (11) HAPG with BGN; (12) HAPG with rhBMP-2 and BGN. A double labeling technique was used for epifluorescence microscopy. Specimens were analyzed by microtomography and processed for histology to evaluate bone formation. Results: Defects were filled by more than 37% of their initial volume after 3 weeks, except for carriers only and control groups. HAPG carriers appear to have a more uniform and compact mineralization compared to groups with PLGA. Groups loaded with rhBMP-2 and BGN have an additive effect on the volume of newly formed bone. Conclusions: The use of HAP/Gemosil® loaded with rhBMP-2 and biglycan can potentially constitute a good strategy for bone regeneration, but further increase in sample size is mandatory. This defect model was found practical although needs refinements.

(P 15) A Novel Thermoresponsive Copolymer As a Suitable Substrate for Tissue Reconstruction

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Introduction: For the reconstruction of tissues and organs, thermoresponsive polymers like Poly(N-isopropylacrylamide(NIPAAm)) play an important role in the detachment of cell layers with undamaged cell-cell and cell-ECM interactions. PIPAAm coated surfaces were usually prepared using electron beam irradiation or plasma polymerisation, both of which are not usually available for every researcher. We aimed to prepare a thermoresponsive copolymer that is easy to prepare and favours cell growth. In this study, a copolymer of NIPAAm and Methyl Methacrylate was prepared and its cytocompatibility was assessed.

Methods: Cytotoxicity and cytocompatibility of the copolymer was analysed in comparison to tissue culture polystyrene using cell lines. To demonstrate thermoresponsiveness, both primary and continuous cell lines were detached from copolymer under its lower critical solution temperature. To analyse specific cytocompatibility at molecular level, secretome of a corneal cell line cultured on both substrates is being studied.

Results: Copolymerisation and hydrophilic/hydrophobic transition at different temperatures was confirmed using fourier transform infrared spectroscopy and differential scanning calorimetry, respectively. Tritiated thymidine uptake assay and MTT assay results showed that copolymer favoured cell attachment, growth and proliferation. Actin staining demonstrated good cytoskeletal organisation and subsequent cell spreading on copolymer. The cell sheet detachment from the copolymer confirmed its thermoresponsiveness and its use in creating tissue structures. Secretome analysis revealed unique protein expressions on both substrates.

Conclusion: Our study proved that a copolymer of NIPAAM can be easily prepared with minimum facilities and the good cytocompatibility along with thermoresponsiveness make the copolymer a suitable candidate as a tissue engineering substrate.

(P 16) A Novel Tissue Prefabrication Model in Rabbit using Polyacrylamide Cryogel Scaffolds

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The purpose of this study is to develop a new, productive and reproducible prefabrication model in rabbit and to test the efficiency of this model with a non-degradable polyacrylamide based cryogel scaffold for studying vascularization and new tissue formation. Twelve rabbits were used for this study. Bilateral thoracodorsal and deep circumflex iliac vascular pedicles of the animals were prepared to simulate prefabrication and sandwiched between two hemispheres of the scaffolds with a radius of 1 cm forming a sphere. In each animal, four spheres were implanted on pedicles as study groups and one sphere per animal was implanted at the back as control. The implants were elevated at 2 (group 1), 4 (group 2) and 6 (group 3) weeks on corresponding pedicles and replaced again. After two weeks, vascularization of the constructs, composite of the new tissue formed and inflammatory responses were evaluated by means of direct observation, microangiography, and histology. Four implants in three animals were rejected due to inflammatory response. All other constructs maintained their initial shape and dimensions. Angiogenesis was evident in 4 weeks and 6 weeks groups of experimental constructs with intense neocapillary formation within the scaffolds. 2 week groups of experimental constructs were less vascularized while control constructs remained relatively non-vascularized. The newly formed tissue was mainly composed of granulation tissue with inflammatory components. This study, offering a new prefabrication model with an economical non-biodegradable scaffold, may accelerate and facilitate the future work on vascularization of in-vivo engineered tissues.

(P 17) A Surface-Modified Electrospun Elastomeric Scaffold As a Small Diameter Vascular Graft in a Rat Model

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There remains a great need for vascular substitutes for coronary artery bypass and peripheral vascular applications. Synthetic materials have proven unsuccessful to replace small diameter vessels. The use of an elastomeric biodegradable material that enables in-vivo remodeling could be beneficial to this purpose.

Tubular grafts (1.3 mm internal diameter) were obtained by electrospinning a polyester urethane urea (ePEUU) solution onto a rotating mandrel. The inner surface was modified with poly (2-methacryloyloxyethylphosphorylcholine-co-methacryl acid) (PMA). Non-coated conduits were used as controls. The conduits were implanted as interposition grafts in the abdominal aorta in a rat model (Coated $n = 12$, Non-coated $n = 25$).

Angiograms at 8 weeks showed 91% patency rate in the coated group vs. 40% in the non-coated. No signs of dilation were evidenced in any group up to 24 weeks. Dynamic compliance testing was performed within the physiological range (Day 0: $2.23 \pm 0.40 \times 10^{-4}$ mmHg^{-1}; 4 wks: $1.34 \pm 0.54 \times 10^{-4}$ mmHg^{-1}; 8 wks: 2.22×10^{-4} mmHg^{-1}; Native aorta: $1.36 \pm 0.33 \times 10^{-3}$ mmHg^{-1}). Ultimate tensile stress (Day 0: $1.74 \pm 0.72 \times 10^{7}$ Pa; 4 wks: $5.70 \pm 3.66 \times 10^{6}$ Pa; 8 wks: 3.60×10^{6} Pa; Native aorta $= 4.58 \pm 0.63 \times 10^{6}$ Pa) and the elastic modulus (Day 0: 2.75 ± 0.64 MPa; 4 wks: 0.44 ± 0.17 MPa; 8 wks: 0.54 MPa; Native aorta: 0.24 ± 0.1 MPa) were measured by uniaxial tensile testing. Histology showed formation of an external collagen capsule and an endothelial-like layer at 8 weeks.

Mechanical properties following implantation suggests partial degradation of the polymer resembling the aorta in strength and elasticity. Surface modification with PMA copolymers significantly increases patency rate. These constructs suggest a good balance between strength, biocompatibility and degradability of this vascular graft.

(P 18) A Tissue Engineered Strategy for Meniscus Bonding: an *In Vivo* Study

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The purpose of this work is to create an *in vivo* tissue engineered model for meniscus bonding by using a neocartilaginous tissue produced by isolated swine articular chondrocytes embedded in fibrin glue as biological glue between two meniscal explants.

Swine menisci were harvested under sterile conditions. Samples were devitalized by freeze/thaw cycles and dissected by radial transections. Swine articular chondrocytes were enzimatically isolated and suspended in fibrinogen. The solution was placed onto the flat cross-section of a meniscus specimen. Then, thrombin was added in order to form fibrin glue embedding chondrocytes. Immediately before gel polymerization, a second meniscus specimen was placed atop. The composites were then wrapped in acellular fibrin glue and transplanted in the back of nude mice for 4 weeks. Similar control samples were prepared using acellular fibrin glue also between the meniscal samples. At the end of the experimental time, samples were macroscopically analyzed for bonding and processed for histological evaluation.

Data showed a macroscopic integration of the experimental samples, while the controls did not achieve any bonding. Histology showed cartilage like tissue maturing within the fibrin glue scaffold, able to bond the devitalized meniscal tissues.

The results of this study demostrate that isolated chondrocytes, seeded onto fibrin glue, produce a cartilage-like matrix that integrates with meniscal tissues. This tissue engineered approach could represent a valuable model for further studies on the repair of meniscus lesions.

(P 19) Acceleration of Spinal Fusion with Adipose Tissue Derived Adult Stem Cells

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Posterolateral spinal fusion is a standard treatment for spinal stabilization. This study was designed to test the hypothesis that syngeneic and allogeneic adult adipose tissue-derived stem cells (ASCs) on a biomaterial scaffold accelerate spinal fusion in a rat model. ASCs from male Fischer and ACI rats were loaded onto collagen/tricalcium phosphate blocks and cultured in stromal media for 48 hours. Male Fisher rats were assigned to 4 cohorts ($n = 16$/cohort): 1) No treatment; 2) Scaffold only; 3) Scaffold + syngeneic ASCs; or 4) Scaffold + allogeneic ASCs. Bilateral L4, L5 transverse processes were decorticated. Scaffold blocks with or without ASCs were placed at the L4-L5 intervertebral space in scaffold treatment groups while none were placed in the no treatment cohort. Half of each cohort was harvested 4 or 8 weeks after surgery. Spinal fusion was evaluated with radiographs, microcomputed tomography, and light microscopy. Spines without scaffolds did not develop callus during the study period. There were no significant differences between scaffold cohorts with or without cells 4 weeks after surgery. Callus formation was more highly organized in the ASC cohorts compared to the scaffold alone cohort 8 weeks after surgery. Callus calcification was evident in ASC cohorts but minimal in the scaffold alone cohort 8 weeks after surgery. These results support ASC acceleration of posterior lumbar spinal fusion in a rat model. Both syngeneic and allogeneic ASCs appeared to accelerate the spinal fusion process equally. The use of ASCs to accelerate spinal fusion has potential to significantly reduce morbidity and complications.

(P 20) Acellular Collagen Matrix for Fascial Tissue Reconstruction

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Various synthetic and naturally derived materials have been proposed as possible materials for fascial reconstruction. Acellular collagen matrices have been used experimentally and clinically for several applications, including urethra and bladder reconstruction. We investigated the feasibility of using a naturally derived, nonimmunogenic, biodegradable, collagen-based matrix derived from porcine bladders as an off-the-shelf biomaterial for fascial tissue repair in 3 different tissue systems. To demonstrate the biocompatibility of the biomaterial, cell viability, mitochondrial metabolic activity, and apoptosis assays were performed. In addition, the acellular collagen matrices were used as a sling material for the treatment of incontinence, for the treatment of abdominal hernias, and as penile tunica reconstruction in 24 rabbits. Retrieved tissues were assessed grossly and histologically. The direct cytotoxicity and mitochondrial metabolic activity assays showed that the acellular matrix did not induce significant changes in cell viability when compared with the controls. The apoptotic activity was also minimal. After implantation, the acellular matrices remained intact at their respective implantation sites and demonstrated maintenance of fascial tissue function. At the time of retrieval there was no evidence of inflammation or infection and minimal adhesion was noted. The biomechanical properties of the collagen matrices were similar to the normal controls. Histologically, the matrix structure was intact, demonstrating the longitudinal collagen bundles. There was only a minimal inflammatory response which gradually decreased over time. The cell density decreased over time and a shift from dominance of lymphocytes to dominance of fibroblasts was observed.

(P 21) Adhesion Strength of Human M[illegible]senchymal Stem Cells on Biodegradable Polymers for Ti[illegible]generation

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Abstract Withdrawn

Current tissue engin[illegible]ng approaches utilize mesenchymal stem cells grown on biomaterials to promote bone regeneration, however it is unknown how the strength of MSC-substrate interactions influences osteoblast differentiation. In this study, we have correlated polymer surface properties with MSC adhesion and differentiation. Methods: Immortalized human MSC were seeded for 1 hr on polymer-cast glass slides. The slides were then inserted into a parallel plate fluid shear device and subjected to a shear stress of 0-25 Pa for 30 cycles. Cell numbers were quantified and the shear required for 50% cellular detachment (tau 50) was determined. To create films, poly-lactide-co-glycolide and polycaprolactone were dissolved in chloroform and cast on glass plates. Alginate was made by crosslinking alginic acid with a $CaCO_3$-GDL acid combination and cast into cell assemblies. PLGA and PCL films were cut and fit into assemblies. Contact angles of polymers were measured using a sessile drop method and bulk elastic modulus was determined using confined compression force measurements.

Results: Shear loading cells on the various polymer films resulted in tau 50 of <1 to 25Pa, where cells on high-molecular weight PLGA demonstrated the greatest adhesion strength. A positive correlation was found comparing elastic modulus and contact angle with adhesion strength of MSC. Both material stiffness and surface chemistry affect the adhesion strength of MSC to the polymers. Preliminary studies suggest an inverse relationship between osteogenic differentiation and adhesion strength.

(P 22) Adipose Tissue Engineering using Adipose-Derived Stem Cells and Fibrin

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The treatment of soft tissue defects still poses a clinical problem in reconstructive medicine e.g. after surgical trauma. Adipose-derived stem cells have already been used for several tissue engineering approaches including adipose tissue engineering. Fibrin has already been reported to be a suitable matrix to support several cell types. This study evaluates the influence of fibrin composition on the formation of an adipose tissue-equivalent *in vitro* using adipose-derived stem cells (ASC). Undifferentiated ASC were mixed with thrombin and added to the fibrinogen component. Subsequently, fibrin constructs were either cultivated in control or adipogenic induction medium and weekly analyzed. The influence of fibrinogen concentration on fibrin structure was evaluated by scanning electron microscopy, its influence on cell viability was demonstrated by CalceinAM/DAPI staining and quantified by lactate-dehydrogenase (LDH) measurement. Adipogenic conversion was demonstrated by Oil Red O staining after cryosectioning, by leptin ELISA and by quantitative RT-PCR for the adipogenic markers PPAR-α, FABP-4 and leptin. Fibrin propagates ASC growth and adipogenic differentiation. The highest cell density was observed when 50 mg/mL fibrinogen were used whereas adipogenic conversion was more pronounced when lower fibrinogen concentrations were applied. Interestingly, adding ASC to the fibrin matrix led to a stabilization of the construct by influencing the structure. Combining fibrin and ASC is a suitable approach for soft tissue regeneration. The fibrinogen concentration has an influence on matrix structure, cell morphology and density. However, adipogenic differentiation is also affected by donor variability.

Acknowledgments: HIPPOCRATES (NMP3-CT-2003-505758), Lorenz Boehler Fonds, EXPERTISSUES (NMP3-CT-2004-500283).

(P 23) All-Trans Retinoic Acid Release From Biodegradable Polyester Microcapsules

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Tissue engineering strategies frequently include a scaffold, living cells and bioactive substances to promote cell growth and guide cell differentiation. The encapsulation in carriers enables protects the bioactivity of those substances upon implantation, avoids being transported by the body fluids and also allows controlling the release rate. Encapsulation materials are preferably biodegradable polymers. In this work, all-trans retinoic acid (atRA) was used as a model bioactive agent; since it was shown that atRA enhances the expression of osteocalcin, a specific osteogenic marker.

atRA was encapsulated into polyester microcapsules by a water/oil/water double emulsion/solvent evaporation technique. The polyesters used in this study are poly(butylene succinate) (PBS), poly(butylene succinate-co-adipate) (PBSA) and poly(butylene terephthalate-co-adipate) (PBTA). The kinetics of atRA release was studied in phosphate buffered saline solution of pH 7.4 at 37°C under orbital shaking.

Encapsulation efficiencies (EE) of 94.0%, 95.4% and 95.8% were obtained for PBS, PBSA and PBTA, respectively. The high EE is probably due to the low water solubility of atRA which is entrapped in the organic phase and does not dissolve into the aqueous phase.

atRA was released from PBS and PBSA steadily with an average release of 12.6% and 9.9%, respectively, after 4 weeks. Release from PBTA showed a burst of 12.8% within the first 24 hours, followed by a slower release stage, reaching 34.0% after 4 weeks.

The results obtained in this study demonstrate the interest of this strategy and show the potential of the proposed microcapsules as carriers for the controlled release of bioactive agents.

(P 24) Altered Neural Function on the Muscle Growth: Implication for the Muscle Generation

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Changes in muscular ubiquitin gene and protein expression in accordance with nerve injury and regeneration process were evaluated.

Sciatic nerve of 12 week old SD rat was induced nerve paralysis. Gastrocnemius muscle of both intact and injured lower extremity were harvested at 5, 10, 20 and 40 days post injury. Contractility and wet weight of the gastrocnemius muscle was measured and compared with the normal muscle. RT-PCR was done for ubiquitin gene detection, and ubiqutin protein expression in the harvested tissue was also checked by immunohistochemisty for the evaluation of expression pattern as the time sequence.

The muscle weight decreased starting the 10th day and was at the lowest at 20th day. A recovery in weight was evident starting 40th day. Pre-harvest degree of muscle contraction showed no changes in the first 5 days. At 10th day, partial recovery was noted and at 20th day, same degree of contraction was noted compared to the normal muscle. Degree of ubiquitin expression from the extracted mRNA showed most activity at the 10th day. At 40th day, the degree of expression was about the same as the normal muscle. The degree increased starting the 10th day and at 20th day, much ubiquitin stained myofibers emerged and the recovery was noted at the 40th day.

Ubiquitin proteolysis seems to be involved in muscle wasting in the reversible nerve injury. It was observed that this particular gene and protein increased in expression during a muscle wasting process.

(P 25) Alveolar Bone Regeneration in a Gingivoperiosteoplasty Model

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Introduction: Primary alveolar cleft repair treating congenital alveolar clefts with gingivoperiosteoplasty (GPP) has a 60% success rate. The remaining 40% have persistent alveolar defects requiring secondary grafting, potentially complicated by donor-site morbidity, graft resorption, and infection. Herein, we describe a novel critical-size defect GPP model using tissue-engineered scaffolds to improve bone healing and circumvent secondary grafting.

Methods: A 7×4×3-mm complete alveolar defect was surgically created in sixty 8-week old rats. Four scaffolds were tested within the defect: absorbable collagen sponge (ACS), ACS+BMP, hydroxyapatite-tricalcium phosphate (HA-TCP), HA-TCP+BMP, and no scaffold as control. Animals were sacrificed at post-operative weeks 4, 8, and 12. New bone was assessed histmorphometrically via micro CT. Decalcified sections were processed for histology.

Results: Negative control groups grew to $43 \pm 10\%$ at 4 weeks, but plateaued to $53 \pm 17\%$ at 8 weeks. ACS groups showed $50 \pm 12\%$ at 4 weeks and $79 \pm 19\%$ at 8 weeks. ACS + BMP showed $49 \pm 4\%$ and $71 \pm 13\%$, respectively. HA-TCP displayed $69 \pm 20\%$ bone growth at 4 wks and plateaued to $86 \pm 6\%$ ($p < 0.05$) at 8 wks, while adding BMP yielded $55 \pm 29\%$ and $91 \pm 5\%$ ($p < 0.05$) at 4 and 8 weeks. 12-week samples were similar to 8-weeks preliminarily. Histologically, HA-TCP groups stimulated appositional bone growth around scaffold granules.

Conclusions: HA-TCP shows greater osteogenic capability than ACS. BMP did not affect significant bone growth increases, perhaps due to lack of a sustained release system. Inherent osteoconductive properties of HA-TCP and its ability to provide for tissue guided regeneration (as opposed to an absorbable matrix) may have contributed to defect healing.

(P 26) Amniotic Epithelial Cells Have Liver-Specific Function of Ammonia Removal and Urea Synthesis

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Recently, under hepatogenic condition, they expressed most of the liver-specific proteins including HNF3-gamma, albumin, alpha-fetoprotein, alpha1-antitrypsin, transthyretin, glutamine synthase, CYP2C9, CYP2D6, CYP3A4, CK18, PEPCK. However, they did not express important genes involved in the urea cycle and gluconeogenesis despite of the presence of stimulating factors such as hepatocyte growth factor, fibroblast growth factor-2, oncostatin M, dexamethasone.

In this study, we tried to differentiate them into hepatocyte-like cells with urea synthesis activity using media modification. During culture, AECs could synthesize urea and the quantity increased with culture time. Also, urea synthesis significantly increased after treatment of 1 mM ammnonia. So that, they had ammonia removal activity. They also expressed cytochrome P1A1/2 activity in the presence of its inducer, 3-MC. This pattern in cell culture was similar to that in tissue culture.

This result shows that their liver-specific functions deeply correlate to the *in vitro* culture condition, and this fact can explain the uncertainty of their hepatogenic differentiation capacity arising from previous results.

(P 27) An Emulsion Preparation for Novel Micro-Porous Polymeric Hemi-Shells for Use in Tissue Engineering and Drug Delivery

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A modified oil-in-water (O/W) emulsion process was developed to produce novel micro-porous hemi-spherical polycaprolactone (PCL) micro-particles called ''hemi-shells''. By addition of a porogen such as sodium bicarbonate ($NaHCO_3$) into the PCL-dichloromethane (DCM) oil phase and emulsification in an acidic polyvinyl alcohol (PVA) aqueous phase, micro-porous hemi-shells formed as solvent evaporated. CO_2 gas evolution from the porogen created particles with an externally micro-porous shell containing a central opening with a large internal cavity. The hemi-shells were characterized by SEM and optical microscopy. The number-average particle yield in the 50-200 μm range was 84%. The number-average hemi-shell yield in the same size range was 41%. These novel hemi-shells could potentially be applied in tissue bulking and regeneration of sphincter muscles in the treatment of reflux, and incontinence. Other potential applications include cosmetic dermatology and drug delivery.

(P 28) An Innovative Vibrating System To Increase the Muscle Growth in Newborn Mice

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The aim of our study is to investigate the relationship between high frequency, low amplitude vibrations on the growth of muscolar fibers in newborn mice. From our previous studies on *in vitro* cells, we observed that the frequency of 30 Hz increases proliferation and differentiation of the cells. So we designed and realized an ad-hoc bioreactor, that can produce vibrations between 1 and 120 Hz and with a sensoring system composed by an accelerometer in order to control the stability of the imposed signals. We treated 10 newborn mice and we used other 10 newborn mice as a control. We divided the ten treated mice in 5 groups and we treated them one hour a day for 1, 2, 3, 4 and 5 weeks, respectively. We sacrificed two mice a week from the control group and other two from the treated group. We took a sample of tibial muscle and one from quadricep muscle from every group. We analyzed the morphometry of all the samples and we compared the perimeter and the area of the muscolar fibers get from the treated groups and the ones get from the control group. We used the statistical test ANOVA-2 to evaluate the confidence of our data. The results showed a strong differences between treated and controls. For instance the mice that received two weeks of treatment showed a higher number of muscolar fibers (48% more than the controls, with a $p < 0.001$) and their fibers have an area 40% less than the control.

(P 29) Angiogenic Potential of the Early Fracture Haematoma Is Increased By Mechanical Stimulation

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Mechanical stimulation and angiogenesis play important roles in the bone healing process. The increased secretion of matrix metalloproteases (MMPs) and paracrine stimulation of angiogenesis by mechanical loading of mesenchymal stem cells have been described earlier. However, the exact interaction of angiogenesis and mechanical loading remains unclear. This study aimed to investigate the angiogenic potential of the human fracture haematoma itself and how this changes with mechanical stimulation, as well as identify molecules potentially involved.

Fracture haematomas were isolated during surgery max. 24 hours post-trauma. They were embedded in a fibrin matrix and mechanically stimulated in a bioreactor simulating the *in vivo*-conditions in the early phase of bone healing (3 days, 1 Hz, 10 kPa).

Both MMP-2 active and proform and MMP-9 proform were detected in haematomas by gelatine zymography. *In vitro* angiogenesis assays of endothelial cells (HMEC-1) showed increased tube formation when stimulated with conditioned medium from mechanically stimulated haematoma. Proliferation of HMEC-1 cells stimulated by haematoma suggests the presence of stimulatory molecules.

In summary, extracellular, pro-angiogenic MMPs are present in the early human fracture haematoma and mechanical loading appears to enhance its pro-angiogenic potential. Their regulation could be a bridge between mechanical stimulation and angiogenesis. Such link would open new therapeutic strategies in bone healing process by stimulating blood vessels formation.

(P 30) Apoptotic Induction in Primary Cell Cultures of Human Umbilical Vein Endothelial Cells (Huvec)

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Introduction: Previous studies carried out by our research group demonstrated that primary cells cultures of human umbilical vein endothelial cells (HUVEC) could display lower cell viability than cells corresponding to the second and third cell passages. However, the mechanisms associated to these findings are unknown. In this work, we carried out a comprehensive gene expression analysis in primary cultures of HUVEC to quantify the expression of genes associated to apoptosis induction or inhibition.

Materials and Methods: Primary HUVEC cultures were isolated from human umbilical cords using collagenase I and cultured in M-199 medium with 10% fetal calf serum and endothelial growth factor. Total RNA was isolated from 3 different subconfluent primary cell cultures and hybridized to Affymetrix U133 plus oligonucleotide microarrays. Genes with a role in apoptosis were selected, and genes whose expression was present in all samples analyzed were considered overexpressed.

Results and Discussion: Primary HUVEC cultures showed high expression of a number of genes related to cell apoptosis, including BCL2A1, BCL2L12, BNIP2, CASP1, CASP6, CASP7, CASP9, CASP12, FIS1, IER3, NCKAP1, PDCD10, PDCD5, PHLDA1, TNFAIP8, TNFRSF1 and TNFRSF21, for example. Expression of several genes with a role in inhibition of apoptosis were expressed in HUVEC cells, including, among others, API5, AVEN, BIRC4, CIAPIN1, FAIM, FKSG2, RTKN, TEGT. These results imply that decreased cell viability found in primary HUVEC could be related to a balance between apoptosis inductor and inhibitor gene pathways.

Supported by FIS PI061784.

(P 31) Application of Confocal Laser Scanning Microscopy to Estimate Spatial Cell Distribution in Cultured Tissues

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This clinical advantage of cultured tissues should be ensured by its quality control (QC) in manufacturing. Many techniques for the QC were developed mainly for safety of cultured tissues. From a viewpoint of engineering, therefore, the strategic construction to estimate the QC for efficacy is inevitable. We have focused on the tool development for spatial estimation of cells in cultured tissues.

The cultured cartilages of chondrocytes embedded in collagen gel were prepared to estimate the spatial cell densities as well as size of cell aggregation by double staining of whole nuclei and living cells. The stained cultured cartilages were set on a confocal laser scanning microscope to capture three-dimensional images. The data analysis based on the image processing revealed that the spatial heterogeneity in cell distribution occurred due to cell aggregation in the gel during culture.

This imaging technique was applied to estimate the proliferative cells in myoblast sheets. The multilayer sheets of human myoblast cells were prepared to estimate the densities of total and proliferative cells by staining of whole nuclei and proliferative nuclei, respectively. We tried to obtain the time profile of myoblast sheet properties. The frequency of proliferative cells decreased sharply, being less than 20% within 1 day. However, the frequency was maintained to be about 10% up to 2 weeks, while keeping a steady state of sheets. This suggests that the frequency of proliferative cells is one of the important indicators to evaluate the QC for efficacy of cultured sheets.

(P 32) Application of Plasma Treatment in the Optimisation of Tissue Engineering using Ceramic Scaffolds

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Introduction: The aim of this study was to determine whether or not plasma treatments increased the wettability of porous ceramic scaffolds, and then to investigate seeding with mesenchymal stem cells.

Materials and Methods: Hydroxyapatite (HA) scaffolds were placed into 3 groups; non-treated, H_2O plasma treated and O_2 plasma treated. Water contact angles ($n = 4$), dye penetration ($n = 5$) and cell penetration ($n = 3$) were all investigated, and X-ray photoelectron spectroscopy (XPS) was used to detect surface changes.

Results and Discussion: Plasma treatment of dense hydroxyapatite reduced contact angles from 16° to a value too low to be detected. Likewise, plasma treatment of porous hydroxyapatite dramatically increased the rate of dye penetration. Studies of the

effects of plasma treatment on mesenchymal stem cell penetration were inconclusive. XPS analysis showed that there was little difference between treated and non-treated materials.

Conclusion: It was concluded that plasma treatment, using both H_2O and O_2 sources, clearly improves the wettability of both dense and porous hydroxyapatite. This was demonstrated by the reduction of contact angles and the increase in dye penetration in treated vs. non-treated HA.

Acknowledgements: The author gratefully acknowledges funding of a CASE studentship from EPSRC and Ceramisys Ltd.

(P 33) Application of PLGA/Keratin Scaffold for Tissue Engineered Articular Cartilage

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Articular cartilage that is difficult to recovery when damaged needs to tissue engineering. Keratins are the intermediate filament proteins that form a dense meshwork of filaments throughout the cytoplasm of epithelial cells. Keratins are generally expressed in particular pairs of type I and type II keratin proteins in a tissue-specific and cellular differentiation-specific manner. In this study, we are developing an alternative approach that consists of generating chondrocytes anchored to poly(L-lactide-co-glycolide) (PLGA) scaffolds impregnated keratin(PLGA/Keratin) using tissue-engineering principles.

We prepared PLGA and PLGA/Keratin scaffolds using solvent casting/salt leaching method. Chondrocytes were isolated from the articular cartilage of New Zealand white rabbit and cultured with DMEM/Ham's F-12 supplemented with 10 % FBS, 1 % penicillin streptomycin, 200 mM L-glutamin, 50μg/ml of ascorbic acid and 15 mM HEPES buffer 1M. Cell proliferation activity was measured at 1, 3, 7, 14 and 21 days by MTT assay. Morphological observation, histology, biological assay for collagen and sGAG, and PCR were performed at each time point 1, 2 and 3 weeks.

The cell viability and the quantity of collagen and sGAG were better PLGA/Keratin scaffolds than PLGA scaffolds. Specific mRNA, collagen type II and aggrecan, for chondrocyte expressed significantly highly in PLGA/keratin scaffold while dedifferentiation marker, collagen type X, did not appear.

PLGA/Keratin scaffold promotes *in vitro* chondrocyte of rabbit articular chondrocytes. This study suggests that PLGA/Keratin scaffold may serve as a potential cell delivery vehicle and a structural basis for *in vitro* tissue engineered articular cartilage.

Acknowledgements: This research was supported by KMOHW (0405-BO01-0204-0006) and SCRC(SC3100).

(P 34) Articular Rehabilitation Controlled By Image Processing-Study on Animals

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The paper presents the investigation strategy in a study developed on rabbits, aimed towards the rehabilitation of the knee joint using bone marrow derived mesenchymal stem cells (MSCs) combined to a scaffold made from resorbable polyglycolic acid felt and sodium-hyaluronat. The cells were previously *in vitro* expanded. After two passages MSCs were seeded on to the scaffold.

A set of 10 rabbits were scanned in two cases: healthy and having an induced joint trauma. The engineering approach was directed to the 3D reconstruction of the articulation area starting from a set of slides taken on a CT. The 3D reconstructed area was generated with special software. The reconstruction software can separate different tissues and build separate masks for the investigated area. The investigation revealed the implant area and defined the shape of the scaffold. It also defined the cutting planes for the implantation process. After the CT investigations and the 3D reconstructions the subjects were submitted to surgery and implanted the scaffolds having pre-determined shape. The actual study will continue with the observation of the subjects during the rehabilitation process.

(P 35) Artificial Vascular Grafts Out of Flexible, Biocompatible Photopolymers

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Cardiovascular disease is one of the leading causes of mortality in the western hemisphere. Autologous vessels are the preferred material to reconstruct vessel segments with diameters less than 5 mm but the number of appropriate grafts is limited due to insufficient native vessels or previous harvest. So the demand for prosthetic substitutes is a continuing concern in coronary and peripheral vascular surgery. Currently available synthetic conduits, like Dacron or ePTFE show generally excellent long-term results for large-caliber arterial reconstruction (aorta, iliac vessels) but when used for small diameter.

We aim at developing suitable blood vessel substitutes out of biocompatible photopolymers which can be printed by rapid prototyping that permits the production of compliant scaffolds that mimic the structure of the extracellular matrix.

A typical resin formulation consists of reactive diluents, special co-monomers and cross-linkers that determine the degradation behaviour. Test specimens were produced by UV-curing and biocompatibility was evaluated by in-vitro cell culture tests using human endothelial cells.

By using appropriate reactive diluents and crosslinkers photopolymerizable biopolymers with elastomer-like behaviour similar to natural blood vessels (E-modulus 0.5 MPa; strain at break 130%) were developed. Endothelial cell attachment and growth was easily achieved without pre-coating the elastomer surface.

The resin systems developed are highly reactive and thus suitable for rapid prototyping of small diameter vascular grafts. The newly developed graft materials show excellent mechanical properties and favor both spontaneous attachment and proliferation of human endothelial cells.

(P 36) Autologous MSCS and Osteoprogenitor Cells for Treatment of Non-Unions

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Non-unions are one of the most severe complications in bone healing. The aim of this study was to examine the therapeutic potential of locally transplanted MSCs or osteoprogenitor cells (OPCs) in non-unions. 76 rats were investigated histologically at day 14, except the control group (C-group), and biomechanically at day 56. Autologous MSCs were cultured in DMEM or osteogenic medium. A femoral osteotomy was stabilized with an external fixator. Except for the C group, a non-union was induced by cauterization of the periosteum and bone marrow removal. Two days later, these animals received an injection of DMEM in the gap containing MSCs (MSC-group), OPCs (OPC-group) or no cells (NU-group). Histology at day 14 demonstrated no bony bridging of the gap. The MSC- and OPC-group showed a significantly larger cartilage area ($p \leq 0.03$) compared to the NU-group. Immunostaining revealed the greatest collagen type II area in the OPC-group. At day 56 the OPC-group showed a higher torsional stiffness than the NU-group ($p = 0.02$). Compared to the C-group the torsional stiffness of the NU-and both cell-groups was significantly lower ($p < 0.01$). Locally applied MSCs and OPCs slightly improved the healing in this model. The MSCs were less effective compared to the OPCs. The less than expected healing improvement of both cell treatments may be related to an unfavourable microenvironment at the application time. An explanation for the superior outcome of the OPCs might be that the OPCs may be protected by macroscopically visible matrix at the transplantation time point.

(P 37) Behaviour of Encapsulated Vascular Smooth Muscle Cells and Endothelial Cell

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PVA hydrogels are good candidates for vascular tissue engineering because of their soft tissue-like mechanical properties. However, they are highly hydrophilic which hinders cell attachment. In this study, PVA-based composite hydrogels which contain cell adhesion promoting macromolecules (Chitosan, Starch, Gelatin) were produced via a two step gelation technique composed of a freeze/thaw cycle and coagulation bath treatment. Physical characterization of the hydrogels were done by, water content, contact angle, percentage shrinkage, protein adsorption measurements, degradation and tensile strength tests, SEM and phase contrast microscopy. Hydrogels were seeded with Bovine vascular endothelial and smooth muscle cells and cell attachment and proliferation was assessed by DAPI, FITC-Phalloidin staining and Alamar Blue assay. Also smooth muscle cells were encapsulated within the hydrogels during one step freeze-thaw gelation in the presence of cryopreservatives and encapsulation parameters (Viscosity, cryopreservative concentration, gel stability) were determined. Presence of the additives did not compromise the mechanical properties. Coagulation bath treatment signifincatly improved tensile strength and resistance to degradation. Both endothelial and smooth muscle cells attached and proliferated on the hydrogels, with slightly better results on PVA/Gelatin hydrogels. Encapsulation was more successful in PVA/Gelatin hydrogels too. Our results showed that, PVA based hydrogels can be utilized for co-culturing of endothelial and smooth muscle cells for imitation of thin arteries (500 μm).

(P 38) Behaviour of Osteoblastic-Like MG-63 Cells Cultured with Hydroxyapatite and Biphasic (Hap/β-Tcp) Porous Granules

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Hydroxyapatite (HAP) and β-tricalcium phosphate (β-TCP) have high potential for bone replacement, particularly HAP and β-TCP composites which combine the bioactivity of HAP with β-TCP reabsorbability. The main motivation of the present study was to investigate the possible impact of newly developed biphasic granules as compared to HAP granules on osteoblastic cell viability and proliferation.

Porous granules with a donut shape were obtained by spray drying hydroxyapatite suspensions. Heat treatment at 800°C of spray dried granules (SGD) produced biphasic granules (HSDG) composed by β-TCP and HAP. MG-63 osteoblastic cells were cultured in the presence of the two types of granules (50 μ/ml −5 mg/ml) for 3 and 6 days, and the system was evaluated for cell viability/proliferation, cell morphology, particle internalisation and cell-induced alterations on the granules. MTS assay showed a dose-dependent effect of the granules on cell viability/proliferation, with a stimulatory effect at low concentrations and deleterious effects at higher levels. Optical microscopy, SEM and EDS X-ray mapping showed a significant internalisation of the particles and at high particle concentration, a trend for increased cytoplasmic volume. Compared to SDG, HSDG caused higher positive effects on cell growth, at 50 μ/ml, and presented lower cytotoxicity at high levels. In addition, at 6 days of culture, the HSDG granules exhibited a new morphology. In conclusion, SDG and HSDG are internalised by growing MG-63 cells, while promoting cell proliferation at selected concentrations. The two materials exhibit differences regarding cell growth rate and, also, culture-induced alterations on the granules morphology.

(P 39) Behaviour of SAOS-2 Cells and Human Periosteum Derived Progenitor Cells in Fibrin Gels

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Both *in vitro* and *in vivo* studies have shown that fibrin sealants provide a promising carrier for bone engineering. The present study compares viability, proliferation and osteogenic differentiation of human osteoblast-like cells (SaOS-2) and Human Periosteum Derived Progenitor Cells (HPDCs) within a fibrin hydrogel.

Fibrin carriers (17 mg/ml fibrinogen, 1 U/ml thrombin; Tisseel VH SD) were prepared with a cell density of 1 million cells/ml. One or two days after construct preparation, growth medium was replaced with osteogenic medium supplemented with/without antifibrinolytic agents. Subsequently, cell-gel constructs were cultured *in vitro*. Cell behaviour was characterized by live/dead staining, DNA quantification, RT-qPCR and histology.

HPDCs seeded fibrin gels degraded faster compared to SaOS-2 seeded gels. Initially, cells were distributed homogeneously throughout the carrier. After 28 days, proliferating SaOS-2 cells had formed a thick cell layer on the surface of the gels. In the center of the carrier substantial cell death was observed, while surviving cells clustered. In contrast, HPDCs remained viable throughout the whole scaffold (average viability of 76%) and formed a thin cell layer on the gel surface. Their total number per gel remained unchanged. Interestingly, HPDCs secreted extracellular matrix and remodeled the inner fibrin matrix in the absence of antifibrinolytics. However, HPDCs did not differentiate into the osteoblastic lineage as indicated by gene expression and histology.

This study illustrates that SaOS-2 cells and primary HPDCs behave differently in a fibrin gel. The use of fibrin gels to support *in vitro* osteogenic differentiation of HPDCs remains to be demonstrated.

(P 40) Behind Functional Scaffold: Biomimetic Concept

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Many tissues are anatomically separated from neighboring tissues/organs often by means of non-specific tissue such as fascia, and other tissues merge into neighbouring tissues. Attachment of the two tissues/organs by such "connector" tissues in the form of gradient property could generates a new physiological function that is lost when the connection between the two tissues/organs is severed.

This paper report a biomimetic diffusion method for the fabrication of compositionally and structurally graded collagen/nano-hydroxyapatite (HA) composite scaffold. The method is diffusion based and produce the precipitation of nano-HA crystallites *in situ*. A collagen scaffold acts as a membrane through which calcium ions (Ca^{2+}) and phosphate ions (PO_4^{3-}) diffusion and precipitate a non-stoichiometric hydroxyapatite. It was observed that prism needle-like nano-HA crystallites (about $2\times2\times20$ nm) precipitated in the interior of collagen scaffold onto the fibrils. Chemical and microstructure analysis revealed a gradient of the Ca to P ratio across the width the scaffold membrane. This lead to the formation of a Ca-rich side and a Ca-depleted side of scaffold. The Ca-rich side featured low porosity and agglomerates of the nano-HA crystallites; while Ca-depleted side featured higher porosity and nano-HA crystallites integrated with collagen fibrils to form a porous network structure. The reported graded scaffold has potential applications in co-culture of tissues and would lead to better understanding of cellular requirements for regeneration of bone.

(P 41) Bioactive Poly(ethylene Glycol) Hydrogels for Modulation of the Smooth Muscle Cell Phenotype

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The formation of scar tissue due to dedifferentiation of smooth muscle cells (SMCs) is one of the major issues faced when engineering bladder tissue. Furthermore, cell sources for regenerating the SMC layer are limiting. Here we explore whether human mesenchymal stem cells (MCSs), cultured in enzymatically degradable poly(ethylene glycol) (PEG) hydrogel scaffolds can be differentiated into SMC-like cells. Synthetic hydrogels are formed by the reaction of cystein-containing adhesion peptides and matrix metallo-proteinase (MMP) sensitive peptides to four-armed PEG-Vinyl Sulfone. Cells are mixed within the gel, attach and are able to migrate within the scaffolds by degrading the matrix via secreted MMPs. As opposed to cultures on traditional tissue culture plastic, we found that MSCs cultured within the PEG hydrogel scaffolds upregulate SMC specific markers, such as a-smooth muscle actin and myosin. MSCs also down-regulate $\alpha5$ integrin and THY-1, that are associated with a synthetic SMC phenotype. Furthermore, we show that MSCs cultured in the PEG hydrogels are able to proliferate and express matrix metalloproteinases for up to 21 d in culture, the full duration of the study. This study addresses the importance of the cellular microenvironment on cell fate, and proposes synthetic instructive biomaterials as a means to direct cell differentiation and circumvent scar tissue formation during bladder reconstruction.

(P 42) Bioactive Polymeric Scaffolds and Nanoformulates in Pain Management and Tissue Engineering

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Today pain management and tissue engineering are two important medical issues. Pain control problems include inefficacy of conventional drugs, serious side effects and tolerance. Naturally derived hydrogels have frequently been used in drug delivery technology and tissue engineering, due to their good biocompatibility and similarity to the natural extracellular matrix.

In this work commercial alginate and ulvan, an alginic type material obtained from renewable resources, were investigated for applications in pain management and neural/hepatic tissue engineering. The undertaken strategy consists in the set up of a polymeric system able to sustain viable and metabolically active PC-12 cell line, which retains chromaffin cell characteristic such as the synthesis and secrection of endogenous opioids, in order to combine a good pain control with reduced side effects.

Alginate beads, loaded with PC-12 were prepared using microprecipitation technique. Cells loaded into alginate beads showed a higher proliferation in respect to controls, representing a good model of bioactive scaffolds. Ongoing investigations are devoted to the identification and quantification of secreted morphine, by means of HPLC.

However, ionically crosslinked alginate has limited mechanical stability under physiological conditions. For this reason, both polymers were functionalized with phenol moieties and successfully processed into enzymatically crosslinked hydrogels by a peroxidase-catalyzed oxidative coupling, obtaining an enhanced stability in physiological environment.

Preliminary results indicate that these promising hydrogels can be employed for both neural tissue regeneration, using SK-N-SH neuroblastoma cell line, opportunely induced to differentiate toward a neuronal phenotype, and for bio-artificial liver support systems, using HepG2 hepatoblastoma cell line.

(P 43) Biocompatibility and Degradation of Elastomeric Nanomaterials for Soft Tissue Engineering

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Nanometric constructs, containing inorganic fillers in different polymer matrices are becoming an emerging type of biomaterials for soft tissue regeneration. On the other hand, elastomeric polymers derived from monomers from renewable resources are becoming very important class of materials. Recently, we produced an elastomeric polymer matrices, which combined with nanometer-size ceramic particles showed various interesting mechanical properties and degradation profile. In this work, we present the results of evaluation of the degradation process *in vitro* and tissue response after implantation of polymer/ceramic materials into soft tissue.

Material composed of poly(ethylene terephthalate) hard segments and dilinoleic acid sequences in soft segments was synthesized in a presence of TiO_2 nanoparticles. Polymer/ceramic materials were implanted into muscles of Wistar rats weighing 200–250 g. After implantation cellular detail, morphology was investigated by light microscopy. Observations were compared with those obtained from polymer without TiO_2. Medical grade poly(dimethylsiloxane) was used as a control.

In vitro degradation studies showed that TiO_2 nanoparticles demonstrated a strong influence on water absorption, crystallinity and molecular weight of nanocomposites. The decrease in Mn after 6 months was 69% for the neat copolymer, whereas 41% was found for the nanocomposite, thus suggesting that nanoparticles indeed contributes to the nanoreinforcing effect. The predominant cell type found after long term implantation were fibroblasts. Lymphocytes were in smaller amount as compared to silicone control.

This work has been financed from the research project DWM/18/POL/2005.

(P 44) Biocompatibility of an Acellular Porcine Meniscal Scaffold

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Background: Meniscal damage is associated with detrimental changes in the knee which may lead to osteoarthritis. There is a need for meniscal replacements which can provide long-term chondroprotection. An acellular porcine meniscal scaffold (APMS) has been previously developed which has the potential to provide an acceptable solution. The aims of this study were to further assess the biocompatibility of the APMS through studies of cell attachment *in vitro* and implantation of APMS tissue in mice.

Methods: Decellularization was performed by subjecting tissue to freeze thaw cycles in combination with sequential incubation in hypotonic buffer/0.1% (w/v) sodium dodecyl sulphate. Cell attachment was investigated *in vitro* using primary porcine meniscal cells (PMMCs) and primary human dermal fibroblasts (PHDFs) cultured on APMS. Cell attachment and morphology was determined by SEM and histology following 7 day culture. The APMS and fresh tissue were implanted subcutaneously in GTKO mice and explanted at 3 months for histological and immunohistochemical analysis to determine any immunological reaction.

Results and conclusion: Both cell types readily attached to the APMS following 7 day culture. Cells showed no signs of cytotoxity and took on a flattened appearance. In the case of PHDFs, cells were shown to infiltrate the scaffold. Analysis of mouse explants revealed a thin capsule surrounding the APMS tissue. Cell phenotyping showed no evidence of a specific immune response. Macrophages, endothelial and fibroblast-like cell infiltrated the tissue indicating a possible remodelling response. This study confirmed the excellent potential of the APMS for use as a tissue engineered meniscal replacement.

(P 45) Biocompatibility, Osteo-Compatibility and Mechanical Evaluations of Novel PLDLLA/TcP Scaffolds

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Composites comprising of biopolymers and bioceramics are useful as scaffolds for bone tissue engineering, because of they could functionally mimic native bone, hold potential osteoinductivity, as well as relative ease of controling mechanical properties, degradation and resorption rates, through composition variations. The aim of our work was to design and fabricate a novel composite scaffolds for bone tissue engineering in medium/high load bearing applications.

Novel composite scaffolds were fabricated by blending Poly (L-lactide-co-D,L-lactide) (PLDLLA) a copolymer with a ceramic-tricalcium phosphate (TCP). The extrusion deposition method was used to fabricate the scaffold with high open porosity. Scaffold architecture and porosity was analysed by scanning electron microscopy and nano-computed tomography. Mechanical properties were analysed by compressive tests of dry and wet composite scaffolds. Porcine bone marrow stromal cells (BMSC) were seeded onto the scaffolds and cultured *in vitro* for up to 8 weeks. BMSC cultured on 2D polystyrene served as positive control. Cell proliferation was studied by microscopic observations and DNA quantification. To assess cell viability, Alamar Blue metabolic assay and FDA/PI fluorescent staining with confocal observation were performed. Osteogenic differentiation of BMSC was analysed by Western blot of osteopontin.

Mechanical tests showed encouraging features of composite scaffolds. BMSC expressed intensive proliferation, metabolic activity and osteogenic differentiation capability on the PLDLLA/TCP scaffolds *in vitro*. From these results composite PLDLLA/TCP scaffolds exhibit potential for bone tissue engineering.

(P 46) Biocompatible Polyurethane Films Incorporation with Antibacterial Silver Loaded Zeolites

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Polyurethanes are polymers which are commonly used in blood contacting devices since they have inherent antithrombogenic and excellent mechanical properties. It is important that, devices like catheters should not cause infections because of adherence of bacteria on the surfaces. There are some techniques for surface modifications such as addition of antibiotics or antibacterial substances. In this study, micron size nanoporous crystalline aluminosilicate zeolites are synthesized and modified with silver ions in molecular level, and combined with polyurethane forming antibacterial composite films. Polyurethane films were synthesized in a closed vacuum system in medical purity by using toluene diisocynate and polypropylene ethylene glycol without adding any other ingredients such as solvent or chain extender. Micron size zeolites were synthesized hydrothermally from gel solutions in three composition; as zeolites; A, X and Beta with low, intermediate and high SiO_2/Al_2O_3 ratios, respectively, and loaded with silver ions by cation exchange process. Then prepared zeolite crystals were added on prepolymer of polyurethane and cured. The composite films were examined by SEM and tensile tester instrument. Addition of zeolite particles into the structure improved the mechanical properties of the composite films compared to the unloaded polyurethane films. Antibacterial zeolite-PU composite films were placed on agar plates containing Escherichia coli and the bacterial growth inhibition zones were examined for all types of composite films. About 1-2 mm clear inhibition zones which show the presence of antibacterial activity were observed around composite films.

Acknowledgements: This project was supported by METU-BAP and European NoE EXPERTISSUES Projects.

(P 47) Biodegradable Magnesium Alloys for Tissue Engineering and Other Biological Applications: Unexpected Findings From a Mouse Model

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Magnesium and its alloys had been investigated for use as an absorbable implant since the beginning of the 20th century. However, rapid gaseous evolution hindered its further use. Renewed interest of it as biomaterial has re-emerged recently on orthopedic and cardiovascular applications, but the use on tissue engineering was rarely explored despite its good biocompatibility. Degradation properties and host response of AM50A, AM60B, AZ91 alloys and 99.95% Mg are investigated in this paper. Polished Mg and Mg alloys treated with concentrated NaOH and DMEM were implanted subcutaneously into a mouse for 6 months. X-ray investigations were conducted twice a week for the first two weeks and weekly thereafter, but there was no significant gas formation observed for AM50A, AM60B and AZ91D. After implant retrieval, some gas bubbles were detected under the fibrous layer covering the implants. This implies that a lack of gas pocket on an X-ray image is insufficient for the declaration of a lack of gaseous accumulation. Moreover it could be observed that the Mg alloys under investigation did not degrade fast in the body. In fact there was nearly no observable dimensional change for the alloys (except the 99.95% Mg) after implantation. AZ91, a commonly used alloy on literatures, was found with much more pitting corrosion than commercially available AM50A and AM60B alloys. This suggests that the use of AZ91 to illustrate the relatively better corrosion profile of another alloy may be misleading.

(P 48) Biodegradable Poly(L-Lactic Acid) Scaffolds with Internal Hyaluronic Acid Coating. Biological Response *In Vitro*

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Poly(L-lactic acid), a synthetic biodegradable polyester, is widely accepted for many tissue engineering applications. Hyaluronic acid (HA), as a polysaccharide of the extra cellular matrix (ECM), besides exhibiting an excellent biocompatibility, influences cell signaling, growth and differentiation. A combination between these materials might be interesting for tissue engineering studies.

However, HA must be chemically modified for this use because of its easy dissolution in water and quick degradation in biological environments. Glutaraldehyde, GA, has been proposed as crosslinking agent to produce HA hydrogels. However, accordingly to the literature, there are contradictory results about the experimental protocol to be used, and relating to the cytotoxicity caused by glutaraldehyde remaining in the sample after crosslinking reactions.

In this study, crosslinking was performed by immersion of HA in water-acetone mixtures containing GA. Reaction was performed in several steps with increasing water/acetone ratios. Higher GA concentrations and higher crosslinking reaction times than the literature were necessary in the addition to the multistep procedure to obtain HA samples that do not dissolve in water within *in vitro* culture times. Poly(L-lactic acid) tridimensional scaffolds were made by compression moulding followed by particulate leaching, and after the scaffolds' impregnation with soluble HA and drying, there was a subsequent crosslinking reaction with glutaraldehyde with the procedure described above.

A morphological study and physical characterization of the hybrid scaffolds, and preliminary results with human fibroblasts and human dental pulp mesenchymal cells show the ability of these scaffolds for tissue engineering applications.

(P 49) Biological Evaluation of Macroporous Scaffolds with Different Surface Energies for Regeneration of the Central Nervous System

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Central nervous system trauma and neurodegenerative diseases involve massive neuronal and glial cell death and loss of the three-dimensional spatial organization and connectivity of the neuronal networks. In this work we employ a biostable model system to study differentiation and viability of neural precursors in 3D scaffolds.

Polymer scaffolds with interconnected porous with 90 microns of pore size were produced varying the hydrophobic-hydrophilic monomeric units ratio along the polymer chain. The materials studied, biocompatible and biostable, were polymer or copolymer networks based on the hydrophobic homopolymer poly(ethyl acrylate), PEA, and its copolymers with hydroxyethyl acrylate, p(EA-co-HEA) and methacrylic acid, p(EA-co-MAAc). In these biomaterials, the survival of differentiated functional neurons derived from cultured subventricular zone (SVZ) postnatal neural stem cells was investigated.

The tissues obtained from the SVZ of postnatal rats were plated in DMEM/F12 basal medium, with EGF and FGF as single suspensions. The formed spheres (neurospheres) were then collected and dissociated mechanically, the new microspheres formed were dissociated again and the process was repeated two times. After dissociation at the third passage, cells were seeded onto the different biomaterials with a differentiation medium of neurospheres.

The neuronal cells were identified by immunocytochemical labeling for the neuronal marker TUJ1, and for the glial marker GFAP. The results with DAPI showed that in all the materials, but mainly in p(EA-co-MAAc) scaffolds, cells survived inside the pores of the biomaterial. These are promising results that prove that these constructs have great potential in neuronal tissue engineering.

(P 50) Biomechanical Stimulation of Hl-1 Cardiomyocytes Cultured in Poly-(1,8-Octanediol-Co-Citric Acid) [Poc] Scaffolds

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Cardiac diseases are the main cause of death in Western countries and one of the most expensive diseases to treat for the health services. Current therapies do not offer a complete cure, with the exception of heart transplantations; however, the shortage of donors limits the number of patients who can benefit from this treatment. Cardiac tissue engineering works towards the creation of an engineered muscle by providing cardiomyocytes with physical support and biochemical factors. This approach aims to

ensure the survival, proliferation and differentiation *in vitro* as well as a fully integration into the prospective host by offering high cellular retention within the implantation. The possible applications of such construct would not only be in clinical therapies but also as a substitute of animal models for drug screening and tests.

It has been shown that constructs seeded with cardiac cells and cultured under mechanical stress have an increased protein expression, differentiation and survival time compared to those cultured without mechanical stimuli. TENCELL is a mechanical bioreactor which allows applying either compressive or stretching load in a controlled environment. Poly-(1,8-octanediol-co-citric acid) [POC] has been reported previously as a flexible biomaterial suitable for cardiomyocyte culture. In this study HL-1 cells were seeded in POC scaffolds and mechanically stimulated to assess whether there was any effect caused by the uni-axial stretching load applied to the constructs. Variations in the seeding conditions as well as in the stretching regimen were also explored.

(P 51) Bioreactor System for Bioengineered Cartilage Grafts

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It has become evident that complex culture systems are necessary for the cultivation of appropriate articular cartilage equivalents with acceptable biological and mechanical properties. It is apparent that the first requirement—the application of mechanical loading to chondrocytes—is essential for the tissue engineering of cartilage substitutes. Perfusion has been identified as the second requirement when designing a bioreactor system for cartilage grafts since a fluid environment ensures a supply of nutrients and waste products are removed efficiently. Finally, there is a third requirement—compliant to cGMP regulations—which any system used commercially for the cultivation and stimulation of tissue engineered cartilage would have to satisfy. With these key requirements in mind, we set out to develop a completely new system for the tissue engineering of matrix-coupled autologous chondrocyte transplants (MACTs) intended for both research and clinical applications.

The designed bioreactor station for simultaneous loading and perfusion of MACTs in a sealed, autoclavable, twin chamber culture device with a magnet-driven actuator for the cyclical mechanical compression of substitutes reflects a novel biotechnological outcome. Uniaxial construct deformations are guided by external magnetic fields that were feedback-controlled in real time by the established innovative sensing concept that is based on accurate, reliable measurements of a platform load cell and an inductive proximity sensor. Those devices also allow for contact-less monitoring of the tissue development or rather the elastic property of the grafts. Additionally, the system enables dynamic medium supplementation, sterile sample removal, and incorporates online measurement of relevant biochemical and biomechanical parameters.

(P 52) Bioresorbable Polymer Scaffolds Reinforced with In-Situ Generated Hydroxyapatite for Tissue Engineering Applications.

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In this work biodegradable polymer scaffolds, for potential application in the reconstruction of damaged bone tissues, were prepared with policaprolactone (PCL) reinforced with hydroxyapatite (HA) generated *in-situ* thruogh a sol-gel method. This preparation method is proposed as a possible solution to issues related with the intrinsic composite nature of these organic-inorganic scaffolds, since it allows the preparation of a continuous inorganic network of HA having a controlled porosity, directly inside the polymer matrix.

The synthetic route is presented for the obtainment of a pure HA porous crystalline phase by a mild solution process, alone or in the presence of the polymer matrix. HA was proven to be obtained in a completely pure crystalline phase by means of XRD analysis, and results are reported. The morphology of the polymer scaffolds reinforced with different amounts of HA was investigated through scanning electron microscopy, and was demonstrated to be highly tunable by changing the experimental conditions for the sol-gel reaction.

(P 53) Bone Marrow Stem Cells on Different Wettability Surfaces

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The effect of surface wettability in the view of the cell-surface interaction has been extensively studied because of its importance in the design of implant scaffolds. Therefore, the cell-surface interaction is one of the most important factors in the development of implantable scaffolds. The objective of this study was to investigate the adhesion and proliferation behavior of human bone marrow stem cells (hBMSCs) on gradient wettable polyethylene (PE) surfaces prepared by corona discharge treatment.

Corona discharge treatment was applied to modify the surface of PE. The wettability of PE surface was gradually increased by power increase of a corona treatment along the PE length, indicating that the hydrophilicity of PE surface increased gradually. The adhesion and proliferation behavior of hBMSCs on the gradient PE surface was evaluated. We found that hBMSCs were adhered to and proliferated on better highly hydrophilic than hydrophobic surfaces. The plot of proliferation rate vs. the water contact angles was parabolic.

In conclusion, we found that the adhesion and proliferation behavior of hBMSCs depends strongly on surface wettability in agreement with previous reports.

(P 54) Bone Marrow Stromal Cells Differentated into Cardiomyocytes within Three-Dimensional Collagen Scaffolds

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Bone marrow stromal cells (MSCs) have been thought to be an attractive candidate for treatment and application of cardiac diseases. However, they are limited in the clinical application because of their poor differentiated ability into cardiomyocytes in two-dimensional culture conditions. As the cells are more viable in the appropriate microenvironment, we hypothesized that MSCs might be easy to differentiate into cardiomyocytes within three-dimensional collagen scaffolds. In this study, the primary MSCs were isolated from Sprague-Dawley rats and expanded in proliferated medium. Their phenotypes were identified with flow cytometer. For subsequent three-dimensional study *in vitro*, MSCs seeded into three-dimensional collagen scaffolds and treated with 5-azacitidine for 24 hours were regarded as experiment group, while those cultured in T-flask were regarded as control group 1, and the MSCs/Collagen scaffolds without treatment with 5-azacitidine were the control group 2. After 2 weeks, immunofluorescence staining against connexin-43, myosin heavy chain(MHC), and cardiac troponin-T(cTn-T) for cardiomyocyte were performed to the section of scaffolds. The results showed that immunostainings were positive against connexin-43, MHC, and cTn-T on the experiment group section and control group 1; as contrast, no connexin-43, MHC, and cTn-T expression was observed in the control group 2. The positive expression of experiment group is stronger than that in control group 1. It is concluded that after being treated with 5-azacitidine, MSCs could differentiate into cardiomyocytes, and MSCs within three-dimensional collagen scaffolds have higher differentiated ratio than that in T-flasks.

(P 55) Bridging Critical Size Defects Applying Tissue Engineering

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Critical size defects of bone cannot be expected to heal by de novo bone formation within the physiological bone healing mechanisms. Here we applied macro porous polylactid glycolid/CaP (PLGA/CaP) composite foam matrices (Osteo Scaf TM) additionally coated with nano particulate CaP using a modified biomimetic method. Geometry and pore size of the scaffolds mimicked the architecture of human trabecular bone.

The animal study was licensed (license number 35/9185.81-3) by the regional council Tübingen according to EU ISO 10993-6. The strain chinchilla bastard was used because these animals are known to be less stress susceptible and have more stable bones.

Non seeded scaffolds and scaffolds seeded with allogenic rabbit bone marrow cells (aspired from iliac crest) were implanted in 18 mm defects of chinchilla diaphyseal femur. The scaffolds were seeded with bone marrow cells using a recently developed bioreactor technology.

The cell seeded scaffolds effectively enabled the bridging of the neighbouring bone parts in this critical defect.

This project was supported by the EU-Project G5RD-CT-2000-00282.

(P 56) C-Terminal Epitope Tagging of Bone Morphogenetic Proteins Interferes with Bioactivity

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Terminal epitope tags are widely used in protein expression and purification approaches due to their unique properties for detection and affinity purification. The aim of this study was to generate C-terminal tagged versions of human BMP2 and BMP7 for purification after expression in eukaryotic cell lines (C2C12, CHO).

BMP2 was tagged with a FLAG-tag (-DYKDDDDKG) and BMP7 was tagged with a 6× HIS-tag (-HHHHHH). C2C12 cells were transfected with plasmids harboring the full length cDNAs of either tagged or untagged BMPs by lipofection. Osteogenic differentiation of C2C12 cells was analyzed after expression-culture in DMEM containing only 1% fetal calf serum to reduce background signals potentially generated by osteogenic factors present in the added serum. We employed alkaline phosphatase assays, RT-PCR, morphology and von Kossa staining as criteria for osteogenic differentiation to compare the bioactivity of the produced growth factors. Positive over-expression was confirmed by RT-PCR for human BMP2 and BMP7 as well as by immunological detection methods for the tagged variants (Western-blotting and Immunocytochemistry).

Our results indicate that C-terminal FLAG- or HIS-tagging of human BMPs reduces or even abolishes their bioactivity *in vitro* compared to untagged variants probably by interfering with secondary or quaternary structure. Nevertheless, non-reducing Western-blotting for FLAG-tagged hBMP2 confirmed successful dimer formation. These findings support the use of protease cleavage-sites for removal of C-terminal protein tags in eukaryotic or prokaryotic expression-systems.

This work was supported by the European projects
Hippocrates (NMP3-CT-2003-505758) and Expertissues (NMP-CT-2004-500283).

(P 57) Calcium Phosphate Formation Causes an Inflammatory Response To a Prototype Biomaterial Scaffold

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Calcium ions are used for cross-bridging in a variety of biomaterials and have been incorporated into a dermal replacement scaffold under development in our laboratory. *In vivo* evaluations of these scaffolds in a porcine full thickness wound model revealed a degree of inflammatory response. The aim of this study was to identify and characterise the cause of this inflammation. Endotoxin assay ruled this out as a contributory factor. Inflammatory potential of individual scaffold components and supernatants from whole scaffolds soaked for 24 hours in buffered saline solutions were assayed *in vitro* using neutrophil CD18 activation. Inflammatory response *in vivo* was measured using a histological scoring system. In vitro studies established that of all the components and degradation products of the scaffolds, only calcium chloride in PBS resulted in significant CD18 activation, suggesting a potential source of inflammatory stimulus. Further studies showed this effect depends on phosphate and could be reduced by centrifugation. PBS solutions containing calcium also stimulated release of neutrophil elastase, and the cytokines IL-8 and TNF-alpha in whole blood cultures, suggesting a self-stimulating mechanism driving the inflammatory response. *In vivo* histology showed progressive inflammation in scaffolds was dependent on the concentration of calcium used to make them. Calcium phosphate precipitation and deposition may cause inflammation and should be carefully monitored and controlled in biomaterials. We were unable to demonstrate marked effects in supernatants but these data suggest that noxious effects of calcium localised to the cell-biomaterial interface could drive an inflammatory response.

(P 58) Can Functional Epidermal Keratinocytes be Derived From Eccrine Sweat Glands?

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Our previous research shows that human eccrine sweat gland cells can form a functional epidermis *in vivo* (1). Expression of various keratinocyte-specific markers is similar in epidermal constructs of both keratinocytes and sweat gland cells. We wanted to see, if sweat gland cell-derived keratinocytes could also adopt a more specific task of human keratinocytes, namely the uptake of melanosomes into their cytoplasm.

We compared engineered skin substitutes derived from human epidermal cells to substitutes derived from human eccrine sweat gland cells. Human melanocytes were added to both cell types in a ratio of 1:5 and were then grown on collagen hydrogels containing human dermal fibroblasts. These constructs were transplanted onto full-thickness skin defects created on immuno-incompetent rats and were examined after 3 weeks.

This demonstrated that sweat gland cells can transform into a stratified epidermis with melanocytes scattered in the basal layer. However, early after transplantation these cells do not show an interaction with melanocytes, as keratinocytes would.

We conclude that sweat gland cells cannot fully act like naturally born keratinocytes in every perspective, at least not after a time period of 3 weeks. We hypothesize that sweat gland cells may need more time to be able to adapt to this new function, which is to differentiate into true keratinocytes and incorporate melanosomes. Studies to prove this are now being undertaken.

References:
(1) T.Biedermann *et al.*, 2008

(P 59) Carrageenan-Based Hydrogels As Potential Systems for the Delivery of PDGF in Bone Tissue Regeneration Strategies

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One of the major drawbacks found in most bone tissue engineering (TE) approaches developed so far consists in the lack of strategies to promote vascularization. Some studies have addressed different issues that may enhance vascularization in TE constructs, most of them involving the use of growth factors that are involved in the restitution of the vascularity in a damaged zone. The use of sustained delivery systems might also play an important role in the re-establishment of angiogenesis. In this study, κ-carrageenan, a natural polymer was used to develop hydrogel beads with the ability to incorporate growth factors with the purpose of establishing an effective angiogenesis mechanism. Some processing parameters were studied and their influence on the final beads properties was evaluated. A model protein—ovalbumin (OVA)—was encapsulated in the beads to optimize the procedure before using the growth factor of interest, i.e., the Platelet Derived Growth Factor (PDGF), selected as the angiogenic factor. The results demonstrate that the developed system is mild and adequate for protein incorporation and that an efficient encapsulation and protein loading were achieved, as well as release profiles matching those usually described for a typical hydrogel behaviour. Moreover, carrageenan shows a huge potential for application in the biomedical field, namely in the development of injectable systems, due to its gelling abilities. In general, the obtained results demonstrate the potential of this system for bone TE applications.

Acknowledgments: This work was supported by the European NoE EXPERTISSUES (NMP3-CT-2004-500283) and the European STREP HIPPOCRATES (NMP3-CT-2003-505758).

(P 60) Carrier-Filled Solid Scaffolds with Time-Released Porosity for Endogenous Bone Engineering

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Introduction: While scaffolds employed in skeletal repair today have excellent osteoconductivity, their porous structure permits

soft-tissue ingrowth. To address these problems, a novel hydroxyapatite (HA) and tri-calcium phosphate (TCP) scaffold was designed with filled internal porosity for *in vitro* and *in vivo* testing.

Methods: A 3D microprinting process was used to create microstructural lattices (11 mm diameter, 3 mm thickness, 250 μm struts, 200 μm pore size, HA:TCP ratio 60/40 or 15/85) that were either left porous or filled with calcium sulfate/chitosan. To assess for scaffold dissolution over time, porous and solid scaffolds were placed in DMEM + 10%FBS and weighed daily for 64 days. *In vivo*, porous and solid scafffolds were implanted in a rabbit critical-size calvarial defect model for 8 and 16 weeks and analyzed by micro-CT, histology, and SEM.

Results: Porous scaffolds retained $97 \pm 2\%$ of their weight at 64 days. When filled by calcium sulfate, mass decreased linearly (0.44%/day, $R^2 = 0.92$), correlating with gradual centripetal exposure of the internal porosity at 1mm/wk. In contrast, chitosan filling of the scaffolds was irregular in its dissolution. By 8 weeks *in vivo*, scaffolds conducted bone across critical defects ($85 \pm 18\%$) compared to non-healed controls ($p < 0.05$). Bony ingrowth corresponded to the degradation rate of the calcium sulfate. Scaffold filling also prevented ingrowth of soft tissue. Sixteen week samples are being processed.

Conclusions: Filling with calcium sulfate successfully limited bloody infiltration and soft tissue ingrowth but retained osteoconductivity. Present work is focused on improving scaffold osteoinductivity by using these fillers to encase bioactive molecules for rate-controlled release.

(P 61) Cartilage Tissue Engineering using Native and Recombinant Spider Silk Scaffolds.

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There is evidence that biological or biomimetic polymers may prove superior to traditional biomaterials for fabrication of tissue engineering scaffolds. The aim of this study was to evaluate the suitability of silk produced by Nephila edulis and a recombinant spider silk for use in cartilage tissue engineering. Cylindriform silk was used to form 3D scaffolds which were evaluated for chondrocyte adhesion and cartilage tissue engineering using published methods (Hatton & Hollander 2003, Biopolymer Methods in Tissue Engineering. Humana Methods in Molecular Biology Series). Untreated spider silk scaffolds provided excellent substrates for cell adhesion, but appeared not to support differentiation. Simple pre-treatments resulted in scaffolds that supported tissue engineering of a hyaline-like cartilage, and the results were improved further by separating the scaffold into inner and outer portions. Recombinant silk was not available in sufficient quantities to fabricate a full scaffold, but chondrocyte pellet culture on fibres suggested that they had comparable properties to the native materials. Overall, the results showed that relatively crude spider silk scaffolds were capable of supporting the formation of a hyaline-like cartilage with similar histological appearance and biochemistry to that formed on commercially available PGA scaffolds. All spider silks provided comparable data to the PGA reference biomaterials, and we therefore concluded that they have potential for use as scaffolds in tissue engineering.

Acknowledgements: We acknowledge the EC for FP5 funding (GRD1-2001-40464, SPIDERMAN) and contributions of our partners including Vollrath (Oxford, UK), Engström and Johansson (Uppsala, Sweden). The authors are members of EXPERTISSUES (NMP3-CT-2004-500283).

(P 62) Cell Encapsulation System using Beads and Fibres From Natural Origin Polymers for Cartilage Tissue Engineering Applications

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The ideal cell-carrier material for cartilage regeneration should closely mimic the natural environment in a living articular cartilage matrix.

Natural based hydrogel like alginate-carrageenan and chitosan (Alg-Car-Ch) can provide excellent supports for chondrocyte encapsulation due to their adequate mechanical strength to maintain the initial shape of the implanted scaffold and sustain cells proliferation and differentiation. To test this hypothesis, we investigated the encapsulation behavior of a chondrocyte cell line ATCD5 into Alg-Car and Alg-Car coated with chitosan polymer. Alginate beads and fibres were used as controls.

Spherical beads have been prepared by an extrusion technique and the fibres were prepared using a coagulation bath. Different formulations of beads and fibers, and different coagulation bath were considered in order to determine the best conditions.

The formulations used in this study were characterized by dynamic mechanical analysis (DMA) and rheometer measurements.

Preliminary biological tests were performed by encapsulating ATCD5 (1×10^6 cells/ml) onto the developed beads and fibres for 1, 3, 7, 14 day of culturing. Cells-beads/fibres were characterized in terms of viability (MTS assay), proliferation (DNA) and specific markers for cartilage. The obtained results indicated that the different formulations showed good cellular viability and proliferation after 14 days both in beads and fibres. On the basis of these results, we conclude that alginate-carrageenan chitosan polymer formulations both in spherical beads and fibers have a considerable potential as a cell-carrier material for cartilage tissue engineering application.

(P 63) Cell Transplantation from Bench to Bedside: Implant Evaluation for Clinical Tissue Engineering

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Tissue engineering has emerged as a promising perspective in orthopaedic surgery. In contrast to mechanical prosthesis, tissue engineered products represent living implants interacting with the recipient tissue. Careful assessment of biocompatibility is therefore crucial for transplantation success. Parts of these assessments can be performed in a model as simple as a chicken egg.

Various biomaterials (collagen sponge, Chondro-Gide®, Chondrocell A®, Bio-Gide®) seeded with fibrochondrocytes were transplanted onto the chorionallantoic membrane. Tissue compatibility, angiogenesis and early degradation were evaluated. Cell loss during transplantation, cell migration and tissue formation at the implantation site were studied.

The highest biocompatibility ranking was achieved by Chondro-Gide® and Bio-Gide® followed by Chondrocell A. The collagen sponge showed significantly poorer biocompatibility patterns. Average angiogenesis was 10 vessels per mm^2 for Chondro-Gide®, 8 vessels per mm^2 for Bio-Gide®, 7 for the collagen sponge and 3 for Chondrocell A. High cell seeding densities and short *in vitro* incubation led to instable constructs and increased cell migration. Even the colonisation of blood vessels was observed. Increasing the *in vitro* incubation period led to stable constructs and sufficient tissue formation at the transplantation site.

Sufficient cell delivery to the transplantation site is dependant on the preliminary incubation period rather than the initial cell load. High cell seeding densities lead to increased cell migration and carry a risk of blood vessel colonisation with subsequent vessel thrombosis. Construct stability as well as tissue compatibility and angiogenetic patterns can be evaluated using the chick chorionallantoic membrane as a rapid and cost efficient testing environment.

(P 64) Channel-Like Pores in Hap-Containing Scaffolds for Bone Engineering: Hydrogels Vs. Ceramics

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Micro-structured scaffolds with channel-like pores were produced by ionotropic gelation of alginate, using a well-known sol-gel-transition mechanism. Adapting the processing to biomedicine, suitable scaffolds for bone regeneration could be obtained by adding hydroxyapatite (HAP) [1]. Three types of constructs were obtained ordered by increasing energy impact by heat treatment: + As prepared—composite of hydrogel reinforced by HAP [1], + Brown body (HAP after thermic polymer removal at 650°C) or + HAP ceramic sintered at 1200°C.

Finding in all three cases very stable constructs with compression strength in lower MPa-range even for brown bodies, we can freely choose between hydrogel and ceramic by heat treatment.

The pore structure and channels surface were characterized by SEM. Temperature exposer led to shrinkage of the pore diameter and grain coarsening seen as surface smoothening. Crystallites were growing from 22 nm to 41 nm or 238 nm, respectivly, which was proven by XRD becoming maybe important for resorption of scaffolds by osteoclasts. Biocompatibility was evaluated by *in vitro* cultivation of human mesenchymal stem cells (hMSC) on the materials. Cell proliferation was monitored by DNA quantification at different time points of cultivations showing 2.5 times increase after three weeks for all material types. Osteogenic differentiation of hMSC was confirmed via measurement of specific alkaline phosphatase (ALP) activity. Maximum was reached at day 14 for brown body and ceramic but only at day 21 for the hydrogel composite. Cell distribution and morphology were studied by cLSM.

[1]J. Amer. Ceram. Soc. 2007, 90, 1703.

(P 65) Characteristics of Human Adipose-Derived Stem Cells Submitted To Short-, Medium- and Long-Term Cultivation.

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The adipose tissue contains a stromal population composed of microvascular endothelial cells, smooth muscle cells and stem cells, which can be enzymatically isolated. The adherent, mesenchymal-like cells that establish under appropriate culture conditions, termed adipose tissue-derived stem cells (ADSCs), have extensive proliferative potential and may undergo multilineage differentiation. They are currently considered as one of the most promising type of adult stem cell for therapeutic applications. In this study, we characterized human ADSCs submitted to different periods of culture: short-term (1-9 passages), medium-term (10-19 passages) and long-term (>20 passages) ($n = 5$ cultures each). The oldest culture analyzed had been maintained for 35 passages. Doubling population indices were significantly higher for long-term and particularly for medium-term cultures when compared to short-term cultures. The clonogenic potential of the cultures, analyzed by limiting dilution, was lower in older cultures. Cell plasticity was analyzed by inducing adipogenic, chondrogenic and osteogenic differentiation of cultures. The differentiation potential was similar in short- and medium-term cultures, but showed a decrease in cultures older than 22 passages. The immunophenotype, typical of ADSCs, was the same in the three types of culture. The DNA content was analyzed by flow cytometry, and was also unaltered (2n) in all cultures. These results show that human ADSCs can be maintained in culture for prolonged periods of time, but that long-term cultures loose some of the potential for therapeutic applications, as shown by decreased plasticity and clonogenic potential.

(P 66) Characterization of a Bioactive Scaffold for Cartilage Repair

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Introduction: The aim of this study was to determine the matrix-synthesis, gene-expression and growth characteristics of human articular chondrocytes (hACs) loaded on bioactive MPEG-PLGA (metoxypolyethyleneglycol-block-co-poly(lactide-co-glycolide) scaffolds. A variety of glycosaminoglycans (GAGs) were incorporated into the scaffold structure leading to a proposed chondrogenic bioactivity.

Methods: hACs (passage 1) were loaded on bioactive MPEG-PLGA scaffolds and after 1, 2, and 4 weeks, RT-PCR, histology and ELISA analyses were performed. Furthermore immunohistochemistry (IHC) was performed using monoclonal antibodies against Col2, Ag and Collagen Type 1. hAC monolayer cultures and MPEG-PLGA without GAG incorporation were used as controls.

Results: hACs adhered to the scaffold and proliferated at a rate similar to the control cultures. RT-PCR analysis demonstrated an upregulation of the chondrogenic markers, S0X9, Col2 and Ag, compared to control cultures. The presence of Col2 and Ag was further verified on the protein-level by IHC. Toluidine Blue O -and Safranin O staining demonstrated an elevated synthesis of proteoglycans.

Discussion: Using the novel scaffold platform, MPEG-PLGA, with a variety of GAGs incorporated, we analyzed the ability of hACs to synthesis a cartilage matrix, by using gene expression analysis, IHC, histology, and ELISA. Significant differences in the analysis results were found, depending on the actual types of incorporated GAG. We conclude that this novel bioactive scaffold is a powerful *in vitro* environment for culturing hACs for tissue engineering. A goat study is now in progress to study this system under *in vivo* conditions.

(P 67) Characterization of Human Pterygium As Source of Mesenchymal Indifferentiated Cells

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Background: Destruction of the limbal epithelium barrier is the most important mechanism of pterygium formation (conjunctiva proliferation, encroaching onto the cornea). It is thought to arise from activated and proliferating limbal epithelial stem cells. The objective of this study is to study cells extracted from human pterygium to evaluate their potential as mesenquimal indifferentiated cells.

Material and methods: Cells from human pterygium were isolated by explantation and placed in culture with Amniomax medium. Once the monolayer was reached the cells were seeded onto 24 well microplates. The cells were studied in the second passage. The immunohistochemical expression of the different embryonic stem cell markers: SSEA-1, SSEA-4, PODXL, CD-9, Nanog and SOX2, was analyzed.

The differentiated phenotypes were characterized with the monoclonal antibodies anti-CD31, alfa-actin and vimentin.

Results: All the cells were positives for SOX-2 at a cytoplasm level, but only around 1% was also positive at a nuclear level. Nanog marker was present in all the population in the nucleus of the cells. The majority of the cell population was positive for vimentin, and CD-9. About 50% of the pterygium cells showed the SSEA-4 mesenchymal stem cell marker. There were no positive cells for the SSEA-1 marker. PODXL marker was only present in 10% of the total population. Less of the 1% of the cells were positive for CD31 and alfa-actin markers.

Conclusions: The pleomorphic cell population obtained from pterygium at the second passage expressed markers for an undifferentiated phenotype compatible to mesenquimal indifferentiated cells.

(P 68) Characterization of NGFR$^+$ and CD34$^+$ Hasc Subpopulations: Comparison with Unselected Hascs (Pa-Hascs)

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Human plastic-adherent adipose-derived stem cells (PA-hASCs) have been extensively characterized for their functional potentials *in vitro*. In this study, we compared positive-selected CD34$^+$ and L-NGFR$^+$ (low-affinity Nerve Growth Factor Receptor) subpopulations with the whole PA-hASCs. In bone marrow, NGFR antigen defines a subset of early MSC with a high proliferative potential, a high degree of clonogenic efficiency and able to differentiate into multiple mesodermal tissues. Phenotypic profile of freshly purified subpopulations was assessed for CD45, CD133, CD34, NGFR, CD117, GlyA, KDR, P1h12 and CD105 expression. Most of the NGFR$^+$-hASCs is CD34$^+$, whereas selected CD34$^+$-hASCs only partially coexpress NGFR. Proliferation and clonogenic efficiency of these three hASCs fractions were tested, and MSC specific markers expression was monitored over time during culture. NGFR$^+$- and CD34$^+$-cells show a culture-dependent mild decrease of NGFR and CD34 expression. In addition, the NGFR$^+$ and CD34$^+$ subpopulations showed a higher proliferation rate compared to the PA one. Testing their differentiation potential, preliminary data indicate that NGFR$^+$-hASCs differentiate more efficiently into adipocyte- and endothelial-like cells; in contrast CD34$^+$-hASCs appear to better differentiate into osteoblast- and chondrocytes-like cells. Indeed, osteogenic differentiated CD34$^+$ and NGFR$^+$ hASCs produce a more abundant extracellular calcified matrix, respect to the PA cells. Pellet-culture of chondrogenic differentiated CD34$^+$ and NGFR$^+$-hASC show a glycosaminoglycan production which is significantly greater than in PA pellet-culture differentiated cells. We suggest that the NGFR$^+$ and CD34$^+$ purified hASCs are two purified homogeneous populations, with a mild different differentiation potential, which is more efficient than the PA unselected cell population one.

(P 69) Characterization of PLGA/Demineralized Bone Particle Composites as Scaffolds

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Intervertebral discs (IVD) are fibrocartilaginous tissues put between vertebral bodies in the spine. In this study, we developed that composites scaffolds by combining poly (lactide-co-glycolide) (PLGA) and demineralized bone particle (DBP). We evaluated pore structure, porosity, water absorption ability of scaffolds for the application of IVD.

PLGA scaffolds were prepared by solvent casting/salt leaching method and were immersed in DBP acidic solution under a vacuum so that the scaffold pores filled with DBP solution. The structure morphology of scaffolds was observed by SEM. Porosity were analyzed by mercury intrusion. We performed FTIR to confirm whether DBP solution impregnated into PLGA scaffolds. Water absorption ability was determined by measuring the weight of scaffolds for 12 days.

We confirmed that DBP solution was impregnated into the pores of PLGA scaffold. FTIR peaks appeared weakly in PLGA/DBP scaffold because the amount of DBP solution put into PLGA scaffold was limited. Water wettability test showed DBP solution permeated into PLGA scaffold, thus the surface of DBP enriched PLGA scaffold was easily wetted due to hydrophilicity of DBP. The ability to absorb water was increased in PLGA/DBP scaffold than in PLGA scaffold. This result suggested that high water absorption ability of PLGA/DBP scaffold would help interaction with cells seeded in scaffolds.

We developed DBP sponge and PLGA/DBP composite scaffolds manufactured by mixing of PLGA solution and DBP powder. A novel scaffold was manufactured to ensure the possibility of application of degenerated IVD.

Acknowledgements: This research was supported by KMOHW (0405-BO01-0204-0006) and SCRC (SC3100).

(P 70) Chitosan Gel as an *In situ*-forming Scaffold for Rat Bone Marrow Stem Cells *In vivo*

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In situ forming gel scaffold is attractive because it can trap the various growth factors, genes, and cells within the gel for the repair and regeneration of damaged tissues. Chitosan-based matrixes are widely used in the biomedical field such as cell encapsulation, drug delivery, cell culture, and wound healing. In this study, we describe an injectable gel carrier which consists of chitosan and glycerol phosphate (GP) as an *in vivo* scaffold for rat muscle derived stem cells (rBMSCs).

First, the phase transition behaviors of chitosan solutions formulated with and without GP were characterized as a function of temperature. Chitosan solutions containing >20 wt% GP became a gel at 37 °C and maintained this form for 28 days *in vitro* and *in vivo*. Next, we examined whether the chitosan gel could act as a suitable biocompatible substrate for the attachment and proliferation of rBMSCs. Immunohistochemistry clearly demonstrated that rBMSCs survived well on the scaffold created by *in situ*-forming chitosan gel in rats. Injection of chitosan gel alone induced macrophage accumulation in the host tissue and at the edge of the chitosan, whereas injection of chitosan gel containing rBMSCs was associated with decreased macrophage accumulation, indicating immunosuppression by the transplanted rBMSCs.

We herein prepared an *in situ*-forming chitosan gel and showed that it could be used as a biocompatible *in vivo* scaffold for rBMSCs in Fisher rats.

(P 71) Chitosan Microparticles as Injectable Scaffolds for Tissue Engineering Applications

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Microparticles may be used as a support for the adhesion and proliferation of cells. Therefore, the combination of isolated particles and previously incubated cells on their surface may have potential to be used, in the form of a suspension with media, as an injectable scaffold in the context of tissue regeneration: on expects that the particles might agglomerate after the implantation as a consequence of cells proliferation and extracellular matrix production. With this aim, chitosan microspheres were prepared by an emulsion procedure involving cross-linking with natural chemicals. Aqueous chitosan solution was added to mineral oil, containing a surfactant to form the w/o emulsion. Then, genipin solution was added as crosslinking agent. Varying the concentrations of the different solutions involved in the process, genipin-chitosan crosslinked microparticles with diameters ranging between 150 and 200 μm were obtained. A 3D construct of these microparticles and GBMCs (goat bone marrow stromal cells) were used for "*in vitro*" cell culture. Viability, growth, adhesion, and morphology of GBMCs were evaluated. The obtained results demonstrated that the microparticles are a suitable support for cell proliferation. The best results were obtained with microparticles crosslinked with 20 mM genipin.

(P 72) Chitosan Scaffold as a Structural Base Material for Tissue Engineering

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This study is to develop a novel method for fabrication of the chitosan scaffold improved their biocompatibility, based on a thermally induced phase separation technique. The porous scaffolds were basically fabricated by solid-liquid separation and subsequent sublimation of solvent. The effects of addition of n-butanol, as non-solvent, into aqueous chitosan solution on pore formation of chitosan scaffold were estimated. The pore diameters of chitosan scaffolds could be controlled within the range $4 \sim 100$ μm, with adjusting to ratio of n-butanol concentration. The inter-

connectivity between pores over resulting chitosan scaffolds was improved without any surface skin layer, compared to control scaffold. We performed further examination of the changes in the biocompatibility of the chitosan scaffold on human dermal fibroblasts (HDFs) after the addition of non-solvent by evaluating the initial cell binding capacity and cell growth rate. The initial cell adhesion on the new prepared chitosan scaffold was 190% higher than control chitosan scaffold. The proliferation rate of HDFs in the new prepared scaffold was 1.82-fold increase than that of control chitosan scaffold, after 3 days culture. The new prepared chitosan scaffold had the specific surface area enough for cell attachment and tissue ingrowth.

(P 73) Chitosan Scaffold for Bile Duct Reconstructive Surgery

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This work aims at the preparation of a novel biodegradable chitosan tube-shaped scaffold intended for bile duct reconstruction. The scope implies the development of a structure, with low permeability to bile, possessing suitable mechanical resistance and potentially able to act as drug delivery system for antimicrobial agents.

The goal is pursued by setting up a multilayer tube where one layer is a crosslinked chitosan hydrogel, imposing mechanical resistance and low permeability to bile, while the second layer is a soft biomaterial acting as optimal cell substrate. The latter is constituted by chitosan and excipients (such as sugars and/or phosphate salts) added to a chitosan solutions. These excipients are not recovered in the scaffold upon solidification, however, they interact with chitosan during the hydrogel formation process. For comparative purpose, monolayer scaffolds were prepared as well. The physical properties and the biological features of the prepared scaffolds have been investigated. The new scaffold shows marked different structure and higher biocompatibility in comparison to that obtained from the sole chitosan solution.

Multilayer scaffolds are less permeable to bile than monolayer ones. This is due to the more compact structure of the multilayer scaffolds as indicated by their lower porosity.

This last characteristic positively influences also the mechanical properties in particular the resistance to surgical suture.

The novel material shows beneficial effects on cell culturing: higher rate of cell attachment and doubling, lower cell detachment even 15 days after seeding, a higher cell yield, and an adequate differentiation morphology.

(P 74) Chondrocyte Redifferentiation in Alginate-Agarose Hydrogel is not Enhanced by Rotating-Wall Bioreactor

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Autologous Chondrocyte Implantation method (ACI) has been introduced for healing focal articular cartilage defects in the knee. To obtain the necessary cell number for construct preparation, harvested chondrocytes are expanded *in vitro* in a monolayer culture system and subsequently seeded into alginate-agarose hydrogel. Three-dimensional environment of the hydrogel enables redifferentiation of previously dedifferentiated chondrocytes. As rotating-wall-vessel (RWV) bioreactor has been shown to stimulate chondrogenesis, we wanted to examine its effect on gene expression of chondrocytes assigned for ACI.

Four samples of human articular chondrocytes were cultured in parallel in RWV bioreactor and static culture. After 14 days, expression of collagen type I, II, aggrecan and versican was assayed by qPCR.

Results showed no significant differences in gene expression between tested culture systems.

(P 75) Chondrogenesis Begins with Condensation: Tissue Engineering Recapitulates Embryonic Development

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In embryonic development the first stage of chondrogenesis is the formation of a mesenchymal condensation, expressing N-cadherin. To tissue engineer cartilage-like constructs of Mesenchymal Stem Cells (MSCs), in-vitro culture models are used with a high cell density, such as pellet cultures. The aim of the presented study was to study whether in-vitro chondrogenesis in the pellet culture is preceded by a condensation stage such as embryonic chondrogenesis.

Therefore, we differentiated Mesenchymal Stem Cells (MSCs) from 4 different donors on chondrogenic medium, with and without dexamethasone, which is known to accelerate chondrogenesis. Pellets were cultured for 5 weeks and harvested on different culture days and processed for immunohistochemistry on collagen II, as a marker for chondrogenic differentiation, and N-cadherin.

Pellets cultured with dexamethasone produced a collagen II rich matrix. In these pellets, N-cadherin expression increased in the first 7-10 days of culture in the centre of the pellets and decreased before collagen type II production in week 2-3. On the other hand, pellets cultured without dexamethasone did not produce any collagen II within 5 weeks. In these pellets, N-cadherin expression was high in weeks 2-3, and did not decrease until the end of culture.

In conclusion, chondrogenic differentiation in *in-vitro* pellet cultures is preceded by a condensation stage, characterized by N-cadherin. Time of onset of collagen II production seems to be related to the duration of this condensation stage. Therefore, N-cadherin seems to be a good marker to monitor progression during chondrogenesis before collagen II production.

(P 76) Chondrogenesis of Human Mesenchymal Stem Cells (hMSCs) Affected by 17ß-Estradiol

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Introduction: Tissue engineering (TE) using bone marrow derived hMSCs is a potent strategy to repair defects of articular cartilage, which have been shown to be a sex hormone sensitive tissue. We investigate the effects of 17β-estradiol on the chondrogenic differentiation of hMSCs *in vitro*.

Methods: Male hMSCs proliferated as a monolayer in serum-containing medium. Using the harvested cells we created aggregates and cultured them under serum-free conditions. First we added Dehydroepiandosterone (DHEA), testosterone (T) and 17β-estradiol (E2) in different concentrations in addition to the standard medium containing dexamethasone. Afterwards we tested the E2 effects in several periods of differentiation. After 21 days we examined the aggregates macroscopically, by histology and immunhistology. Type II collagen content (RT-PCR, ELISA), DNA and sulphated-glycosaminoglycan (sGAG) concentrations were measured.

Results: Aggregates treated with DHEA and T did not show significant differences in the size and extracellular matrix production compared to the standard group, however we found significant smaller aggregates, lower type II collagen and sGAG content after E2-addition. The DNA content, which was equivalent to the cell count, did not vary between the groups. The sequential addition of E2 showed that the inhibitory effects of E2 occur during the first 7 days of differentiation.

Conclusions: We found that chondrogenesis of hMSCs was repressed by E2. Our results may provide important approaches for the improvement of TE-construct quality for cartilage replacement. Elucidating the detailed processes of E2-inhibition influencing the initial phase of chondrogenic differentiation of hMSCs is the aim of our current and future studies.

(P 77) Chondrogenesis using Mesenchymal Stem Cells and PCL Scaffolds

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Mesenchymal stem cells are considered as candidate cells for cartilage tissue engineering by virtue of their ability to undergo chondrogenesis *in vitro*. The purpose of this study is to test the feasibility of PCL scaffold manufactured from melt-molding particulate-leaching method as a suitable material for cartilage tissue engineering from MSCs. Porous polycaprolactone scaffolds were fabricated by a melt-molding particulate-leaching method. Pluronic F127 was blended with PCL to promote cell attachment by enhancing hydrophilic property of scaffold. Collagen was also added to PCL scaffold to enhance cell attachment in other samples. MSCs at passage 3 were seeded into scaffolds and cultivated for 1 week under chondrogenic medium containing 10mg/ml of TGF-b2. Then the cell-scaffold complex was prepared for histological examination by paraffin block. Chondrogenesis of bone marrow-derived cells can be reliably induced with TGF-b2 treatments, for cultures seeded in all groups. The proliferation of DNA content increased with PCL/collagen and PCL/F127/collagen scaffolds. These scaffolds were effective in inducing cell proliferation. GAG analysis showed enhanced level with PCL/collagen and PCL/F127/collagen scaffolds. The findings from Safranin-O and type II collagen staining closely paralleled the finding from GAG analysis. We have shown by DNA contents, GAG analysis, histology and immunohistochemistry that chondrogenesis of bone marrow-derived cells can be reliably induced with PCL/collagen and PCL/F127/collagen scaffolds. These findings suggest that the three-dimensional PCL/collagen and PCL/F127/collagen scaffolds are a potential candidate bioactive scaffold for cartilage tissue engineering applications.

(P 78) Chondrogenic Differentiation of Adipose Derived Stem Cells—a Bumpy Road

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Adipose derived stem cells (ASC) show multilineage differentiation potential, however chondrogenic differentiation is challenging. Aiming to substitute chondrocytes in autologous chondrocyte transplantation (ACT) this study focuses on optimization of chondrogenic differentiation of ASC.

We evaluated the influence of 3 approaches (growth factors, co-cultures, scaffolds) to improve chondrogenic differentiation of ASC. First, media containing the growth factors TGF-b3, BMP-6 or FGF-2 were tested for their differentiation inducing capacity in ASC pellet cultures. In the second approach we investigated synergic effects between cell types by co-culturing ASC with native chondrocytes at different ratios. Finally, the role of different biomaterials was studied and cells were seeded in fibrin or a collagen I scaffold. Results were evaluated histologically, biochemically and by qRT-PCR.

Glycosaminoglycan expression of pellet cultures could be induced by TGF-b3/BMP-6/FGF-2. However, quantitative RT-PCR data demonstrate a strong inhibitory effect on chondrogenic marker gene expression in presence of FGF-2, whereas TGF-b3 and BMP-6 act as an inducer. Although TGF-b3 led to high COL2A1 expression on the mRNA level, BMP-6 was necessary to detect collagen II immunohistologically. Interestingly, collagen X expression exceeded all other markers. So far, chondrogenic differentiation was not enhanced in co-culture with chondrocytes. When comparing fibrin and collagen I scaffolds the results were quite controversial between different marker genes.

With a special growth factor repertoire ASC are able to differentiate towards a chondrogenic phenotype. After optimizing the combination of growth factors and cell density both matrices should be suitable for cartilage regeneration using ASC.

(P 79) Chondrogenic Differentiations of Adipose Tissue- Derived Mesenchymal Stem Cells: the Effects of Higher Doses of Growth Factors

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There has been a controversy on the chondrogenic potentials of adipose tissue- derived mesenchymal stem cells (ATMSCs) as compared to bone marrow derived mesenchymal stem cells (BMMSCs). This study examined the combination of growth factors that would induce effective chondrogenesis from adipose tissue- derived mesenchymal stem cells (ATMSCs). Chondrogenesis was induced by culturing the ATMSCs in pellets with the following growth factors: #1, without growth factors (negative control) : #2, 5 ng/ml of TGF-b2; #3, 15 ng/ml of TGF-b2; #4, 25 ng/ml of TGF-b2; #5, 5 ng/ml of TGF-b2 and 100 ng/ml of IGF-I ; #6, 15 ng/ml of TGF-b2 and 300 ng/ml of IGF-I ; #7, 25 ng/ml of TGF-b2 and 500 ng/ml of IGF-I. The BMMSCs cultured under 5 ng/ml of TGF-b2 was used the positive control. The proliferation of DNA content increased with higher doses of TGF-b2 and IGF-I combinations. Higher doses of TGF-b2 and IGF-I combinations were effective in inducing cell proliferation. GAG analysis showed enhanced level with the combination of higher doses of TGF-b2 and IGF-I combinations. We confirmed the effects of these growth factor through quantitative RT-PCR after inducing chondrogenesis. Adding higher doses of TGF-b2 and IGF-I combinations made the expression of type II and sox-9, maker of Chondrogenesis increase.The findings from Safranin-O and immunohistochemical staining closely paralleled the finding from GAG analysis. Higher doses of TGF-b2 and IGF-I combinations, may be used to enhance chondrogenesis in the chondrogenic differntiation from MSCs.

(P 80) Circulating Progenitor Cells Obtained from Valvular Disease Patients: Potential Applications for Engineering Heart Valves

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One of the persistent problems with commercially available bioprosthetic cardiac valves is the formation of thrombosis and calcification. This is mainly caused by deficiency of endothelial cell coverage on the valve surface. We previously have shown that it is possible to isolate a population of progenitor cells from circulating blood that give rise to endothelial and muscle cells. However, the use of progenitor cells for tissue engineering has not been studied extensively. In this study, circulating progenitor cells were isolated and expanded from blood samples of patients with valvular disease. Progenitor populations were analyzed by fluorescent-activated cell sorter (FACS) with endothelial cell (EC) and smooth muscle cell (SMC) specific markers before and after differentiation. The expanded cells were induced into endothelial and smooth muscle cell lineages followed by immunohistochemical, biochemical, and physiological analyses. Populations of circulating progenitor cells were successfully isolated using Anti-CD133 and Anti-KDR antibodies. CD133+/KDR+ cells were grown and expanded *in vitro*. After EC differentiation, cells stained positively for CD31, VE-Cadherine and vonWillebrand factor (vWf). After muscle cell differentiation, cells stained positively for SM a -actin and caldesmon. EC also formed capillaries in Matrigel™. The proliferation of EC was higher than that of tissue derived EC. EC cells seeded on these matrices attached, proliferated and formed defined cell layers. These engineered valve tissues possessed similar biomechanical properties as normal valve tissues. The degree of coagulation was minimal in cell seeded valve surfaces when compared to unseeded decellularized valve matrices.

(P 81) Clonally Expanded Sca-1$^{pos\&}$ Mesenchymal Stem Cells Spontaneously Generate Heterogeneous Phenotypes *In vitro*

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Mesenchymal stem cells represent a population of multipotent progenitors residing within the bone marrow, whose ability to generate different phenotypes *in vitro* and *in vivo* has been compellingly demonstrated. Despite the great interest they raise as possible candidates for cell therapy, the understanding of the mechanisms controlling mesenchymal stem cell homeostasis remains poor. In fact, several lines of evidence showed that among MSC population, Sca-1+, c-kit+, Islet-1+ cells are present, but the interactions between these subsets of progenitors are currently unknown. To improve the knowledge on stem cell behaviour, a stable murine Sca-1+ MSC stem cell line (Sca-1 mTERT-MSC) was generated by mTERT ectopic transduction and single cell cloning. This cell line displayed typical stem cell morphology, phenotype and multipotency and preserved these features until at least the 150 passage in culture. When the immortalized Sca-1 mTERT-MSC line was subjected to single cell cloning, few clones grew in culture. The analysis of clone morphology, phenotype and differentiation ability clearly demonstrated that they had distinct features: in particular, while clone 1 and 3 continuously expressed Sca-1 and preserved their multipotency, clone 4 spontaneously lost Sca-1 expression and multipotential capacity. Finally, clone 10 spontaneously proceeded to adipogenic differentiation in standard culture conditions. These data strongly suggest that mesenchymal stem cell compartment is intrinsically heterogeneous. These conditions are likely to constantly occur in stem cell niches. These evidences imply that very accurate protocols must be set up to envisage a factual use of stem cells in the fabrication of engineered bio-substitutes.

(P 82) Co-Culture of Endothelial and Smooth Muscle Cells for the Development of Blood Vessel Constructs

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Diseases of the blood vessels, particularly of small diameter arteries are responsible for most of deaths in developing and developed countries. The number of operations using arterial

prostheses and of small calibre vascular bypass reaches enormous values every year. With the limited availability of functional autologous vessels the need for new strategies to develop artificial blood vessels is increasing. The aim of this work is to develop constructs composed of a polymeric support with two different surfaces, displaying distinct surface properties, to seed endothelial (ECs) and smooth muscle cells (SMCs) with the purpose of obtaining an artificial blood vessel substitute.

Novel polycaprolactone (PCL) 2D bi-layer membranes fabricated by means of electrospinning and solvent casting, were developed. ECs and SMCs were isolated from human umbilical cord vein. After confirmation of the phenotype of the isolated cells by immunocytochemistry, static co-cultures were established by seeding ECs and SMCs respectively on the electrospinning and solvent cast layer. Cell proliferation and collagen and glycosaminoglycans (GAGs) production were assessed on the co-cultures and on the isolated cultures established as controls.

The results revealed that the bi-layer structure enhanced the proliferation rate and the collagen production in the co-culture while no differences in GAGs deposition were observed. Moreover the established co-culture system permitted to conclude that one cell type was not negatively influencing the other. In this context, the proposed structure constitutes an interesting strategy for assembling a blood vessel substitute.

(P 83) Coating Human Bone Allograft Surfaces Markedly Increases Mesenchymal Stem Cell Attachment and Proliferation *In vitro*

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Lyophilized human bone allograft is regularly used for providing mechanical stability and a scaffold for bone re-growth in cases of bone loss. Several reasons are responsible for the very slow rebuilding of the allografts, one of these is the low rate of mesenchymal stem cell recruitment. We designed a novel coating technique which is able to increase the attachment and proliferation capacity of stem cells. Human lyophilized bone chips were preincubated with fibronectin, albumin, fetal calf serum or collagen I solutions and used immediately or lyophilized before use. Mesenchymal stem cells were cultured from human bone marrow and seeded onto the constructs. Cell attachment and proliferation was evaluated by confocal microscopy 3, 10 and 18 days after seeding. The un-treated allograft barely attracted any cells and the constructs were devoid of cells after 18 days. Coating the bone with fibronectin increased the attachment, however, proliferation and long-term survival was still not achievable. Pre-incubating the bone with human albumin, especially when it was lyophilized onto the bone resulted in very good stem cell attachment and proliferation. Collagen I and fetal calf serum were also effective. *In vivo* biocompatibility assays in rats showed survival of the graft after 4 weeks of implantation. This novel coating technique markedly increased the number and proliferation potential of stem cells on allograft matrices. This procedure may be applied to create implantable bone substitutes which have a faster rebuilding time *in vivo*.

Supported by OTKA T049621, AÖU 66öu5, Bolyai and Oveges Fellowships.

(P 84) Collagen-Chitosan Polymeric Scaffolds for 3D Culture of Adipose Tissue-Derived Stem Cells

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Introduction: To culture cells in a three-dimensional fashion, scaffolding architecture and biomaterial used are vital. We have proved that adipose tissue-derived stem cell (ADSC) had limited proliferation ability in plate and our developed collagen/chitosan scaffold had good biocompatibility with ADSC. Thus it is necessary to investigate the proliferation ability of ADSC in collagen/chitosan scaffold.

Methods: hADSCs were isolated and cultivated in plates and in collagen/chitosan scaffolds for 14 days respectively. During the culture period, the growth dynamic parameters and viability of ADSCs were examined by CCK-8 kit every other day; the metabolic rates of glucose and lactic acid were analyzed every day. After 14 days, the cells in scaffolds were evaluated with scanning electron microscope (SEM) for histological examination of the aggregates. The surface markers (CD13, CD29, CD34, CD44, CD45, CD105, CD166 and HLA-DR), the specific transcription factors (Oct-4, Sox-2, Rex-1) and the multi-differentiation potential (adipogenic, osteogenic and chondrogenic) were also assayed to identify the stemness of proliferated cells.

Results: The results showed that hADSCs in scaffolds could be expanded by more than twenty times, and they presented better morphology and vitality and stronger differentiation ability than the cells cultivated in plates. All cells maintained stem cell characteristics after expansion.

Discussion and Conclusions: Our developed collage/chitosan scaffolds can promote ADSCs adhesion, template to organize and direct the expansion of cells, and maintenance of differentiated function. So it is a favorable scaffold for the proliferation and differentiation of adipose tissue-derived stem cells.

(P 85) Combined Bioimplants for Articular Defects Healing

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The aim of the present study was the optimization of the *in vitro* seeding procedures for mesenchymal stem cells (MSCs) culture on 3D matrices and evaluation of the *in vivo* behavior of these combined implants.

MSCs were isolated from bone marrow of 15 dogs and seeded on to collagen- polyglycolic and collagen- polylactic matrices. The *in vitro* adherence of MSCs was evaluated. For the *in vivo* study, the cartilage defects on the femoral condyls were surgically

induced. The defect shape and size was assessed by CT scan and 3D reconstruction. The healing process after 110 days was investigated by clinical, radiology (CT) and arthroscopic examination.

The results indicated good cells viability without significant differences between scaffold types or seeding procedure. Regarding the adherence the better results were noticed in case of collagen-polyglycolic matrices. After 24 h incubation period, the cell attachment rate was $70 \pm 5.86\%$, but after only 2h of cell-material contact was noticed a percentage of $63 \pm 7.54\%$ attached cells, suggesting that the main interaction were developed in the first hours. In 75% subjects of the experimental groups, the cartilage was partially restored in the area of implant, compared with control group in which was noticed lack of cartilage healing, only a slight beginning of this process from the periphery. Histological aspects are concordant with the clinical and imaging data.

(P 86) Combining Engineered Growth Factors, Adult Stem Cells and Bioabsorbable Materials for Skeletal Repair and Regeneration

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Due to the limited regenerating ability of human skeletal tissues, severe injuries to the bones often leave life-lasting sequels. Tissue Engineering has emerged as a promising alternative to the traditional inert orthopedic devices. Engineered bones are being developed by combining biomaterials with osteogenic cells and/or osteoinductive factors. Our group is dedicated to the study of skeletal regeneration, with a focus on stem cell-based skeletal tissue engineering.

PSCs: Our work on bone marrow progenitor stem cells (BM-PSCs) focuses on a patented methodology that combines a 3D-collagen scaffold with home-engineered growth factors to isolate, propagate and differentiate BM-PSCs into de chondrogenic and osteogenic pathways (Patent PCT/ES2005/000287). We are currently characterizing this method.

Engineered growth factors: The TGFß superfamily (especially BMPs) have proven to be the most powerful osteogenic molecules among growth factors, but their low affinity for collagen and short half-life hamper them for therapeutic use. For this reason we are engineering specific growth factors of the TGFß-superfamily to carry a collagen binding domain (CBD). The resulting molecules should get trapped in the tissues extracellular matrix and be slowly released from there. We have engineered two growth factors in this way: rhBMP2-CBD and rhBMP6-CBD. Their stability and osteoinductive properties are currently being tested.

Biomaterials: We are testing the performance of composites made up of our chondro/osteoinduced cells and specific biomaterials. Preliminary results show that our cells can be successfully combined with hidroxiapatite. We are presently testing a novel biomaterial based on the SBA-15 ternary system (patent number P200702694).

(P 87) Comparative Characterization of Cultured Human Term Amnion Epithelial and Mesenchymal Stromal Cells for Application in Cell Therapy

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Emerging evidence suggests human amnion as a source of two types of pluripotent cells, amnion epithelial cells (hAECs) and mesenchymal stromal cells (hAMSCs), for applications in cell therapy. We focused on first side-by-side characterization of hAECs versus hAMSCs regarding their expansion and stemness after isolation and culture. We looked at amnion cell isolates from 27 term placentas. Primary yields were 6.3×10^6 hAECs and 1.7×10^6 hAMSCs per gram amnion. All 27 cases gave vital cultures of hAMSCs, while one third of cases (9 of 27) failed to give adherent cultures of hAECs. Primary cultures contained significantly more proliferating than apoptotic cells. Neither hAECs nor hAMSCs were clonogenic. They showed slow proliferation that almost stopped beyond passage 5. hAEC morphology changed towards mesenchymal phenotype over several passages. Primary and secondary cultures of hAECs and hAMSCs showed expression of mesenchymal progenitor markers CD73, CD90, CD105 and CD166, and the embryonic stem cell markers SSEA-3 and -4. Transcripts of Oct-3/4 and stem cell factor were detected in primary and secondary cultures, but no of telomerase reverse transcriptase. Immunocytochemistry confirmed translation into Oct-3/4 protein in part of hAEC cultures, but not in hAMSCs. Both amnion cell types stained for CD90 and SSEA-4. Osteogenic induction studies showed significantly stronger differentiation of hAECs than hAMSCs; this capacity to differentiate varied among cases. In conclusion, hAECs and hAMSCs in culture exhibit and maintain a similar marker profile of mesenchymal progenitors. hAECs were found as less reliable source than hAMSCs and altered morphology during subculture.

(P 88) Comparative Study of Cell Seeding and Bioreactor Culture Methods for Bone Engineering Scaffolds

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Bone tissue engineering (TE) offers a promising strategy to heal severe bone injuries by using cell-seeded scaffolds. In order to evolve towards clinical practice it is essential to firstly guarantee a high, consistent and uniform cell-seeding and secondly culture these cell-seeded scaffolds in controlled bioreactors. Because most static seeding techniques do not meet these requirements

this study evaluated various cell seeding methods in bone TE Titanium scaffolds (diameter 5 mm, height 3 mm). Two Million Human Saos-2 Osteoblasts were seeded using different ratios of cell suspension volume versus scaffold volume (0.68, 1.01, 1.69) with different pre-seeding times (30 min, 2h, 4h) for static culture, and different dynamic culture conditions (rotatory, perfusion, oscillatory perfusion). The total DNA in the scaffolds, in the media and in the vessels was quantified to determine the cell number and distribution. The samples were stained with LIVE/DEAD viability kit and analysed using fluorescence stereomicroscope. It was shown that the 0.68 ratio group resulted in the highest cell proportion adhering to the scaffold. The dynamic method had a lower seeding efficiency (~38%) compared with static methods (~64%). However the cell distribution in static group is less homogeneous. It was concluded, when aiming at consistent cell-seeding, that for static seeding a small volume ratio is required followed by culturing in an initial 2h pre-seeding period. For dynamic seeding, a precise controlled oscillatory seeding strategy is preferred. Further optimisation of these different methods is required in order to obtain sufficiently high seeding efficiencies that would allow a future transfer towards clinical practice.

(P 89) Comparative Study of Slow Freezing and Vitrification of Mouse Osteoblasts in Suspension and in Monolayer

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The influence of two cryopreservation methods, slow freezing and vitrification, on the survival of osteoblasts cryopreserved as single cells in suspension and adherent cells in monolayer was investigated. Cells suspension and cell monolayer were exposed to 10% Me_2SO with or without 10% FBS in DMEM at 4°C, and then the cells were cooled to -80°C at controlled rate of 1°C/min for overnight and stored in liquid nitrogen. A vitrification cocktail of Me_2SO, formamide and 1,2-propanediol was loaded stepwise into single cells in suspension and adherent cells in monolayer and then quenched into the liquid nitrogen. After rapid thawing in a 37°C water bath and serial removal of cryoprotectants, cell survival rate was judged by trypan blue and Ho/PI staining. Cell morphology, alkaline phosphatase (ALP) activity was also determined. The viability of single cells in suspension after slow freezing was 92%, but after vitrification no single cell was recovered. Surviving cells after slow freezing quickly recovered and exhibited a morphology and alkaline phosphatase (ALP) activity indistinguishable from noncryopreserved cells. The survival rate of the adherent cells in monolayer after the slow-freezing and vitrification was very low. The results showed that the viability of osteoblasts in monolayer decreased significantly compared with the viability of cells in suspension under the slow-freezing cryopreservation procedure. Cell-cell, cell-matrix interaction may alter the cryobiological properties of cells. It is indicated that adherent cells were subject to more severe damage than single cells. The conventional slow-freezing method of cryopreservation of cells in suspension is reliable and effective.

(P 90) Comparison of Different Stem Cell Sources and Biomimetic Scaffolds for Bone Tissue Engineering

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Properties of stem cells together with tissue engineering approaches can be used to overcome bone degeneration and its loss in dentistry, plastic surgery and orthopedics. We have compared the seeding of progenitor cells from 3 different sources (adult periosteum, adult subcutaneous fat tissue and fetal stem cells) on 2 types of scaffolds, traditionally used in bone tissue engineering: β-tricalcium phosphate (ChronOs™, Synthes GmbH, Switzerland) and hydroxyapatite. Human adult fibroblasts were used as control cell type in all experiments. The highly porous matrices support the induction of osteogenic differentiation of stem cells, confirmed by positive staining with monoclonal antibodies to osteocalcin. These scaffolds also create and maintain a space that facilitates progenitor cell survival, proliferation, and migration, shown on scanning electron microscopy pictures. All of the cell types were successfully seeded on both types of matrices, though, showed different proliferation and migration results. The best result on seeding, proliferation and survival was reached with the use of cells from adult periosteum and β-tricalcium phosphate porous blocks. Thus, we suggest to use periosteal cells as the appropriate source of cells-progenitors for autologous bone tissue engineering techniques. This approach will help to solve problems in regenerative medicine, associated with such areas as wound healing and the

(P 91) Comparison of Flow Cytometry *vs* Haematology Analyzers in Cell Enumeration for Haematopoietic Transplantation

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Over the last two decades peripheral blood progenitor cells (PBPC) have replaced bone marrow (BM) as the preferred source of haematopoietic stem cells (HSC) for both autologous and allogeneic transplants. It is known that the number of infused CD34+ cells in patients determines the success of transplants. This quantification is routinely measured by flow cytometry, but it is known that both CD34+ and CD34- haematopoietic progenitor cells (HPC) cells are essential to transplants' success. Last generation haematology analyzers such as the Sysmex XE-2100 with the ''immature information'' (IMI) channel offer an alternative method to count HPC present in a sample. Several studies have proved the correlation between CD34+ counted by flow cytometry and HPC counted by automated haematology analyzer. From January to the end of July 2007, 21 healthy donors and 30 patients with haematologic malignancies underwent G-CSF mobilization for collection of PBPC. On the 5th day of mobilization total leucocyte and HPC counts were assessed by the Sysmex XE-2100 analyzer in EDTA

peripheral blood (PB) samples and PBPC grafts. CD34+ cell enumeration was performed in the same samples by standard flow cytometric assay. Our data showed that for both healthy donors and patients, PB HPC counts correlated with CD34+ cell counts predicting the PBPC yield and that HPC counts were significantly higher than CD34+ counts (2.0 fold for PB and 2.6 fold for PBPC). In summary, it remains to be determined the role of HPC content of the graft on the haematopoietic recovery of patients.

(P 92) Comparison of Grafting with Buccal Mucosa Versus Bladder Submucosa (BSM) in Patients with Complex Urethral Structures

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Urethral strictures represent a longstanding reconstructive dilemma due to the limited availability of tissue substitutes and incidence of recurrence. The goal of this study was to compare bladder derived collagen-based matrices (bladder submucosa; BSM) to another commonly used tissue substitute, buccal mucosa, in patients with complex urethral strictures. To this end, BSM was obtained from cadaveric donors and prepared as an "off the shelf" material for use in 30 stricture patients (age ranging from 21–59 years (ave: 36.2)). Patients were randomized into two groups and treated with an onlay procedure (avg. stricture length of 6.9 cm). All patients were followed for 18–36 months (mean: 25 months). In patients with a healthy urethral bed (< 2 prior op), the success rate of buccal mucosa grafts (10/10) was similar to the BSM grafts (8/9) in terms of patency. In patients with an unhealthy urethral bed (> 2 prior op), 2/6 patients with a BSM graft were successful, whereas 5/5 patients with a buccal mucosa graft had a patent urethra. Postoperative uroflowmetry showed a significant voiding improvement in both groups. Histology of graft biopsies showed normal urethral tissue characteristics. These findings show that BSM can be used for urethral repair in stricture patients with a healthy urethral bed (2 or less prior surgeries), and furthermore, as an "off the shelf" material it eliminates the problems associated with donor site morbidity. Nonetheless, the buccal mucosa remains a preferable graft material for patients with 3 or more prior surgeries (i.e., unhealthy urethral beds).

(P 93) Comparison of Mesenchymal Stem Cells Derived from Bone Marrow and Adipose Tissue of Minipigs

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For testing the reconstruction of osteochondral defects *in vivo* by tissue engineering approaches large animal models like pig have to be used to ensure that similar physical forces as in humans act on the constructs. To circumvent the need of immunosuppressive agents for future *in vivo* studies autologous cells should be used. Hence, bone marrow and adipose tissue from Göttinger minipigs were compared as mesenchymal stem cell sources regarding cell yield, proliferation capacity, surface marker expression and differentiation ability to the adipogenic, osteogenic and chondrogenic lineage.

Proliferation capacity was examined by calculating population doubling time (PDT). FACS analysis was performed for typical MSC surface markers. Differentiation experiments were done as described for human MSC and lineage conversion was checked by Oil Red O staining (adipogenic), alkaline phosphatase activity (osteogenic), Alcian blue staining and GAG quantification (both chondrogenic).

PDT of ASC during the first 8 passages was 57 hours whereas BMSC needed 108.4 hours for doubling. This could be decreased to 58 hours by supplementing the culture medium with 1 ng/mL bFGF. Adipogenic and osteogenic differentiation was observed in 90% of BMSC and ASC experiments performed whereas chondrogenic differentiation was found in all BMSC but only in 1 of 5 ASC approaches.

Porcine BMSC were found to be superior to ASC regarding their chondrogenic differentiation potential *in vitro*. If this is due to *in vitro* conditions or inherent characteristics that can also be observed *in vivo* has to be elucidated.

Acknowledgments: HIPPOCRATES (NMP3-CT-2003-505758), Lorenz Boehler Fonds, EXPERTISSUES (NMP3-CT-2004-500283).

(P 94) Comparison of Schwann Cell and Olfactory Ensheathing Cell Influence in Peripheral Nerve Regeneration

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Introduction: The use of cell filled conduits in bridging nerve defects has become one of the most promising alternatives in the reconstruction of complex nerve injuries. Our group used a biogenically created conduit filled with Schwann Cells (SC), Olfactory Enesheathing Cells (OEC) and Fibrin alone to bridge a 15 mm gap in the sciatic nerve of the rat.

Materials and Methods: We obtained biogenic conduits in a two-step operation. The following groups were investigated: autologous nerve transplantation NT, biogenic conduit filled with a three-dimensional fibrin matrix BC, F, biogenic conduit and OEC in fibrin matrix BC, F, OEC, biogenic conduit and SC in fibrin matrix BC, F, SC. Survival time was 4 and 16 weeks. Regeneration was clinically tested using the sciatic functional index. The number of axons per cross section and the axon myelin ratio were determined on semi thin sections.

Results: The axon myelin ratio of the operated sides of all groups showed significant worse myelinisation than the healthy control group after 4 and 16 weeks. The SC myelinisation was significantly better than the OEC myelinisation. The axons in the fibrin filled conduit showed better myelinisation than the SC and OEC groups. Clinical function after 16 weeks showed identical results for the fibrin filled and cell filled conduits.

Conclusion: Biogenic conduits for gap bridging are a suitable alternative to artifical conduits. Paradoxically the results demonstrate an advantage of only fibrin filled conduits compared to cell filled conduits, while the use of OEC showed no advantage to the use of SC.

(P 95) Comparisons Between Sources of Mesenchymal Stem Cells in the Wharton's Jelly of the Human Umbilical Cord and Bone Marrow

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The Wharton's jelly of the umbilical cord contains mucoid connective tissue surrounding two arteries and one vein. The phenotypic stromal cells in the Wharton's jelly are fibroblast-like ones. The differentiation may be manipulated by the culture medium. However the potential of umbilical stromal cells (UMSC) is required to be compared with bone marrow mesenchymal stem cells (BMSC).

Initiation of Wharton's Jelly Matrix Cell Cultures to obtain human UMSC from the umbilical cord, when expanded under suitable culture conditions, the differentiation ability will be compared with human BMSC. The cellular markers were studied by flowcytometry, western blotting and immunocytochemistry.

Wharton's jelly matrix has very little collagen, and there are multiple mesenchymal cells. Using flow cytometric analysis, we found that UMSC express matrix receptors (CD44, CD105) and integrin markers (CD29, CD51) but not hematopoietic lineage markers (CD34, CD45). Interestingly, these cells also express significant amounts of mesenchymal stem cell marker (CD73). We therefore investigated the potential of these cells to differentiate into multilineage potential under suitable culture conditions, are able to differentiate into cells of the hepatogenic, adipogenic, chondrogenic and osteogenic lineages.

We reasoned that umbilical cord matrix could be an affluent source of UMSC based on the primitive cell types seen in Wharton's jelly. There are no major differences between UMSC and BMSC. These findings may have a noteworthy impact on studies of stem cell plasticity, multi-lineage differentiation, functional genomics, pharmacological testing, cell therapy, cell bank and tissue engineering by assisting to get rid of troubled ethical and technical issues.

(P 96) Complexity of Approach and Demand for Esophagus Tissue Engineering

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There is an enormous demand for esophageal tissue not only in the pediatric population but also the adult population. Since esophagus is an organ that transverses 3 anatomic planes, the neck, thorax and the abdomen its anatomy varies according to the type of function. The three main broad functions of the esophagus involve active swallowing, transportation of the swallowed food bolus and resisting the gastric reflux. Although the esophagus in geometric terms is a tube the histology of the organ and the tissue organization vary along its entire length.

The aim of this literature based review is to identify pathologies and pathological states that require esophageal tissue replacement. Furthermore, an estimation of the population affected by esophageal pathologies was calculated and to evaluation the morbidity due to esophageal tissue loss was categorized. Also, the surgical techniques such as gastric transposition, ileal conduit and colon transposition presently used to treat esophageal pathlogies have been evaluated and their morbidities estimated.

The major demand for esophageal tissue replacement in the field of pediatric surgeryis in the area of the thoracic esophagus. Technical difficulties in the repair of esophageal atresia according to the classification identifies the demand for this malformation. The results show the spectrum of esophageal loss and requirement from the clinical perspective inorder to provide objective guidelines for tissue engineering research in esophagus.

(P 97) Composite Calcium Phosphate Scaffolds for Bone Tissue Engineering

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Tissue grafts engineered to facilitate healing of bone defects require scaffolds capable of bearing load and supporting the growth of osteoprogenitor cells. Hydroxyapatite (HAP) is a calcium phosphate that has been widely used as a scaffold for bone grafts due to its osteoconductivity and biocompatibility. However, HAP lacks osteoinductive properties to stimulate osteogenesis and is resistant to biodegradation.

In contrast, amorphous calcium phosphate (ACP) is a mineral that solubilizes under aqueous conditions, releasing calcium and phosphate ions. We propose that the immobilization of ACP particles within a biodegradable PLGA scaffold will enhance the stiffness and osteoconductivity of the scaffold while providing calcium and phosphate ions to stimulate osteogenic differentiation.

PLGA microspheres were fabricated using an oil-in-water emulsion technique. Microspheres were then fused with 0.5% (w/v) HAP or ACP particles at 70°C. Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were used to determine pore size and calcium phosphate distribution on the scaffold surface. Scaffolds were tested in compression to determine the effects of calcium phosphate addition on scaffold stiffness.

Osteogenic differentiation on the scaffolds was assessed by culturing pre-osteoblasts on the surface of HAP and ACP scaffolds. Cell number and alkaline phosphatase activity were measured at days 7 and 10. Late stage osteogenic markers were measured by quantitative PCR after 14 days of culture.

Preliminary SEM and EDS have confirmed the presence of minerals on the scaffold surface. In addition, cells cultured on scaffolds containing ACP demonstrated increased ALP activity.

(P 98) Comprehensive *In vitro* Model for the Engineering of Microvasculature—Evaluation of Morphological and Functional Data

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Objectives: The engineering of functional microvasculature has special impact for voluminous and metabolically active tissues. In order to quantify formation of coherent vascular networks, appropriate 3D imaging and image processing is needed. We present a model for *in vitro* engineering, imaging and comprehensive analysis of microvessel-like structures.

Material and methods: Small-diameter vessel equivalents were fabricated from collagenous scaffolds, ATSC and endothelial cells. Specimens were compared after 16 days of rotation or pulsatile perfusion with regard to ''capillary'' density, recruitment of α-actin-positive cells and branching from the central lumen. Algorithms were developed for 3D measurement of characteristic quantities of capillary-like structures and for pericytal recruitment from CLSM (confocal laser scanning microscopy) data.

Results: The aggregates were imaged and measured three-dimensionally by use of CLSM and image analysis. Capillary density and the maturation of capillary-like structures ascertained by the recruitment of á-actin-positive cells reached the highest degree in the luminal portion of the perfused specimens. Manually acquired histomorphometrical data were compared with computed CLSM derived data and agree with results from automated 3D measurement.

Conclusion: Application of hydrodynamic forces (flow and pressure) is essential for microvascular engineering *in vitro* and has impact for the engineering of ''feeder donor vessel'' with the ability to supply surrounding tissues for the purposes of plastic and reconstructive surgery.

(P 99) Conjugation of Fibroin and Starch to Chitosan for Increasing Cell Proliferation Capacity

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In this study, chitosan conjugates with starch and fibroin were produced for increasing degradability in the presence of physiological enzymes and cell proliferation capacities of biomaterials. The degradation profile was monitored over prolonged time periods and characterization of chemical changes during degradation periods were investigated by spectroscopic methods. Various ratios of starch, fibroin and chitosan (%, (weight/weight)) were prepared. The *in vitro* cell culture studies were conducted to evaluate biocompatibility and proliferation capacities of conjugate materials. The DNA content of cells at certain time points of cell culture was measured for their proliferation potentials. The cell morphologies such as cell area and maximum cell length were measured over a large cell population. The spectrum traces suggest that the weight loss was primarily from starch degradation by α-amylase. The absorption bands after protease degradation showed no significant changes and this result can indicate that the enzyme activity was impaired or inhibited by extensive crosslinking between oxidized starch and fibroin or chitosan (a natural substrate of protease) biopolymers. The DNA quantities of conjugate materials after cell culture were increased linearly with higher fibroin ratios. This result proves that fibroin incorporation into chitosan and starch matrix is improving adsorption and proliferation properties of conjugate materials. The unique cell features were detected for each type of conjugate material. In general, the cells on pure fibroin and chitosan had a higher cell area than conjugates. Higher fibroin content steadily increased the cell area and cell length.

(P 100) Construction and Characterisation of a New Vascular Composite Bioscaffold

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Introduction: Atherosclerotic disease is the leading cause of death in Western society. Most patients do not have suitable/sufficient autologous tissue as bypass material. The main natural materials constituting the arterial wall are type I collagen (for strength) and elastin (for elasticity). Therefore, the goal of this study is to use highly-purified type I collagen and elastin to create tubular grafts.

Materials & Methods: Elastin fibres in diluted acetic acid were moulded around a mandrel, frozen and lyophilised. Subsequently, type I collagen in diluted acetic acid was moulded around the elastin sleeve and mandrel, frozen and lyophilised. Mono- and dual-layered scaffolds were chemically crosslinked (EDC/NHS), with or without heparin. The growth factors VEGF and FGF-2 were bound to heparin by dipping techniques. The morphology and ultrastructure were studied with SEM and TEM. The degree of crosslinking and the amount of covalently bound heparin were measured. Proteins and growth factors within the scaffold were examined by (immuno) histology.

Results: Tubular mono- and dual-layered constructs were made. Histology and TEM clearly showed attachment of the collagen layer to the elastin layer. Crosslinking efficiency was 60%. Up to 20% of heparin was covalently attached to the scaffolds. Immunohistology demonstrated that heparin, VEGF and FGF-2 were present throughout the scaffold.

Conclusion: These preliminary results are encouraging for the construction of completely natural grafts, as (1) type I collagen and elastin are the main components of the arterial wall, (2) this graft is molecularly-defined and (3) glycosaminoglycans and growth factors can be added easily.

(P 101) Construction of Dendric Structure Carrying RGDS and PHSRN Peptide Cell-Adhesion Motifs

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Solid-phase signals from biomaterial surface to adhering cells can be provided by adsorbed adhesion proteins of extracellular matrix or their fragments or even simple peptide motifs, capable of interaction with the cell transmembrane proteins—integrins. Not only the presence of the adhesion structures but also their proper distance and orientation at nanometer scale is important for the signal transfer efficiency. The work presents the design and synthesis of a dendron construct that can carry finbronectin-derived peptide sequences, such as RGDS and PHSRN peptide motifs of the 10^{th} type III repeat of fibronectin and to present them in an optimum distance and orientation for effective synergic binding to the alpha5beta1 cell integrin[1]. Series of constructs was designed based on lysine-core dendron structures with oligo 2-amino(ethoxy-acetic) acid[2] (AEAA) arms, containing an orthogonally protected reactive groups, allowing selective dendron modification by fibronectin based peptides. The dendrons with variable length of the oligo-AEAA arms were prepared using solid-phase peptide synthesis technique. Average distance between N-terminals of the dendron arms was studied using nonradiative excitation energy transfer (NRET) fluorescence spectroscopy with tryptophane (Try) and 4-dimethylamino-1-naftalenesulfonyl (Dns) as donor and acceptor, respectively.

Acknowledgement: Support by Academy of Sciences CR (No.: 1QS00110564) and Center for Cell Therapy and Tissue Repair (Ministry of Education CR, No.: 1M0021620803) is acknowledged.

[1]T.A. Petrie, J.R. Capadona, C.D.Reyes, A.J. García:, Biomaterials 27 5459–5470 (2006).
[2]V. Proks, L. Machová, F. Rypáèek: J. Pept.Sci., 2006, 12, 240 Suppl. S

(P 102) Controlling the Architecture and Mechanical Properties of Supercritical Fluid Foamed Poly D,L-Lactic Acid Scaffolds

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Supercritical CO_2 ($scCO_2$) scaffold formation is a solvent free, low temperature process which produces open cell, inter-connected foamed structures. Successful applications of this technique include controlled release of proteins (Howdle *et al.*, 2001), promotion of bone formation *in vitro* and *in vivo* (Yang *et al.*, 2004) and the induction of angiogenesis *in vitro* (Kanczler *et al.*, 2007). Scaffolds of divergent pore size and structure can be produced and hence this study sought to elucidate the effects of processing conditions on the architecture and mechanical properties of the scaffolds.

Three molecular weights of poly(D,L-lactic acid (15 kDa, 24 kDa and 57 kDa) were used to form scaffolds under different depressurisation rates. Scaffold morphology was characterized by micro X-ray computed tomography and mechanical properties were tested using a TA.HD Texture Analyser.

57 kDa PDLLA scaffolds displayed a typical stress-strain curve for elastomeric open cell foams. The Young's modulus, elastic collapse and ultimate stresses were increased by using shorter depressurisation rates during fabrication; the ultimate stress ranged from 5.1 MPa using a 10 minute vent to 2.8 MPa for a 45 minute vent.

The failure behaviour of 15 kDa and 24 kDa PDLLA scaffolds was more typical of porous brittle materials (Gibson, 2005). The porosity of the scaffolds increased with decreased depressurization rates; the maximum stress for the 15 kDa and 24 kDa scaffolds (0.29 MPa and 0.60 MPa respectively) occurred with a 10 minute vent.

The architecture and mechanical properties of PDLLA scaffolds can be tailored by adjusting the processing conditions and the molecular weight of the polymer.

(P 103) Conversion of Corallina Officinalis into Calcium Phosphates by using Hydrothermal Treatment and their Potential for Bone Tissue Engineering Application

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Calcium phosphates (CaP) such as hydroxyapatite (HA) and tricalcium phosphate are widely used in bone Tissue Engineering, due to their biocompatibility, osteoinductive and/or osteoconductive properties. The aim of this work is to obtain CaP through the hydrothermal conversion of the calcite (HCC) present in the alga C. officinalis. To promote the HCC into CaP, the alga was immersed in a phosphate solution (Ps) and subjected to a hydrothermal treatment. Different Ps concentrations were used to test the influence of this parameter on the HCC into CaP and on the type of CaP formed. FTIR, EDS and XRD analyses demonstrated the successful conversion of the C. officinalis calcite into HA, after treatment with a 0.1M Ps, and into a mixture of HA and whitlockite, after treatment with a 0.5M Ps. Moreover, the SEM analysis showed that this treatment allowed the preservation of the original porous structure of this alga. A cellular study was also performed in order to evaluate the capacity of these CaPs to support the proliferation and differentiation of goat bone marrow cells (GBMCs) into osteoblasts. GBMCs were seeded onto PCL membranes with the CaP granules obtained by HCC. Cells were cultured under static conditions in osteogenic medium for 7, 14 and 21 days. GBMCs seeded on C. officinalis-PCL membranes showed a higher cell viability and proliferation when compared with GBMCs seeded onto PCL (polycaprolactone) membranes without CaPs, the positive control, indicating that the obtained CaPs are suitable for bone tissue engineering applications.

(P 104) Cryogel Scaffolds with Stem Cells for Bone Tissue Engineering: an Animal Model

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Lactide-dextran based swellable cross-linked cryogels with supermacro porosities were produced by cryogelation. Mesencyhmal stem cells were derived from rat bone marrow. 8-mm-diameter critical size cranial defects were created in rat cranium as animal model for bone tissue engineering. Cryogel scaffolds (8 mm in diameter and 1 mm in thickness) were implanted in the cranial defects with or without stem cells. In a parallel group of study, stem cells were injected into the defects without scaffold. Samples from the implant sites were removed after 1, 3 and 6 months postimplantation and bone regeneration and tissue response were followed by histological tests. The *in vivo* results exhibited osseous tissue integration within the implant and mineralized functionally stable bone restoration of the cranial defects. Tissue formation started in the macrospores of the scaffold starting from periphery to the center. A significant ingrowth of connective tissue cells and new blood vessels allowed new bone formation. Histological data clearly demonstrated that the more successful results were observed in the group that we have used cryogel scaffolds with mesenchymal stem cells.

(P 105) Cryopreservation of Neural Stem Cells Encapsulated in Rat Tail Collagen Type I

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The cryopreservation of neural stem cells (NSCs) for clinical medicine transplantation and the establishment of stem cell bank are of important significance. This study followed the traditional slow-cooling method with the NSCs encapsulated in rat tail collagen type I to develop a new cryopreservation protocol. DMSO is the cryoprotectants. The concentration of rat tail collagen type I is 0.5mg/ml,1.0 mg/ml and 1.5 mg/ml respectively, and the concentration of DMSO is 10%(v/v), 15%(v/v), 20%(v/v) and 25% (v/v). From the variation of the concentration of DMSO at the external system after loading, the average concentration of DMSO solution within the collagen system can be calculated. And then the average internal concentrations of 8% to 15% were selected for the cryopreservation experiments and the mechanical function of collagen was tested. The NSCs are assayed by flow cytometry for the apoptosis of 0h and 72h after freeze-thawing and the ATP enzyme activity. The NSCs stem cells after freeze-thawing were assayed by immunofluorescence. The results show that neural stem cells in the collagen keeps their good shape, six groups of experimental conditions were tested for the slow-cooling cryopreservition of NSCs. The survival rate of the encapsulated NSCs after freezing and thawing operations could be about 85%, which is higher than that of cryopreservation of cell suspension. Its ATP enzyme activity is also higher than that of the traditional method. The NSCs are assayed by immunofluorescence after freeze-thawing, the Nestin-positive cells were found. The cultured NSCs could be differentiated into neuron (αtubulinIII-positive), astrocyte (GFAP-positive) and oligodendrocyte (RIP-positive).

(P 106) CT-Assisted Virtual Endoscopy: an Innovative Imaging Tool for the Assessment of Engineered Tissue

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Introduction: Computerized Tomography (CT) has available for many decades. Although the capabilities of conventional CT scanners is limited by visualizing anatomical structures, technological advances in hardware improvement and sophisticated software development have allowed for expanded utilization. We examined the capabilities of a three-dimensional CT scanner and enabling software to provide a virtual endoscopic tool for assessment of tabularized engineered tissue *in vivo*.

Methods: We used a Toshiba 32-Slice Aquilion scanner, capable of imaging 0.5 mm-thick tissue slices. Double imaging could provide visualization every 0.25 mm. The scanned images were processed with the TeraRecon Aquarius Work station, which provides 2–4 dimensional viewing capabilities. CT-guided urethrocystographic examinations were performed in 12 male beagles that underwent urethral surgery using a 6 cm-long tabularized engineered construct. Pre and post-operative scanning was performed in conjunction with retrograde urethrograms and voiding cystourethrography for comparison, and animals were sacrificed at predetermined time points. Urethral diameter, length and area of lumen were measured.

Results: Scanned images were converted into movie files, which allowed for accurate analyses from various angles. The pre and post-operative scans of each animal correlated with retrograde urethrograms and cystourethrography. The surgical sites were readily identified in detail. Gross examination of the retrieved urethras confirmed all the anatomical details visualized by virtual urethroscopy.

Conclusions: These findings show that CT-assisted virtual urethroscopy allows for accurate visualization of the urethral lumen, and provides an accurate assessment of tissue integrity. This technological non-invasive tool may play an important role in the evaluation of tabularized engineered tissues *in vivo*.

(P 107) Culture Substrate Geometry Influences Chondrogenic Phenotype *In Vitro*

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Availability of a sufficient number of cells with an appropriate phenotype is a major challenge especially for cartilage regenera-

tion. Microcarrier culture systems usually employ dynamic seeding on round-shaped support materials and seem to preserve the chondrogenic phenotype or even improve it (re-differentiation).

Interestingly, this holds as long as the microcarrier is approximately round, regardless of its diameter and substrate composition. Therefore, we aimed to investigate the effect of substrate curvature (flat vs. round) on cells in static (regular culture wells) or dynamic (spinner flask) cultures.

We cultured primary chondrocytes from different passages in a spinner-flask. Using microcarriers of identical chemical composition, but with two different surface geometries, either round or cubic (flat), we showed that not seeding or culturing dynamics (i.e. ''shear'') prevents chondrogenic de-differentiation in bioreactor systems.

Same cells were kept in regular static 2D culture on custom-made, fibronection-coated silicon rubber scaffolds with different surface curvatures. Chondrocytes spontaneously stretched out once the arc of the surface (i.e. the radius of the curved semisphere) became too long. Based on marker gene expression (aggrecan; versican; collagen types I, II, and IX; and SOX9), biochemical GAG and collagen analyses and cytoskeletal stainings, we showed that the curvature radius (or arc length) of the culture surface is the crucial driving force preserving a proper chondrogenic cell shape and phenotype.

Currently, we are investigating the optimal radius for serum- and growth factor-free cultures in order to maximize proliferation and re-differentiation capacity. Our insights might lead to innovative culture methods for cartilage tissue regeneration.

(P 108) Cytokeratin Expression Profiling as a Quality Control of Oral Mucosa Substitutes Generated by Tissue Engineering

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Introduction: Different techniques have been used to evaluate the quality and efficiency of oral tissues developed by tissue engineering. In this work, we have assessed the usefulness of our model of artificial human oral mucosa by *in vivo* grafting and evaluation of cytokeratin expression at both *in vitro* and *in vivo* levels.

Materials and Methods: Human oral mucosa substitutes were generated from primary cell cultures of human oral fibroblasts and keratinocytes using fibrin-agarose scaffolds. Air-liquid culture technique was used to induce stratification and differentiation of the epithelial layer. *In vivo* evaluation was carried out by implantation of the oral mucosa substitutes at the back of nine Fox1nu/nu immunodeficient athymic mice for 4 weeks. Expression of several cytokeratins and proliferating cell nuclear antigen (PCNA) was determined by microarray and immunohistochemistry.

Results and Discussion: Our results showed that the oral mucosa substitutes kept *in vitro* developed a multilayered epithelium that expressed PCNA and some markers of simple epithelia (cytokeratins 7, 8 and 18) and stratified epithelia (cytokeratins 5 and 13). Then, bioengineered tissues grafted onto nude mice exhibited complete biointegration, showing a cytokeratin expression pattern that was very similar to normal native oral mucosa controls, with the formation of rete ridges and neovascularization. Our results suggest that *in vitro* and *in vivo* cytokeratin profiling is a good quality control for human tissue substitutes generated by tissue engineering and imply that our model of fibrin-agarose oral mucosa substitute could have potential clinical value.

Supported by CTS-06-2191 and CM2005/011 from Junta de Andalucia.

(P 109) DC, NK and NKT Cells Present in PBPC Allografts Influence the Onset of Acute GVHD and the Immune Recovery

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Allogeneic haematopoietic stem cell transplant (HSCT) has evolved from a tool to rescue patients from the pancytopenia caused by aggressive chemotherapy regimes to a form of anti-neoplasic immunotherapy. Peripheral blood progenitor cells (PBPC) grafts are composed of not only $CD34^+$ progenitor cells, but also of large number of immunocompetent cells, namely natural killer cells (NK), natural killer T lymphocytes (NKT) and dendritic cells (DC), which can exert a potent anti-tumour effect.

We studied the composition of the PBPC allografts, from HLA matched relatives, used to transplant 25 patients with haematological diseases between August 2004 and August 2006. The time to engraftment and incidence of acute Graft versus Host Disease (aGVHD) in the patients were evaluated in order to investigate DC (both DC1 and DC2 subpopulations), NK and NKT influence on the clinical outcome.

Of the populations and ratios studied, only the DC population, in particular the DC1 subpopulation, correlated with the onset of aGVHD. We also found that the $CD34^+$ and NKT cell number in the graft, as well as the NKT/DC ratio, correlated with the peripheral blood (PB) lymphocyte count at day 15 post transplant, and the NK/DC ratio correlated with the time to a PB lymphocyte count of 500/uL, both important parameters in immune recovery.

From this ongoing work we have evidence that the composition and dose of the PBPC graft affects the outcome of allogeneic HSCT. Understanding how each population and their interaction affect the outcome of HSCT will hopefully allow for future tailor made PBPC grafts.

(P 110) Defined Biomatrix with Growth Factors for Bladder Wall Regeneration

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Introduction: Bladder augmentation through tissue engineering currently involves autologous tissue transplantation, e.g. insertion of small intestinal tissue. We evaluated four acellular collagen biomatrices for bladder wall regeneration in a rabbit model.

Material & Methods: Rabbits underwent bladder wall augmentation with a circular biochemically defined single (SL) or dual layer (DL) collagen biomatrix. The SL or DL matrices were composed of collagen (COL) + heparin (HEP) or COL + HEP + Growth Factors (GF). The following GF were used: Epithelial Growth Factor (EGF), Vascular Epithelial Growth Factor (VEGF) and b-Fibroblast Growth Factor (b-FGF). The regenerated bladder walls were harvested at 2, 4 and 12 weeks after implantation. Tissue regeneration was evaluated using routine histology.

Results: Biomatrix encrustation was observed in 30/48 (63%) of animals. All biomatrices showed considerable influx of inflammatory cells in the first 4 weeks, gradual decreasing in time. At all time points, we found collagen remnants in the regenerated bladder. The SL-HEP-GF, DL-HEP and DL-HEP-GF matrices showed earlier epithelialization and neovascularization in comparison with the SL-HEP biomatrix at 2 weeks. In the biomatrices with growth factors, only single muscle cells were present at the peripheral area of the regenerated graft material at 12 weeks.

Conclusions: Tissue regeneration velocity was higher with the use of dual layer or Growth Factors-modified biomatrices. This clearly shows that bioactive modifications with the use of special coatings and growth factors improves bladder wall regeneration for tissue engineering. The dual-layer biomatrix containing heparin and growth factors have a high capacity for bladder regeneration.

(P 111) Defining Physiological Parameters for Engineering a Vascular Media Model

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Engineering a blood vessel requires an understanding of the parameters governing the survival of resident vascular smooth muscle cells. We have developed an *in vitro* collagen-based vascular media model to test the correlation between cell density, O_2 requirements and cell viability.

Collagen type I constructs seeded with porcine Pulmonary Artery Smooth Muscle Cells (PASMCs) at different densities, low (11×10^6 cells/ml) and high (23×10^6 cells/ml) were compressed to produce 100 μm thick, dense collagen sheets. These were subsequently spiraled around a mandrel to mimic tubular structures and cultured for up to 72 hrs *in vitro* under static conditions. O_2 levels were quantified in the core of these constructs and correlated to cell viability.

The starting O_2 level was 140 mmHg.Within 24 hours O_2 levels in the core of constructs with low cell density dropped to 80 mmHg. For the high cell density, levels dropped to 10 mmHg and remained at this level for up to 3 days. Cell viability in high density constructs was 99% at 24 hrs both in the core and on the surface. This decreased to 80% ($p < 0.05$) in the core after 3 days, but remained high (95%) on the surface, indicating that physiological hypoxia might reduce cell viability. At low cell density, increasing diffusion distance by 33% (increasing collagen layers) resulted in a significant reduction of core O_2 levels to 50 mmHg within 24 hrs ($p < 0.05$).

This study provides a useful model for examining physiological parameters dictating vascular smooth muscle cell survival and behaviour in engineered vascular structures.

(P 112) Degradable Particulate Composite Reinforced with Nanofibre Meshes for Biomedical Applications

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In the biomaterials field, nanofibre based structures and its composites are promising materials to produce scaffolds mimicking the architecture of the extracellular matrix (ECM). The main purpose of this work was to develop a novel composite structure which combines polymeric microfibres reinforced by nanofibres. This combination was obtained by melting extrusion of a composite with a natural polymer, Chitosan particles, and a biodegradable polymer, poly(butylene succinate) (50:50 wt), reinforced with chitosan nanofibre meshes (0.05% wt). The chitosan meshes were produced by electrospinning. The nanofibre reinforced microfibres were analysed by SEM demonstrated a considerable alignment of the nanofibres along the longitudinal axis of the microfibres.

Tensile mechanical properties revealed that the introduction of the reinforcement in the microfibres composite increased the tensile modulus until 295.7 ± 16.2 MPa. This improvement is around 70% since the tensile modulus of microfibres without the nanofibre reinforcement was 175.6 ± 32.7 MPa. Various structures were subjected to swelling and degradation tests, in an isotonic saline solution at 37°C. The presence of chitosan nanofibres in the microfibres enhanced the water uptake in up to 24%. The weight loss was also increased, reaching a maximum of 7.4% at the third day of the degradation tests.

The combination of good mechanical properties and enhanced degradability of the developed fibres may have a great potential to produce 3D fiber meshes scaffolds.

Human bone marrow-derived stromal cells (hBMSCs) were seeded on those 3D fiber meshes scaffolds reinforced by nanofibers and sustain osteogenic differentiation, are adequate for bone tissue engineering.

(P 113) Development and Plasticity in Networks of Neurons Grown onto Micropatterned Substrates

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Various molecules, during development of the central nervous system, act on axons as guidance factors involved in the dynamic rearrangements of actin cytoskeleton and promoting the formation of neuronal networks. In order to characterize aspects of synaptic transmission and plasticity, we generated artificial networks of primary neurons onto substrates patterned with adhesion and guidance molecules by using micro-contact printing (microCP). We used poly-dimethylsiloxane stamps to pattern poly-D-lysine in various geometries, with the aim to optimize adhesion conditions for neurons on substrates. We evaluated different specific markers for structure and synaptic function to assess how different patterning geometries influence neuronal growth and network development. The structural organization of the network was analyzed by using immunofluorescence and atomic force microscopy. The functional parameters of synaptic transmission of micropatterned neuronal networks was assessed by patch-clamp techniques. Since the drying step in conventional microCP could induce protein denaturation and loss of function, we applied an indirect microCP by which the surface was patterned with an intermediate drying resistant molecule (protein A) that binds with high affinity and specificity to appropriate chimeras of the guidance cues, expressed as recombinant proteins (i.e. L1-Fc, NCAM-Fc). We found that glutathione and streptavidin could be used as bridging molecules because of their ability to resist to the drying step and bind with high affinity Glutathione-S-Transferase and biotin. This approach makes it possible to pattern more than one factor on the same surface and to study the simultaneous effects of multiple molecules on the differentiation of the neuronal network.

(P 114) Development and Validation of Bioactive Surfaces to Modulate Micro-Vessel Formation

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Introduction: The reconstitution of damaged tissues or organs represents a challenge for our aging society. Tissue engineering, at present, is facing as a major limitation to construct viable tissues, the lack of suitable vascular network to support its nutrient exchanges, growth and functionality.

In the present work, we propose the use of cross-linked gelatin to study HUVEC specific adhesion to bioactive niches immobilized on a low-fouling surface, aiming to stimulate and to control the formation of a vascular network in an adequate three dimensional system.

Materials and Methods: Glass substrates were surface-modified by plasma polymerization of n-heptylamine, in a custom-built plasma reactor. The exposed amine groups, resulted from n-heptylamine plasma polymer (HApp) layers, were used to covalently graft carboxy-methyl-dextran (CMD) layers by carboiimide chemistry.

Afterwards, synthetic Arg-Gly-Asp (RGD) peptides, were dispensed on activated CMD surfaces by using the Biochip Arrayer, a non-contact dispensing robot, with arraying capabilities such as those used to produce DNA and proteins chips.

These surfaces bearing RGD arrays were thereafter exposed to HUVEC in a gel, prepared through cross linking of acid porcine skin gelatin with transglutaminase (TGase).

Cells responses toward these spots will be presented.

Results: Based on this study, we found that the optimal conditions for gelatin gel preparation were 5% gelatin and 2U/mL of TGase, avoiding cells proliferation all over the gel, excepting those who selectively attached to bioactive niches. The results show a good perspective to produce controlled and directed micro-vessel networks.

(P 115) Development of a Growth Factor-Embedded Scaffold with Controllable Pore Size and Distribution using Micro-Stereolithography

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Introduction: Conventional scaffold fabrication methods have the demerit that they cannot control the inner/outer shapes of structures. By contrast, SFF(solid free-form fabrication) can control structure shapes freely. Therefore, the interest in SFF has increased. Among SFF technologies, MSTL(micro-stereolithography) does not use a heat source during fabrication and has the highest resolution. In addition, many researchers have reported on the effect of growth factors in cell differentiation. Therefore, we fabricated 3D (three-dimensional) scaffolds containing PLGA(polylactide glycolic acid) microspheres incorporating the growth factor BMP-2 (bone morphogenetic protein-2) using MSTL and observed the effects on cell differentiation.

Methods: Poly(propylene fumarate) (PPF) was synthesized via a condensation reaction. Then, diethyl fumarate(DEF) was added to reduce the viscosity. Finally, a photoinitiator was mixed with the synthesized photopolymer. BMP-2 was encapsulated in the PLGA using a double emulsion technique and the fabricated microspheres were centrifuged and freeze dried. Then, a MSTL system consisting of an Ar ion laser, an x–y–z stage, and optical components was used to fabricate 3D scaffolds by solidifying it in layers.

Results/Discussion: A scaffold containing PLGA microspheres that incorporated the growth factor BMP-2 was fabricated using the MSTL system and PPF/DEF photopolymer. The pores and lines of the fabricated scaffold were regular and all of the pores were connected. PLGA microspheres were located both within and on the surface of the scaffold. After scaffold fabrication, MC3T3-E1 cells were seeded on the scaffold and cell adhesion and differentiation were observed. This growth factor-embedded scaffold will be a good candidate as a bone scaffold in tissue engineering.

(P 116) Development of a Strategy to Functionalize Dextrin-Based Hydrogel for Animal Cell Cultures

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Several approaches can be used to functionalize biomaterials for biomedical applications, such as hydrogels. One of the molecules mostly used to improve cells adhesion to the biomaterials is the Arg-Gly-Asp (RGD). The RGD sequence is the ligand for integrin-mediator cell adhesion, which is present in several proteins from the extracellular matrix (ECM); in many cases, this sequence was described as being the major functional group responsible for cellular adhesion. The covalent binding and incorporation of this molecule during biomaterial production are the procedures mostly used. Another approach is to bind RGD molecules on the biomaterials surface by fusing proteins with affinity to the biomaterial. The present work shows the successful functionalization of a dextrin-based hydrogel using a fusion protein, containing a C-terminal starch binding domain (SBD) and an N-terminal RGD sequence. The SBD belongs to an α-amylase from Bacillus sp TS-23. The recombinant proteins were cloned, expressed, purified and tested in *in vitro* assays. The evaluation of cell attachment, spreading and proliferation were performed on the polystyrene and dextrin-based hydrogel, using mouse embryo fibroblasts 3T3. The results show that, in both cases, the presence of RGD-SBD protein highly improves cells adhesion and spreading. In fact, cell differentiation on the hydrogel surface was observed to occur only in the presence of the RGD-SBD.

(P 117) Development of Bio-Nanocomposite Hydrogels Based on Silicate Cross-Linked Peo for Tissue Engineering

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The goal of our work is to develop and refine engineered synthetic hydrogels with appropriate structural and mechanical properties that can be readily applied to the creation of replacement tissues. Our approach is predicated upon the development of a silicate cross-linked polyethylene oxide (PEO) hydrogel containing polysaccharides. We have developed a new technology to produce hydrogels that can be both viscoelastic and injectable or elastomeric-like tough and compliant. Although PEO-based materials have gained recognition for good biocompatibility, the use of these materials remains limited due to their relatively inferior mechanical properties. Our approach overcomes this major deficiency by using silicate nanoparticles to cross-link the PEO. In addition, these silicate cross-linkers provide exfoliated, dissolution, and degradation properties. By the addition of polysaccharides, we can create a host of interpenetrating hydrogel networks with synthetic variations in structure and mechanical strength. Most importantly, the reversible cross-linking of the interpenetrating polymer networks allows for the ability to tune mechanical properties to those of cartilage or other tissues. We will present preliminary results that show how to synthesize and formulate optimal nanocomposite hydrogels that maintain mechanical strength, biocompatibility and degradability. We will show how the physical properties of scaffolding materials will be optimized to provide a suitable extracellular matrix (ECM) for cell growth and how the biological properties will be developed for repair of cartilage.

(P 118) Development of Bioactive and Biodegradable Chitosan-Based Thermo-Responsive Hydrogels

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There is increasing interest on the development of new tissue engineering strategies to deliver cells and bioactive agents encapsulated in a biodegradable matrix through minimally invasive procedures. The present work proposes to develop a combination of chitosan—β-glycerophosphate salt formulation with bioactive glass (BG) nanoparticles in order to conceive novel injectable thermoresponsive hydrogels to be employed in different orthopedic applications. This work is inspired on natural biomineralization process and bone structure to create injectable bionanocomposites that combine thermal inducing gelation, biocompatibility, biodegradability and bioactivity.

The BG particles presented a spherical shape with nanometric scale diameters, which guarantee its efficient injection through small diameter needles into bone defects. The rheological properties and gelation points of the developed nanocomposite systems revealed to be adequate for intra-corporal injection. *In vitro* bioactivity tests, using incubation protocols in a simulated body fluid, SBF, with ion concentration approximately equal to those of human blood plasma, allowed the observation of bone-like apatite deposition in the hydrogel formulations containing bioactive nanoparticles. The density of apatite formed increased when increasing BG content and soaking time in SBF. These results suggest that such smart hydrogels could be used as temporary injectable scaffolds in bone tissue engineering applications. Besides, they can also be combined with more robust tri-dimensional scaffolds in bone tissue engineering strategies to guarantee adequate bone's mechanical features. Finally, the proposed osteoconductive injectable biodegradable materials have potential to be used in different orthopedic reconstructive and regenerative applications and stimulate the development of other biocompatible formulations.

(P 119) Development of Bioartificial Hair Papilla Tissue Employing Mesenchymal Stem Cells

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The hair follicle is small, but complex and a dynamic organ. In humans, it plays an important role from the esthetic aspect of social life. In the past,alopecia was treated typically by various methods of implanting artificial hair into hair follicle root bulbs of the scalp, but such artificial hair implant methods had led to some serious problems, and such methods are now banned. Currently, there are two methods employed to treat alopecia: drug or natural substance therapy, and human hair transplantation. Although the transplanted hair settles at the transplant area as a complete hair follicle and becomes a permanent hair that undergoes a normal growth cycle, the number of hair to be transplanted is severely limited, and in case of transplanting about 2,000 hair strands per one operation, it is generally not possible to perform more than three such operations. Thus, the methods currently used for treating alopecia have a number of limitations, and to overcome such problems, many re-

searchers have attempted to revive hair follicles by *in vitro* culturing hair follicle cells and implanting them in the treatment area. In our study, by utilizing culture-expanded mesenchymal stem cells (MSCs) which don't have aggregative activity, cell-aggregated spheroidal DP tissues were produced by a special culture condition *in vitro*, and hair follicle inductive capacity pertinent to the aggregative activity was evaluated.

(P 120) Development of Biomimetic and Smart Coatings Based on Elastin-Like Polymers Containing RGD Cell Attachment Sequences

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Elastin-like polymers (ELPs) are based on repeating sequences found in the natural elastin. They show remarkable combination of properties, such as a large range of mechanical properties, excellent biocompatibility, and smart behaviour and self-assembly towards temperature, pH and often different stimuli. Around a certain temperature, known as inverse transition temperature (ITT) these polymers show phase transitions aqueous solutions: under the ITT, the polymer chains are disordered and in a relatively extended state, remaining soluble; above the ITT, the chains suffer a transition to a helical ordered state and aggregate. Nowadays, through recombinant genetic engineering it is possible to construct and produce recombinant ELPs with specific cell attachment sequences from human fibronectin and elastin.

In this study, an ELP containing six monomers of RGD sequence was obtained by fermentation of a genetically modified Escherichi coli (E. coli) stock. After purification, the final bioproduction yield was over 120 mg/L. Electrophoreris and mass spectroscopy were performed for assessment of RGD purity. Molecular weight was of about 60 kDa as expected. Differential scanning calorimetry (DSC) tests were done to evaluate the variations of ITT as a function of pH.

The objective is to use the properties of recombinant ELPs containing RGD in the development of coatings for medical implants with improved biocompatibility and biomimetic behaviour regarding cell attachment, through technologies such as layer-by-layer and spin-coating.

(P 121) Development of Enhanced Low Serum Culture Strategies for Human Amniotic Fluid Stem Cells

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The role of stem cells in tissue engineering and regenerative medicine is evolving rapidly, namely the use of mesenchymal stem cells (MSCs) due to their potentially immune privilege. These cells can be isolated from different sources such as bone marrow, adipose tissue, umbilical cord and more recently amniotic fluid. Although the amniotic fluid cells have been used for prenatal diagnosis since 1950s in a well established routine technique, little is known about the origin and properties of these cells. In this study, we aimed at developing a method of isolation and expansion of purified cultures of adult stem cells from amniotic fluid to be used as a tool for regenerative medicine. We isolated hAFSCs (Human Amniotic Fluid Stem Cells) from day 6 supernatant of the cultures of amniotic fluid obtained from amniocentesis. The cell pellet was reseeded in expansion medium and cultured until confluence. The effect of cell density plating, basic fibroblast growth factor (bFGF) and foetal bovine serum (FBS) concentrations, in the culture medium were tested and the proliferative potential was evaluated by cell counts at various time points. The osteogenic and chondrogenic differentiative potential were also evaluated. Parameters as cell surface markers of ''stemness'', telomerase activity and stem cells transcripts expression of specific lineages were evaluated by flow cytometry and RT-PCR techniques. With this work we intended to contribute to unfold the potential of stem cells populations from amniotic fluid by decreasing FBS concentration, number of cell-passages and culture time needed to obtain a homogeneous stem cell culture.

(P 122) Development of Novel Carrageenan Scaffolds for Tissue Engineering using Rapid Prototyping

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In the Tissue Engineering (TE) field, great attention has been given devoted to the use of rapid prototyping (RP). The combined use of Computer Assisted Design (CAD) with advanced RP techniques enables the design and fabrication in a reproducible way of patient adapted scaffolds featuring complex 3D architectures. In this context, Carrageenan is a natural polymer that exhibits a very high biomedical potential. Carrageenan is a sulphated hydrocolloid capable of forming hydrogels with very different behaviours and properties depending on the experimental conditions.

In this preliminary work, novel 3D porous structures of carrageenan were for the first time prepared using RP, by using a 3D bioplotting (Bioplotter®). The study aimed to establish a relationship between the scaffold architectural and crosslinking parameters and the subsequent physicochemical properties. In this perspective, the conjugation of diverse architectural arrangements and post-processing methods, including chemical crosslinking techniques, were explored in order to obtain a diverse range of scaffold architectures.

The properties of the developed scaffolds were assessed by both dynamic and quasi-static characterization tools. The relationships between mechanical properties and architectural parameters and

degree/type of crosslinking were established. Furthermore, cytotoxicity testing using fibroblasts (L929) and direct contact assays using osteoblast-like cells (SaOs-2) were performed in order to assess the effect of crosslinking on cell behavior. Preliminary results confirm the potential of carrageenan scaffolds to be used in bone TE.

(P 123) Development of PLA and PCL Based Textile Structures as Scaffolds for Tissue Engineering

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Textile structures are particularly attractive to tissue engineering because of their ability to tailor a broad spectrum of scaffolds with a wide range of properties. Our initial efforts have been successful in designing textile based biostable polyester scaffolds for urinary bladder reconstruction. We have been working on the development of biodegradable scaffolds for the development of blood vessels in our laboratory. The inherent problems of PLA in terms of rigid structure and high crystallinity were overcome by using a blended composition. The PLA was blended with the poly(lactic acid-co-glycolic acid) (PLCL) and was subsequently spun into filaments of 40–60 microns diameter. Alternatively, PCL filaments were produced by the melt spinning process. The spun filaments were subjected to the drawing and heat setting operations so that required strength in filaments is achieved. The blending leads to significant alteration in the inherent PLA structures. The filaments thus produced have good tensile strength and high elongation. It is important to mention here that the blending leads to significant reduction in the crystallinity of the filaments. The PLA and PCL filaments are being made into the textile structures so that the tubular structures of 5–6 mm may be produced. Characterization of these filaments and structures is being carried out.

(P 124) Development of Textile Scaffolds for Bone Tissue Engineering

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Introduction: The development of textile scaffolds for bone regeneration should take into consideration the effect of its composition and structure on cell function. The ideal scaffold should be biocompatible, biodegradable, and promote cellular interactions and bone tissue development. The scaffolds architecture should initially permit, and promote, cell colonization and subsequently drive the population towards osteoblastic differentiation.

This study aims at defining of optimal physical character of fibrous mesh scaffolds mesh for bone tissue engineering. This is conducted by elucidating the influence of fibre diameter, mesh size, and fibre crossing angle on cell migration and proliferation.

Materials and Methods: Reaggregates of primary adult trabecular bone derived human osteoblasts, and of human skin fibro-blasts, were used to assess the capacity of the cell population in covering the textile scaffold as function of fibre diameter and mesh gap size. PET and PA textile scaffolds of various fibre diameters, mesh size and fibre crossing angle were used. Cell reaggregates were seeded onto the scaffolds and samples were examined using light microscope. Subsequently, cell spreading was monitored by assessing the evolution of the total textile area covered by cells.

Results and Discussion: The results obtained in this study strongly suggested that fibre diameter, and mesh gap size, significantly influenced cell outgrowth out of the reaggregate. Furthermore, the cell population response to the physical characteristic of the scaffolds appeared to be cell type dependent. Further work is undergoing to substantiate these results by quantifying cell migration and proliferation rate on these scaffolds.

(P 125) Development of Three Dimensional Culture Systems for Embryonic Stem Cells

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The capacity of pluripotent, self-renewing embryonic stem (ES) cells to differentiate into any cell type of the adult body holds great potential for their use in tissue engineering and cellular therapy. However, current two dimensional (2D) culture techniques are hindering their large scale culture and efficient differentiation. Three dimensional (3D) scaffolds can mimic the *in vivo* microenvironment of ES cells, leading to sustained pluripotency and enhanced differentiation into mature cell phenotypes.

We are focusing on the development and application of electrospun poly(lactic-co-glycolic acid) (PLGA) scaffolds and self assembling Fluorenylmethoxycarbonyl (Fmoc) peptide hydrogels to the culture of ES cells. Parameters for electrospinning 20% PLGA in Hexafluoroisopropanol (HFIP) have been established. The resulting non woven meshes were comprised of fibres ranging between 0.2 and 1.5 μm in diameter. Mouse ES cells cultured on the surface of RGD functionalised dipeptide hydrogels formed colonies reminiscent of those observed in standard 2D culture conditions. Cells seeded within the gels remained viable after 24 hours.

Future work will focus on assessing the survival of ES cells within the Fmoc-dipeptide hydrogels over a longer time period, and the successful application of electrospun PLGA to ES cell culture. Long term aims include modification of both 3D culture environments with oligosaccharides or extracellular matrix proteins in order to better replicate the environment stem cells experience *in vivo*.

(P 126) Differentiation and Proliferation of T17b Murine Embryonal Endothelial Progenitor Cells with Angiogenic Potential in a Fibrin Matrix

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Introduction: Vascularisation of bioartificial matrices is an important goal in tissue engineering. T17b murine embryonal en-

dothelial progenitor cells (EPC) (Hatzopoulos, 1998) can differentiate and support formation of new blood vessels *in vivo*. Therefore, the aim of our study was to investigate their properties in a fibrin matrix *in vitro*.

Materials and Methods: EPC were cultured in a fibrin matrix or on chamber slides and overlayed with basal medium (BM) or differentiation medium (DM). Constructs were harvested after 3 or 8 days and immunohistochemically assessed for EPC proliferation (Ki-67) and differentiation (von Willebrand Factor (vWF)). VWF gene expression was investigated by PCR. VEGF secretion in chamber slides supernatants was quantified by ELISA.

Results: Under BM incubation EPC formed multicellular clusters containing lumen-like structures and displayed positive Ki-67 staining in the fibrin matrix. Addition of DM resulted in a reduced cell number, absence of Ki-67 expression and upregulated vWF gene expression, indicating successful differentiation of EPC. In chamber slide cultures, hypoxia/BM induced significant increases in proliferation and vWF gene expression as well as a 6-fold increase in VEGF secretion compared normoxia/BM where vWF staining was negative and cell proliferation less pronounced. Normoxia/DM resulted in the highest levels of vWF gene expression but no proliferation.

Conclusion: EPC exhibited proliferation and differentiation and formed multicellular lumen-containing clusters in fibrin matrices. Hypoxia stimulated cell proliferation more than normoxia and induced differentiation and VEGF secretion. Taken together, these findings indicate that T17b EPC may deserve further investigation as a tool to enhance vascularisation of bioartificial matrices.

(P 127) Differentiation of Bone Marrow-Derived Stromal Cells using Co-Culture System with Annulus Fibrosus Cells

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Healing damage of intervertebral disc(IVD) dose not naturally occur and degenerative processes may lead to herniation of the annulus fibrosus(AF), resulting in pain. One major aim is the identification of suitable cell populations with the capacity to regenerate IVD tissue. So, we have focused bone marrow stromal cells (BMSCs) as an alternative cell source. In this study, we evaluated the possibility of differentiation of BMSC into AF-like cell by the co-culture system of BMSC and AF cells.

We isolated AF cells from the spine of New Zealand white rabbit and cultured cells using DMEM/Ham's F-12 supplemented with 10% FBS and 1% penicillin streptomycin. For BMSCs isolation, it was aspirated from femoral region of rabbit and cultured using α-MEM contained 10% FBS and 1% PS. We established three experimental group; (A)BMSCs, (B)AF and (C)BMSCs cocultured with AF cells. The gene expression of type I collagen was examined by RT-PCR. We assayed DNA and sGAG contents. And safranin-O staining and immunohistochemistry for type I collagen were performed.

We could observe the typical morphologies of fibroblast-like BMSCs and polygonal AF cell in group (A) and (B), respectively. In group (C), it was observed that BMSC morphology changed into polygonal AF-like cell. The RT-PCR result showed significant upregulation of type I collagen gene expression.

These results revealed that BMSCs were differentiated to AF-like cell showing character of AF cell. We concluded the differentiation of BMSCs into AF-like cells is possible through coculture system.

Acknowledgements: This research was supported by KMOHW (0405-BO01-0204-0006) and SCRC (SC3100).

(P 128) Differentiation of Umbilical Cord Lining Membrane Stem Cells into Hepatic-Like Cells

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We have successfully isolated both epithelial and mesenchymal stem cells from the outer amniotic lining membrane of the umbilical cord with easily accessibility and significantly high yield. Interestingly, these cells possess embryonic stem cell characteristics, and could be differentiated into specific cells such as skin, bone, and fat. In the present study, we examined the *in-vitro* hepatic differentiation of umbilical cord lining derived epithelial stem cells (CLECs) and mesenchymal stem cells (CLMCs). These cells were cultured under 2-step protocol with the use of hepatocyte culture medium (HCM) and hepatocyte maturation medium (HMM). Expression of a variety of hepatic makers was analyzed by RT-PCR, Western blot, and immunocytochemistry. The functionality of differentiated cells was assessed by their ability to produce albumin, secrete urea, store glycogen, and uptake low-density lipoprotein. After 7 days of induction, the cells were morphologically transformed into hepatocyte-like cells with increased mRNA expression of most hepatic markers. Moreover, increased protein expression of Albumin and cytokeratin-18, as well as decreased protein expression of alpha-fetoprotein and cytokeratin-19 was also observed. Differentiated cells further demonstrated *in vitro* functions characteristic of liver cells, including albumin production, glycogen storage, urea secretion, uptake of low-density lipoprotein. In conclusion, CLECs and CLMCs will be good cell sources for hepatocyte transplantation for the treatment of metabolic disorder or bioartificial liver system.

(P 129) Direct Laser Writing of Cad-Designed 3D Scaffolds for Tissue Engineering

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Currently, there is a great demand in the ability to mimic the *in vivo* cellular tissue environment in the laboratory conditions. The systematic study of cell interaction and tissue growth in 3D relies on the ability to fabricate scaffolds, supporting the cell growth, in a

reproducible manner. In this contribution, our recent advances on laser microfabrication of three-dimensional (3D) scaffolds are presented.

By applying multi-photon illumination technique, arbitrary CAD-designed 3D objects can be fabricated in a single step. Taking its origin in multiphoton microscopy, two-photon polymerization technology is now becoming an important rapid prototyping tool. In our work, near-infrared femtosecond laser pulses are applied for 3D material processing. When tightly focused into the volume of a UV-photosensitive material, they interact with material through two-photon absorption. As a result a highly localized material modification appears. This can be, for example, polymerization process, transferring liquid into the solid state. By moving the laser focus in 3D a trace of modified material is produced, resulting in a fabrication computer generated 3D structure by direct laser ''recording'' into the volume of photosensitive material. After the illumination, the unmodified material is removed by an appropriate developer, and the fabricated structure is revealed. Such scaffold structures are used to support cell growth, in order to artificially produce tissue of a desired shape. Application of 2PP for fabrication of 3D scaffolds directly targets such common issues as vascularization of large *in vitro* grown tissues, and demand for patient specific scaffold production.

(P 130) Does Administration of Immunosuppressive Drugs Impact on the Immunomodulatory Properties of Mesenchymal Stem Cells (MSC): Insights from an *In vitro* Study

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Background: Mesenchymal stem cells (MSC) down-modulate immune responses *in vitro* and clinical studies currently test the role of allogeneic MSC in immunosuppressed patients suffering from graft versus host disease (GVHD). We tested *in vitro* the interaction of different immunosuppressive drugs (IS) on the immuno-modulatory properties of MSC.

Methods: Both calcineurin inhibitors: cyclosporine (CsA) and tacrolimus (Tac), and mTOR inhibitors : sirolimus (Si) and everolimus (Ev) were tested.

MSC proliferation was assessed on day 5 & 7. For *in vitro* experiments, the effect of IS drugs on the immune modulation by MSC was measured in proliferation assays of modified mixed lymphocyte reaction where MSC were added (ratio 1:2) with decreasing concentration of IS drugs (C100 to C0).

Results: On day 5, no significant difference in proliferation was noticed among IS drugs. On day 7 of culture, all IS drugs except tacrolimus significantly decreased cell proliferation compared to IS-free culture medium (CsA, -43.6%; Si, -14%, Ev, -17%).

In dose-dependant PBMC-MSC co-cultures, the immunomodulatory properties of allogeneic MSC were maintained independantly of the concentration of IS drug used.

At C50 for Tac (5 ng/ml) and Si (0.3 ng/ml), the down-modulation by MSC was still present since the degree of inhibition, for a 1:2 MSC/PBMC ratio, was 96% and 94%, respectively.

Conclusions: The concomitant use of immunosuppressive drugs significantly interacts on the proliferation of MSC *in vitro*.

Among their class, Tacrolimus and Sirolimus were the drugs with the least interaction and at C50 neither had detrimental effect on the immuno-modulatory properties of MSC.

(P 131) Dose-Dependent Effect of Plasma-Rich in Growth Factors (PRGF) in the Proliferation of Rat Bone Marrow Stromal Cells

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Plasma-rich in growth factors (PRGF) is a blood derived fraction containing high concentrations of autologous growth factors. PRGF application as been reported as an effective way to accelerate and stimulate hard and soft tissue repair. The main goal of this work is to evaluate the effect of autologous PRGF on the behavior of rat bone marrow stromal cells. Cell behavior in the presence of fetal bovine serum (FBS) was also assessed.

PRGF was obtained by cardiac puncture from Wistar rats using a centrifugation methodology. Bone marrow cells were isolated from the femurs of the same animals and cultured in ∞-MEM, 10% SBF, in standard conditions. At 70–80% confluence, cells were released and the cell suspension was cultured (10^4 cell/cm^2) for 10 days in the presence of (i) 0.5 and 5% PRGF and (ii) 0.5 and 5% FBS. Cultures were characterized for cell morphology and proliferation.

PRGF treated cultures presented a higher cell growth rate throughout the incubation time. FBS treated cultures showed a similar proliferation pattern. Despite the similar profile, both conditions caused a dose-dependent effect on cell growth, an effect more marked in condition (i). Differences regarding cell morphology were also apparent, with FBS treated cells presenting more pronounced cytoplasmic extensions.

In conclusion, results showed that the cultures supplemented with autologous PRGF presented a high proliferation rate at very low PRGF concentrations, compared to FBS. Thus, combination of autologous stem cells and PRGF might provide an optimized system for *ex vivo* cell expansion regarding bone tissue engineering applications.

(P 132) Dynamic Cell Culture Prevents Core Necrosis and Creates Meaningful Osteoblastic Networks in Thick 3D Tissue-Engineered Constructs

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Introduction: Bone lacuno-canalicular fluid flow ensures chemotransportation and provides a mechanical stimulus to cells. A novel flow-perfusion bioreactor was used to provide chemotransportation through a thick 3D scaffold *in vitro*. We hypothesize that flow perfusion will improve cellular distribution and viability throughout the construct and that osteoblasts will form meaningful osteoblastic networks.

Methods: 3D bioprinted cylindrical hydroxyapatite-tricalcium-phosphate scaffolds (24×6 mm, 200×200 μm pore size) were seeded with MC3T3-E1 cells (4×10^6 cells/cm^3) and subjected to dynamic cell culture (0.4 dyn/cm^2) in osteogenic medium. Control scaffolds remained in static culture. A dye-transfer technique using diI and calcein AM was used to label cells to check for gap junction formation. Samples were harvested at 2, 4, 6, 10, 14, and 21 days, plastic-embedded, and analyzed by confocal microscopy and scanning electron microscopy.

Results: Histologic analysis confirmed uniform cell distribution after seeding and that >90% of cells survived the time allotted for cellular adherence. By 6 days in static culture, 80% of cells in the core of the scaffolds were non-viable, while 90% of those at the peripheral crust survived. In contrast, scaffolds subjected to flow perfusion demonstrated uniform cell density and 95% viability throughout the construct at all time points. Osteoblasts assumed characteristic dendritic phenotype and extended podocytes toward other cells, with evidence of gap junction formation.

Conclusion: Flow perfusion ensured adequate chemo-transportation to all regions of a thick 3D scaffold, such that cellular viability and distribution were preserved. The 3D environment was conducive to the formation of meaningful osteoblastic networks.

(P 133) Dynamic Seeding and Culture of Urothelial Cells on Tubular Type I Collagen Scaffolds for Urogenital Tissue Engineering Applications

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Purpose: Urethral reconstruction still leads to significant complication rates and remains a challenge within the Urology. Tissue engineering raised our expectations of changing this situation. Our study aims to evaluate different cell seeding methods on collagen based tubular scaffolds for urethra reconstruction.

Methods: Three different urothelial cell seeding procedures were compared on highly porous type I collagen tubular scaffolds. In the static method, cells were suspended into the lumen of the scaffold and cultured for one or seven days. This method was compared with two dynamic cell seeding procedures; the open method (Xo) in which the flow was directed through the lumen of the construct, and the sealed method (Xs) in which the flow was perfused through the wall of the tubular scaffold. In the Xs method we also compared two different flow-rates (1 ml/min and 1 ml/h). After one or seven days culture the constructs were evaluated with histology and electron microscope.

Results: With the Xo method and the static method, cells were seen in the lumen of the tubular scaffold. However, with the static method, cells were also observed on the outside of the scaffold. Interestingly, the Xo method resulted in cell distribution throughout the wall of the scaffold. No cells could be observed with the 1 ml/min Xs method. No distinctive differences between 1 and 7 days time-points were observed.

Conclusion: Although cells were not always evenly distributed, both dynamic methods resulted in a localized cell growth (lumen versus wall) with a high density.

(P 134) Effect of Keratin/PLGA Scaffold on the Neural Differentiation of Rat BMSCs

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Bone marrow stromal cells(BMSCs) exhibit multiple traits of a stem cell population, and they can expand many times *in vitro* and be induced to differentiate into multiple cell types. Keratin is the major structural fibrous protein providing outer covering such as wool, hair, and nail. Keratin is useful as natural biomaterial. We examined the effect of Keratin/PLGA scaffold on the neural differentiation of rat BMSCs.

We developed the keratin loaded poly(L-lactide-co-glycolide) (PLGA) scaffold (Keratin/PLGA). Keratin/PLGA (Keratin contents: 0%, 10% and 20% of PLGA weight) scaffolds were prepared by solvent casting/salt leaching method. BMSCs were harvested from the femurs of adult female Fischer rat (rBMSC). These cells were seeded in Keratin/PLGA scaffold and cultured with Medium 1(50 ml DMEM , 2% DMSO, 200 μM BHA, 25 μM valproic acid, 10 μM forskolin, 1 μM hydrocotisone, 5 μg/ml insulin)and Medium 2 (50 ml DMEM, 10% FBS, 1% PS) for 14 days. rBMSC viability in keratin/PLGA were assessed using the MTT assay. mRNA expression using RT-PCR to neural maker such as NF and NSE was conducted to confirm the effect of Keratin/PLGA scaffold on the neural differentiation of rBMSCs. In addition, we evaluated NF and NSE protein on Keratin/PLGA scaffold seeded rBMSC by immunohistochemisty.

We confirmed that effect of Keratin/PLGA scaffold on the neural differentiation of rat BMSCs. According to our results, 10 % and 20 % keratin/PLGA scaffold have good cell compatibility and the expression of neural markers was higher. In conclusion, Neural differentiation of rat BMSCs is possible on Keratin/PLGA scaffold.

This research was supported by SCRC(SC3100).

(P 135) Effect of Mechanical Stimulation on the Differentiation of Human Dental Pulp Cells

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Recently, there are lots of periodontal diseases and one of them is a defect of alveolar bone considered as a serious problem. Hence, researchers have studied to treat these problems using tissue engineering technique. Periodontal tissues are exposed to mechanical

stress caused by occlusion and mastication, and both the cells and extracellular matrix in these tissues undergo architectural modification to equilibrate the applied stress. Therefore, in this study we are willing to recognize the effect of the mechanical tension on the osteogenesis of human periodontal dental pulp cells.

To identify the osteogenesis by mechanical stress in the dental pulp, we examined the effects of the tension on human periodontal dental pulp cells (HDPCs). We evaluated the effects of mechanical stimuli for the osteogenesis of human dental pulp cells on the silk scaffold using a bioreactor with 10% tension. The tension was applied with 0.2 Hz during 5 days and continuously applied during 10 days. We evaluated the differentiation of cells by RT-PCR, Western blot and immunohistochemistry. Application of 10% tension culture reported in increases in collagen type I, Fibronectin, osteoprotegerin, bone sialoprotein, and decreases in alpha-smooth muscle actin. These data suggest that mechanical stimulation can promote osteogenesis of human dental pulp cells.

(P 136) Effect of Radiation on Tissue-Engineered Mucosa

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Tissue-engineered mucosa (TEM) can be used to reconstruct defects that remain after oncological resection. After resection, most patients receive radiotherapy. Radiation primarily affects proliferating cells. Therefore, mucosa, in particular the basal layer of the epidermis, is susceptible to radiation damage due to its physiological high cell turnover rate. Radiation damage results in DNA double strand breaks (DSBs).Our goal was to determine the effect of various radiation doses on TEM.

Human keratinocytes and fibroblasts were isolated from buccal mucosa. Keratinocytes were seeded onto a scaffold of acellular human donor skin and fibroblasts were seeded onto a polyester membrane insert. TEM irradiated with 0, 0.5, 1, 2, 4, 6 and 12 Gy and harvested after 6 hours. Radiation damage and repair was quantified by determining the number and size of ionizing radiation-induced foci (IRIF) for damage marker 53BP1, and repair markers Mre11 and Rad51. Cell proliferation was determined by immunolocalization of Ki-67.

Overall morphology did not change significantly up to 4 Gy. At 6 Gy the TEM showed picnotic cells. At 12 Gy the epidermis was severely damaged, resulting in vast destruction of the morphology.

A significant increase of 53BP1 positive cells was observed as the rediation dose increased.The size of IRIF of both Mre11 and Rad51 gradually expanded with increasing radiation dose. Increasing radiation dose resulted in a decrease in cell proliferation.

To our knowledge, this is the first study to use a 3-dimensional mucosal construct to study radiation damage. Our results strongly indicate that TEM can repair DNA DSBs caused by radiation.

(P 137) Effect of Solvent-Dependent Viscoelastic Properties of Chitosan Membranes on the Permeation of Low Molecular Weight Drugs

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Chitosan (CTS), a widely used biopolymer in different biomedical applications, is a derivative of chitin, the most abundant polysaccharide found in the marine world.

This work aimed at providing relevant information about the use of CTS membranes in separation applications or in sustained release systems of therapeutic molecules. Moreover, the mechanical characterization may be also very important in such kind of applications, especially if the materials are tested in adequate physiological conditions.

Chitosan membranes, both non-crosslinked and crosslinked with genipin, were characterized by dynamic mechanical analysis, swelling and permeability experiments using a model molecule. The membranes were tested immersed in different mixtures of water/ethanol. The swelling equilibrium varied linearly with the volumetric composition of the solvent mixture. The mechanical properties of CTS increased with the enhancement of the crosslinking density. A peak of the loss factor appeared at 24.5% of water attributed to the α-relaxation of chitosan and simultaneously a reduction of the storage modulus was observed. This was the first time that the glass transition (Tg) dynamics was monitored in a polymer in immersion conditions, where the plasticizer composition in the bath changed in a controlled way. Permeability decreased sharply until it reached very small values around the Tg.

We hypothesise that conformational mobility of the polymeric chains may play an important role in the diffusion properties of molecules through polymer matrices. Results may elucidate some aspects regarding to relationships between glass transition and transport properties that may be important in the use of CTS in TE strategies.

(P 138) Effective Action Distance Between Umbilical Cord Blood-Derived Hematopoietic Stem Cells and Human Adipose Derived Stem Cells

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Abstract: In order to investigate the optimal communication distance between umbilical cord blood (UCB) derived hematopoietic stem cells (HSCs) and human adipose derived stem cells (ADSCs), transwell co-culture protocol was applied and the distance between the two culture chambers was adjusted. UCB mononuclear cells (MNCs) were isolated by density gradient centrifugation over Ficoll (1.077 g/ml, proportion of 1:1~2 between the Ficoll and UCB) and centrifuged horizontally at 2500 r/min for 30 minutes at room temperature. The HSCs were cultured in the transwell with the inoculated density of 5×10^5 cells/ml supported with the ADSCs of 1×10^5 cells/ml inoculated density in the 6 well-plate for 7 days. IMDM medium was used supplemented with 12.5% horse serum (GIBCO) and 12.5% fetal calf serum (GIBCO). The total cell number was counted and the cell morphology was viewed every 24 h by the hemacytometer and inverted microscope. The results show that there exists an optimal communication distance about

370 μm between these two kinds of stem cells during their co-culture. At this distance the expansion fold of the UCB MNCs becomes the maximum of 27.29 ± 1.62, and the CD34+ cells can be expanded by 10.92 ± 0.65.

Keywords: Adipocyte derived stem cells, hematopoietic stem cells, transwell, co-culture, umbilical cord blood.

(P 139) Effective Expansion of Umbilical Cord Blood HSPCs by Regulating of Microencapsulated Osteoblasts Under Hypoxia Condition

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This study was aimed to construct a three-dimensional static hypoxic culture system by using encapsulated osteoblasts (OBs) and to investigate its support effect on *ex vivo* expansion of hematopoietic stem/progenior cells (HSPCs) from umbilical cord blood. The rat osteoblasts were isolated by tissue culture method and were cultured in HG-DMEM containing 10% newborn bovine serum. The osteoblasts were encapsulated in gelatin-alginate-chitosan (GAC) microcapsules by the polyelectrolyte-complexation method to create the special culture environment. Hematopoietic mononuclear cells (hMNCs) were isolated from fresh umbilical cord blood by density gradient centrifugation and seeded into the 6 well plates together with the encapsulated OBs for a co-culture *ex vivo* in CO_2 incubator under 5% and 20% oxygen tension respectively. The expansion of HSPCs was evaluated by counting the hMNC number and by using colony-forming assay and CD34+ flow cytometric analysis. Meanwhile the pH value, the glucose and lactic acid concentrations were detected everyday. After 7 days' culture, the expansion of hMNCs co-cultured with encapsuled osteoblasts under hypoxia condition was 18.7 folds, the percentage of CD34+ cells increased from 2.0% to 2.5%, and the CD34+ cells was expanded by 23.4 folds. The co-culture system with low oxygen level displayed more significant support effect compared with other culture systems, especially on long-term HSPCs survival *ex vivo*. It is concluded that the supporting capacities of microencapsuled osteoblasts in *ex vivo* expansion of umbilical cord blood HSPCs is notable under hypoxia condition. Hypoxia and osteoblasts play a crucial synergistic role in regulation of HSPCs proliferation.

(P 140) Effects of Common Sterilization Methods on the Structure and Properties of 3D Silk Fibroin Scaffolds

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Scaffold development for tissue engineering applications requires careful choice of macroscale properties, such as porosity, interconnectivity and biodegradation. The micro- and nanoscale properties of a scaffold are another important design criterion as these influence cell adhesion, proliferation and differentiation. Standard sterilization methods such as autoclaving, ethylene oxide treatment or gamma irradiation have been shown to be unsuitable for many polymer scaffolds because of deformation from elevated temperatures, lengthy degassing, and deterioration due to decreased molecular weights. Apart from being autoclavable, 3D silk fibroin scaffolds have previously been demonstrated to offer exceptional benefits over conventional synthetic and natural biomaterials in generating functional tissue replacements. It would be interesting to investigate whether topographical changes on the scaffold surface caused by sterilization methods could be used as a tool to influence cellular behaviour. This study sought to determine the effects of sterilization with different autoclaving protocols (dry, humid, wet), ethanol, ethylene oxide treatment or aseptic preparation on the structure of silk fibroin scaffolds. All methods resulted in scaffold sterilization, and scanning electron microscopy revealed deformations on the scaffold surface. The extent of topographical alterations was various, including flattened structures, increased roughness, indentation or a mixture of those structures on the microscale level, depending on the sterilization method used. None of the methods had an influence on the crystalline structure or the degree of crystallinity of the silk fibroin after treatment with 90% methanol as verified by FTIR. Future studies will show to what extent those topographical differences influence cell adhesion, proliferation and differentiation.

(P 141) Effects of Fibrin Component Compositions on Fibrin Structure and Cell Viability

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Introduction: Fibrin is a natural polymer proposed as a cell scaffold for numerous tissue engineering purposes. Studies on fibrin optimisation for cell scaffolds have mainly focused on fibrinogen and thrombin content. However, other factors, such as factor XIII (FXIII), calcium, and chloride ions contained in fibrinogen and thrombin formulations, are also known to affect fibrin structure. In this study, such effects on cell viability during culturing were assessed.

Materials and Methods: Different FXIII, calcium, and chloride concentration combinations were used to polymerize fibrin resulting in various amounts of cross-linking. The relative amounts of cross-links were estimated by gel transparency measurements ($n = 3$/conditions). Two compositions resulting in high and low amounts of cross-linking were then tested for cell viability. These carriers were seeded with C3H10T1/2 cell line (0.6 million cells/ml) and cultured for 7 days (medium + 5%FBS). Cell number, morphology and distribution on histological sections were assessed at day0 (D0) and day7 (D7), ($n = 3$/carrier-type/time/test). All fibrin carriers were synthesized as cylinder (8 mm diameter, 4 mm height) containing 20mg/ml fibrinogen and 13.5U/ml thrombin.

Results: Chloride increased gel cross-links, as well as FXIII (133+/-1U, high transparency). Calcium decreased cross-links (60+/-6U, low transparency) and countered the effect of FXIII. Cell proliferation at D7 was higher for lower (227+/−26% of initial cell number) than for higher cross-linking (120+/−1%). Cell spreading was observed in both types of carrier, but clusters were only seen for lower cross-linking.

Conclusion: These results showed that FXIII, calcium and chloride affects fibrin structure and that less cross-linked fibrin carriers allowed better proliferation of mesenchymal cells.

(P 142) Effects of Frequency and Loading Duration on Mechanically Modulated Collagen Production by Osteoblasts in Bone Tissue Engineering

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Bone cells are known to respond to mechanical stimulation, therefore mechanical conditioning is used to modulate matrix formation in bone tissue engineering. Mechanotransduction is the signalling process where external an mechanical signal is translated into a controlled cascade of biological events. In this study we compared the effects of different loading regimens on cell proliferation and matrix production. MLO-A5, late stage osteoblast-like cells were seeded in cylindrical polyurethane (PU) scaffolds, 10 mm thick and 10 mm diameter. Dynamic compressive loading to a maximum of 5% strain was applied to the cell seeded scaffolds in a sterile medium-filled BioDynamic chamber on days 5, and 7 of culture at 1 hz and 2 hz frequencies and durations (30, 60 and 120 mins). Between loading cycles, scaffolds were cultured in an incubator in standard conditions. Cells survived and cell number increased over a 12 day time course of the experiment. Cells in PU scaffolds responded differently to loading in a frequency/ duration dependent manner. 60 minutes of loading at 1 Hz increased final cell number but had no effect on total collagen production. Increasing either the duration of the loading period or the frequency of loading eliminated the effect of loading on cell number but total collagen content was higher. This study demonstrates that the frequency of load and the number of loading cycles applied during the processing of constructs influences the production of bone matrix. These results indicate that mechanical stimuli in dynamic bioreactor culture should be carefully modulated to produce the required product.

(P 143) Effects of Human Amniotic Fluid Derived Stem Cells on Cardiac Function After Myocardial Infarction

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Cell therapy has been proposed as a means to promote the regeneration of injured heart muscle. We have established lines of multipotent stem cells derived from primitive fetal cells present in human amniotic fluid (hAFS) cells. In this study, we hypothesize that AFS cells can differentiate into cardiac cells and be used for cardiac tissue regeneration. AFS cells were treated with 5-aza-2-deoxycytidine to induced cardiogenic differentiation. The cells were analyzed for expression of cardiogenic markers. We used an ischemic injury model in rats to test the ability of the hAFS cells to survive and integrate after direct injection into the myocardium. Immediately after ligation, 5 million AFS cells were injected into the heart. Echocardiography was performed at 1, 2, and 3 months, and invasive hemodynamic studies at 3 months. The hearts were removed and analyzed for integration and morphology. We observed that by 20 days after induction, AFS cells stained positively for cardiomyocyte markers including cardiac troponin T and troponin I. PCR analysis revealed expression of cardiomyocyte markers, including Nkx 2.5 and Mlc2v. Histology indicated that AFS cells integrated into the injured myocardium. Echocardiographic analysis and invasive hemodynamics suggested that AFS cells improve cardiac function after myocardial infarction.These results demonstrate that stem cells derived from amniotic fluid can differentiate *in vitro* and express cardiac markers. *In vivo* studies indicated that AFS cells have a potential to improve cardiac function after myocardial infarction. These cells represent a promising novel source of cells for therapy of a variety of cardiac diseases.

(P 144) Effects of Hypoxia on Rat Mesenchymal Stem Cell Expansion in Hollow-Fiber Membrane Bioreactor

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Rat mesenchymal stem cell (MSC) represents a small portion of the cells in the stromal compartment of bone marrow with many virtues. Low oxygen tension exists natively for MSCs in bone marrow microenvironment, ranging from 1 to 7%. So it is necessary to investigate the effect of low oxygen tension close to normal physiologic levels on the growth of MSCs, under the well controlled conditions in bioreactors. rMSCs were cultured under physiologically relevant oxygen environments (5% O_2) on the hollow-fiber membrane constructs for up to 8 days. The cell growth curves were obtained and colony counts were carried out.The expanded cells were induced to differentiate toward osteoblasts, cartilage cells and adipocytes. Flow cytmoetry analysis was also applied to examine the capacity of the rMSCs both in self-renew and in maintaining their pluripotentiality.The results showed that hypoxic rMSCs exhibited a short lag phase ,but they subsequently proliferated continuously throughout the culture period and at the end we observed a significant increase in cell number. Moreover, rMSCs grown at 5%O_2 consistently showed a nearly two-fold increase compared to those at other pO_2 . In 5%O_2 condition rMSCs on hollow-fiber membranes can maintain significantly higher colony-forming unit capabilities than normoxic controls, approximately 115 and 45 respectively. Hypoxic rMSCs also expressed higher levels of HIF-1α, $CD44^+$, $CD\ 29^+$, $CD\ 13^+$, markers than normoxic controls.These findings demonstrate that low oxygen conditions may selectively facilitate the survival of more primitive cells and hypoxia can promote the proliferation of rMSCs on hollow-fiber membranes *in vitro*.

(P 145) Effects of Lactate on MSCs Survival, Proliferation and Differentiation

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Local supply of mesenchymal stem cells (MSC) has been proposed for the repair or replacement of damaged tissue such as bone and cartilage. *In vivo* transplantation of MSCs into avascular tissue leads to oxygen deprivation, anaerobic respiration and lactate accumulation. Moreover, it was reported that lactate could reach 10 mM in inflammatory environment. The purpose of this study was to evaluate, the effects of lactate concentration on MSC viability and differentiation capability.

MSCs were cultured either in the absence or in the presence of 5 to 50 mM lactate (pH 7.4) over 15 days; MSCs proliferation was assessed by DNA quantification every 3 days. Lactate effects on MSC differentiation were studied by culturing MSCs either in the absence or in the presence of 1 to 50 mM lactate over 30 days in osteo-inductive or adipogenic induction media. Osteoblastic differentiation was assed by alkaline phosphatase (ALP) quantification and Von Kossa staining; adipogenic differentiation was assessed, by Oil Red O staining and quantification.

At low (1–5 mM) concentrations, lactate had no effects on cell proliferation over 15 days, nor modified ALP activity, calcium deposition or adipogenic differentiation. In contrast, above 10 mM lactate, MSC proliferation was inhibited after just 3 days of culture as were both osteoblastic and adipogenic differentiation after 10 days.

This study demonstrated that MSCs are very sensitive to high lactate concentrations. Improved culture methods are therefore needed to enable MSCs to conserve their proliferation capabilities as well as multi-lineage potential in such conditions.

(P 146) Efficient Cell Encapsulation Based on Biodegradable Stimuli-Responsive Hydrogels

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Stimuli-responsive hydrogels are chemically or physically cross-linked polymer networks that can swell extensively in aqueous environments while they do not dissolve in water. They are further characterized by their ability to show drastic changes in the degree of swelling in response to physical or chemical stimuli. Responsive hydrogels have been investigated for use in targeted drug delivery, controlled drug release systems and polymer actuators. Here we report on the use of bio-degradable stimuli responsive hydrogels for the efficient encapsulation of eukaryotic cells. The novel method facilitates the use of conventional 2D cell culturing techniques on a substrate provided with flat polymeric structures. The subsequent application of a physical stimulus results in the release of these polymer structures from the substrates and automatic encapsulation of the cultured cells. In this contribution we describe the cell encapsulation mechanism based on stimuli-responsive hydrogels, we discuss the biodegradability of the chemically cross-linked polymer network, we present test results that indicate that the materials used in these investigations show no direct or indirect cytotoxic properties towards eukaryotic cells and finally, we show the first examples of the encapsulation of human carcinoma epidermoid cells.

The cell-filled polymer microcontainers, resulting from this novel encapsulation method, offer advantages for use in cell therapies as well as tissue engineering applications.

(P 147) Electro-Spinning of Collagen Nano-Fibres—Myths and Reality

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Electro-spinning has been recently introduced to fabricate fibrous nano-scale scaffolds for tissue engineering applications. The process involves suspension of polymers in a highly volatile solvent (usually 1,1,1,3,3,3-hexafluoro-2-propanol) and extrusion of the mixture within a strong electric field. Upon application of the high voltage (10–30 KV), the solvent evaporates and the nano-fibrous meshes are collected. Collagen, an abundant structural protein, has been used extensively for tissue engineering due to its superior mechanical strength and low immunogenicity. Since the *in vivo* diameter of collagen fibres ranges from a few nanometres to several micrometers, the combination of technology and material attracted much attention in order to fabricate biocompatible scaffolds that would mimic the extracellular matrix. However, we showed recently that suspending collagen in fluoroalcohols yields scaffolds that are water soluble, pepsin-sensitive, not triple-helical, lack the characteristic D-periodicity of collagen, and exhibit low denaturation temperatures ($<40^{\circ}$C). These are all characteristics of gelatin and not collagenous supramolecular assemblies. Therefore, such scaffolds fail to mimic the extracellular matrix. In our attempt to produce actual collagen nano-scaffolds, we evaluated electrospinning of collagen-polyethylene oxide suspension out of dilute acidic solutions. The resultant nano-scaffolds formed a mixed bead/fibre landscape and exhibited the characteristic ultra-structural D-periodicity of native collagen. They were also water-insoluble, pepsin-resistant, triple-helical and of high denaturation temperature ($>60^{\circ}$C). All these confirm typical *in vitro* collagenous supramolecular assemblies. Overall herein we demonstrate that electrospinning of collagen-polyethylene oxide out of dilute acidic solutions preserves the native characteristics of collagen and consequently biomimetic scaffolds can be produced.

(P 148) Electrospinning of Highly Porous Scaffolds for Cartilage Tissue Engineering

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Deep articular cartilage defects are a rather common and painful condition and it is well established that once destroyed the articular cartilage has limited regenerative capacity. In order to ease the pain and restore joint function cartilage repair techniques has been developed to recruit or deliver reparative cells to the defect, with or without a three-dimensional scaffold.

In this study we describe a new and innovative technique for creating highly porous scaffolds with a suitable combination of nano- and microfibers. With this new technique the nanofiber-coated microfibers can be formed into scaffolds with tailored porosity for cellular infiltration and nanostructured surface morphology for cell growth.

The nanofiber-coating was obtained by using a grounded collector rotating around the microfiber forcing the nanofibers to collide with, and be collected upon, the microfiber. Additionally, a Teflon tube surrounding fibers and collector was used to force the nanofibers to the microfiber. The scaffolds were fabricated using polycaprolactone nanofibers and polylactic acid microfibers. The nanofiber-coated microfibers were manually formed into scaffolds with porosities $>95\%$. A preliminary cell study using human articular chondrocytes was performed comparing purely nanofibers, nanofiber-coated microfibers and pure microfibers scaffolds.

Considerably enhanced cellular infiltration in scaffolds of nanofiber-coated microfibers compared to scaffolds of only nanofibers was observed. Additionally, the study showed that the porosity of the scaffolds containing nanofiber-coated microfibers could be carefully modified, an important feature in creating scaffolds for cartilage tissue engineering.

(P 149) Electrospinning of Hydroxyapatite/Chitosan Nanocomposite Nanofibers

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The basic building blocks of bone are the hydroxyapatite-nanocrystals incorporated collagen nanocomposite nanofibers. This innate nanofibrous structure has inspired researchers to develop structural biomimicking bone-repairing materials by such techniques as electrospinning. Although numerous previous studies have suggested that the inorganic-organic hybrid of hydroxyapatite/chitosan (HA/CS) can be used as one of the most promising biocomposites for engineering load-bearing bone tissue, electrospinning HA/CS nanocomposites into nanofibers has been a challenging task to perform because of its limited usable solvents and poor electrospinnability. In this paper, we present for the first time a successful electrospinning of HA/CS nanocomposite, which was dissolved in a mixed solvent system of acetic acid and dimethyl sulfoxide and used a small amount of ultrahigh-molecular-weight poly(ethylene oxide) as an effective fiber-forming facilitating additive. The used HA/CS nanocomposites with the HA mass ratio of 30% were prior synthesized by a wet chemical co-precipitation method so as to attain homogenous dispersion of the spindle-shaped HA nanoparticles (ca. 100×30 nm) within the chitosan biopolymer. SEM and TEM observation indicates that continuous and uniform HA/CS nanofibers with a diameters of several hundreds nanometers had been produced successfully and the HA nanoparticles with aggregations to some extent were incorporated discretely along the electrospun nanofibers. Further XRD and SAED analysis revealed that the crystalline nature of the HA remains and had survived the acetic acid-dominant solvent system. Our currently developed nanocomposite nanofibers of HA/CS may be employed as a novel biomimetic materials for bone tissue engineering application.

(P 150) Electrospun Polyurethane Scaffolds for Mechanical Stimulation of Cells in Bone Tissue Engineering

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Polyurethanes (PU) are regarded as a suitable biomaterial for musculoskeletal tissue engineering due to their biocompatibility and durability. Here we have electrospun a 3D microfibrous scaffold using the medical grade PU Tecoflex® SG80A (Lubrizol) for use in mechanical conditioning experiments. Scaffolds were electrospun using a electrospinning rig (Linari) from a 10% solution (w/v) of Tecoflex in 70:30 THF:DMF, for 3.5 hours, flow rate 5 ml/hr. Mechanical properties were analysed in tension (Instron). Scaffold thickness ranged from 653 to 1950 microns, with mean breaking strain 464.18 +/−30.76%, mean breaking stress 3.785 +/− 0.39 MPa and Young's modulus 0.88+/−0.05 MPa.

Scaffolds were analysed for cytotoxicity by a conditioned medium test using the osteoblast cell line MC-3T3-E1 which indicated that there were no soluble toxic factors. Cell attachment was analysed by seeding cells on 5 cm discs of scaffold and culturing for 7 days. There was good attachment as seen by the high number of viable cells present on day 7 (MTT assay) as compared to another electrospun 'home-made' PU scaffold.

For mechanical conditioning 'Dogbone' shaped scaffolds were seeded with 100,000 cells and cultured for 12 days. Scaffolds were loaded in dynamic tension at 2% strain for 2 hrs, 1 Hz on days 7 and 9 of culture in a sterile media-filled Bose biodynamic chamber. There was an increase in the number of viable cells and total collagen production (Sirius red) on stimulated samples compared with non-loaded controls. In conclusion: we have electrospun a polyurethane which is suitable for mechanical conditioning experiments in musculoskeletal tissue engineering.

(P 151) Electrospun Scaffold for an *In Vitro* Tissue Engineered Blood-Brain Barrier Model

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The *in vitro* blood-brain barrier (BBB) model, based on co-culture of endothelial cells and astrocytes on opposite sides of a membrane, is an isolated system allowing the selective study of physiology, pharmacology and pathophysiology. Therapeutic approaches for neurological disorders treatment, based on *in vitro* effective central nervous system (CNS) drugs, show several limitations, being excluded *in vivo* by the BBB. Therefore, a reliable BBB model that allows for inexpensive and mass testing of putative CNS therapeutic agents is a relevant issue to be addressed. Several BBB *in vitro* models have been studied, based on commercially available polymer membranes. However, these membranes lack certain attributes preventing a reasonable modelling of *in vivo* situation. This work is based on the hypothesis that co-culturing endothelial and astrocyte cells on opposite sides of a biodegradable, fibrous membrane will allow the development of an *in vivo* resembling BBB model. Electrospun poly(e-caprolactone) (PCL) micrometric and sub-micrometric fibrous mats (average fiber diameter 3.6 ± 0.8 vs 0.8 ± 0.2 microm, respectively.

(P 152) Electrospun Spiral-Wounded Polycaprolactone Scaffolds with or without Stem Cells for Cranial Bone Defects:an Animal Model

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PCL with two different molecular weights (high and low) were synthesized, and the blends of these two were electrospun into nonwoven membranes composed of nanofibers/micropores. The matrices were then spiral-wounded to produce scaffolds with 3D-structures having both macro- and microchannels. 8-mm-diameter critical size (non-self-healing size) cranial defects were created in rats, and scaffolds in the same size of the defects were implanted into these defects. In a parallel group of study, mesencyhmal stem cells derived from rat bone marrow stem were injected into the defects with or without scaffold. The test animals were sacrificed after 1, 3 and 6 months of post-implantation. Soft and hard tissue specimens were removed and bone regeneration and tissue response were followed by histological tests. A significant ingrowth of connective tissue cells and new blood vessels allowed new bone formation, especially with the macrochannels of the spiral-wounded PCL membranes. Regenerations were better when results were observed in the group scaffolds used with mesenchymal stem cells.

(P 153) Endothelial Cell Differentiation of EPC Is Increased by Interaction Between CD34-Positive Cells and CD14-Positive Cells

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Introduction: Neovascularization induced by endothelial progenitor cells (EPC) for the treatment of ischemic diseases, has been a topic of intense research. The rare CD34-positive stem cell is often designated as the archetype EPC, because it contributes to repair of ischemic injuries. However, incorporation of CD34-positive cells into the neovasculature is limited, suggesting a paracrine mode of action. CD14-positive cells can also differentiate towards an endothelial cell phenotype. However, the low proliferative capacity of monocyte-derived endothelial cells hampers their use as therapeutic cells. We hypothesized that an interaction between CD34-positive and CD14-positive cells augments endothelial differentiation of the latter cell type. The combined properties of CD34-positive and CD14-positive cells may therefore render these cells suitable to treat ischemic diseases.

Results: *In vitro*, 63% of the cultured CD14-positive cells adopted an endothelial phenotype, while in CD34/CD14 co-cultures 95% of the cells differentiated into endothelial cells. Furthermore, cell proliferation was increased by 5–12% in the CD34/CD14 co-cultures compared to both monocultures. CD34-cell conditioned medium also increased endothelial differentiation of CD14-positive cells; this effect was diminished by HGF-neutralizing antibodies.

Conclusion: Endothelial differentiation of CD14-positive cells increases through co-cultivation with CD34-positive cells. Furthermore, interaction between CD34-positive and CD14-positive cells results in increased cell proliferation. Increases in differentiation and proliferation of CD14-positive cells did not depend on cell-cell contact, but on paracrine signaling through HGF. Taken together, the combination of CD34-positive and CD14-positive cells is a suitable cell source that may augment therapeutic neovascularization and can be applied as cell therapeutic for treatment of ischemic diseases.

(P 154) Endurance Strength of Artificial Ligament Materials

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Although replacement of injured anterior cruciate ligament with allo- and autografts is currently performed on a routine basis, there are still a number of problems related to explant morbiditiy, limited

availability of autologous material, the loss of stability due to remodelling processes of the new ligament or disease transmissions in case of taking an allograft. Artificial ligaments could offer substantial advantages in this respect.

The goal of this work is to assess various potential candidates for artificial ligament materials (PET, polyester, PE, UHMWPE, Aramid, alumina ceramics, PEEK) to figure out, whether one meet the enormous requirements, that a human anterior ligament achieves. All materials were tested with a pneumatically driven knee joint simulator which can strain the ligaments similar to the natural situation. In order to evaluate the influence of tribological parameters, two different feed-through-systems (made of titanium or PEEK, respectively) were investigated. Depending on the stiffness of the material we reached max. values of 670 N in full extension.

The time to failure at the above mentioned conditions varied between 100.000 and 300.000 cycles, depending on ligament material and feed-through material. The ligaments failed by an excessive amount of abrasion and/or fatigue. By considering that a knee is bent in average 4 million times a year, it can be concluded that currently available materials by far miss the longevity which would be required for an artificial ligament replacement. New replacement strategies beyond the currently known routes based on autologous material thus have to rely on tissue engineering approaches.

(P 155) Engineering Muscularized Vascular Conduits

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Although the presence of a confluent monolayer of endothelial cells on the luminal surface has been shown to prolong the patency rates of implanted grafts in a variety of cardiovascular applications, the functional performance of these grafts over time still remains suboptimal compared to the native artery. A potential cause for the current deficiencies in bioengineered vascular grafts is the lack of medial smooth muscle cell layer upon implantation. In fact, vascular Smooth muscle cells (VSMCs) impart important functional characteristics in the native artery, and therefore, should logically be incorporated in the development of tissue engineered blood vessels designed for vascular repair or replacement. However, the architecture and low porosity of both naturally derived (i.e., decellularized vessels) and many synthetic biomaterials has impeded efforts to incorporate a VSMC layer in tissue engineered blood vessels. To this end, the goal of our work is to optimize methods for seeding, proliferation and maturation of VSMCs *in vitro* for subsequent implantation *in vivo*. Thus far we have focused our efforts on surface modification of decellularized scaffolds, as well as electrospinning of synthetic constructs comprised of PCL/collagen/elastin that would promote/enhance subsequent proliferation and formation of a VSMC layer during bioreactor preconditioning *in vitro*. This approach provides improved VSMC content on biocompatible and biodegradable scaffolds that can be further remodeled *in vivo*. The overall goal is to derive engineered constructs *in vitro* that more closely mimic native vasculature. Our initial work indicates that this approach provides accelerated maturation of tissue engineered blood vessels *in vivo*.

(P 156) Enhanced Control of Porosity in Electrospun Nanofiber Meshes

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Electrospinning has gained popularity as a simple and versatile technique to produce synthetic polymeric ultrafine fibers. This technique allows the production of non-woven meshes with fiber diameters in the nanometer range, which results in a high surface area-to-volume ratio and high porosity. Additionaly, these nanofiber meshes can mimic the extracellular matrix of human tissues and, therefore, can be used as scaffolds for Tissue Engineering (TE) applications. However, electrospun nanofibers meshes have an important drawback to this type of application. The obtained pore size is tipically too small to allow cell penetration into the inner regions of the nanofibrous scaffold. To overcome this problem, PCL and PEO solutions were electrospun simultaneously to obtain a dual composition nanofiber mesh. Then,a selective dissolution of PEO nanofibers was performed.

These structures were characterized in terms of morphology, mechanical properties and cellular response. PCL nanofiber meshes with comparable volume of material were used as control. The dual composition electrospun nanofiber meshes showed an increased porosity when compared with PCL meshes. The biologic assays were conducted with human osteoblast-like cells (Saos-2 cell line). By SEM and confocal microscopy, it was shown that the cells can penetrate into the nanofibrous structure, forming a fully cellularized construct appropriated for TE applications.

(P 157) Enhanced Vascularisation Induced by Endothelial Progenitor Cells (EPCs) Improves Matrix Organisation in Wound Healing

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Introduction: We previously demonstrated the increase in vascularisation by a subset of EPCs, called Blood Outgrowth Endothelial Cells (BOECs), in secondary wound healing. Here, we investigated a potential link between vascularisation and quality of dermal regeneration.

Materials & Methods: Wounds were made on the back of athymic nude mice ($n = 60$) and were treated either with saline ($n = 15$), human BOECs ($n = 14$), laminated human fibroblast sheets ($n = 14$) or a combination of both ($n = 17$). One group of animals ($n = 24$) was sacrificed on day 5, another group ($n = 36$) on day 10 for histological evaluation of vascularisation (CD31, α-SMA) and matrix composition (fibrillar collagen I and III) of the dermis within the wound area.

Results: Histological examination at day 5 revealed a significant increase in CD31+ vessels at the wound borders in the conditions

treated with BOECs. Moreover, a significant increase in vascular density was visible in the entire wound area on day 10. Conditions treated with BOECs revealed more smooth muscle cell-coated vessels, indicative of a more functional revascularization. Importantly, analysis of the dermis revealed a higher collagen content in the conditions treated with the fibroblast sheet, but the percentage of organized (red-orange birefringent) collagen was higher in both BOEC treated conditions.

Conclusion: Here we prove the link between an induced increase of vascularisation by BOECs and the quality of the dermal matrix organisation. Since BOECs and dermal fibroblast sheets can easily be derived from any patient, they can be considered as powerful tools for the creation of an autologous vascularised dermal template.

(P 158) Enzyme-Triggered Injectable Hydrogel of Tetronic-Tyramine Conjugates for Tissue Regeneration

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Over the past few decades, hydrogels have been an issue of intensive research for various biomedical applications. Especially, injectable system has been paid much attention. Such a system makes cell, drug and bioactive molecules easily entrapped *in situ* by a simple syringe injection of their aqueous solutions at target sites. In these systems, recently enzyme-triggered *in situ* hydrogel system has been studied. Enzymatic cross-linking systems have some advantages, such as biocompatible and fast reaction in mild condition. In this study, we report enzyme-triggered injectable hydrogel of Tetronic-Tyramine (Tetronic-TA) conjugates.Tetronic-TA conjugates were prepared by first reacting Tetronic with succinic anhydride and subsequent conjugating with tyramine (TA) using DCC/NHS. To confirm their chemical structure, ^{1}H NMR and FT-IR spectra were measured. Tetronic-TA hydrogel was formed above 10 wt% in the presence of horseradish peroxidase (HRP) and H_2O_2 under physiological conditions. Their mechanical properties, gelation time, swelling ratio and degradation time were evaluated depending on the concentration of polymer, HRP and H_2O_2. In addition, *in vitro* cell test was investigated using osteoblast to confirm its cytotoxicity. The cells were cultured in Tetronic-TA hydrogel matrix (3D culture) and on its surface (2D culture) for 24 hrs. The cultured cells in the hydrogel were observed by Live/Dead assay. The hydrogel showed low cytotoxicity. The obtained results demonstrated that Tetronic-TA hydrogel has potentials of injectable hydrogel for tissue regeneration.

Acknowledgement: This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health&Welfare, Republic of Korea (02-PJ3-PG6-EV11-0002).

(P 159) Esophageal Replacement By Aortic Allograft in a Porcine Model: Preliminary Results

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Background: Usual techniques of esophageal reconstruction carry a high morbi-mortality. The objective of this study was to evaluate the feasibility of circumferential replacement of the esophagus by aortic allograft in a porcine model.

Methods: In 21 minipigs, 3 centimeters of cervical esophagus were replaced by a fresh aortic allograft. Animals were divided in two groups according to the endoscopic insertion of a removable stent or not. No immunosuppressive therapy was given. In case of stent migration or stenosis following stent removal at one month, a new stent was placed. Histological analysis of the graft was performed at 1 and 3 months.

Results: All pigs without stent insertion ($n = 3$) experienced an early death because of anastomotic leakage, leading to interruption of this approach. In pigs with stent insertion ($n = 18$), one-month mortality rate was 33%, due to hematoma, small bowel obstruction, pneumonia, or technical failure during stent insertion. Among the 12 survivors, 10 experienced a stent migration or an obstructive granuloma. Stent removal systematically carried a necrotic aortic allograft with the occurrence of a stenosis (5–7 days). Histological analysis of the grafted area, showed a scar retraction with malpighian epithelium covering a fibrotic tissue, without muscular cell.

Conclusions: After circumferential esophageal replacement by an aortic allograft, one-month survival is 66%. Esophageal stenting is necessary at least for 3 months to avoid anastomotic leakage and stenosis. The aortic graft is subject to early necrosis and leave place to a fibrotic tissue covered by a malpighian epithelium, without muscular cell at 3 months.

(P 160) Evaluation of *In Vitro* Elution Profile, Antimicrobial Efficacy and Cytotoxicity of 3D Bioactive Polycaprolactone Honeycomb Scaffold

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Secondary infections and inflammations are common problems faced after an occurrence of trauma. These incidents could bring about undesirable or even disastrous implications when there is inadequate treatment and attention rendered. Therefore the development of a 3D antibiotics incorporated-bioactive polycaprolactone honeycomb scaffold could prove beneficial for such cases. Three different percentages (5wt%, 15wt% and 25wt%) of antibiotics incorporations were tested in this study. The efficacy of the eluted antimicrobial drugs was evaluated using the log reduction method on two bacteria strains (Staphylococcus aeurus and Pseudomonas aeruginosa). The elution profiles of the drug incorporated scaffolds were also evaluated at various timepoints up to a period of 1 week. After which *in vitro* cytotoxicity test was carried out, noting the human dermal fibroblasts metabolic activity and proliferation when exposed to scaffold with impregnated gentamicin

sulfate as a model drug. It is observed that the 3D bioactive polycaprolactone honeycomb scaffold is an efficient delivery system for antibiotic drugs, fast and effective in the elimination of both strains of bacteria and without significant cytotoxic effect thus suitable for many different applications and implantations.

(P 161) Evaluation of Mesenchymal Stem Cells and Anterior Cruciate Ligament Fibroblasts as Cell Sources for Tissue-Engineered Ligament

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The aim of this study was to compare the cellular responses of bone marrow-derived mesenchymal stem cells (BMSCs) and anterior cruciate ligament fibroblasts (ACLFs) on combined silk scaffolds for ligament tissue engineering application. Rabbit BMSCs and ACLFs were isolated and cultured *in vitro* for two weeks after seeding on the silk scaffolds. Samples were evaluated and compared for their cellular morphology, proliferation, gene and protein expression of tenascin-C, type I and type III collagen. In addition, the two cell types were transfected with green fluorescent protein (GFP) to trace their fate in the knee joints. Preliminary results comparing cell proliferation indicated that BMSCs proliferated faster than ACLFs. Gene expression of the phenotypic markers measured using real-time reverse transcription polymerase chain reaction (RT-PCR) indicated the transcript levels of BMSCs were significantly increased after two weeks of culture, whereas those of ACLFs had no significant difference. The protein levels and localization were determined by western blots and immunohistochemical staining, the results showed more production of ligament-related extracellular matrix (ECM) by BMSCs as compared to ACLFs. Moreover, 4 weeks postoperatively, more fluorescent cells were presented in BMSC-loaded constructs than in ACLF-loaded constructs. Therefore based on the cellular response *in vitro* and *in vivo*, BMSCs were found to be a better cell source than ACLFs for the further study of ACL tissue engineering.

(P 162) Evaluation of Nerve Regeneration Across using a Fibroblast Nerve Conduit Filled with a Collagen-Based Biomaterial to Bridge Peripheral Nerve Gaps

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The autograft has long been the gold standard solution for nerve gap repair although it inflicts a sensory deficit at the donor site and rarely adapts perfectly to receiver site anatomy. Our current technology for nerve repair using autologous nerve grafts is limited by the availability of donor tissue and its related morbidity.

The aim of this work is to prepare a nerve conduit made of fibroblasts that will serve as a nerve guide to enhance recovery of sensory and motor functions. Fibroblast nerve guide tubes, empty (control) or filled with a collagen matrix enriched or not with NGF will be used to bridge a 20 mm gap of sectioned rat sciatic nerves. Tubes will be transplanted for 120 days. The quantitative analysis or regenerating nerve fibers will be assessed through serial histochemical and immunohistochemical analysis. Functional motor reinnervation will be tested using the Walking track analysis and Relative gastrocnemius muscle weight indexes.

The use of tissue engineered autologous nerve grafts associated with neurotrophins could enhance and replace current treatment modalities used for iatrogenic, post-traumatic or neoplastic nerve gap repair.

(P 163) Evaluation of the Influence of the Mesenchymal Stem Cells (MSC) Density Seeded on Coralline Scaffolds on Bone Formation after Implantation in Sheep

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Aim of the study

Pathological events such as trauma, inflammation, and surgical treatment of tumors are often associated with extensive bone loss. Reconstruction of these defects represents a significant clinical problem in the those cases.

Present therapeutic approaches include transplantation of bone grafts (autologous, homologous or heterologous grafts) and implantation of different biomaterials. However, none of these have proven to be fully satisfactory. The present study aimed at evaluating the influence of the mesenchymal stem cells (MSC) density seeded on coralline scaffolds (acropora) on the bone formation after 8 weeks in sheep.

Materials and Methods: Prior to implantation, three-dimensional coralline scaffolds ($3\times3\times3$ mm) seeded with sheep MSCs (biohybrids) (1 million cells/ml) were cultured (i) in static conditions for 24 hours, 1 week or 3 weeks or (ii) in dynamic conditions in a perfusion bioreactor for1 or 3 weeks. The Biohybrids were implanted subcutaneously in sheep for 8 weeks.

The harvested biohybrids were embedded in methacrylate 2%, incubated at 17 °C 4 weeks. Sections were surface-stained with picrofuchsin and analysed (matlab).

Results: A positive correlation between bone neoformation and the initial number of cells seeded on the biomaterial was found. The osteogenic ability of biohybrids was significantly higher compared with nude scaffolds. The increased bone formation found in the scaffolds cultured for 1 or 3 weeks was independent of the applied culture condition.

Conclusion: The present study confirmed the osteogenic ability of biohybrids composed of MSCs and an osteoconductive scaffold. Biohybrids engineering is a valuable alternative to repair large segmental defects.

(P 164) Evaluation of the Integration and Neovascularisation of a Novel Fibrin-Based Dermal Scaffold

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Enhanced migration of endothelial cells *in vitro* has been demonstrated in fibrin gels when compared with collagen. Based on this potential pro-angiogenic property, a fibrin-based dermal scaffold (Smart Matrix) has been developed with a view to treating full-thickness skin loss. We aimed to establish evidence of *in vivo* integration of the smart matrix and wound bed, through cellularisation and new vessel formation within the neodermis.

Scaffold integration was evaluated *in vivo* using a porcine full-thickness wound chamber model. Punch biopsies of scaffolds were taken for histological and immunocytochemical analysis (CD31, vWF, VE-cadherin) at days 3, 7, 14 and 21. Neovascularisation was assessed by examination, and neovessel profile counts per field at day 7. Stability of these structures was inferred by extent of associated pericytes (α-SM-actin +ve) staining.

Cellular ingress from the wound bed occurred more rapidly into Smart Matrix than Integra. However, ingress into Smart Matrix, but not Integra was associated with a variable inflammatory (predominantly neutrophil) infiltration. The degree was mainly dependent on the concentration of calcium used in the scaffold manufacture process, being significant at >12 mM. The rate and extent of neovascularisation was distinct between Integra and Smart Matrix. Whereas capillary ingress into Integra was via angiogenesis, Smart Matrix displayed evidence of vasculogenesis through differentiation of granulation tissue, and the profile of endothelial differentiation marker expression. The vascular density in Integra at day 21 remained relatively low.

Fibrin-based matrices appear to offer a more favourable environment for accelerated tissue re-growth compared to conventional collagen-based scaffolds.

(P 165) Expanded Clinical Indications for a Tissue Engineered Blood Vessel

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We have developed a completely autologous tissue engineered blood vessel (TEBV) using a process called sheet-based tissue engineering. In this approach, we depart from the usual tissue engineering strategy by eliminating the need for synthetic scaffolds. In initial clinical trials, we implanted the TEBVs as arteriovenous shunts for hemodialysis access and demonstrated patency at time points beyond 12 months. In an effort to justify evolution to coronary use, we have expanded our mechanical testing to include dynamic fatigue, low pressure static fatigue, and step-wise fatigue testing. TEBVs were built from cells harvested from patients with advanced cardiovascular disease as described previously. Static fatigue tests were run by pressurizing the vessels to 250 mmHg for 5 days and then increasing pressure to burst. Dynamic fatigue testing was performed by cycling the pressure between either 120/80 or 600/400 mmHg for 14 and 3 days respectively (1 Hz). Vessels were then pressurized to burst. Step-wise fatigue testing was performed by pressurizing internal membranes (TEBVs with only 3 decellularized plies) to1200 mmHg for 15 minutes and then increasing pressure by 200 mmHg increments and holding for an additional 15 minutes or until failure. TEBVs demonstrate burst pressures in excess of 3000 mmHg. Burst pressure did not diminish significantly after fatigue loading relative to unloaded controls. These mechanical results, coupled with positive initial results in peripheral indications, would appear to support the evolution to coronary use. We have enrolled patients for coronary implant and will report clinical updates for both lower limb and coronary indications.

(P 166) Expansion of Cord Blood CD34+ve Cell Fraction using Chromatin Modifying Agents, Different Supporting Stromal Cells and Different 3D Scaffolds.

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Cord blood provides an easily accessible source of stem cells. Transplantation is however limited by the number of cells per cord blood unit, which are insufficient for adequate haematopoietic restoration of many adults.

Attempts to ameliorate this have included incomplete myeloablation, multiple unit transfusion and *ex-vivo* expansion. We present our studies in the latter.

Following consent, the mononuclear cell fraction was isolated from cord blood samples by density gradient centrifugation and immediately cryopreserved. Upon thawing, samples were pooled and cultured for a week with or without DNA-modifying agents and/or supporting cells. They were then analysed by flow cytometry to assess the CD34+ cell fraction.

Demethylating agent 5-aza-cytidine followed by Histone deacetylase inhibitor trichostatin A resulted in the greater increase, confirming previous studies.

Co-culturing the mononuclear cells with skin-fibroblasts, cells derived from the cord stroma and from the term placenta also increased the CD34+ fraction.

A further, statistically significant increase was seen upon co-culture with a mixture of all three cell-types indicating a possible synergistic effect in supporting progenitor expansion.

This optimised 2-D co-culture cell mixture was then compared with the same mixture grown in 3-D scaffolds of bovine cancellous bone (Surgibone), adjacent glass beads and non-woven fabrics. Bone matrix appeared to best support CD34+ cell fraction expansion.

DNA modifying agents largely obviated the effects of co-culture, possibly due to the effect of the chemical modifiers on the stromal cells themselves. Using these two sets of response modifiers in a consecutive manner may therefore produce the best effects.

(P 167) Expression and Purification of Recombinant Aprotinin for Enhanced Stability of Fibrin Biomaterials

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Fibrin is an integral component of the clot during wound healing and can be harvested autologously for creation of biologically active, biocompatible, hydrogel matrices. However, fibrin gels can lose utility due to their rapid rate of degradation via the actions of plasmin, especially in the context of the *in vivo* environment. Covalent-binding of plasmin-inhibitors to the matrix would allow for prolonged stability of fibrin while bypassing any alterations in its structure. Previous studies have successfully bound bioactive factors to fibrin matrices via a transglutaminase (TG) factor XIIIa substrate sequence tag, derived from α2-plasmin inhibitor. Using standand cloning techniques, a recombinant protein, aprotininTG, was created by fusing the TG-substrate sequence onto the C-terminus of the plasmin inhibitor, aprotinin. AprotininTG was then expressed in Escherichia coli and was purified from crude cell lysate using GST affinity chromatography. Bioactivity of the recombinant protein was assessed via a plasmin-inhibition assay. Thus, a mutant form of aprotinin has been created as a fusion protein which allows for efficient covalent cross-linking of the plasmin inhibitor into fibrin matrices. Use of aprotininTG within fibrin matrices may reduce their rate of degradation and therefore further augment their utility as biomaterials.

(P 168) Expression, Purification and Bioactivity of Recombinant Human Bone Morphogenetic Protein-4, -9, -10, -11 and -14 Produced in Escherichia Coli for Tissue Engineering Applications

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Bone morphogenetic proteins (BMPs) are cytokines from the TGF-β superfamily, with important roles during embryonic development and in inducing bone and cartilage in the adult body. In this contribution, we report the expression of recombinant human BMP-4, BMP-9, BMP-10, BMP-11 (or growth differentiation factor-11, GDF-11) and BMP-14 (GDF-5), using Escherichia coli pET-25b expression system. The BMPs were purified by affinity chromatography and its bioactivity accessed in C2C12 cell line, by screening the expression of osteogenic markers with RT-PCR. The expression of Smad-1, Smad-3, Smad-5, Runx2/cbfa1, Osterix, Bone sialoprotein, Osteopontin and ALP was increased with some or all of these BMPs, while Smad-7 was down-regulated, coinciding with the different changes in cell morphology. No cytotoxicity was observed and around 200 mg of BMPs were purified per Liter of batch. Ongoing work includes bioactivity assays in human adipose stem cells and with the effect of heparin on the BMP activity. The approach described here is a promising method for producing large scale amounts of recombinant BMPs, in pure and bioactive form, for novel biomedical applications.

Acknowledgments: Fundação para a Ciência e Tecnologia for PhD grant SFRH/BD/17049/2004 and project ElastM POCI/CTM/57177/2004 supported by FEDER and the Fundação para a Ciência e Tecnologia; European STREP Project HIPPOCRATES (NMP3-CT-2003-505758). The work was carried out under the scope of the European NoE EXPERTISSUES (NMP3-CT-2004-500283).

(P 169) Extracorporeal Shockwave Treatment of Early Spontaneous Osteonecrosis of the Knee

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This work reported of eleven cases of early spontaneous osteonecrosis of the knee successfully treated with a novel extracorporeal shockwave treatment.

Traumatic and vascular theories have been proposed as a causative factor of spontaneous osteonecrosis of the knee, but precise etiology still remains speculative.

ESWT, thanks to its neo-angiogenetic effect, can be suggested as an effective regenerative treatment for spontaneous osteonecrosis of the knee.

Ten patients with medial femoral condyle spontaneous osteonecrosis of the knee were evaluated. Exclusion criteria was evidence of structural collapse of subchondral bone. Two patients had received femoro-popliteal by-pass, while others five presented deficit of the vascular axis of the homolateral lower limb documented by an eco-colordoppler. Clinical evaluation was taken at diagnosis using KSS, McGill Pain Questionnaire.

Patients were treated with a cycle of three ESWT performed with 2000 pulses of 0,28 mJ/mm^2 with Wolf Piezoson 300 with 6,5 MHz ultrasounds for three times in a month.

Clinical evaluation was performed at first and at third month after treatment and a RMI evaluation was performed at fourth month after the treatment.

Clinical evaluation showed significant improvement of symptoms ($p < 0.001$) and articular functionality ($p < 0.001$). MRI of all cases revealed the continuity of the cartilage with a reduction in bone marrow edema and no collapse of lesion; in one case the total recover up to a normal signal in the subcondral bone was documented.

Therefore, a single cycle of ESWT produced an improvement of clinical and MRI aspects in eleven cases of spontaneous osteonecrosis of the knee.

(P 170) Fabrication and Characterization of Carbon Nanotubes Based Coatings for the Repair of Cartilage Tissue

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The possibility to develop novel biomaterials is critical in order to improve the success of any tissue repair strategy. An ideal biomaterial for bone tissue regeneration should possess chemical-physical properties similar to the cartilage being regenerated and good biocompatibility with surrounding tissue. The engineering of the surface properties of biomaterials at the molecular level is then critical to develop bioactive devices with which to elicit appropriate cellular responses (1). In this work biocompatible ultra-thin film coatings, with specific and predetermined properties, were prepared on a silicon substrate by the layer by layer (LBL) self assembly technique (2). Specifically multilayered coatings containing funtionalized single walled carbon nanotubes (SWCNTs), growth factors and polyelectrolytes were assembled, structurally characterized and are currently tested for the growth of chondrocytes, in order to evaluate their potential for the treatment of materials employed in cartilage tissue engineering. Quartz crystal microbalance technique and atomic force microscopy showed the reliability and reproducibility of the multilayered coatings fabrication procedure. Preliminary results on cell response characterization showed that the developed coatings have a high potential for the engineering of the surface properties of biomedical implants.

[1]T.A Desai (2000): "Micro- and nanoscale structures for tissue engineering constructs". Med Eng Phys. Vol. 22: 595–606.
[2]F. Caneva Soumetz, L. Pastorino, C. Ruggiero (2008): "Human osteoblast-like cells response to nanofunctionalised surfaces for tissue engineering". Journal of Biomedical Materials Research: Part B—Applied Biomaterials. Vol. 84: 249–255.

(P 171) Fabrication of Blended 3D PCL/PLGA Scaffolds using a Precise Multi-Head Deposition System Based on Solid Free-Form Technology

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We present a novel approach to manufacturing biodegradable poly-caprolactone/polylactic-co-glycolic acid (PCL/PLGA) scaffolds using SFF technology. In this study, blended 3D PCL/PLGA scaffolds with a uniform pore size of 600 μm and a line width of 200 μm were fabricated using a precise multi-head deposition system (PMDS) that we have developed. We developed the PMDS for fabricating 3D scaffolds using SFF technology. This system made it possible to create a repeatable 3D microstructure with a resolution of several tens of micrometers. The fabrication of the scaffold required the integration of multiple technologies, including 3-axis motion control, thermal control, pneumatic control, and CAD/CAM software. PLGA (Mw = 50,000–75,000) is a well-known biodegradable material for bone and cartilage regeneration. However, it is brittle and has low mechanical strength. In this study, PCL (Mw = 65,000) and PLGA polymer were well blended at a ratio of 1:1 under a melting temperature no higher than 150°C to compensate for the shortcomings of pure PLGA. The PCL/PLGA scaffolds were fabricated at a fixed pressure of 650 kPa and a temperature of 130°C. The pore size and line width were manipulated by changing the speed of the X-Y stage. Stacked scaffolds with a staggered shape were constructed using a layer-by-layer process to achieve an overall scaffold size of 7.4×7.4×3.2 mm. In addition, mechanical testing and cell interaction experiments were conducted to evaluate the performance of the scaffolds. we have demonstrated that the PMDS can be successfully used to fabricate blended 3D PCL/PLGA scaffolds.

(P 172) Fabrication of Well-Aligned Nanofibrous Scaffolds by a Novel Electrospinning Method

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An ideal scaffold should mimic the structural and functional properties of the native extracellular matrices. Native ECMs are typically fibrous structures with various nano-sized diameters. Electrospinning is a well known technique to produce nanofibers and has been utilized by many researchers to make nanofibrous matrices for tissue engineering applications. Most native ECMs found in tissues or organs, however, have regular or defined orientation architecture, which is significant for tissue function. Efforts are being made in various research groups to produce aligned nanofibrous structures all over the world and some breakthroughs have been obtained.

In the present work, a modified electrospinning system with a high level of control over the alignment of the nanofibers is introduced. Applying this new mechanism, it's not only possible to control the extent and pattern of alignment precisely but also one could easily produce two/three-dimensional hybrid structures composed of well-aligned nanofibers. In this method, the nanofibers are aligned between the two parallel disc-shaped rotating electrodes and guided to the collector, where they have no choice but being collected without any changes in their alignment. It's worth mentioning that the movement of the collector in any desired direction and with any desired speed(s) can lead to the formation of two/three-dimensional structures of nanofibers each layer of which can have the same or different materials and/or alignments. The new mechanism has been successfully applied for most of the biocompatible polymers and a vast range of well-aligned two/three-dimensional nanofibrous structures have been created.

(P 173) Fast Responding PNIPAAm Hydrogels

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Limited cell source and the reliability of the conventional cell culturing technique are some of the major challenges facing tissue engineering. The use of Poly(N-Isopropylacrylamide) (PNIPAAm) as a temperature-induced molecular switch for cell adhesion and cell release, has revolutionised the cell culturing fraternity. The use of

PNIPAAm as a cell culturing substrate allows viable tissue-like cell constructs to be cultured *in vitro* without requiring destructive enzymes. PNIPAAm is a temperature sensitive polymer which changes its surface properties reversibly between hydrophobic and hydrophilic states at temperatures above or below its lower critical solution temperature (LCST) (~32–33°C). PNIPAAm, however displays poor mechanical properties, and slow reswelling properties due to strong hydrophobic interactions between the isopropyl groups in its backbone. In this study a variety of PNIPAAm hydrogels were prepared by free radical polymerisation in water, THF:water or acetone:water mixtures. PNIPAAm hydrogels were crosslinked either with methylene-bisacrylamide (BIS) or polyethylene glycol dimethacrylate. When BIS was used, a feed crosslinker content of 1 to 9 molar percent was investigated with a NIPAAm/BIS molar ratio (R) varying from 91 to 10 respectively. Crosslinked gels were characterised by gravimetric swelling ratios, gravimetric deswelling kinetics, LCST, environmental scanning electron microscopy, and dynamic oscillating rheology. Preliminary results indicate that the choice of polymerising solvent greatly influenced the swelling capability, porosity and heterogeneity of the gels. The swelling capacity and porosity of the gels decreased at both 25°C and 37°C as the crosslink density increased. All gels maintained their phase transition temperatures irrespective of the BIS content investigated.

(P 174) Ferrimagnetic Glass-Ceramics for Magnetic Induction Hyperthermia

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Magnetic induction hyperthermia is one of the therapies employed in cancer treatment. This method involves biocompatible magnetic materials, which generate heat under an alternating magnetic field. These biomaterials are implanted into a neoplastic tissue and the patient is placed in an external magnetic field. The magnetic materials will heat, due to various loss mechanisms, producing tissue necrosis or thermoablation. The heating temperature depends on the materials properties, magnetic field parameters (intensity, frequency) and tissue characteristics (blood flow, tissue density, type of tumoral cells, etc.

The aim of this work is the preparation and characterization of innovative bioactive ferrimagnetic glass-ceramics in the system SiO_2–Na_2O–CaO–P_2O_5–FeO–Fe_2O_3

These biomaterials contain different amounts of nanometric or sub-micrometric magnetite crystals, homogeneously distributed in an amorphous matrix. They are bioactive, making them also suitable for bone substitutions. The hysteresis loss and the specific power loss are compatible with the temperatures required for hyperthermic treatments of neoplastic tissues. These glass-ceramic materials are biocompatible with the osteoblast cells. The surface of these materials was modified (by a suitable functionalisation process), in order to improve its integration into natural tissue.

(P 175) FGF2-DDS Could Enhance Peripheral Nerve Regeneration and Schwann Cell Proliferation in Bioabsorbable Nerve Conduit

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Introduction: Recently, various biodegrable polymer tubes have been reported. However those results were not satisfying enough for clinical use.

We have already investigated that our biodegradable nerve conduit filled with gelatin incorporating FGF-2 is a reliable drug delivery system(DDS) and it could release FGF-2 for two weeks.

The purpose of this study is to introduce our novel biodegradable polymer tube filled with gelatin incorporating FGF-2 as DDS and to report its effect on peripheral nerve regeneration and angiogenesis.

Materials and Methods: Study #1; effects of DDS of FGF-2 on peripheral nerve regeneration, Schwann cell and angiogenesis :we implanted biodegradable polymer conduit filled gelatin with or without incorporating FGF-2 into 12 mm sciatic nerve gaps in Wistar rats. At 12 weeks after implantation, number of regenerating nerves and vessels in transverse sections at mid-conduit were observed histologically.

Study #2; enhancement of DDS FGF-2 on peripheral nerve regeneration, angiogenesis and Schwann cell migration: In the same series, at 5D, 10D, 15D, 20D post operation, relationship of regenerating nerves, Schwann cells and vessels from proximal stump in longitudinal sections were observed immuno-histologically.

Results: Study #1; Concerning FGF-2, the results of nerve regeneration at 12 weeks obviously showed the advantage of DDS with FGF-2. And the number of vessels and total area of vessels in conduit significantly increased with FGF-2, too.

Study #2; DDS of FGF-2 could enhance nerve regeneration at 15D and 20D, Schwann cell migration throughout 20 days, angiogenesis at 15D and 20D.

Among them, angiogenesis was obviously enhanced.

(P 176) Fibrin-An Injectable Smart Hydrogel

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This study investigates the release of VEGF-165 from fibrin *in vitro* and *in vivo*.

Fibrin (FS) is a two component system, composed of fibrinogen and thrombin.

For the *in vitro* studies different concentrations of VEGF-165 were mixed into fibrinogen solution. During the injection both components (fibrinogen & thrombin) were mixed and crosslinked to fibrin. After polymerization the fibrin was covered by PBS and incubated for 7 days. At specific time points parts of the PBS were collected and replaced by new PBS. The collected supernatants were analyzed by VEGF ELISA. Results showed that the release of VEGF is continuous over the time period of 7 days.

For the *in vivo* studies a hindlimb ischemia/reperfusion injury transgenic mouse model was used. Transgenic VEGFR-2 luciferase knock-in mice were used for non-invasive, real-time assessment of the VEGF-R2 expression. Ischemia was maintained for 2 h with subsequent reperfusion for 24 h. Control animals received no treatment whereas the animals of the FS/VEGF group received an injection of 200 ng VEGF/ final FS clot in their hindlimb subcutaneously, 15 min prior to reperfusion. At different time points bioluminescence detection to observe VEGF-R2 expression was performed. With VEGF in the fibrin biomatrix a significant in-

crease in receptor expression was observed compared to baseline levels as well as to the control group.

We conclude that fibrin, as an injectable smart hydrogel, is effective in continuously releasing VEGF *in vitro* as well *in vivo*.

(P 177) Filling Cranial Defects in Rats: 6 Months Animal Study

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Poly(L-lactide) and poly(ε-caprolactone) with average molecular weights of 200 kDa and 40 kDa, respectively, were synthesized by ring opening polymerization of the respective dimer/monomer, and their blends with a ratio of 50/50 (w/w) were prepared. Scaffolds with highly open pores structures (interconnected) with pore sizes in the range of 250–355 μm were produced by using a two-porogen technique, namely salt leaching followed by supercritical fluid treatment. Discs with a diameter of 8 mm and a thickness of 1 mm were cut from the microporous sheets. Scaffolds were investigated by SEM and micro-CT. Micro-CT analyses showed 94 percent porosity. Defects with the aforementioned diameters (critical defect size) were created in female Sprague Dawley rats ($n = 96$). Animals were randomly assigned into three groups as follow: (i) Only scaffold group ($n = 32$); (ii) scaffold + stem cell (mesenchymal stem cells from rat bone marrow) group ($n = 32$); and (iii) control group ($n = 32$). Samples were collected from the defect site at days 30, 90 and 180. Bone regeneration was assessed by histological tests.

(P 178) Flexible Micropatterned Porous Scaffolds

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Introduction: In various tissue engineering areas two major issues are extensively studied; i.e. efficient nutrient supply and optimal cell organization. Through scaffold design, both these issues can be addressed:

- inner-porosity to provide nutrient supply,
- micropatterning to induce cell organization.

Micropatterning of the scaffold surface can promote enhanced cell attachment and orientation. Moreover, the micropattern can optionally be used for additional perfusion to improve nutrient supply [1].

Method: Previously [1], we showed fabrication of 3-D multilayer stacked porous micropatterned scaffolds of well-known semi-crystalline poly(l-lactic acid) (PLLA) by one-step fabrication method Phase Separation Micromolding (PSμM). With PSμM, a polymer solution is solidified on a micropatterned mold by immersion precipitation, inducing the micropattern to be replicated into the polymer sheet. The resulting PLLA sheets were highly porous (>80%) and the incorporated micropattern designs induced alignment of mouse pre-myoblasts, C_2C1_2 cells.

Results and Discussion: In this work, we develop highly porous micropatterned sheets from amorphous Poly(trimethylene carbonate) (PTMC). The flexibility of the PTMC sheets can be advantageous in tissue engineering application where high flexibility at the site of implantation is required. By variation of processing conditions (e.g., polymer concentration, additives) we obtain highly porous PTMC sheets with pores in the range of 1–50 μm. Additionally, the micropattern is tailored to obtain various surface topographies. Cell culturing experiments show great potential of these scaffolds for tissue engineering applications concerning cell attachment, viability, proliferation and organization.

[1]Papenburg, B.J, *et al.*, Biomaterials 28(2007), 1998.

(P 179) Functional Reconstruction of Engineered Digits

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Surgical reconstruction of digits injured by trauma are challenging due to limited tissue availability. We explored the feasibility of engineering skeletal muscle and cartilage structures *in vivo* which could be potentially used for functional digit reconstruction. Composite scaffolds consisting of synthetic PGA polymers (1.0× 0.5×0.5 cm) and naturally derived collagen matrices (1.4×0.4× 0.3 cm) were constructed. Chondrocytes were seeded onto the PGA polymer matrices and muscle cells onto the collagen matrices. The scaffolds containing both cell types were analyzed *in vitro* for cell viability and tissue formation. The engineered digits were implanted subcutaneously in athymic mice ($n = 36$) and followed for up to 6 months. The cells seeded on the composite digit constructs readily attached to their designated region of the scaffold and remained viable. Grossly, the implanted scaffolds formed muscle and cartilage tissues adjacent to each other. Each tissue type was confirmed histo- and immunohistochemically using cell specific antibodies. Biomechanical studies showed that the cartilage tissue was elastic and could withstand high degrees of pressure. Physiologic organ bath studies of the retrieved muscle tissues showed adequate contractility in response to electric field stimulation.

(P 180) Gelatin Hydrogel Crosslink by Transglutaminase in Bone Tissus Ingeneering: Application for MSC Survival

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Tumor resection and trauma are often associated with large defects which need to be reconstructed. Autografts are considered as the golden standard in this respect. However, the volume of autogenous bone transplants is rather limited and their harvesting and transplantation might be connected to complications such as infection and donor site pain. Bone cellular therapy using adult mesenchymal stem cells (MSCs) in combination with a osteoconductive biomaterial is gaining interest, but not yet a clinically applicable alternative due to cellular death. The aim of this work was to explore the capability of a hydrogel made of gelatine crosslinked chemically by Transglutaminase (Tgase) for enhancing MSCs survival. In a first time biochemical experiments were assessed on the hydrogel, then in a second time imaging and biological tests were done on MSCs included in gelatin hydrogel. Assessing gelation time and rheological properties resulted in a hydrogel with optimal concentrations of gelatin and Tgase of 5% and 1.5 U/ml respectively. Cell cycle analysis, cell counting and DNA quantification revealed that (i) MSCs did not proliferate but survived inside the hydrogel, (ii) Tgase had no effect on cell proliferation whereas (iii) gelatin inhibited MCS proliferation, freezing the cells in the G0/G1 stage. The present work demonstrated that dense gelatin hydrogels are not suitable for use as proliferation scaffold in bone tissue engineering. Cellular survival for over 10 days on the other hand could be confirmed for the hydrogel used.

(P 181) Gelatin Matrix to Support Angiogenesis

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Objective: To bridge gaps of lesioned nerves after PNS injury we have started to develop a nerve guide implant made of stabilized gelatin. The novel concept is to design an implant material that facilitates angiogenesis which in turn promises to improve neuronal regeneration.

Materials and Methods: Chemical crosslinking together with sponge formation techniques were employed to generate stabilized gelatin sponges. Four different *in vitro* approaches with fibroblasts and endothelial cells in conjunction with immunocytochemistry and an enzyme assay were performed to test biocompatibility/toxicity, cell adhesion, -migration and -proliferation. To analyze angiogenesis *in vivo*, gelatin structures were implanted onto the chorioallantois membrane (CAM) of the chicken egg and subcutaneously into mice.

Results: Preconditioned gelatin specimens were positively tested *in vitro* with regard to all four parameters. Implantation of gelatin sponges onto the CAM induced robust formation of blood vessels. Angiogenesis inside gelatin implants occured more than 200 times faster than in a commercial collagen sponge. After subcutaneous implantation of sponges into mice, immigration of cells and subsequent formation of functional vasculature became evident.

Conclusion: In summary, the novel matrix based on crosslinked gelatin promises to be a valuable component of future implants in order to improve regeneration by concomitant pro-angiogenic stimulation.

Supported by BMBF 0313144A, B.

[1]Dreesmann, L., Ahlers, M, Schlosshauer, B. (2007) The pro-angiogenic characteristics of a cross-linked gelatin matrix. Biomaterials 28, 5536–5543.
[2]Schlosshauer, B., Dreesmann, L., Schaller, H.-E., Sinis, N. (2006) Synthetic nerve guide implants in humans—a comprehensive survey. Neurosurgery 59, 740–748.

(P 182) Gene Expression Regulation During the *In vitro* Development of a Bioengineered Corneal Endothelial Barrier

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Introduction: Strict regulation of the transendothelial ion flux between the corneal stroma and the anterior chamber of the eye is necessary for the cornea to remain transparent and allow the incoming light to reach the retina. Nevertheless, the ion transport regulatory mechanisms that develop during the formation of the corneal endothelial barrier are unknown.

Materials and Methods: In this work, we have quantified the intracellular content of sodium, potassium and chlorine in the confluent and non-confluent rabbit corneal endothelial barrier by electron-probe X-ray microanalysis. Then, we carried out a gene expression profiling at the mRNA and protein levels by RT-PCR, Western-blot and immunocytochemistry on the confluent and non-confluent endothelial barrier.

Results and Discussion: Our results revealed that non-confluent endothelial cells showed higher intracellular contents of potassium and chlorine than confluent cells, with a hypertrophic volume increase. In contrast, the intracellular contents of potassium and chlorine decrease when cells reach confluence and the endothelial barrier forms, showing a decrease in cell volume. Our genetic and protein analysis showed a higher expression of KCNC3 in confluent cells, and of CFTR and CA2 in non-confluent cells. These results suggest that endothelial cell confluence and cell-cell contacts are able to control and regulate the normal transendothelial ionic current that keeps the corneal stroma dehydrated and transparent. For that reason, only confluent corneal endothelial cells should be used to develop artificial corneas by tissue engineering.

Supported by CM2005/011 from Junta de Andalucia.

(P 183) Generation of a Novel Sca-1^{pos} Lin^{neg} Murine Mesenchymal Stem Cell Immortalized Line for Tissue Engineering Applications

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Tissue engineering techniques rely on adult stem cells as a promising source of multipotent progenitors to repair damaged tissues and organs. In this context, the search for the ideal candidate for

stem cell therapy is still ongoing and further *in vitro* investigations are required. Unfortunately, *ex vivo*-derived progenitor cells display high donor variability and limited lifespan in culture, proceeding to senescence within few passages. Recently, a unique Sca-$1^{pos}Lin^{neg}$ mesenchymal stem cell line (Sca-1 mTERT-MSC) was generated by mTERT ectopic transduction of bone marrow purified Sca-1^{pos} MSC. This cell line, obtained by serial passages of single cell cloning, displays spindle-shaped morphology and continuously expresses mesenchymal stem cell markers (Sca-1, c-kit, Nanog, Nestin, Nucleostemin), while retaining the ability to differentiate towards osteogenic, adipogenic and myogenic lineages in standard culture conditions. Moreover, TERT-transduced MSC show enhanced resistance to H_2O_2-induced apoptosis similarly to early passage cells. Finally, when cultured onto three-dimensional biocompatible scaffolds, Sca-1 mTERT-MSC line displays the ability to maintain the typical stem cell phenotype and multipotency, thus suggesting its utilization as a powerful tool for engraftment and *in vitro* differentiation studies.

(P 184) Generation of Immobilised Gradients Without Microfluidics for Adhesion of Neural Cells

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The idea of a chemotatic, haptotatic or morphogen gradient is intimately associated with the concept of positional information. A cell is believed to read its position in a concentration gradient of an extracellular signal factor, and to determine its functional and developmental fate. Biomolecular gradients have been difficult to produce because the distances involved are over a maximum of 10s of cell distances. Today multichannel microfluidic techniques are routinely used to create gradients of various shapes and sizes, but they create cell damage owing to the presence of even a minimal flow over cells. Gradients can be produced using gels in which a temporary gradient is set up by moistening one side of a gel with the molecule of interest, and then using the gel as a stamp. Based on this principle we have developed an immobilised gradient generator, that consists of a 2 chamber device, in which the larger chamber is filled with a liquid containing the ligand at a given concentration, and the smaller chamber is filled with an agarose gel. Given the diffusion constant of the ligand within the gel, it is possible to generate a precise concentration profile within the gel, as a function of time. The gel is then quickly stamped onto a derivatised glass slide.

Using this method, we have printed immobilised polylysine gradients from 0 to 300 microg/ml over 2.4 cm, for the study of mesenchepalic neural stem cell adhesion.

(P 185) Glass-Ceramic as Scaffold for MSCs Growth

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Introduction: A bioactive glass-ceramic (GC) was evaluated about the capacity of mesenchymal stem cells (MSCs) to adhere, proliferate and differentiate into osteoblast (OBs) with or without GC was investigated.

Materials and Methods: The GC was obtained by heat treatment of a bioactive glass (55-SiO_2; 41-CaO; 4-P_2O_5; mol %), obtained by the sol-gel method. The adult MSCs were isolated from bone marrow of adult rabbits obtained by direct aspirations of ileac crest. The OBs used as control were obtained by the method of enzymatic digestion. The behavior of isolated MSCs on the GC, two series of 96-well plates were seeded, with GM and OM. Then 5×10^3 cells were seeded. At 24 hours, 7, 15, 21 and 27 days cell adherence and growth were analyzed. A variance analysis (ANOVA) was carried out with a minimal significance of $p < 0.05$.

Results: The MSCs under the conditions of this study expressed an osteoblastic phenotype and took place by either the action of exposing the MSCs to a MO as well as by the effect of the GC.

Conclusions: The GC has provided a favourable environment to support adhesion, proliferation and differentiation of MSCs to OBs.

(P 186) Heparin-Collagen Coated TCP/HA Granules as a Carrier for BMP-7

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Bone regeneration via the delivery of BMPs (bone morphogenic proteins) often goes with problems in controlling its binding and release. The aim of this study was to attached heparin covalently to type I collagen-coated TCP/HA (tricalcium phosphate hydroxyapatite) granules to specifically bind the heparin binding growth factor BMP-7.

We used TCP/HA granules with a diameter of 2–4 mm. These granules were coated with collagen (COL) by mounting the granules in a 0.5% (w/v) collagen suspension followed by subsequent freezing and freeze drying. Coated granules were cross-linked in the presence and absence of 0.5% (w/v) heparin (HEP) by means of carbodiimide cross-linking. Recombinant human BMP-7 was loaded onto the granules by a two hour incubation in 1.0 μg/ml BMP-7 in PBS (pH 7.4) followed by three washings in PBS. The granules were characterised using scanning electron microscopy (SEM) and immunofluorescent assay (IFA). The free amino groups and heparin content were determined with biochemical assays. The amount of BMP-7 bound to the TCP/HA, TCP/HA-COL and TCP/HA-COL-HEP granules was analysed with SDS-PAGE silver staining.

SEM analysis showed a highly porous collagen network on both TCP/HA-COL and TCP/HA-COL-HEP granules. IFA could localise the heparin and BMP on the granules. On SDS-PAGE the heparin coated granules showed more bound BMP even after 21 days. These heparin coated granules can also be used for the delivery of other heparin binding growth factors such as VEGF, FGF and/or PDGF, which may enhance angiogenesis and osteogenesis. This delivery system may provide a powerful modality for bone regeneration.

(P 187) High-Throughput Biological Screening of Cell-Surface Topography Interactions

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The advent of micro- and nanotechnologies has generated immense possibilities to create substrates with different surface topographies. Also, in the past few years there have been various studies dealing with the effect of biomaterial surface on cell behaviour. It appears that cell shape is an important factor governing cell fate (i.e. migration, growth, differentiation and apoptosis). With the advancements in micro- and nano- technologies it is now possible to generate tools that can influence cell shape and thus cell behaviour. However, most studies in the past have dealt with just a few variations in surface topographies. In the present study we have defined around 8000 variations in surface topography. The topographies were created on a 2×2 cm silicon mould using stereolithography and the mould was used to replicate the features onto PLLA and PEO/PBT polymers, which are refered to as TopoChips. Next, we seeded GFP-positive CHO cells and mouse embryonic stem cells onto TopoChips and analysed GFP and nuclear staining respectively using a fluorescent microscope and a micro-array scanner. Current research is aimed at developing reporter assays to reveal the biological response of cell grown on the array of surface topologies on the TopoChip.

(P 188) How To Optimise the Maturation Conditions in a Bioreactor? Toward an Intelligent Bioreactor for Vascular Tissue Engineering

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The need for bioreactors is well established in tissue engineering. Bioreactors are generally designed to closely recreate specific physiological environments in which cell-colonized scaffolds (constructs) are expected to maturate and achieve functionalities. This process is function of a number of interlinked dynamical parameters. The clear establishment of those parameters (and ranges) susceptible to lead to the optimal construct maturation is not trivial. A number of scientists might be interested in knowing which dynamic regimes should be applied during maturation to ensure expected maturation of tissue engineered constructs. This work aimed to instrument a bioreactor with advanced process-control tools in order to optimize the maturation process. It was hypothesised that a Partially Observed Markov Decision Process (POMDP) will provide the necessary framework to appropriately control the bioreactor. Sensors in the construct under maturation generate the applied parameters and ranges. An output action was expected on the construct. When the consequences of the actions are uncertain and cannot be predicted with certainty, POMDP is appropriate to generate an experience plan, susceptible to translate inputs from sensors to output actions to be executed. A randomized point-based value iteration solver for POMDP, called PERSEUS, was combined with this control system. The bioreactor was instrumented with an optical interferometer to measure tissue growth. A mass-flow/pressure controller was used to dynamically regulate the pressure signal inside the construct, the flow was generated by a peristaltic pump and controlled by a computer. Results confirmed that the POMDP allowed the control of the bioreactor to apply dynamic routine.

(P 189) Human Bone Marrow Mesenchymal Stem Cells Expressing CD105 After *Ex vivo* Replication Defective Adenovirus BMP-2 Mediated Gene Transfer

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Tissue engineering integrating *ex vivo* gene therapy and polymer science may have clinical significance to bone regeneration application. This study aims to understand *in vitro* cellular differentiation post adenoviral infection.

The experimental group was composed of *ex vivo* replication defective adenovirus BMP-2 mediated gene transfer to the expanded human MSCs. The controls were performed using human MSCs. We tested the parameters in flow cytometry as CD166, CD105 and CD34 post infection 2, 3, 6 and 9 days.

Comparison between the experimental and control groups, there were no difference in the expression of CD 34. While CD 166 showed no significant increase in the experimental group. Both groups demonstrated less decrement of CD 105 in the experimental one in flowcytometry and western blotting study.

This data evidenced that *ex vivo* replication defective Adenovirus mediated human BMP-2 gene transfer to MSCs enhances the expression of CD 105 which is an accessory protein of multiple kinase receptor complexes of the TGF-β superfamily. TGF-β is directly implicated in vascular development and thought to control interaction between endothelial cells and smooth muscle cells. BMPs may also be involved in these processes. BMP-2 and -7 can act on vascular smooth muscle cells to inhibit their proliferation without stimulating extracellular matrix synthesis, whereas activin-A has a growth-stimulatory effect.

(P 190) Human Dental Pulp an Abundant Source of Multipotent Adult Progenitor Cells

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Whilst protocols for the isolation of progenitor cells from numerous sources are described in extreme detail, characterisation of individual subpopulations has proved cumbersome. Dental

pulp from both deciduous and adult teeth has been identified as a potential source of primitive cells, which could provide a safe, reliable and abundant supply of progenitor cells.

Isolated pulp was dispersed and cells resuspended in culture medium. Undifferentiated confluent cells were removed and analysed by flow cytometry. Colony forming unit (CFU) analysis was performed using complete MethoCult Media. Cells were cultured in media known to cause lineage differentiation along adipogenic, chondrogenic, osteogenic and neurogenic pathways. Phenotyping by immunostaining, histological staining and western blotting for proteins associated with the desired lineage was carried out at days 7, 14, 21 and 28.

Heterogeneous pulp cells exhibited markers associated with adult MSCs, HSCs and ESCs as observed by flow cytometry. Further analysis of undifferentiated cells ascertained the expression of STRO-1, Oct-4, Sox2 and Tra-1-81 within subsets of the population. Upon introduction into methylcellulose, cells formed CFUs. Individual subpopulations were homogeneously isolated using high-speed FACS sorting based on CD90. CD90+/- cells introduced into adipogenic, chondrogenic, osteogenic and neurogenic differentiation medias exhibited morphological transformations and furthermore demonstrated a gradual increase in proteins associated with the desired lineage, most notably the expression of neurotransmitter Synaptophysin in cells treated with neural differentiation media. Histology confirmed differentiation. The multipotentiality of the CD90- population indicated additional sources of primitive cells within dental pulp, identifying dental pulp as a clinically applicable cell source.

(P 191) Human Dermal Fibroblasts Cultured on Xenogeneic Extracellular Matrix—*In Vitro* Construction of a Potential Skin Substitute.

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Introduction: The use of bioengineered skin constructs has developed owing to the limitations of autologous skin grafting. These constructs feature a matrix, typically collagen, onto which cells important for skin repair are seeded. To successfully induce tissue repair, bioactive constructs must be able to promote adhesion, migration and proliferation of cells. The aim of this study was to investigate the suitability of an emerging acellular xenogeneic extracellular matrix - porcine urinary bladder membrane (UBM) as a substrate for culturing human dermal fibroblasts with a view to creating a novel skin substitute.

Methods: Human dermal fibroblasts were seeded on sheets of UBM, scaffolds were cultured for 16 days and the composites analysed for cellular viability, attachment, propagation and collagen production. Cell viability and mitochondrial activity was assessed using live-dead immunoflourescence and MTT assays respectively. The cellular and matrix morphology was examined using optical and scanning electron microscopy (SEM).

Results: The cell-scaffold composites showed excellent viability with live cells at 16 days. Over 90% cellular adhesion was noted. Cells revealed a time weighed increase in numbers, forming a fully confluent monolayer by day 6. Collagen synthesis was reduced by 16% when compared to the control group.

Conclusions: We have found UBM to be a suitable substrate for adherence and propagation of dermal fibroblasts, while also demonstrating the ability of UBM to modulate the synthesis of collagen by dermal fibroblasts. These results suggest that UBM/dermal fibroblast co-constructs may be suitable for future tissue engineering efforts in the area of wound healing

(P 192) Human Hair Derived Keratins Mediate Schwann Cell Behavior *In Vitro* and Facilitate Rapid Regeneration of Peripheral Nerves *In Vivo*

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Nerve defects are a common result of peripheral nerve injury and present a significant challenge clinically. It has been shown that the insertion of tissue engineering scaffolds into a nerve guidance conduit can enhance regeneration. Keratins extracted from human hair fiber are a novel group of biomaterials that can be processed into hydrogel conduit fillers. In this study, keratin gels were prepared by treating human hair fibers with peracetic acid, followed by extraction and purification to a powder. Hydrogels were prepared by re-hydrating the powder. Micro-architecture of the gels was observed by scanning electron microscopy (SEM). Proliferation, migration, and adhesion of RT4-D6P2T Schwann cells was assessed. To determine the effects of keratin on nerve regeneration *in vivo*, repair of tibial nerve axotomy in mice was assessed. Examination of lyophilized hydrogels by SEM showed a fibrous and highly porous architecture. Keratin was not cytotoxic and increased cell proliferation at concentrations ranging from 0.1 μg/mL to 1 mg/mL over serum containing media control. Migration in the presence of keratins was significantly enhanced at 0.1 and 1.0 mg/mL concentrations. Schwann cell adhesion on keratin coatings was higher than on uncoated and fibronectin coated slides. Six weeks following nerve transaction and repair, latency and amplitude was found to be better in the keratin group compared to both empty conduits and the autograft control. Muscle force testing demonstrated comparable muscle reinnervation in all treatment groups. Histomorphometric analysis showed a significant increase in nerve diameter in the keratin group over empty conduit controls.

(P 193) Human Osteoblastic Response to a Novel Calcium Phosphate / SiO_2-Xerogel Composite *In Vitro*/*In Vivo* for Bone Tissue Engineering

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Novel calcium phosphate / SiO_2-xerogel (BONITmatrix®)—collagen scaffolds (BM-collagen) for bone substitutions were tested for their osteoblastic response in an *in vitro*/*in vivo* study.

Previous *in vitro* studies have shown promising endothelial cell growth, which is important for vascularization. The main goal of this study was to examine osteoblast biocompatibility.

Primary human osteoblasts (HOS) and the human osteoblast-like cell line MG63 were used for this study. In addition, the coculture of HOS and microvascular endothelial cells (HDMEC) and their potential for angiogenesis was studied *in vitro*.

HOS and MG63 demonstrated strong cell proliferation as shown by calcein-AM staining and subsequent confocal microscopy. Cell morphological investigations on BM-collagen, a generous gift of DOT (Rostock), by means of SEM confirmed latter observations. RT-PCR analyses revealed physiological gene expression. A long-term coculture of HOS and HDMEC demonstrated positive endothelialization and an incipient window formation for a potential vascularization.

For *in vivo* studies, 40 female 5-week-old Wistar rats were randomly divided into 2 groups of 20 animals each. From each group 4 animals were used for each of the following time points: 3, 10, 15, 30, and 60 days. Group 1 was treated with BONITmatrix®. Group 2 was a sham group.

At the time points indicated the biomaterials were explanted along with the surrounding peri-implant tissue and fixed for histological, histochemical and electron microscopical analysis. Cells involved in the inflammatory response as well as development of neo-angiogenesis associated with the biomaterial were quantified at the above mentioned 5 time points.

(P 194) Human Primary Osteoblasts Matrices as a Model System for Bone Metastasis Research

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Prostate cancer is the most common cancer in males and the second leading cause of male cancer deaths. Metastasis of prostate cancer cells, specifically to bone, is reliant on the reciprocal interactions between cancer cells and the bone niche/microenvironment. These interactions are dependent on factors such as cytokines, growth factors, serine proteases and bone related proteins.

The production of suitable matrices to study metastasis, carcinogenesis and in particular prostate cancer/bone microenvironment interaction has been limited to specific protein matrices or matrix secreted by immortalised cell lines. The latter are limited since they are produced from a homogeneous population of cells and tend to be locked into a particular stage of differentiation retaining a set of characteristics representative of that phenotype. As such, they may have undergone transformation processes altering signalling pathways and modifying gene or receptor expression.

We have used matrices formed from human bone explant cultures. Primary human osteoblasts were cultured for 5 weeks in osteogenic medium to allow secretion of a mineralized matrix. Cells were removed with ammonium hydroxide leaving the matrix intact. Prostate cancer cells (eg PC3, LNCaP) were seeded onto the osteoblast matrix and allowed to grow. Prostate cancer cells adhered strongly to the matrix, were resistant to EDTA-mediated detachment and displayed altered morphology when compared to conventional coatings such as collagen I. Analyses of gene and protein expression are currently underway.

We show that matrices produced by primary osteoblasts are an excellent paradigm to study tumour/bone interaction such as those observed in bone metastasis.

(P 195) Hybrid Polymer/Ceramic Nanofibre Scaffolds for Soft Tissue Engineering

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From a biological viewpoint, almost all of the human tissues and organs are deposited in nanofibers forms or structures, including dentin, collagen, cartilage, and skin. All of them are characterized by well organized hierarchical fibrous structures realigning in nanometer scale. As such, current research in electrospun polymer nanofibers has focused one of their major applications on bioengineering. Their promising potential can be seen in various biomedical areas, including three dimensional scaffolds for cartilage regenerations. The materials used include segmented polyuretanes, Pluronics, poly(glycolic acid), poly(lactic acid), and their copolymers.

The development of new biomaterials for various engineering applications requires different systems, including polymer/ceramic composite materials with their interesting combination of specific physico-chemical, mechanical and processing properties. Combining unique properties of the organic component (oligomer or polymer) and the inorganic one (inorganic particles or aggregates, including ceramics), they fulfill the highest requirements of modern materials for medical applications.

New organic/inorganic hybrid materials prepared from multiblock terpolymer as a polymeric matrix containing phthalic acid segments (PBT) as hard block and hydrogenated dimer fatty acid (DLA) as hydrophobic soft block, and poly(ethylene glycol) (PEG) as second soft block of hydrophilic nature are presented in this work. Two types of nancrystalline hydroxyapatites (non-calcined, HAP I, and sintered, HAP III) were used in amount of 0.5wt% as inorganic phase within hydrophilic/hydrophobic multiblock terpolymer matrix. The results related to the preparation of electrospun nanofibers for cartilage regeneration will be presented.

(P 196) Hydrostatic Pressure Culture System for Human Chondrocyte Cultivation and Stimulation in Gellan Gum Hydrogel Disks

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Cartilage tissue loss, resulting from injury or disease, is a major health problem worldwide. Tissue Engineering (TE) has a great potential for the treatment of vast number of pathologies. In this regard, bioreactor systems are used as frameworks for studying fundamental aspects of cell response and tissue development, as well as scale–up platforms for industrial application. It has been

shown that mechanotransduction mechanisms modulate cell functioning, including cell differentiation, proliferation and extracellular matrix (ECM) deposition, and that chondrocytes are mechano–sensitive cells. Hydrogels are often used in TE to encapsulate chondrocytes and engineer cartilage–like constructs, which are incompatible with flow perfusion bioreactors. In this work, hydrostatic pressure (HP) was used as an alternative to mechanically signal human chondrocytes encapsulated in gellan gum hydrogel discs, as it has a profound effect on cartilage metabolism in normal and pathological conditions. HP was applied in both static and dynamic regimens. Dynamic conditions were used in order to mimic the non–continuous *in vivo* loading of articular cartilage and induce the production of cartilage–like ECM. The effects of HP over chondrocytes was assessed by morphological and histological characterization of the constructs: detection of Sulphated glycosaminoglycans (GAGs), collagen type I and II; evaluation of mRNA expression by real time RT–PCR analysis, and visualization of the chondrocytes inside the produced matrix by bright–field microscopy. HP was shown to affect the synthetic capacity and viability of chondrocytes, depending on the mode, duration and magnitude of pressure. The properties of the hydrogel were also shown to depend of HP conditions.

(P 197) Hypoxia Exerts Differential Effects on the Chondrogenic and Osteogenic Differentiation of Human Adipose-Derived Mesenchymal Stem Cells

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Human adipose tissue-derived stem cells (hATSC) have been contemplated as reparative cells for cartilage engineering. Chondrogenic differentiation of hATSC can be induced by an enriched culture medium and a three dimensional environment. Given that bone is vascularized and cartilage not, oxygen tension has been suggested as a regulatory factor for osteo-chondrogenic differentiation. Our work aimed at determining whether hypoxia affects the osteo-chondrogenic potential of hATSC. HATSC were cultured in chondrogenic or osteogenic medium for 30 days, in pellets or monolayers, and under 5% or 20% oxygen tension. Cell differentiation was monitored by real-time PCR (COL2A1, aggrecan and osteocalcin). The chondrogenic differentiation was further evaluated by Alcian Blue and immunohistological staining for glycosaminoglycans (GAG) and type II collagen respectively. The osteogenic differentiation was also assessed by the staining of mineralized matrix (Alizarin Red) and measurement of alkaline phosphatase activity (ALP). The expression of chondrogenic markers was upregulated when hATSC were exposed to hypoxia in chondrogenic medium. Conversely, osteocalcin expression, mineralization and ALP activity were severely reduced under hypoxic condition even in the presence of osteogenic medium. Our data strongly suggest that hypoxia favors the chondrogenic differentiation of hATSC as evidenced by the expression of the chondrogenic markers, whereas it could alter their osteogenic potential. Our results highlight the differential regulatory role of hypoxia on the chondrogenic and osteogenic differentiation process of hATSC. These data could help us exploit the potential of tissue engineering and stem cells to replace or restore the function of osteoarticular tissues.

(P 198) Hypoxia Modulates Matrix Metalloproteinase-9 Secretion on Tissue-Engineered Mucosa

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Oral cancer is the sixth most common cancer in the world. After oncological resection the defect is reconstructed using free skin flaps. Tissue-engineered mucosa (TEM) can be used to reconstruct the defects after resection. Upon transplantation, a period of hypoxia occurs resulting in a limited cellular respiration which can lead to graft rejection. Therefore the aim of this study was to determine the effect of hypoxia on TEM.

Human keratinocytes and fibroblasts were isolated from buccal mucosa and seeded onto a scaffold of acellular human donor skin. After 14 days of culture at the air-liquid interfase TEM was exposed to 1.5% and 20% O_2 during 6, 12 and 24h. After each incubation period, media was harvested and TEM was snapfrozen or embedded in paraffin. The effect of hypoxia on TEM was visualized using hematoxiline/eosine (HE) staining and a Hydroxiprobe-1 kit. The harvested media was analyzed for MMP-9 activity. TIMP-1, TIMP-2 and HIF-1α proteins were analyzed by western blot. Secretion of VEGF and PDGF was assessed by ELISA.

TEM exposed to hypoxia showed presence of hypoxic cells and a disturbed basal cell layer compared to TEM exposed to normoxia. Significantly higher levels of MMP-9 activity were detected in supernatant of hypoxic TEM. An increased expression of TIMP-1, TIMP-2 and HIF-1α was found in TEM cultured under hypoxic conditions. In addition, the secretion of VEGF and PDGF was enhanced on TEM exposed to hypoxia.

Our results suggest that hypoxia preconditioning of TEM may favour wound healing and angiogenesis upon transplantation.

(P 199) Hypoxic Culture and Expansion of Mesenchymal Stem Cells in Air-Lift Loop Hollow Fiber Membrane Bioreactor

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Mesenchymal stem cells (MSCs) are the promising ''seeding cells'' for tissue engineering and excellent tools for gene therapy. However, MSCs are in limited quantity and their viability decreases with the individual age increases, which makes it necessary for them to be expanded *in vitro*. Many researches indicated that hypoxia accelerated MSCs proliferation and differentiation, but all were in two dimension and static conditions. So in this paper, MSCs were cultured and expanded in hypoxic, three-dimensional and dynamic conditions *in vitro*.

MSCs of P4 mixed with type I collagen were innoculated into hollow fiber membranes (HFM), where the gel with MSCs formed and then was innoculated into an air-lift loop hollow fiber membrane bioreactor (ALHFMB) for the dynamic culture in an incubator.

During the experiments, the OD value of medium was detected every 24 hr to calculate cell number and to obtain cell growth curves, and the metabolic parameters, such as glucose, lactic acid, ammonia, glutamine, pH, O_2 and CO_2 were also detected to evaluate the cells growth.

Moreover, the expanded cells were identified through antibodies CD29, CD44 and CD45 and through inducing them into osteoblasts, chondrocytes and adipocytes.

The results showed that in the ALHFMB, O2 concentration kept constant; MSCs metabolized robustly and expanded about 50-fold within 7 days in hypoxic condition, more than that in normoxic conditions; most of the expanded cells were CD29 and CD44 positive and CD45 negative; after being induced, the expanded cells still reserved the strong muti-differentiation potential of becoming bone, cartilage and adipose.

(P 200) Immobilization of Biomolecules on Chitosan Surface for Selective Recruitment, Controlled Growth and Differentiation of Cells from Mixed Populations

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One of the issues for the future of the art in Tissue Engineering is the inadequate initial interaction between polymer/surfaces and cells leading to *in vivo* adverse body reactions. The selectivity of biomaterial surfaces for a particular cell type present in a mixed cell population can be achieved by engineering a polymer surface with specific molecules.

In the present work, for the precise control of cell adhesion, growth and differentiation, we modified the surface of chitosan by means of covalent immobilization of different biomolecules. With this purpose, specific proteins and antibodies were activated using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) and the reaction as stabilized with N-hydroxysuccinimide (NHS). The reaction was optimized by manipulating multiple variables like time incubation, pH, protein concentration and protein: EDC:NHS ratio, in order to control the activation of the carboxyl group of the proteins and thus its covalent binding to the amino groups of the chitosan surface. Quantification of immobilized albumin and fibronectin was performed by colorimetric and fluorescence methods. Fluorescence microscopy using labelled antibodies and confocal microscopy were also performed. Adhesion and viability of leukocytes and osteoblast like cells were assessed by MTS and DNA assays. The results revealed that the use of EDC/NHS activated the immobilization of the different molecules on chitosan surfaces. Membranes modified with adhesive and non-adhesive proteins revealed distinctive cell recruitment profiles as assessed by cell adhesion and proliferation rates.

(P 201) Impact of Physical and Chemical Treatments on *In Vitro* and *In Vivo* Biocompatibility of Human Bone Tissues

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Objective: Chemical and physical treatments were developed to reduce the immunogenicity and risk of virus/bacteria/prion transmission. This work studies the impact of treatments on *in vitro* and *in vivo* biocompatibility properties of tissues.

Methods: Three types of tissues were tested: i) fresh-frozen bone (FFB) as control, ii) freeze-dried treated irradiated bone (FDTB) and iii) frozen treated irradiated bone (FTB).

In vitro testing was performed by indirect (MTS, LDH) and direct (cell attachment, cell adhesion) toxicity assays with Saos2 cell line. For *in vivo* testing, each group was subcutaneously implanted in immunocompetent Wistar rats to assess the immunological (CD3, CD68), recolonization (Masson's Trichrom) and revascularization (vWF) processes at 2 / 4 weeks post implantation.

Results: For FFB as well as FDTB, no modification of cell viability (MTS/LDH) was observed for Saos2 in contact with tissue extract. In contrast, a significant reduction of cell viability (MTS) was observed for FTB ($p < 0.05$). After 3 hours of direct contact between Saos2 and bone tissues, a significant decrease of adhesion was demonstrated in case of FTB in comparison to FFB and FDTB. At 24h of contact, cellular adhesion was significantly decreased for FDTB and FTB ($p < 0.05$). A significant higher immunological reaction (lymphocytes/macrophages recruitment and bone resorption) was found for FFB, after subcutaneous implantation in rats, in comparison to FDTB and FTB. In contrast, decellularized (FDTB/FTB) tissues significantly improved neo-vascularization in connective matrix after 15 days of implantation.

Conclusion: Human bone processing with defatting and decellularization promotes *in vitro* biocompatibility and *in vivo* tissue tolerance.

(P 202) Impaired Survival of Bone Marrow Stromal Cells in Hydrogels

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Introduction: Bone marrow stromal cells (BMSC) are often used for cell based tissue repair, cultured in a hydrogel. For substantial tissue regeneration, BMSCs need to survive in this 3D scaffold. We hypothesize that undifferentiated BMSCs will not thrive within these gels in contrast to articular chondrocytes (ACs) and nucleus pulposus cells (NPs), but that early chondrogenic differentiation of the former will increase survival.

Materials and Methods: BMSCs, ACs and NPs were isolated from five calves. Primary NPs, ACs and passaged BMSCs (P2) were cast in 1.2% alginate or 2% agarose (4 mm×2 mm) and cultured for 21 days in high-glucose DMEM with 10% FCS. Half the BMSCs were cultured in chondrogenic DMEM with 1% ITS+,

10ng/m TGFb1 and 10-7M Dexamethasone. Living cell number (Live/Dead), DNA (relative to day 0) and gene-expression was measured on days 0, 7 and 21.

Results: By day 21, NPs and ACs proliferated in both scaffolds. In contrast, DNA content of BMSC constructs decreased in both alginate (20%) and agarose (60%). However, the addition of TGFb1 stimulated cell survival in alginate (116%) and in agarose (400%) while pushing them towards chondrogenic lineage. NPs and ACs both maintained their phenotype.

Discussion: These hydrogels appear to provide an appropriate environment for NPs and ACs but much less so for undifferentiated BMSCs. Since cell number and survival are key factors for tissue regeneration, cell survival of BMSCs within hydrogels should be improved. Preconditioning of BMSCs using TGFβ1 could be a worthwhile strategy.

(P 203) Improving Endothelial Cell Adhesion and Proliferation on Titanium by Sol-Gel Derived Oxide Coating

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In-stent restenosis becomes increasingly prevalent as a difficult-to-treat disease. An alternative therapeutic strategy to drug-eluting stents for that is enhancing, rather than inhibiting, endothelialization of metallic stent surfaces.

In this light, the present study attempted to modify surface chemistry and topography of commercial pure titanium (cp-Ti) by different oxides (TiO_2, SiO_2, SiO_2/TiO_2 and Nb_2O_5) coating via the sol-gel process that can probably improve endothelial cell adhesion and proliferation, therefore stimulate endothelialization without using any bioactive agents. The modified surfaces were then physicochemically characterized in the thickness, nano-scale topography and wettability, and biologically evaluated with the HPMEC endothelial cell line by cell counting, cell morphology and expression of focal adhesion molecules.

Ellipsometry determined the thickness of oxide layers to approximately 100 nm; atomic force microscope revealed a nanoporous structure in the TiO_2 and Nb_2O_5 layers and the Ra values ranged from 20 to 50 nm. The surface energy determined by sessile-drop method showed the highest polar component for SiO_2 (30.7 mJ/m^2) and the lowest for cp-Ti and 316L stainless steel (6.7 mJ/m^2). In 3-day culture, the higher endothelial cell proliferation rates were found on SiO_2/TiO_2, Nb_2O_5 and TiO_2 than cp-Ti. TiO_2 showed a higher cell adhesion rate than cp-Ti in kinetic adhesion assay.

These results offer an insight into that certain oxide coating processing could significantly improve endothelial cell adhesion and proliferation especially in early culture period, which will favor the endothelialization before the formation of smooth muscle capsules.

(P 204) *In Vitro* Biocompatibility and Cristallinity of Polylactides

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Thermoplastic aliphatic biodegradable polyesters based on lactid acid (polylactides or PLAs) are attractive candidates as scaffold for tissue engineering because they are biodegradable, bioresorbable and biocompatible. A remarkable feature of polylactides is their crystal polymorphism that affects cell response to roughness and surface morphology. In this work, we carry out a biological evaluation of polylactide scaffolds of different composition and crystalline characteristics (PLLA, PDLA and the equimolar blend of PLLA/PDLA) in terms of citotoxicity assays and scanning electronic microscope studies with four types of cells: L929 fibroblasts, human neonatal keratinocytes, human neonatal fibroblasts and osteoblasts. For citotoxicity studies, we used the extract assay. Cells were plated in 96-well clusters. The extract solutions were thawed and prepared into full culture medium adequate for each cell type. A positive control of full medium containing 0.015% sodium dodecyl sulphate was also assessed. The plates were incubated at 37°C for 24, 48, and 72 h. and toxicity determined by a MTT method. For scannig electronic microscope studies, cells were plated onto the films and, after 48h, processed and observed in a Hitachi SEM. No toxicity was observed between PLLA and PLLA/PDLA extracts in all cell types. PDLA produced a significant decrease in proliferative activity of cells. Direct contact between cells and materials did not evoke any adverse effect at the SEM level. No differences were found between composition and crystalline characteristics of polylactides, nor between cell types in cell monolayer formed.

Grants by Dpt Sanidad (PI2005111043) and Industria (ETORTEK) Gobierno Vasco.

(P 205) *In Vitro* Biocompatibility of Plain and Doped Silicon Nanowires

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The unique capabilities of one dimensional nanomaterials make them potential candidates for catalysts, biological and chemical

biosensors, drug carrier systems and even tissue engineering applications. However, interactions of nanomaterials with biological systems and the environment could lead to side effects. While there have been reports on the cytotoxicity of carbon nanotubes, quantum dots and gold nanoparticles, not so many studies have yet appeared on the biological effects of silicon nanowires, which are becoming increasingly important as a nanostructure. Also, Si nanowires have advantages over polycrystalline metals and most importantly, the degradation products of Si nanomaterials, principally in the form of $Si(OH)_4$ are metabolically tolerant *in vivo*. In this study, cytotoxic behaviors of doped and plain Si nanowires were investigated for possible biomedical use. The plain and doped Si nanowires were synthesized via gold catalyzed CVD method. For investigation the biocompatibility of the materials, MTT viability, cell proliferation, PI/AO staining and blood biocompatibility tests were performed. According to these standard tests, there were no significant cytotoxic effects for both the plain and dopped silicon nanowires. Cells have shown quite high viability as above 80% within 48 hrs for both types of nanowires. Also, these nanostructures have shown no considerable negative blood response with respect to control group. Therefore, it was concluded that these plain and doped nanowires can be used for medical applications safely.

(P 206) *In Vitro* Characterization of Bidirectional Tet-Inducible Expression Systems for Bone Tissue Engineering

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Gene medicine approaches for osteoinduction could provide potent alternatives to current growth factor or stem-cell based approaches. Our aim was to design Tet-inducible single vector systems for the simultaneous bidirectional over-expression of osteoinductive growth- and transcription-factors in target cells.

These new system mediate inducible expression of two different genes of interest and constitutive expression of the reversed Tet-activator in only one plasmid.

In vitro characterization was carried out in C_2C_{12} cells transfected with the described TetON systems at different doxycycline concentrations. Osteogenic differentiation was detected by alkaline phosphatase assays, RT-PCR and von Kossa staining. Time and dose dependent kinetics were studied for 10 days monitoring the expression of fluorescent reportergenes.

Osteogenic differentiation was confirmed for all therapeutic expression systems. No expression was detectable without doxycycline. The combined expression of BMP2 and BMP7 generated the most potent osteogenic response probably due to BMP2/7 heterodimer formation. Initial expression activation was observed 12 hours after doxy application in a dose dependent manner decreasing within 10 days.

Our *in vitro* data strongly supports the therapeutic potential of bidirectional expression systems to induce osteogenic differentiation. We conclude from our *in vitro* results that the constructed single vector, bidirectional inducible expression systems provide a promising and safe alternative to currently employed growth factor therapies. Furthermore, linked expression of therapeutic genes with suitable reportergenes in this new system will allow monitoring of tet-regulated gene-expression levels by *in vivo* imaging methods.

This work was supported by the European projects.

Hippocrates (NMP3-CT-2003-505758) and Expertissues (NMP-CT-2004-500283)

(P 207) *In Vitro* Differentiation of Osteoblast Like Cells from Peripheral Blood

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The aim of the present study was to evaluate the applicability of peripheral blood monocytes for *in vitro* generation of osteoblasts.

Monocytes obtained from venous blood were cultivated on fibronectin-coated cell-culture dishes without any addition of cytokines. Differentiation of monocytes to osteoblast-like cells was initiated by cultivating them in differentiating-medium containing dexamethasone, ß-glycerophosphate and ascorbic acid. Osteogenic differentiation of the cells was confirmed by detection of alkaline phosphatase activity and detection of mineralized extracellular matrix production by van Kossa-staining. Additionally, we analyzed the ability of monocytes proliferation *in vitro* and the influence of different pretreatments to differentiate into osteoblast-like cells.

We were able to confirm a successful differentiation of monocytes into osteoblast-like cells by detection of alkaline phosphatase and mineralized, extracellular matrix. We found, that pretreatment of monocytes with medium conditioned by CD14-negative blood cells increased the efficacy of differentiation compared to a pretreatment with dedifferentiation-medium, containing IL3, ß-mercaptoethanol and M-CSF followed by our differentiation medium. Examination of cultures clearly revealed that monocytes have a rather low *in vitro* proliferation capacity compared to mesenchymal stem cells or primary osteoblasts.

Our work confirms the ability of monocytes to differentiate into cells with osteoblast characteristics and the effectiveness of osteogenic differentiation can be increased by pretreatment with conditioned medium. Nevertheless, before monocytes can be used as a source for tissue engineering the proliferation capacity of monocytes must be further improved.

(P 208) *In Vitro* Evaluation of Chromosomal Stability of Rabbit Fibroblasts for the Development of Oral Mucosal Analogous on Collagen Scaffolds

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One of the tissue engineering goals is to reduce the impact caused by the difficulty in obtaining enough autologous tissue grafts when healing and repair of extent defects are required. In order to develop this kind of tissue, cells are seeded and cultured *in vitro*,

growing in over several orders of magnitude that there is a loose in the chromosome stability (CS).

In the present study we used conventional cytogenetic techniques (Sister Chromatid Exchange [SCE]), chromosomal aberrations) and toxicological cytogenetic techniques (Micronucleus Test [MN]) to evaluate the behavior of primary rabbit oral mucosal fibroblasts between passages 2 and 5, expanded and cultured in plastic flasks or seeded in a collagen scaffold to investigate whether the cell management or its interaction over different supports has an effect on CS.

Chromosomal instability, defined as an increase in structural and numerical chromosomal changes, was observed as a result of extensive cellular manipulation over time. Our observations showed nucleoplasmic bridges in binucleated cells, increase of SCE number, and presence of MN scored in binucleated cells. In addition, a polyploidy cell constitution was evident when passage 4 took place. Instead, no significant effect was found on rabbit oral mucosal fibroblasts on CS when seeded over collagen scaffolds or plastic flasks.

These findings suggest that passage number over time in rabbit oral mucosal fibroblasts culture is a determinating factor on CS. Further studies *in vivo* are required to evaluate the clinical efficiency of collagen scaffolds with seeded fibroblasts in the repair of extent oral mucosal defects.

(P 209) *In Vitro* Generation of an Endothelialized Adipose Tissue Construct for the Engineering of Vascularized Adipose Tissue

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Cell survival is a major limitation in the use of larger tissue-engineered adipose tissue constructs for implantation. Ingrowth of blood vessels from the host into the construct is a time consuming process resulting in hypoxia and nutrient limitations. Adding endothelial cells to adipose tissue constructs *in vitro* could contribute to the formation of a vascular network inside the construct once implanted. We tested the feasibility of generating a spheroïd coculture in which human umbilical vein endothelial cells (HUVEC) are able to maintain viability, while adipose tissue derived stromal vascular cells (ADSC) differentiate towards adipocytes.

For spheroïd formation 95%, 80% and 60% ADSC and 5%, 20% and 40% HUVEC were pooled to a total of 2×10^5 cells, centrifuged and cultured on a carefully preselected 1:1 mix of adipogenic medium and endothelial growth medium. Flow cytometry showed that on average $35\% \pm 9\%$ of the HUVEC survived after 7 days of culture and were still present at day 14. Oil-Red-O staining and CD31 staining of spheroïd cross-sections revealed that the ADSC in the spheroïds accumulated lipid droplets in their cytoplasm while the HUVEC were present as round, individual cells throughout the spheroïds. The distribution of HUVEC in the spheroïds was similar for all concentrations of HUVEC seeded.

In conclusion, we succeeded to generate spheroïd cocultures of ADSC and HUVEC, and to differentiate ADSC towards adipocytes while maintaining significant numbers of endothelial cells. This approach potentially accelerates the vascularization of adipose constructs upon implantation. This will be tested shortly with *in vivo* experiments.

(P 210) *In Vitro* Model of Angiogenesis by Adult Bone Marrow-Derived Stem Cells and Adipose-Derived Stem Cells in a Fibrin Matrix

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In vivo degradation and cellular necrosis at the centre of *in vitro* engineered tissue constructs is a major problem caused by the lack of sufficient vascularisation. The starting point of our investigation was the question whether adult human bone marrow derived stem cells (MSC) and adipose-derived stem cells (ADSC) could be used for *in vitro* angiogenesis purposes using an *in vitro* angiogenesis model.

An angiogenesis model was developed consisting of MSC or ADSC pellets in a fibrin gel. These pellets were cultured on growth factor supplemented endothelial medium for 14 days. Pellet morphology was observed by light microscopy and the secretion of important angiogenic factors was assessed by multiplex ELISA. Extensive capillary-like outgrowth was observed in both MSC and ADSC cultures already after 3 days of culture. Secreted protein levels of PDGFB, TPO, ANG-2 and HGF of both MSC and ADSC were comparable to that of human umbilical vein endothelial cell pellets when used in the same model. The next phase of our investigation is to understand the contribution of different cell types to the formation of the observed capillary-like structures. It is possible that these outgrowths consist of not only cells differentiated towards the endothelial lineage, but also the original cell type acting in a supporting fashion. Identifying the role of differentiated and undifferentiated stem cells in the development of premature vessel networks *in vitro* may help to improve vascularisation of tissue engineered equivalents *in vivo*.

(P 211) *In Vitro* Reactivity of a Porous SI-MG-CA-P Glass Scaffold

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A scaffold for tissue engineering should provide a template and support tissue growth. An ideal scaffold should have interconnected pores, micro and macroporosity and adequate surface activity. One key reason that makes bioactive glasses a relevant scaffold material is the possibility of controlling a range of chemical properties and thus the rate of bioresorption. Thus, the adjustment of solubility seems to be of utmost importance for the manufacturing of implant materials with resorption rates matching the growth rates of bone.

In this work porous Si-Mg-Ca-P glass scaffolds were developed by the salt sintering process. Mixtures of NaCl (200 to 300 μm) and glass powders (20 μm average particle size) were sintered up to 800°C and then the salty phase leached by water. Weight percents of salt from 30 to 70 were tested. The resulting porous scaffolds had pore volume between 57 and 85%, average pore diameters between 150 and 400 ìm and specific surface areas varying from 3.4 to 11 m^2/g depending on the glass to salt ratio in the mixture. *In vitro* reactivity studies carried out in SBF up to 14 days showed that degradability of the scaffolds generally increased with increasing surface area. Dissolution of Ca and P, as assessed by ICP, additionally indicated the bioactive character of the glass scaffolds. Solubility tests according to DIN ISO719 complemented the SBF results. The present study is believed to provide further understanding of the mechanisms regulating surface reactivity of porous glass scaffolds for tissue engineering exhibiting both biodegradable and bioactive behaviours.

(P 212) *In Vitro* Reconstruction of Actinic Skin

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The identification of genetic changes has led to an increased understanding of tumorigenesis, identifying the involvement of putative oncogenes and tumor-suppressor genes. Such approaches are equally applicable to nonmelanoma skin cancer (NMSC), such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC).

NMSCs represent the most common types of cancer in white population and the incidence of skin cancer shows a steadily increase, reaching epidemic proportions. It could be therefore possible to investigate the key genetic changes and molecular characteristics of each stage of progression of SCC development.

DNA copy number changes represent molecular fingerprints of solid tumors and are as such relevant for better understanding of tumor development and progression. In this study, we applied CGH array to identify gene-specific DNA copy number changes in chromosomal skin cancers.

Up to now, many studies on tumor cells have been performed under standard *in vitro* cultures.

In standard culture conditions, neoplastic cells, as expected, can grow only in a two-dimensional array that differs from their native three-dimensional organization in nodules or masses and although interesting results have been obtained, three-dimensional scaffolds would better allow the tumor cells to organize into nodules or masses. With the present project we cultured keratinocytes isolated from skin cancer in 3D conditions by using scaffolds made with hyaluronic acid derivatives. These three-dimensional tumor cell cultures were be used to study genetic changes and in particular to define genetic profile, cell spatial organization, cell/cell and cell/extracellular matrix interactions of skin cancer keratinocytes.

(P 213) *In Vitro* Study of the Effect of Some Blood Components Embedded on the Implants Surface Upon Proliferation Ane Synthetic Activity of Bone Cells

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The blood tissue is the site of the first connection between the implant and the live tissue. Therefore, the effect of blood components on response of cells growing in the implant immediate vicinity should be of the particular interest to understand the healing process.

The coating by human serum (an albumin-rich medium), by an activated human plasma (a fibrin-rich medium) and a platelet-rich plasma (PRP, a fibrinogen and platelet-rich medium) were used for the surface modification of selected commercially available bone implant materials (Ti-HA, Ti-Etching, Ti-Plasma spray, zirconium ceramics ,Carbon composit). The effect of the surface modification on proliferation and synthetic activity of osteoblasts was studied. The platelet adhesion and activation were followed as well.

All coatings led to higher cell proliferation than observed on non-coated surfaces. The serum coating had a minor stimulation effect on osteoblasts viability whereas the plasma coating led to uniform surfaces exhibiting a similar high proliferation (with no respect to the surface origin) comaparable with the TCPS control. The PRP coating caused a significantly higher proliferation only in the case of Ti-HA and Ti-Etching surfaces.

There was observed an increasing tendency in productions of TNF-alpha and BAP for coated surfaces as well as for surfaces with lower cell proliferation. The coatings also decreased the production of the chemokine IL-8. Interestingly, the plasma coating led to uniform surfaces with the lowest IL-8 production, probably as a result of the fibrin mesh present on the surface.

This work was supported by IGA MH CR project No.8829-3/2006.

(P 214) *In Vitro* Therapy of Cartilage Defects using Human Osteoarthritic Condyles and Chondrogenic Spheroids as Transplants

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Objectives: Cartilage defects after traumatic injuries or also in degenerative diseases like osteoarthritis lack appropriate self-repair and require therefore cell based therapies for tissue regeneration. To avoid the application of dedifferentiated single cells as therapy basis pre-differentiated cell aggregates should be used as transplants. To meet this objective *in vitro* tissues from culture expanded human chondrocytes were engineered to be used as transplants. Cartilage defects to be repaired *in vitro* were fissures and fluffy cartilage of condyles from OA patients after total knee joint replacement. To evaluate the *in vitro* therapy process the integration of the transplants into the OA defects as well as repair tissue formation, morphology, and maturation was examined.

Material and methods: Three-dimensional cartilage-like spheroids were cultured in medium supplemented only with human serum or further additives like TGF-ß and/or ascorbic acid. Predifferentiated cell aggregates were transferred to the naturally formed OA-defects on human condyles. The tissue regeneration process *in vitro* was analysed using histology (HE, Safranin-O) as well as fluorescence-based immunohistology (collagen type I and type II, S100) of cryosections.

Results: The cartilage-like spheroids integrated well into the OA-defects and set up a repairing process. The level of transplant integration, cell migration into fissures and repair tissue maturation is dependend on the pre-differentiation via human serum, TGF-ß and/or ascorbic acid. On one hand, integration and migration are dominating, on the other hand there is a higher differentiation degree of the pre-formed tissues after a special time point of *in vitro* therapy.

(P 215) *In Vitro* Toxicity of Novel Bone Cement PMMA-co-EHA

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Poly(methylmethacrylate) (PMMA) bone cement is largely employed in orthopaedic surgery since it was developed by Charnley and Smith in 1960. However, it is known to promote bone necrosis at the site of implantation due to its exothermal polymerization and local effects of methyl methacrylate monomer leaching out of the material as it cures *in situ*. There are also some mechanical problems associated with this cement, such as brittleness and poor fatigue life.

In the present work we evaluated the *in vitro* toxicity of a novel bone cement, PMMA-co-ethylhexylacrylate (EHA) (1:1) developed as an attempt to improve some properties of conventional polyacrylate. Human osteoblast-like MG63 cells were exposed to cement extract dilutions and the cell metabolic function was determined by MTT assay. A new flow cytometry (FCM) protocol was also used to monitor cell cycle progression during exposure time, measuring DNA content by propidium iodide uptake.

MTT results demonstrated that the most concentrate extract exerted some reduction on culture growth ($41.1\% \pm 3.1$). However, the analysis of cell cycle suggests that the damage produced by the cement extract is not irreversible and the surviving cells maintain their proliferation capability.

This preliminary study indicates that PMMA-co-EHA has potential to be used as bone cement.

(P 216) *In Vivo* Cardiac Tissue Engineering in Vascularised Chambers

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Vascularised chambers have proven to be effective for cardiac tissue engineering *in vivo*, enabling cardiomyocyte suspensions to assemble into contractile tissue and grow in a protected environment. Here we report a simplified model of *in vivo* tissue engineering with a minced muscle preparation that avoids enzymatic cell isolation and culture protocols, and improves efficiency. We have used this model to compare outcomes from neonatal or adult rat tissue and to investigate tissue growth mechanisms. Minced rat heart (neonatal or adult) in Matrigel was seeded into a vascularised chamber and harvested between 2–10 weeks later. Neonatal cardiac tissue structure was maintained and from 6 weeks all constructs had discrete focal areas of spontaneous contractile function. Vascular and lymphatic vessels were evident throughout the tissue and cells expressed myocyte and gap junction proteins. Cardiomyocytes increased in size and were positive for proliferation markers Ki67 and PCNA. Adult rat heart muscle however showed no spontaneous contractile activity and tissue structure was lost over time. In chambers which were prevascularised for one week before tissue implantation the cardiac tissue was larger (100% increase at 2 weeks.

(P 217) *In-Vitro* Analog of Human Bone Marrow From 3D Scaffolds with Inverted Colloidal Crystal Topology

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In-vitro replicas of bone marrow could provide a continuous source of blood cells for transplantation or serve as a laboratory model to examine immune system dysfunctions and drug toxicology. Here we report the development of an *in vitro* artificial bone marrow based on a 3D scaffold with inverted colloidal crystal (ICC) geometry mimicking the structural topology of actual bone marrow matrix. To facilitate adhesion of cells, scaffolds were coated with a layer of transparent nanocomposite. After seeding with hematopoietic stem cells (HSC), ICC scaffolds were capable of supporting expansion of CD34+ HSCs with B-lymphocyte differentiation. 3D organization was shown to be critical for production of B cells and antibody. Functionality of bone marrow constructs was confirmed by implantation of matrices containing human CD34+ cells onto the backs of SCID mice with generation of human immune cells. Animals were sacrificed after 2 weeks and then the implanted matrix, mouse bone marrow and spleens were collected for leukocyte subset phentotyping. Examination of the cells populating the spleens of these mice indicated that the majority of cells (89%) were also of human origin as indicated by evaluation of MHC class I co-staining and the predominant cell type found was CD19+ IgD+IgM positive. Median engraftment time for bone marrow reconstitution by transplant of bone marrow or cord blood is typically 18–26 days. One can suggest that engraftment of HSCs in the artificial bone marrow construct with continued production of CD34+ cells is similar to that seen in human or murine reconstitution of irradiated marrow.

(P 218) Increase in Cell Migration and Angiogenesis in a Composite Silk Scaffold for Tissue-Engineered Ligaments

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The purpose of this study was to evaluate the biocompatibility of silk and collagen-hyaluronan (HA) *in vitro* by assessing ACL cell and T lymphocyte cultures on scaffolds. The use of composite scaffolds as artificial ligaments in ACL reconstruction and their effects on angiogenesis were evaluated *in vivo*. The immune response was higher in both scaffolds after 72 h compared with the control culture condition without scaffolding, as assessed by T lymphocyte cultures *in vitro*. There was no significant difference in the immune response *in vitro* between the silk and composite silk scaffolds.

Silk and composite silk scaffolds were implanted as artificial ligaments in ACLs removed from the knees of dogs, and they were harvested six weeks after implantation. On gross examination, the onset of an inflammatory tissue reaction, such as synovitis, was seen in both the silk scaffold and the composite silk scaffold groups. A histological evaluation of the artificial ligament implants revealed the presence of monocytes in the silk composite scaffold and the absence of giant cells in all cases. MT staining in the composite silk scaffold-grafted group showed granulation tissue consisting of fibroblasts, lymphocytes, monocytes, and newly formed collagen fibers. In addition, CD31 staining revealed the formation of new blood vessels. On the other hand, no reparative tissues, such as blood vessels, collagen, and cells, were observed in the silk scaffold-grafted group. These results suggest that the lyophilized collagen-HA substrate is biocompatible *in vitro* and enhances new blood vessel and collagen formation *in vivo*.

(P 219) Influence of Bioimplant Modification on Gene Expression of Human Chondrocytes "*in vitro*".

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Introduction: Cycloolefin copolymer (COC) and cycloolefin copolymer/polyethylene (COC-blend) were selected based on our previous experiments as materials suitable for skeletal implants. In this study, we evaluated biotolerance of abovementioned materials in culture of chondrocytes *in vitro*.

Material and methods: Two types of materials (COC and COC-blend) either coated with collagen type II or uncoated were tested *in vitro*. For better adhesion of collagen, the surface was modified with N and O ions. Commercially available chondocyte cell lines (Normal Human Articular Chondrocytes, Knee cryopreserved, DIAGENES) were used for *in vitro* experiments. After 48 hours of exposure to these materials, total RNA from chondrocytes was isolated and reverse transcribed to cDNA. Gene expressions of several metalloproteinases (MMP-1,-3,-13), pro-inflammatory cytokines (IL-1, TNF-alpha) and apoptotic molecules (BAX, Bcl-2) were studied by qRT-PCR.

Result & Discussion: All tested materials did not significantly influence gene expression of tested pro-inflammatory cytokines and apoptotic molecules by chondrocytes. Nevertheless, materials with plasmatically modified surface and application of collagen type II were shown to induce expression of several MMPs (2–7 fold increase) by chondrocytes *in vitro*. To be sure that modified materials can be efficacious for artificial substitution of osteochondral defects in humans, *in vivo* experiments are necessary.

This work was supported by Grant Agency Czech Republic, project No. 106/06/0761

(P 220) Influence of Erosions on Levels of Collagen Degradation Biomarkers and Inflammation in Hand Osteoarthritis

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Introduction: This study demonstrate the influence of inflammatory and degradation processes in small eroded joints in hand osteoarthritis (OA) on levels of selected bone, cartilage and synovial biomarkers and their role in differentiation between erosive (EOA) and non-erosive (NEOA) forms.

Patients: Serum and urine samples from 89 clinically and radiologically examined patients (56 with EOA, 33 with NEOA) who fulfilled ACR hand OA criteria were analyzed. Between EOA/NEOA were no differences in age (64/62 years), sex or disease duration.

Methods: Urinary deoxypyridinoline (DPD), bone turnover biomarker was determined using IMMULITE, pentosidine (PEN) indicating rather cartilage degradation was measured in serum and urine by HPLC. Inflammatory parameters were analyzed in serum: hyaluronic acid (HA) by ELISA, C-reactive protein (CRP) using high-sensitive immunoturbidimetry.

Results: Significantly higher HA was found in EOA in comparison with NEOA (100.78 vs. 58.11 ng/ml; $p = 0.02$), but no difference was in CRP. Collagen catabolic processes in bone and cartilage were reflected by insignificant DPD and PEN increase in EOA too. Moreover, we found positive correlation of HA and both cross-links with age, urinary DPD with PEN and serum and urinary PEN. Follow-up of laboratory biomarkers is intended to see the response to metotrexate 1-year therapy.

Conclusions: The results, particularly HA levels suggest that erosions and age in hand EOA may be the reason for local joint inflammation and slightly increased collagen catabolism as reflected by mild elevation of both DPD and PEN in EOA.

Supported by Czech Ministry of Health, projects Nos. NR/8447-4 and 00023728.

(P 221) Influence of Thickness on the Transmittance in Fibrin-Agarose Corneal Constructs

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Introduction: Previous characterization of the optical properties of biomaterials used for construction of corneal substitutes by tissue engineering is essential to ensure a proper functionality of the bioengineered constructs. The aim of this work was the study of transmittance (the material property of allowing light to pass through) in matured fibrin-agarose constructs and its dependence with thickness and incident light wavelength.

Methods: In this work, we developed an artificial substitute of the human corneal stroma by using fibrin-agarose biomaterials with human keratocytes immersed within. After 28 days in culture, we evaluated the spectral radiance of the artificial tissues using a spectroradiometer PR-704 (Photoresearch) under geometry CIE 45°d illuminating condition. The transmittance for 5 mm constructs thickness and the prediction for other thickness values (from 4.5 to 0.5 mm in 0.5 mm steps) were calculated within the visible spectrum (400–700 nm) using the theory of Kubelka-Munk.

Results: The spectral transmittance for 5 mm construct was 0.383 for 400 nm, increasing in a progressive manner to 0.660 for 700 nm. This transmittance improved up to 0.90–0.95 for a thickness similar to the one of central human cornea. Statistically significant differences were found between the short and large wavelengths, being smaller for the first ones.

Discussion and Conclusions: Transmittance is in inverse relationship with thickness and direct relationship with wavelength, with differential behaviour for small and large wavelengths. Spectral transmittance of these constructs is expected to be high at the physiological thickness of the human cornea.

Supported by PI-0132/2007 and P06-CTS-2191 from Junta de Andalucia.

(P 222) Injectable Alginate Gel Formulations with Controlled Degradability

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Alginates are biocompatible polymers with the unique property to form gel structures under physiologic relevant conditions. The successful use of alginates as implantable biostructures may in several cases, however, rely on the ability to control *in vivo* degradation of the polymer network. In this work, a recently developed internal alginate gelling system allowing gels to be moulded *in vitro* or injected before gelling, was studied with respect to degradability. Gel formulations were prepared by mixing sodium alginate with calcium alginate particles. In order to test gel stability *in vitro*, several different formulations were prepared and compared in a long term study. For this, gel formulations were moulded and stored in physiologic solutions at 37°C and gel stability was recorded with time. In a selected test solution the different gel formulations were found to completely degrade from within two weeks up to months. Lower gel stability was generally obtained by using alginates with low Mw, high mannuronic acid content and lower degree of calcium cross-linking. One alginate formulation with high biodegradability was also tested by subcutaneous or intramuscular administration into rats. At both sites the injected alginate composition was found to disappear within short time but considerably faster at the intramuscular site. Our data supports that specific alginate qualities and gelling formulations with controlled degradation profiles may be successfully designed as implantable or injectable matrices for cells or other.

(P 223) Injectable Hydrogels Based on Chitosan

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Oligo-D-glucosamine (''oligomer'' of chitosan) seems well suited for injectable, biodegradable systems (IBS), due to their solubility in water, easier funtionalization and the possibility of working at high concentrations.

Chitosan was enzymatically degraded with a commercial enzyme (Multifect Pectinase FE) at 50°C and pH 5.5 during 17h, adapting a previous procedure. The oligomers were precipitated in ethanol and analysed by MALDI-TOF/MS and FTIR. They were then functionalized by reaction with methacrylic anhydride (MethA), varying ratio of amount of substance of MethA : amount of substance of -NH2 groups in the oligomer. The obtained methacrylamide-oligomers were polymerized with a potassium persulfate/vitamin C initiation system. Both the modified oligomers and the polymerized product were analysed by FTIR and NMR.

In FTIR, there was the appearance, on the spectrum of the acrylic ester oligomer, of bands atributted to $=CH_2$ stretching ($\sim 3100\,cm^{-1}$) and the strengthening and splitting in two of the bands of amide I ($\sim 1650\,cm^{-1}$), due to the reaction of carbonyl from the anhydride with amine of chitosan. After polymerization with the above mentioned system, the methacrylamide oligomers became insoluble due to the crosslinking at the double bonds of the acrylic side groups, and the spectrum shows notable changes.

We have shown that injectable systems based on chitosan oligomers may be easily prepared. These materials have varying properties depending on the amount of methacrylamide groups incorporated in the oligomer chains.

LF Boesel acknowledges the support of the Marie Curie actions (Renewinj project).

(P 224) Inkjet Gene Printing: A Novel Approach to Achieve Gene Modified Cells for Tissue Engineering

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Gene modification of cells has been used in various areas of research for improving cell and tissue function. Although there are many established methods to deliver genes into cells, application of the existing techniques requires additional steps toward building

new tissues for therapy. We have developed a novel method that allows for safe delivery of genes into cells during the process of building tissue constructs using the inkjet technology.

Methods: HP Desktop printers were used. Porcine aortal endothelial cell lines (EC) and pmaxGFP plasmids were loaded into ink cartridges and printed together onto collagen gel-coated substrates. To evaluate the effects of *in vivo* gene transfection, a fibrin solution and a mixture of EC cells and pmaxGFP plasmid were directly printed into the subcutaneous tissues in athymic mice.

Results: Gene transfection *in vitro*: After gene printing, the cells expressed GFP gene in culture over a 10-day period. Compared with the liposome and physical methods, the inkjet gene printing method showed significantly higher cell viability after transfection. Over 90% of the cells were intact during the transfection process. Approximately 12% of the printed cells were transfected with the plasmid genes. These data suggest that the inkjet process, which creates heat and shear shock, facilitates the entry of plasmids into cells.

Conclusions: This study shows that safe delivery of genes into living cells can be achieved using the inkjet printing technology. Organ printing technology, combined with gene transfer, may allow for enhanced tissue formation with target function achievement.

(P 225) Integrative Capacity and Functional Competence of Detergent-Decellularized Xenogeneic Pulmonary Valves

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Introduction: The regenerative ability of implanted xenogeneic decellularized heart valves is still discussed controversial. The aim of this study was to evaluate the *in-vivo* integration capacity of decellularized xenogeneic porcine pulmonary valves (PV) in comparison to allogeneic ovine grafts.

Methods: Six porcine and six ovine PV were decellularized using detergents (0.5%Sodium-deoxycholate/0.5%SDS). The valves were implanted into the orthotopic position in sheep as conduits. Three of each were echocardiographically investigated and harvested after 3 and 6 months, respectively. For morphological evaluation of explanted PV, H&E-, Elastica van Gieson-, Pentachrome-, von Kossa, collagen-IV-, perlecan-, eNOS-, α-actin-, procollagen-I-immunostaining, and scanning electron microscopy were performed.

Results: All implanted PV were functionally competent without insufficiency, stenosis, significant macroscopic degeneration or thrombosis. Microscopic evaluation showed a similar *in-vivo* endothelial and interstitial scaffold reseeding in both group with distinct increase of reseeding extension after 6 months. Significantly higher expansion of the neo-intima hyperplasia was observed in xenogeneic PV as compared to allogeneic grafts. No significant calcification was found in both groups.

Conclusion: Integrative ability and functionality of xenogeneic decellularized PV were similar up to 6 months as compared to allogeneic valves. Whether the significantly higher neo-intima hyperplasia in xenogeneic valve further increases and interferes with late function has to be investigated in future long-term animal experiments.

(P 226) Integrin Alphav Subunit—Membrane Marker Mediating Differentiation of Human Bone Derived Cells?

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Integrins are a family of adhesion receptors which may regulate many cellular functions, like adhesion, motility, phenotype and cell maturation. Recent studies indicated that integrin beta1 plays a role in osteoblastic differentiation[1]. As for alphaV and beta1 integrins, it was shown that they are characteristic molecules for osteoclast functions[2]. The aim of our study was to investigate whether the alphaV integrin subunit is involved in the human osteoblast differentiation process. The localization and level of expression of mRNA for alphaV integrin was determined three times during osteoblast differentiation. AlphaV subunit of integrin was detected by immunofluorescence staining method and observed in a fluorescence microscope. The experiments were performed on culture plastic and on alumina. The results of RT-PCR were analyzed with Gel Doc 2000 using Quantity One software. We found that the alphaV integrin was present in focal contacts and cell cytoplasm at subsequent stages of cell maturation. In addition, we found that the expression of mRNA alphaV integrin differed at various observation periods, and shows the highest level in mature osteoblasts. For better understanding of the integrin function, we treated the cell culture with monoclonal antibodies against human alphaV integrin to block its ligand-binding activity. Blocking this integrin transduction pathway caused changes in cell activity and reduced the expression of alkaline phosphatase. Our results suggest that alphaV integrin is involved as an important receptor mediating human osteoblast differentiation.

Work supported by the Polish Government (3T08A00130).

[1]Liping Wang *et al.*; Biomaterials 27(2006).
[2]Ichiro Makamura *et al.*; J.Bone Miner Metab 25(2007).

(P 227) Intelligent Hydrogels for Chondrogenesis of Human Adipose-Derived Stem Cell

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The composite of Pluronic F127 and hyaluronic acid (HA) was investigated, exploring the benefits of each hydrogel in the chondrogenesis of human adipose-derived stem cell (ASC). F127 was chemically modified through a series of reactions to graft heparin and then dexamethasone (Dex), respectively. Crosslinked HA was used as a physical stabilizer of F127. The modified F127/HA was also physically coupled to TGF-beta1 by the immobilized heparin. We hypothesize that as the physical stability of hydrogels is maintained, chondrogenesis can occur with the aid of the incorporated chondrogenic factors. The sol-gel transition of F127/HA

was found to be similar to that of F127 itself. From *in vivo* test using nude mouse for 4 weeks, the volume of the composite hydrogel seemed to be well-maintained as compared to either F127 or HA alone. DAPI staining showed that ASCs were widely dispersed inside the hydrogel matrix. No significant difference was noticed among the test groups. Immunofluorescent staining of type II collagen indicated that the group, F127/HA/TGF/Dex could sustain more active green fluorescence than F127/HA. This work confirmed that the combination of two hydrogels (F127/HA/Dex and F127/HA/TGF) is possible to make it thermoreversible and physically more stable. In addition, this system proved that the incorporation of chondrogenic inducers was effective in inducing *in situ* directly chondrogenic differentiation of ASC.

(P 228) Interaction of Cells Involved in Wound Repair with Different Fibrin Sealant Matrices

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Commercial fibrin sealants are widely used in various surgical indications as an adjunct to haemostasis and for sealing of soft tissue. Current fibrin sealants are composed primarily of fibrinogen and thrombin, both isolated from human plasma. Depending on the isolation and the production process of the fibrinogen and thrombin component fibrin sealants may contain a number of other proteins, such as growth factors, albumin, Factor XIII, and fibronectin. In recent years fibrin sealants have been used successfully as a vehicle for delivery of endothelial cells, fibroblasts and keratinocytes in different applications such as wound healing and angiogenesis. The usability of a fibrin sealant as delivery matrix and as a scaffold for wound healing will very much depend on the quality of its individual constituents and the overall composition of excipients. Alternations in the fibrin structure and function have implications for the wound healing especially in diseases where the fibrin structure may be modified. The results presented in this paper show significant differences between the commercial fibrin sealants Artiss (Baxter) and Evicel (Omrix) with respect to morphology, adhesion and viability of primary human dermal fibroblasts, human umbilical vein endothelial cells and human epidermal keratinocytes. In contrast to Artiss, viability of cells was significantly decreased on Evicel, indicating that composition and/or structure of this fibrin matrix did not promote fibrin-cell interactions. Artiss with a more physiological clot properties is significantly better in supporting cell adhesion and viability. In conclusion, our study clearly demonstrates differences of commercially available fibrin sealants in cell compatibility.

(P 229) Intraoperative Tissue Engineering for Multilevel Posterolateral Cervical Fusion

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Introduction: The outcomes of posterolateral multilevel spine fusion in difficult clinical settings such as old multi-diseased osteoporotic patient remain unpredictable. The osteoprogenitor cells in bone marrow decrease with aging without losing their osteogenic potential. Bone marrow cells (BMCs) from iliac crest aspiration can be concentrated in the operating room and the platelet-rich fibrin (PRF) can be obtained from peripheral blood as source of autologous ostoprogenitor cells and growth factors respectively.

The objective of this report is to describe the use of autologous BMCs concentrate enriched with PRF on corticocancellous bone allograft to improve multilevel posterolateral cervical fusion.

Method: We presented the case of a 88-year-old men affected by cervical stenosis and subjected to decompression and a posterolateral C3-C7 instrumentation with apposition of posterolateral corticocancellus bone allograft augmented with autologuos BMCs concentrate from iliac crest aspiration enriched with PRF from peripheral blood.

Results: At 6-months follow up the bone allograft shows consolidation signs evaluated by Lateral Dynamic X-Rays and CT scan.

Conclusion: This paper describes a simple and effective method for potentially improving fusion rate in aged osteoporotic patients by using corticocancellus bone allograft augmented with autologus BMCs concentrate from iliac crest enriched with PRF from peripheral blood, rapidly obtained before the surgical procedure. Corticocancellous bone allograft acted as an osteoconductive and osteoinductive structural support for cells and growth factors forming a ''tissue engineered'' construct ready to be used in the operating room.

(P 230) Investigating the Role of Collagen in Osteogenic Differentiation of Human Mesenchymal Stem Cells

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Secretion and subsequent mineralization of extracellular matrix (ECM) by osteoblasts is a hallmark of osteogenic differentiation. Because bone ECM is mainly composed of collagen type I, we investigated its effects in osteogenic differentiation of human mesenchymal stem cells (hMSCs).

First, we grew hMSCs on collagen-coated plates for 7 days and we observed that hMSCs cultured in the presence of basic or osteogenic medium showed no consistent increase on alkaline phosphatase (ALP) expression when compared to control.

Next, we inhibited collagen synthesis by excluding ascorbic acid (AscAc)—a cofactor essential for collagen synthesis—from the culture medium. We consistently observed a decrease in proliferation, ALP expression and calcium deposition.

In contrast, inhibiting collagen crosslinking with β-aminopropionitrile (BAPN) - an irreversible inhibitor of lysyl oxidase—had no effect on proliferation and ALP expression but, to our surprise, it consistently enhanced calcium deposition up to 5-fold.

In vivo bone formation by hMSCs showed a substantial decrease in bone formation in the absence of AscAc whereas BAPN-treated hMSCs produced a comparable amount of bone as cells grown in control medium.

In summary, we showed that collagen, as well as its level of crosslink, controls proliferation and differentiation of hMSCs which could be further exploited to improve hMSCs-based bone tissue engineering.

(P 231) Investigation of Bone Formation on Collagen-GAG Scaffolds with Varying Composition

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Collagen-glycosaminoglycan (GAG) scaffolds are currently being used in many tissue engineering applications including nerve, cartilage and skin (1). The aim of this study was to investigate the process of *in vitro* bone formation on a series of collagen-GAG scaffold variants to find an optimal composition for use in bone tissue engineering. Collagen-GAG scaffolds were made as previously described and crosslinked at 105°C (2). Scaffolds were divided into two groups: [1] Collagen Variants: 0.5%, 0.75% and 1% Collagen (w/v) and [2] GAG Variants: 0%, 0.022%, 0.044% and 0.088% GAG (w/v). Scaffolds were seeded with 2×10^6 MC3T3-E1 cells and pre-cultured for 2 days to allow for proliferation before the addition of ascorbic acid and β-glycerophosphate. Scaffolds were assessed at time points of 1, 3, 7, 14, 21 and 28 days. Cell number, metabolic activity, alkaline phosphatase (pNPP assay) and osteopontin (ELISA) were measured at each time point and scaffolds histologically assessed for mineralisation. Cell number and metabolic activity was highest on the 1% collagen and the 0.088% GAG scaffolds by day 28. Alkaline phosphatase was seen to peak at day 14 although no difference was seen between scaffolds. Nodules of mineralisation within the scaffold stained with alizarin red appeared at day 14 and continued until day 28. The results demonstrate that the 1% collagen and 0.088% GAG scaffolds variants are optimal for bone tissue engineering.

Acknowledgements: Science Foundation Ireland & Integra Life Sciences.

[1]Yannas I.V, *et al.* PNAS USA.1983; 86:933–937.
[2]O'Brien F. J, *et al.* Biomaterials. 2004; 25: 1077–1086.

(P 232) Investigation of Osteoblast Response to Biodegradable Bacterial Cellulose Scaffolds

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Aim of this project is to investigate the ability of bacterial cellulose (BC) and oxidized Bacterial cellulose (OBC) use as a scaffold in tissue engineering. Bacterial cellulose was produced with being provided optimum conditions from Acetobacter xylinus. BC was transformed dialdehyde cellulose (DAC) as biodegradable form by treating with periodate. Oxidation was carried out in aqueous solution at 50°C in the dark for 24 hours. The mole-to-mole ratio of sodium metaperiodate to anhydroglucose repeat unit (AGU) of cellulose was 0.5, 1.0 and 1.5.

In order to test cell response to the developed scaffolds osteoblast-like cells (SaOs-2) were cultured on them during two weeks. Cell adhesion and morphology were analyzed by SEM while the cell viability and proliferation were assessed by MTS test and DNA quantification assays. Cell culturing experiments showed that cells were able to attach on their surfaces. However it was found that oxidation influenced cell viability, showing that there was an optimum oxidation degree for cell attachment. Besides the chemical properties of the surface, the morphology was also found to be dependent of oxidation degree. The one showed better ability for cell attachment (AGU/cellulose: 1.0) has the surface more porous which is a desirable property for a tissue engineering scaffold. Cell attachment and proliferation on these scaffolds were successful, whereas spreading of the cells on the material was not very favorable. Consequently, they can be used with surface modification or with bioactive agent for spreading the cells on the scaffold better.

(P 233) Investigation the Influence of Ar Plasma Treatment on Cell Response for Wet-Spun Starch/Polycaprolactone Fiber Mesh Scaffolds

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In design of a tissue engineering scaffold, surface physicochemistry is one of the most important issues to be considered. The physicochemical properties of the surface directly influence the scaffold performance by affecting the cellular response and ultimately effecting the new tissue formation. In order to improve the cell affinity, the surface hydrophiliticy, surface energy, surface roughness and surface charge can be modify by different methods. Plasma treatment is a versatile method for surface treatment of biodegradable polymers without altering their bulk properties. By this method, it is possible to introduce or graft desired functional groups onto the surface. This study aims to investigate the influence of Ar plasma treatment on osteablast cell response for fiber mesh scaffolds from a starch-polycaprolactone blend. The scaffolds with 77% porosity were successfully produced by a wet spinning technique. The fiber surfaces were then treated by plasma at 30W for 15 min using Argon as a working gas. It was observed that the surface morphology and chemical composition were significantly changed due to the etching and functionalization of the fiber surfaces. XPS analyses showed an increase of the oxygen content of the fiber surfaces after plasma treatment (untreated scaffolds O/C:0.26 and plasma treated scaffolds O/C:0.32). Both untreated and treated scaffolds were examined using human osteoblast-like cells (SaOs-2) during 2 weeks of culture. The cell seeded on wet-spun SPCL fiber mesh scaffolds showed high viability and alkaline phosphatase enzyme activity. Those values were found to be even higher for the cells seeded on the plasma treated scaffolds.

(P 234) Keratinocytes Derived from Human Eccrine Sweat Glands

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In injured interfollicular skin, stem cells from the hair follicle can re-establish a multilayered epidermis. In contrast, palmo-plantar skin does not contain hair follicles. Instead this type of skin is rich in eccrine sweat glands, which are also found throughout almost the entire body. This raises the question if there are also multipotent epithelial stem cells located in eccrine sweat glands.

We employed primary human sweat gland cells in engineered skin substitutes. Cultured sweat gland cells were grown on collagen hydrogels containing human dermal fibroblasts. The dermo-epidermal substitutes were transplanted onto full-thickness skin defects created on immuno-incompetent rats. We found that epithelial cells derived from human eccrine sweat glands can develop into a fully functional stratified and cornified epidermis. We conclude that sweat gland cells can be manipulated to display the characteristics of epidermal keratinocytes. Our data suggest that sweat glands harbor multipotent stem cells that can give rise to a functional epidermis in a wound situation.

(P 235) Kinetics of Chondrocytes Gene Expression Following Bioreactor Stimulation—Towards Optimization of Loading Intervals

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Introduction: Intermittent loading has widely been used to stimulate cell-scaffold constructs for cartilage tissue engineering. The optimal interval between loading cycles may depend on the kinetics of gene expression levels upon mechanical stimulation. The aim of this study is to determine the course of chondrocyte mRNA levels of genes responsive to applied surface motion [1]. Methods: Bovine articular chondrocytes were seeded into polyurethane scaffolds and mechanically stimulated using our loading device that simulates the motion of articulating joints. A ceramic ball dynamically compressed the scaffold and simultaneously articulated over the scaffold at 1 Hz. Scaffolds were loaded during 1h twice a day for 3 days. Gene expression levels were determined immediately, 2 h, 6 h, 12 h, 18 h, 24 h, and 36 h after the last loading period. Results: Proteoglycan-4 (lubricin) and cartilage oligomeric matrix protein mRNA increased after loading and reached highest levels after 12 h. By 36 h the expression of these genes progressively decreased towards control levels. The MMP-13 expression was decreased after loading and remained down-regulated throughout the 36 h observation period. Discussion: This finding of peak expression levels 12h after loading validates our strategy of 2 loading periods per day and may explain previous observations of enhanced responses in cells loaded twice versus once a day. It also suggests that shorter loading intervals may not be advantageous. While kinetics of mRNA levels depend on the gene and on the stimulus, they are valuable not only for optimizing tissue engineering protocols, but also for investigating mechanotransduction mechanisms.

[1]Grad +, Tissue Eng 12(11), 2006.

(P 236) Local Delivery of VEGF165 from Fibrin Biomatrix Significantly Reduces Tissue Necrosis in a Dose Dependent Manner

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Tissue transplantation needs sufficient blood supply to ensure survival of the transplanted tissue and a successful outcome. An intact fibrin network is critical for effective wound healing. Previous studies have suggested that vascular endothelial growth factor (VEGF) is a regulator of physiologic and pathologic angiogenesis and is known to physiologically bind to fibrin. In the current study we evaluated the efficacy of fibrin biomatrix (Tisseel 4U thrombin/ml, Baxter Biosurgery) supplemented with increasing VEGF165 concentrations in prevention of necrosis in a rodent epigastric flap model. After tissue harvesting flaps were randomly assigned to sprayed fibrin biomatrix without or supplemented with VEGF165 at 20, 200, 400, and 800 ng/mL final fibrin clot. Quilting sutures served as controls. Development of necrotic flap tissue was documented by digital photography over a 1 week period and quantified using planimetric analysis. Flap perfusion was measured using a 2D laser Doppler system. Comparing the planimetric analysis of necrotic tissue on day 7 post surgery, it was found that VEGF165 at a concentration of 200 and 400 ng/mL final fibrin clot resulted in a significant reduction in tissue necrosis compared to the control group. The fibrin biomatrix by itself had also trendwise less necrotic areas than the control group. Significantly improved flap perfusion was also found in the 200 and 400 ng/mL VEGF/FS group compared to control. In summary, we found that flap necrosis was substantially reduced and flap perfusion significantly increased when using sprayed fibrin biomatrix with VEGF in a dose dependent manner.

(P 237) Low-Pressure H_2O/O_2 Plasma Assisted Treatment of PCL Membranes for Cell Cultivation

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The aim of this study is to develop novel tissue substituents by using low pressure plasma assisted treatment with biosignals for L929 mouse fibroblast cell cultivation. For this purpose,

biodegradable, PCL (poly-ε-caprolactone) membranes were manufactured by solvent casting method.

Plasma assisted technique was focused on enhancing COOH functionalities on PCL surface using a three step process: H_2O/O_2 plasma treatment; *in situ* or *ex situ* gas/solid reaction to convert OH functionalities into COCl groups; and hydrolysis for final COOH functionalities. Experimental parameters for plasma modifications were optimized according to DoE (Design Expert 7, USA). COOH and OH functionalities on modified surfaces were detected quantitatively by using fluorescent labeling technique and an UVX 300G sensor. Chemical structural information of PCL membranes were acquired using pyrolysis GC/MS analysis. High resolution ESCA spectra showed negligible decarboxylation of PCL samples during the plasma procedure and highly increase in carboxylic functionalities after oxalyl chloride functionalization. By AFM study, it was seen that nanoscale features of the surfaces were dramatically changed during the treatments.

By using fluorescent labeling techniques, the amount of immobilized insulin and heparin were determined as 219.23 $nmol{\cdot}cm^{-2}$ and 271.20 $nmol.cm^{-2}$, respectively.

MTT results showed that heparin immobilized PCL membranes were effective on L929 cell growth, especially, in the late stage of the culture. Consequently, the present study showed that applied plasma treatment method works well for the immobilization of biomolecules onto PCL membranes and these improved PCL membranes can be used as artificial tissue substituents.

(P 238) Low-Serum Conditions for Expansion Culture of Multiple Cell Types

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Serum is commonly used at a concentration of 10–20% for culture of virtually all cell types to allow for their attachment, proliferation and survival *in vitro*. However, serum has several disadvantages for regenerative medicine as it is undefined, highly-variable and of animal origin. The large variability between serum batches dramatically influences cell behaviour, necessitates cell-specific batch selection and makes interstudy comparisons challenging. An alternative medium with low (2%) serum content (Mesenpro) has recently been developed for mesenchymal stem cell (MSC) expansion to eliminate the need for serum batch testing and concomitantly reduce the serum content. We have evaluated the effects of this medium on the growth and differentiation characteristics of several cell types to determine whether it can serve as a standard low-serum condition for general expansion culture.

Primary human MSCs, chondrocytes, osteoblasts, and ovarian and prostate cancer cell lines were cultured for up to 4 weeks in the commonly used medium for the cell type or in Mesenpro. Cell proliferation was measured and gene expression was analyzed by quantitative real-time RT-PCR. Additionally, MSCs and chondrocytes were further cultured in serum-free differentiation conditions and evaluated using lineage-specific stains.

All cell types were successfully expanded using Mesenpro and differences in proliferation and gene expression were found relative to the standard conditions. MSCs were able to differentiate down the adipogenic, osteogenic and chondrogenic pathways and chondrocytes maintained their chondrogenic capacity following Mesenpro expansion. These results indicate that Mesenpro is a viable option for standardized low-serum expansion of several cell types.

(P 239) Macroencapsulation of Human Parathyroid Tissue for Treatment of Hypoparathyroidism: *In Vivo* Study

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Purpose: This work studied the potential of tissue macroencapsulation to cure hypoparathyroidism.

Methods: Human parathyroid glands (from 7 patients, 55 ± 17 yrs old) were mechanically prepared by cutting from hyperplastic parathyroid. Tissue particles (500 μm) were encapsulated in a macrodevice (3 pieces per graft) made of alginate 3%w/v and incubated at 37°C during overnight. The male Wistar rats (6–8 weeks old) underwent subtotal thyroidectomy and total parathyroidectomy. Three experimental groups were designed: rats transplanted with (i) macroencapsulated human tissue ($n = 6$), (ii) non-encapsulated human tissue (Ctrl+, $n = 4$) and (iii) empty macrodevice (Sham, $n = 3$). Serum calcium was measured before the surgery, before transplantation and weekly after transplantation.

Immunohistochemistry analysis was performed to assess immunological response (lymphocyte, CD3/macrophages, CD68) and revascularization (von Willebrandt factor) after graft explantation.

Results: After parathyroidectomy the average starting serum calcium before transplantation was 6.1 ± 1.1 mg/dl. No correction of calcium sera levels was obtained for Ctrl+ and Sham animals until a mean of 40 days post-transplantation (ratio between serum calcium after transplantation and initial calcium level prior transplantation (%): +1.2% and -29% for each expiremental group, respectively). In case of Ctrl+ animals, non-encapsulated human tissue was severely rejected as evidenced by infiltration of CD3 and CD68 positive cells. No degradation of macrocapsules was found in case of Sham animals up to 1 month post-transplantation. In contrast, the serum calcium rose by an average of 20% (of initial values) when macroencapsulated human tissue was implanted subcutaneously at day 29.

Conclusion: Encapsulated human parathyroid tissues can significantly correct hypocalcemia following parathyroidectomy in a xenograft model.

(P 240) Made-to-Order Collagen Matrix: Application of Excluded Volume Effect to Control Quantity, Pattern and Type of Collagen Deposited by Fibroblasts

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The excluded volume effect (EVE) caused by macromolecules crowding in a given volume affects reaction kinetics. The im-

plementation of EVE in fibroblast cultures greatly accelerates the enzymatic conversion of procollagen to collagen.

Fetal lung fibroblasts were treated with ascorbic acid in low-serum medium, with or without crowders: 500 kDa Dextran Sulfate (DxS500), 70 kDa Ficoll (Fc70) and 400 kDa Ficoll (Fc400).

Collagen from the cell layer was extracted with pepsin and analyzed using SDS-PAGE. Under standard culture conditions for 2 days, minimal collagen was found in the cell layer. 2 days of EVE, by Fc70 and Fc400, increased collagen deposition at the cell layer by 10-fold. Under EVE by DxS500, deposition increased by 20-fold.

Immunohistochemical analysis of the cell layer revealed collagen I deposited under standard culture conditions was minimal and in wispy, thin strands. Collagen I deposited under EVE by Fc70 and Fc400 was laid down in an extensive network of fibers. Contrastingly, DxS500-induced collagen I deposition was granular.

Collagen deposited on the cell layer under EVE for 9 days comprised mainly collagen I. When EVE was applied for 2 days and followed by 7 days of standard condition, collagen type composition changed to a mixture of collagen I and V.

Various crowders, DxS 500, Fc 70 and Fc 400, can be used to accelerate quantity and different patterns of collagen deposition. EVE applied in timed pulses controls the type of collagen laid down by the fibroblasts. Hence, EVE allows for more refined control of collagen matrix formation for tissue engineering purposes.

(P 241) Magnetic Carrageenan Nanospheres for Medical Imaging

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Magnetite (Fe_3O_4) nanoparticles due to their low toxicity and magnetic properties have a recognized potential for several biomedical applications including diagnosis and targeting drug delivery [1]. In order to prevent the agglomeration and improve the dispersion in aqueous medium, nanoparticles may be prepared using water compatible stabilizers.In our previous work [2] k-carrageenan, a non toxic sulphated polysaccharide has been successfully used as colloidal stabilizer in the synthesis of magnetite nanoparticles, preventing their spontaneous agglomeration and conferring biocompatibility to the resulting composite.In this work magnetic k-carrageenan nanospheres with an average diameter of ca.75 nm have been prepared using water-in-oil (w/o) microemulsions. The w/o microemulsions were obtained by sonication of a quaternary system comprising n-heptane as the organic phase, cetyltrimethylammonium bromide (CTAB) as surfactant and 1-butanol as the co-surfactant.The aqueous phase contained the biopolymer and magnetite nanoparticles (d~8 nm). The latter were previously prepared by co-precipitation within the k-carrageenan matrix under alkaline conditions [2]. Magnetic measurements showed superparamagnetic magnetite nanoparticles at ambient temperature.The resulting composites are therefore of potential interest as contrast enhancers in magnetic resonance imaging (MRI). Since the average diameter of these nanopheres is lower than 100 nm, blood half-life can be prolonged, allowing for imaging of different tissues. Moreover, since k-carrageenan forms thermoreversible gels, the controlled release of magnetic particles or loaded drugs can be envisaged by both thermal and magnetic stimuli. In addition carrageenan can be further functionalized for the conjugation of biomolecules on the surface of the nanospheres.

[1]A.K. Gupta *et al.*, Biomaterials 2005, 26, 3995.
[2]A.L. Daniel-da-Silva *et al.*,Biomacromolecules 2007, 8, 2350.

(P 242) Magnetic Field Affects Proliferation of Human Cells *In vitro*

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Many physical factors have been examined to allow more effective cultivation and expansion of cells in culture for tissue engineering purposes. One of the possible stimuli to take into consideration is Static Magnetic Field (SMF). Although the literature data is equivocal and the working mechanism is unknown, some authors point to the effects of SMF on the cells' shape, growth or metabolic activity (1).

As our previous experiments demonstrated the influence of SMF on human osteoblast-like cells (MG-63), we decided to confirm this observation and examine the effect of SMF on Human Bone Marrow Derived Mesenchymal Stem Cells.

The experiment was performed in a 96-well tissue culture polystyrene plate (XTT-assay) and in Petri dishes (cell counting). When the cells adhered and spread on the bottom of the wells, the plate was exposed to SMF. Neodymium magnets were placed beneath the tested wells (or dish) and kept there for the next 5–7 days. Finally, cell proliferation assay (XTT), cell count (Bürker camera) and microscopic observations were performed.

SMF enhanced the cell number for both kind of tested cells. This effect was confirmed by both methods used. The morphology of cells was not affected by the application of SMF.

The application of SMF seems a promising tool for the enhancement of cell growth, especially in the situation of a restricted number of available donor cells in tissue engineering applications.

Work supported by Grant No.3 T08A01829, Ministry of Education and Science.

[1]Leszczynski, D. 2005. Prog. Biophys. Mol. Biol 87(2–3):247–53.

(P 243) Magnetic Nanoparticle-Based Gene Trasnfection using Oscillating Magnet Arrays

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Magnetic nanoparticle-based gene transfection has been shown to be effective both in combination with viral vectors and with non-viral agents. In these systems, therapeutic or reporter genes are attached to magnetic nanoparticles which are then focused to the target site/cells via high-field/high-gradient magnets. The technique has been shown to be efficient and rapid for *in vitro* transfection and compares well with cationic lipid-based reagents,

producing good overall transfection levels with lower doses and shorter transfection times. In spite of its potential advantages (particularly for *in vivo* targeting), the overall transfection levels do not generally exceed those of other non-viral agents. In order to improve the overall transfection levels while maintaining the advantages inherent in this technique, we have developed a novel, oscillating magnet array system which adds lateral motion to the particle/gene complex in order to promote transfection. Experimental results indicate that the system significantly enhances overall *in vitro* transfection levels in several cell types compared to both static field techniques ($p < 0.005$) and the cationic lipids ($p < 0.001$) tested. In addition, it has the previously demostrated advantages of magnetic nanoparticle-based transfection—rapid transfection times and requiring lower levels of DNA than cationic lipid-based transfection agents. This method shows great potential for non-viral gene delivery both *in vitro* and *in vivo*.

(P 244) Markers to Evaluate the Quality and Self-Renewing Potential of Engineered Human Skin Substitutes *In Vitro* and after Transplantation

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We screened a series of antibodies for their exclusive binding to the human hair follicle bulge. In a second step these antibodies were to be used to identify basal keratinocytes and potential epithelial stem cells in the human epidermis and in engineered skin substitutes. Of all the antibodies screened, we identified only one, designated C8/144B, that exclusively recognized the hair follicle bulge. However, C8/144B-binding cells were never detected in the human epidermal stratum basale. In the bulge C8/144B-binding cells gave rise to cytokeratin 19-positive cells which were also tracked in the outer root sheath between bulge and the hair follicle matrix. Remarkably, cytokeratin 19-expressing cells were never detected in the hair follicle infundibulum. Yet, cytokeratin 19-expressing keratinocytes were found in the epidermal stratum basale of normal skin as a subpopulation of cytokeratin 15-positive (not C8/144B-positive) basal keratinocytes. cytokeratin 19/cytokeratin 15-positive keratinocytes decreased significantly with age. We suggest that cytokeratin 19-expressing cells represent a subpopulation of basal keratinocytes that is particularly adapted to the lateral expansion of growing skin. Cytokeratin 19-expressing basal keratinocytes may, or may not be unipotent epithelial stem cells. Our data show that cytokeratin 19 in combination with cytokeratin 15 is valuable marker to routinely monitor epidermal homeostasis and (at least indirectly) the self renewing potential, and thus to compare the quality of engineered skin to normal epidermis.

(P 245) Matrix Elasticity Influences Chondrocyte Dedifferentiation

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Cells sense and respond to the mechanical properties of their environment. It has been demonstrated that matrix elasticity plays an important role not only for cell spreading and adhesion but also for cell differentiation.

The aim of this study was to examine the influence of mechanical properties on the differentiation of chondrocytes cultured in monolayer.

We used a 2D-culturing-system in which polyacrylamide gels with different percentages of BIS-Acrylamide were coated with collagen type I. This system allows altering the matrix elasticity while leaving all other matrix-parameters constant.

Matrices with a Young's Modulus of 5 kPa, 10 kPa, 40 kPa and 100 kPa were produced, as determined by atomic force microscopy. Porcine and human chondrocytes were cultivated on matrices in low density culture for up to two weeks. The differentiation of chondrocytes was investigated by measuring collagen type I and II gene expression using RT-PCR and by immunofluorescence staining for collagen type II.

Obvious differences between cells cultivated on the different matrices were monitored especially after the first days of cultivation. Human and porcine chondrocytes grown on stiffer matrices showed a more flattened morphology, lower collagen type II and higher collagen type I expression when compared to cells grown on softer matrices.

Our findings indicate that chondrocytes sense the elasticity of the matrix and respond by dedifferentiating slower on soft matrices than on stiff matrices. These data might be used in the design of novel scaffolds with mechanical properties specifically tailored to chondrocyte differentiation in tissue engineering applications.

This work was supported by EXPERTISSUES.

(P 246) Matrix Metalloproteinase-1 Treatment of Muscle Fibrosis

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The onset of scarring after injury may impede the regeneration and functional recovery of skeletal muscle. Matrix metalloproteinase-1 (MMP-1) hydrolyzes type I collagen and thus may improve muscle regeneration by resolving fibrotic tissue. We examined the effect of recombinant human MMP-1 on fibrosis in the lacerated gastrocnemius muscle of NOD/scid mice, allowing treatment potential to be ascertained in isolation from immune response. The efficacy of proMMP-1 and active MMP-1 were compared with or without poly(ethylene glycol) modification, which was intended to increase the enzyme's stability. Active MMP-1 was most effective in reducing fibrosis, although treatment with proMMP-1 was also beneficial relative to controls. PEG-modified MMP-1 had minimal activity *in vivo*, despite retaining activity towards a thioester substrate. Moreover, the modified enzyme was inactivated by trypsin and subtilisin at rates comparable to that of native MMP-1. These results and those of computational structural studies suggest that modification occurs at the C-terminal hemopexin domain of MMP-1, which plays a critical role in collagen turnover. Site-specific modifications that spares catalytic and substrate binding sites while

protecting susceptible proteolytic digestion sites may be beneficial. We conclude that active MMP-1 can effectively reduce muscle scarring and that its activity is related to the ability of the enzyme to digest collagen, thereby facilitating remodeling of the injured muscle.

(P 247) Measurement of Oxygen Consumption Rate for Aortic Valve Interstitial Cells (VICs): Application to Tissue Engineering

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Objectives: To investigate the factors influencing the metabolism of oxygen by aortic valve interstitial cells (VICs) and in aortic valve leaflet tissue, with a view to generate input parameters to oxygen transport computational models.

Methods: Isolated porcine VICs at 50% and 100% confluency and passages 5 (P5) and 10, or fresh porcine valve leaflets ($n = 4$) were incubated in an oxygen respiration chamber (Strathkelvin Instruments) at 37°C in DMEM. The dissolved oxygen concentration in the medium was continuously recorded at 1 Hz using a Clark-type oxygen electrode and a 782 oxygen meter. The consumption rates at different oxygen concentrations were evaluated from the drop in the recorded oxygen tension over time. The maximum oxygen consumption rate (nmol/million cells/hour) was calculated by employing the Michaelis-Menten equation.

Results: In all cases, the oxygen consumption rate was relatively constant until the concentration dropped to 4% (v/v) and then became approximately logarithmic to the drop in oxygen tension. There was no significant difference between the consumption rate of fresh tissue and freshly isolated VICs (300–380 nmol/million cells/hour). Significant differences were found between cells at P5 and P10, as well as between cells that were 50% and 100% confluent.

Conclusions: This study demonstrated that the metabolic activity of VICs, with regards to oxygen consumption, is dependent upon age and proliferation status. These conclusions provide valuable input to computational models of oxygen transport in tissue engineered valves, as well as to bioreactor optimisation.

(P 248) Mechanical Loading on the Growth Plate: Implication for Endochondral Bone Formation

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Multiple factors that regulate bone cell activities, mechanical environment plays a critical role. There will be a reduction of bone mass and even irreversible changes in the skeleton when human is exposed to the situation where normal weight bearing is prohibited due to medico-surgical diseases. This study was conducted to reveal any histopathological changes occurring in the growth plate when the rats were subjected to be deprived of normal weight bearing using the model of hindlimb unloading.

Thirty male SD rats, aged 6 weeks, were acclimatized with standard conditions. They were divided into two groups according to periods of Hind limb Unloading 3 weeks and of Reloading 1 week, and each control groups were maintained for an identical period in the same conditions. BrdU immunohistochemistry and TUNEL assay were performed. Heat shock protein 47, 70 were immunolocalized to assess the chondrocytic activities in terms of production of stress protein.

The length of the growth plate and the proliferative potential of chondrocytes were decreased in the unloading group than those of control groups. Inter-group differences were more significant in the proliferative and hypertrophic zones. The expression of HSP47 was seen in proliferation zone and those superior to hypertrophy zone. Besides, the expression of HSP47 was the strongest in unloading group.

Alterations in the weight bearing induced changes in the chondrocytic proliferative potential of the growth plate. This may suggest that deprived weight bearing due to various clinical situations hamper normal longitudinal bone growth.

(P 249) Mechanical Properties of Tissue Engineering Scaffolds

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Mechanical properties are of great importance for the clinical use of scaffolds in tissue engineering. Depending on the desired application, the scaffold may have to be very elastic to adapt itself to the size of the explant or quite rigid to support tensions for example. We synthesized polymeric scaffolds with varying porosity and microstructure by mixed porogen leaching/ freeze extraction processes, and drove systematic mechanical properties measurements in compression, in order to characterize the response of scaffolds, and the influence of macro and microporosity on the observed properties. In an attempt to develop a mathematical model based on the cellular solids theory by Ashby and Gibson we designed a gradual compression test to obtain further theoretical knowledge on the collapse process of porous scaffolds.

(P 250) Mechanical Stimulation of an Osteoblastic Cell Line on a Three-Dimensional Collagen Scaffold

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The aim of this work was to study the effect of mechanical strain on the osteogenic differentiation of MG-63 cells in a three dimensional collagen scaffold. The cells were stimulated for 15 minutes, 60 minutes, two hours, four hours and eight hours. The stimulations were performed once and thrice. Furthermore, a continuous stimulation was applied where the previous stimulations were performed in series. Parallel to the stimulations control cells without stimulation were cultivated on the collagen scaffold. After the experiments the viability of the cells was proved with an MTT assay. The viability was not affected by the mechanical stimulation. The mRNA of

the cells was isolated and a PCR was performed to investigate the expression of bone markers. The expression of typical bone markers was determined. It was noticeable that each stimulation scheme had different influence on the cells. The expression of bone markers varied in the different stimulations. The activity of alkaline phosphatase was examined by an AP-activity test and was verified for almost all stimulations. There was no difference between the AP activities of the stimulated und unstrained cells. The synthesis of alkaline phosphatase and RUNX2 were tested with immunological staining. RUNX2 was not detected in any experiment. Alkaline phosphatase was substantiated in the most experiments. The mineralisation of the extracellular matrix was proved with an alizarinred/calcein-double staining. Especially by the threefold stimulations and the unstrained cells an explicit mineralisation was recognized. Recapitulatory, thrice two hours of strain induced strongest effects and enhanced osteogenic differentiation.

(P 251) Mechano-Compatible Collagen-Based Scaffolds for Vascular Tissue Engineering: Low Doses of UV Affect the Viscoelastic Behaviour

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Although collagen shows promising properties, the mechanical properties of materials fabricated from reconstituted collagen are generally far from those of biologic tissue. This mismatch is particularly evident in scaffolds that are supposed to support and stimulate vascular tissue engineering. Therefore, the purpose of this study was to investigate new approaches for improving the mechano-compatibility of collagen-based constructs and to study the effects of low doses UV radiation, which have been shown in previous works to stimulate cell activity. Constructs made of collagen and smooth muscle cells were cultured in a custom-made rotating device. The compliance of the constructs was measured starting after 1w of culture. The microstructure of the resulting collagen and the cell-matrix interactions was investigated by Atomic Force Microscopy and Confocal Laser Scanning Microscopy. Finally, the effects of low UV radiation (0 to 75 J/cm^2) on the viscoelastic properties of collagen scaffolds were investigated. Results show that the mechanical properties of the irradiated scaffolds are encouraging, although still lower then physiological ones. Analyses with AFM and CLSM showed the strong interaction between cells and collagen matrix. In the gel it was possible to detect a binding pattern of 67.4 ± 1.5 nm, which is in agreement with the theoretical one reported in literature. Furthermore, the scaffold appears to be as a random fibrillar matrix. In order to improve these properties, appropriate maturation in a home-made bioreactor under specific mechanical stress and strain are envisaged. The use of silk as reinforcement for the collagen is also under investigation.

(P 252) Mechanobiology in Bone Tissue Engineering: Responses to Fluid Flow

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Matrix production by tissue engineered bone is enhanced when the tissue is subjected to mechanical forces in a bioreactor. For this to occur bone must be able to sense mechanical force and translate this to a matrix producing response. In bone, *in vivo*, mechanosensitivity is proposed to result from the application of shear forces on the cell membrane of osteocytes. However, in tissue engineered bone osteoblasts and their precursors must directly sense and respond to mechanical forces in a less constrained environment. Previously we showed that the cell coat (glycocalyx) is responsible for transmitting fluid flow shear forces in osteocytes. The aim of the current work is to investigate whether the glycocalyx is also a mechanosensor of fluid shear stress in osteoblasts in a tissue engineering environment. Using the late-stage osteoblast cell line MLO-A5 we showed that these matrix synthesising osteoblasts have a glycocalyx rich in Hyaluronic Acid (HA) which can be degraded with hyaluronidase treatment, whilst leaving the cell attached to the surface. As fluid flow experiments are usually conducted in a 2D environment but tissue engineering requires a 3D scaffold we compared cell shape and attachment on the base of a flow chamber and a polyurethane open foam scaffold using actin phalloidin staining and confocal microscopy. As expected there was a marked difference in cytoskeleton organisation and cell shape between 2D and 3D. In conclusion, we have established MLO-A5 will be an good model cell type with which to study the mechanobiology of synthesising ostoblasts.

(P 253) Mesenchymal Stem Cell Behaviour on Topographical Cues

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To mimic natural organization of cells, the control of cell behavior and cell-material surface interactions using physical cues is an important point to be considered in tissue engineering. The scope of this study is to investigate the effect of micropatterned surfaces on the morphology, alignment and proliferation of Wharton's Jelly-derived Mesenchymal Stem Cells (MSC).

A blend of polyesters, poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) and poly(L-D, L-lactic acid) (P(L-D,L)LA) was used to prepare micropatterned (MP) films carrying features such as channels with different dimensions and channel wall inclination. Human Wharton's Jelly MSC were seeded on the sterile micropatterned films and cultured for 4 days in an incubator maintained at 37°C and 5% CO_2. The cells were stained with FITC conjugated phalloidin and DAPI for assessment of cytoskeletal and nuclear alignment.

It was observed that MSCs attached on fibronectin adsorbed micropatterned polymeric films especially in and at edges of the channels and they had a tendency to align along the axis of the channels. The alignment of MSC cytoskeleton and nuclei was more distinct in the narrower channels, those the dimensions of which are closer to that of the MSCs.

Acknowledgments: This study was supported by EU FP6 NoE Project Expertissues and by a grant from METU (BAP-2004-07-02-00-17).

(P 254) Microarray-Determined Potenciality of Wharton's Jelly and Adult Stem Cells

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Introduction: In this work, we have carried out a comprehensive gene expression analysis in monopotent and multipotent epithelial and connective human stem cells to determine which one of these cell types are more suitable for use in tissue engineering protocols.

Materials and Methods: Primary cultures of human Wharton's jelly multipotent stem cells, epithelial stem cells (keratinocytes) and connective stem cells (fibroblasts) were established. RNA was isolated and high-density gene expression profiling was performed by using Affymetrix Human Genome U133 plus 2.0 microarrays. Genes with an average expression above 5000 F.U. were selected for each group, and identification of specific genetic pathways and functions was done by using the program Cytoscape-BiNGO.

Results and Discussion: Wharton's jelly stem cells showed overexpression of 306 genes, corresponding to 109 different cell functions, including extracellular matrix and collagen synthesis and bone and muscle development. Monopotent keratinocytic stem cells overexpressed 280 genes and 74 cell functions, including epidermis development, whereas fibroblast cells showed upregulation of 257 genes corresponding to 72 functions, including extracellular matrix synthesis. All samples overexpressed several common genes (cell growth, proliferation, metabolism, cytoskeleton, biosynthesis, etc.), although some pathways were specific of multipotent Warton's jelly cells (actin and collagens IV and V synthesis, cell motility, Golgi complex synthesis, organs development and regulation). We conclude that adult monopotent stem cells should be used for tissue engineering of epithelial tissues, whereas both multipotent or monopotent stem cells could be used for developing of connective tissues.

Supported by FIS PI061784, CM011/2005 and P06-CTS02191.

(P 255) Microfabricated Hydrogel Scaffold for Regenerative Medicine Applications

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Cellular adhesion, proliferation and differentiation is closely connected with chemical and mechanical properties of the scaffold used, in order to simulate the biological mechanical properties of tissue, the shape of the scaffold is modulated using several parameters (porosity, line width, topology). Traditionally microfabricated scaffolds used in tissue engineering applications are made of synthetic polymers such as PLLA, PLGA or PCL. These polymers present surface properties able to promote cell activities, and the modulation of the scaffold shape can mimic the mechanical properties of biological tissue.

Now we propose a new concept of microfabricated scaffold in order to biomimicry all tissue properties both from the topological to the chemico-mechanical point of view. Using hydrogel it is possible to realize bioispired scaffolds, in particular we analyzed alginate mechanical properties and demonstrate its similitude with several biological tissue. In particular, characterizing the cross-linking reaction between sodium-alginate and calcium-phosphate concentration and their reaction time it is possible to modulate the elastic modulus of obtained material . Using PAM (Pressure Activated Microsyringe) technique we defined the working conditions in order to realize different shape of alginate-hydrogel scaffolds. Using a FEM simulation it was possible to optimize this technique in order to extrude a mixture of cells in alginate hydrogel, realizing a total new concept of engineered tissue. Cellular experiments using murine hepatocyte cell line (HepG2) were performed and the results showed that not only survive but also proliferate and preserve their phenotype.

(P 256) Microfluidic Gradient Maker for Pattern Generation

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First proposed by Whitesides in 1999, the gradient maker has been extensively used by several researchers for generating large concentration gradients over small length scales. Basically, the gradient maker (GM) is a microfluidic device consisting of a series of channels connected to a chamber in which the gradient is formed. Since it was first conceived, the gradient maker has undergone several variations and it is now possible to create time independent solution or surface concentration gradients with complex spatial forms.

One of the main topics under investigation is the study of morphogen or chemotatic gradients which are known to guide cell differentiation and movement.

Here we propose the use of the gradient maker in tissue engineering, to create spatial and temporal information for cell assembly, and as a substitute for traditional tissue engineering scaffolds. The paradigm of development in biology is based on cells assembling and organizing by responding to morphogenetic signals, be they spatial or biochemical, through a process of accumulation and accretion rather than degradation and attrition. Through the use of appropriate activator-inhibitor signals the gradient maker can create repetitive or complex chemical 'scaffolds', in a manner which mimics that of organ and tissue development in living systems. Using fluid-dynamic and transport models of a microfluidic network, we demonstrate how spatial specific chemical information can be imprinted in a cell culture environment either by employing activator-inhibitor complexes as proposed by Meinhardt and Gierer, or using simple diffusion of one or more cell modulators

(P 257) Microparticles for Incorporation in *In Situ*-Forming Implants in Osseous Defect Reconstruction

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Positive results of growth factor administration in bone defect regeneration can be limited by inappropriate release kinetics. It is vital to minimize disease prolonging effects by finding the right release pattern in a suitable dosage form. In this approach, the release from an *in situ*-gelling system shall be adjusted by incorporation of microparticles into the gel matrix.

In situ-forming implants were made from chitosan and beta-glycerophosphate. Chitosan was dissolved in a hydrochloric acid solution and mixed with an aqueous solution of beta-glycerophosphate. Recombinant human Bone Morphogenetic Protein 2 (rhBMP-2) had been added to the chitosan solution.

Microparticles were produced by a W/O/W-double emulsion method using polymers of different hydrophilicity. rhBMP-2 was incorporated in the inner phase with and without stabilizing agent.

Release measurements were carried out by determining the amount of rhBMP-2 in supernatant and quantification with ELISA.

Opaque, stable gels containing rhBMP-2 were produced. A pilot study was carried out to determine release kinetics. By 15 hours, 10 percent of the amount of rhBMP-2 incorporated had been released.

In contrast to the gel, microparticles of the most hydrophobic polymer used did not show any release within two weeks, but after an incubation time of 9 weeks, rhBMP-2 could be found.

The size and shape of the microparticles were investigated. Both were found to depend on the load with rhBMP-2, as well as on the type of the organic phase and the use of stabilizer within the inner aqueous phase.

(P 258) Micropatterned Chitosan Films for Cell Guidance

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Use of polymers in tissue engineering is essential for the design of scaffolds. Natural and biodegradable polymers like gelatin, collagen and chitosan are used for this purpose along with synthetic polymers. A critical issue is the adhesion and proliferation of the cells on the surface. Therefore, modification of the surface chemistry and topography may be needed for optimum results. One approach is the creation of micro or nano patterns on the surface.

In this study, micropattered chitosan films with different deacetylation degrees (75% and 85%) were prepared on micropatterned PDMS templates and crosslinked with epichlorohydrin. Mechanical properties and surface hydrophilicities were examined. The effect of microscale surface features on adhesion behavior of human osteosarcoma cells, SaOs-2, were examined. Some samples were further modified by adsorption of fibrinogen to enhance attachment. Cell morphology and cytoskeletal organization were studied by fluorescence microscopy. MTS assay was used on days 1 and 7 to determine the cell proliferation. It was observed that Saos-2 cells align along the grooves. Fibronectin coating did not improve the cell adhesion. No significant difference in morphology and alignment of Saos-2 cells was observed as a result of chitosan deacetylation, surface morphology and fibronectin coating. Saos-2 proliferation, however, was higher for the 85% deacetylated samples than the 75%.

Acknowledgements: This project was conducted within the scope of the European NoE project EXPERTISSUES and MC RTN project BIOPOLYSURF.

(P 259) Microtomography Characterization of Scaffolds for Bone Regeneration.

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In the last few years the huge steps made by scientific research and the continuous evolution of surgical techniques have allowed the use of bone substitutes, both in dental and orthopaedic field. The use of bone grafts or alloplastic materials is based on the assumption that new bone growth would be stimulated because these materials may: 1) include cells that can rebuild bone (osteogenesis); 2) serve as scaffold, favouring the migration of osteogenic cells and the formation of bone tissue (osteoconduction); 3) contain, in their matrix, substances of bone induction (osteoinduction). Prosthetic materials overcome some of these issues, but their effectiveness is limited by unpredictable graft resorption, infection and structural failure. The search for a reliable implantable material has spurred a new line of research on biocompatible implantable scaffolds. Structure and architecture of scaffolds are very important factors for bone regeneration tissue engineering because they act as a template for cell interactions and as a mechanical framework which osteogenic cells can attach and proliferate on. The aim of the present study is to analyze microarchitectural and morphometric parameters of some biomaterials from different origins using microtomographic technique. X-ray micro-computed tomography (Skyscan 1072, Belgium) is a well known technique used for non-inasive and non-destructive 3D characterization of various materials in different field. Micro-CT analysis allows to accurately quantify scaffold porosity, surface area and 3D measures such as pore size, pore distribution and strut thickness: these data are essential for the success of the graft, being striclty related to bone growth.

(P 260) Modelling Glucose and Oxygen Diffusion in Fibroblast Seeded Collagen Scaffolds Cultured in a Spinner Bioreactor

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The manufacturing of tissue from isolated cells seeded onto biocompatible scaffolds requires studying nutrient transport from the bulk medium to the system which is composed of the submerged scaffold and the fluid medium. The Tissue Engineering Group of

the National University of Colombia made some advances in developing artificial oral connective tissue for grafting wounded oral mucosa. To evaluate some of the group's experimental results, a mathematical model based on principles of transport phenomena has been developed to study glucose and oxygen transport inside oral fibroblast-seeded collagen I scaffolds cultured in a spinner-like bioreactor. The model allows estimates of cellular density as well as glucose and oxygen consumption as a function of scaffold width and diameter. The model also permits establishing the effect of scaffold micro-structural properties and the amount of initial nutrients required for artificial tissue formation. Furthermore, the mathematical model could be used to optimize the scaffold micro-structural properties and the scaling up of artificial connective tissue production.

(P 261) Modification of Polylactide Surfaces with PLA-b-PEO Block Copolymers Deposited from Selective Solvents

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Amphiphilic di-block copolymers composed of polylactide (PLA) and omega-methoxy-poly(ethylene oxide) (mPEO) in selective solvents can associate to micelles and/or with surfaces compatible with PLA block. Formation of surface brush layers based on deposition of PLA-b-PEO copolymers from selective solvents is studied as a method of surface modification of PLA-based biomaterials for tissue engineering. A series of PLA-b-PEO copolymers with different molecular parameters, block lengths and stereoregularity of PLA block (DL or L) were synthesized and characterized by ^{1}H NMR spectroscopy and SEC. The solution properties and self-association of copolymers in various solvent mixtures, such as acetone/methanol, dioxane/methanol, acetone/water, were investigated by dynamic light scattering (DLS) and the size distribution and stability of aggregates were related to molecular parameters of copolymers and mixed solvent composition. Selected compositions of copolymer micelles (nanoparticles) were deposited by spin-casting on poly(L-lactide) surfaces. The surface properties of PLA modified with deposited copolymer films were characterized by contact angle measurements and the stability of films in aqueous environments was evaluated. The efficiency and stability of PLA surface modification, based on wettability changes was related to the solution properties and molecular parameters of copolymers. The most efficient surface modification was achieved by deposition of PLLA-b-PEO with 10 KDa PEO block.

The support by Academy of Sciences of CR (1QS500110564), Center for Cell Therapy and Tissue Repair (MSMT: 1M0021620803) and 6FP-EU NoE ''EXPERTISSUES'' (NMP3-500283-2) is acknowledged.

(P 262) Modification of Surface Chemistry in Conjunction with Defined Media to Replace Recombinant Growth Factors During *In Vitro* Expansion of Mesenchymal Stem Cells

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The *ex vivo* control of cell function and fate relies heavily on the provision of a suitable substratum on which a cell can adhere and then spread thus emulating the native niche in which a cell functions *in vivo*. Mesenchymal stem cell (MSC) culture requires exogenous growth factors and cytokines to modulate proliferation and differentiation. In this study, chemical modifications to the substratum not only guided the growth of cells geometrically but also provided the potential to mimic the native ligands of a plethora of cell surface receptor molecules thus initiating not only conformational, but intracellular signalling responses.

PLLA and PCL substrates were modified with hydroxyl, carboxyl, amino and methyl groups. Human MSCs were characterised then seeded at specified densities and cultured for 7 and 21 days onto these substrates in defined media derived de novo in house and containing no additional growth factors. Phenotype was confirmed using immunohistochemistry and confocal microscopy.

The modified substrates in conjunction with defined media devoid of exogenous growth factors all demonstrated the capacity to support cellular adhesion up to 7 days, the PLLA-based modifications being superior. At 21 days, cells on all but the methyl modified surfaces had predominantly formed rounded aggregates whilst cells exposed to the methyl surface remained spread and expressed Nucleostemin and STRO1.

This research established the fundamental principles for smart materials, developing the hypothesis that controlled modulation of substrate chemistries can be used to remove the need for purified or bacterially derived growth factors during periods of *ex vivo* culture.

(P 263) Modified Collector Geometry for Electrospun Nanofibers

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Introduction: This research aims to investigate the mechanism by which nanofibres attach to various modified collector geometries.

Materials and Methods: Polycaprolactone (PCL) polymer solutions were prepared in a mixed solvent solution, comprising tetrahydrofuran, dimethylformamide and chloroform. Polymer concentrations varied from 3% to 15%. A syringe pump was utilized to maintain consistent flow of polymer solution. A modified collector was constructed from interlocking aluminium sheets, allowing the size and orientation of pores to be manipulated. Polysulphone fibre (ca. 200 μm fibre diameter) was used to bridge large pores as an investigation of the physical effect of materials on nanofibre deposition.

Results and Discussion: As expected, the fibre morphologies changed with regard to polymer concentration, whereby frequent, elongated beading was observed on 3% PCL solutions. 7.5%–15% PCL did not express beading.

By altering the polymer concentration, the deposition of the nanofibres was altered, in some instances causing bridging across large pores, to deposition at the edges of the collector, or penetration through it.

A 7.5% PCL solution resulted in concentrated fibre deposition on the edges of the grid. It is likely that nanofibres formed from higher polymer concentrations provide more significant residual charge and physical presence, leading to alteration in the nanofibre

deposition. Also, higher concentrations of polymer will result in increased chain entanglement, therefore allowing the formation of longer fibres by an increased incidence of bifurcation, creating the observed 'bridges'.

The minimum pore size expressing bridging was predominantly dictated by the concentration of the polymer. 3%PCL showed occasional 'bridging' across smaller pores.

(P 264) Modified Iron Oxide Nanoparticles: a Suitable Tool for Cellular Imaging

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Using stem cells in human medicine remains a distant goal because of the still-considerable risk of tumor formation or differentiation into an improper phenotype. Non-invasive cellular imaging allows the real-time tracking of grafted cells as well as the monitoring of their migration. To improve MR imaging, superparamagnetic iron-oxide nanoparticles (SPION) were modified with different coatings (Poly-L-Lysin—PCSPION, D mannose—MCSPION, polydimethylacrylamid—PDMASPION) and compared to commercial dextran-coated SPION, Endorem(R). Cell viability (measured by the WST-1 assay), labeling efficiency and the MR detection limit were studied in mesenchymal stem cells, olfactory ensheathing cells, chondrocytes and hepatocytes. Depending on cell and SPION type, cell viability decreased by 3–56% after labeling; the best results were obtained with PCSPION and MCSPION. Hepatocytes were viable only after labeling with Endorem. The labeling efficiency was highest with PCSPION. The average iron content per labeled cell ranged from 14.6 pg to 51.7 pg, depending on the SPION type. Cells labeled with DCSPION, PDMASPION or PCSPION possessed higher relaxivity compared to cells labeled with Endorem. MR images of phantoms containing suspensions of MCSPION-, PDMASPION- and PCSPION-labeled cells showed a much stronger hypointense signal than did images of Endorem-labeled cells. In MR images of a rat brain with PCSPION-labeled cells injected intracerebrally, 1000 labeled cells were detected. The better internalization of PCSPION, PDMASPION or MCSPION into cells and their improved resolution on MR images enables the easier detection and tracking of cells in the tissue after transplantation. Supported by AV0Z50390703, KAN201110651, GACR309/06/1594, GACR304/07/1129, 1M0538, LC554.

(P 265) Morphology and Human Bone Cell Interaction of Fibre Reinforced Composite Scaffolds for Bone Regeneration

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The employment of composite scaffolds with a well-organized architecture and multi-scale porosity certainly represents a valuable approach for achieving tissue engineered construct able to reproduce the middle and long-term "*in vitro*" behaviour of hierarchically complex tissues such as spongy bone. In this paper, it was proposed a PCL/PLA composite scaffold (fibres of PLA in PCL matrix), prepared through the synergic use of phase inversion and salt leaching technique, commonly employed in the scaffolding for tissue engineering, and the filament winding technology, traditionally used in the composite material engineering. Continuous fibres integrated with the polymeric matrix allow mimicking the collagen fibres spatial organization of the ECM present in all tissues and, then, also support the stress transfer from the polymer matrix, moving towards mechanical performances like mineralized tissues.

Proposed scaffolds, examined according to the conventional analytical methods (i.e. mercury intrusion porosimetry), shows very interesting pore morphological features in terms of porosity degree and bi-modal pore size distribution which embraces the basic requirements for reaching 1) an adequate supply of nutrients by diffusion into tissue culture media *in vitro* or through newly formed blood vessels *in vivo*, 2) an optimal ingrowth of the healing bone tissue. The attachment and cell proliferation of human bone cells (marrow stromal cells, trabecular osteoblasts) cells confirmed the main contribution of the scaffolds morphology on the cell/material interaction. In detail, changes in polymer fibers induced by the interaction with MSC and HOB *in vitro* adumbrate the potential active role *in vivo* of the scaffold.

(P 266) Mri Depiction and Quantification of Blood-Spinal-Cord-Barrier Permeability After Spinal Cord Contusion Injury

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Objectives: The Blood-Spinal-Cord-Barrier (BSCB) represents a selective physiologic barrier that provides a stable microenviroment within the neuronal tissue. Spinal cord injury (SCI) causes a BSCB breakdown, which results in increased capillary-permeability for plasma molecules that increase neuronal damage as well as for intravenously administered neuroprotective drugs.

Here we used contrast enhanced MRI to determine evolution and duration of BSCB-breakdown after standardized SCI and define the "therapeutic window" for this injury model.

Materials/Methods: In male Sprague-Dawley rats, a laminectomy was carried out at TH11 and a contusion injury was inflicted, using the IH® Impactor with a force of 150 kdyn. Rats were divided into groups with different observation times: 0h ($n = 8$); 24 h, 72 h, 4 d, 6 d, 10 d ($n = 5$ each).

At the end of the observation time each rat received an intravenous injection of 0.8ml/kg gadopentetate dimeglumine (Magnevist®, Bayer HealthCare). Rats were euthanized 10 minutes (0 h group, $n = 3$) or 1 hour after contrast agent administration and MRI was performed on a 3Tesla scanner.

Results: Pronounced signal enhancement at the injury epicenter was measured after observation periods up to 4 days, gradually decreasing in cranial and caudal direction. After 6 days or later, no signal enhancement was visible.

When euthanization and MRI were performed 10 minutes after contrast agent application, signal increase was mainly detectable in the SC arteries, whereas distribution within the neuronal tissue could be observed after 1 hour.

Conclusion: The inflicted SCI increases BSCB-permeability for 4 days. Delayed dispersion of the contrast agent within the neuronal tissue has to be considered.

(P 267) Mri Visualization of Human Mesenchymal Stem Cells Targeting Brain Tumors in an Orthotopic Mouse Model

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An estimated 20,500 people in the United States were diagnosed with primary brain tumors in 2007, with gliomas comprising approximately 60% of these cases. Glioblastoma multiforme (GBM) representing the most malignant and most common glioma. GBM remains virtually untreatable despite extensive surgical resection, radiotherapy and chemotherapy. Treatment difficulty is amplified because of their exceptional migratory nature and their predilection to integrate into normal brain tissue. Recently it has been suggested that adult mesenchymal stem cells have the potential to seek, migrate and integrate into brain tumors. We hypothesize that human mesenchymal stem cells (hMSCs) homing capabilities could be exploited to seek out and delineate brain tumors regardless of their size or location using superparamagnetic/fluorescent particles (FSPMP). This strategy could allow for the detection of newly developing smaller tumors or areas of tumor infiltration that would otherwise go undetected and consequently untreated.

HMSCs were intracellularly labeled with FSPMP and were easily visualized by fluorescence and magnetic resonance imaging for up to 11 weeks; furthermore using *in vitro* studies, we were able to detect the labeled hMSCs at single cell level by MRI. *In vivo* studies using a murine orthotopic GBM xenograft model demonstrated that FSPMP labeled hMSCs were able to migrate towards established intracranial gliomas and were detectable after intracranial injection by both fluorescence and MRI.

In conclusion, our data suggest that hMSCs labeled with superparamagnetic particles are a promising adjunctive strategy for brain tumor therapy that could result in earlier diagnosis and better evaluation of the therapeutic regimen.

(P 268) Multicomponent Scaffold for Osteochondral Defects Tissue Engineering

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In tissue engineering approaches for osteochondral defects repair, different techniques are currently evaluated: cell therapy, the use of active molecules to stimulate the repair process and finally the use of 3-D scaffolds, eventually combined with cells and-or growth factors. An osteochondral graft should distribute and support static and dynamic loads, protect trabecular bone, present a smooth surface and a low coefficient of friction of the cartilage side, and possess adequate wear resistance. Moreover, it should allow growth and proliferation of osteoblasts from the bone side and chondrocytes of the articular cartilage while consenting a close biochemical dialogue between those cell types.

On the basis of the above requirements, a multicomponent asymmetric scaffold made by two silk fibroin-based sponges separated by an electrospun nanometric biodegradable polymer net have been prepared. The aim of the net is to avoid the migration of osteoblasts to the cartilage site and the passage of blood cells from the subchondral vascularized zone. Sponges have been prepared by using fibroin/PEG blends, at different PEG content, for the chondral site and pure fibroin for the subchondral site, being PEG capable of reducing proliferation rate of chondrocytes. Nets have been prepared by electrospinning of PDLA. The single model scaffold components have been characterized by traditional physico-chemical techniques and separately seeded with osteoblasts or chondrocytes in static conditions, while cell culture experiments under dynamic compressive loads have been performed on the assembled scaffold by using a made-in-house bioreactor.

Aknowledgements: Expertissues NoE is aknowledged for the support.

(P 269) Muscular Compensation in Patient with Massive Rotator Cuff Tear: Superficial Electromyography Study

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The shoulder girdle is an extremely mobile joint. Rotator cuff tears alter the existing equilibrium between bony structures and muscles. The "subacromial impingement syndrome" resulting from this unbalance leads to an extension of the rotator cuff lesion.

Many authors have postulated a mechanism of compensation, but its existence still requires evidence. According to this model, the longitudinal muscles of the shoulder and the undamaged mus-

cles of the rotator cuff would be able to functionally compensate, supersede the function of rotator cuff, and reduce symptoms.

The aim of this study was to evaluate muscular activation of the medium fibers of deltoid, the superior fibers of pectoralis major, the latissimus dorsi and the infraspinatus by a superficial electromyographic study (EMG) and the analysis of kinematics in patients with a massive rotator cuff tear.

We evaluated 30 subjects: 15 had pauci-symptomatic massive rotator cuff tear (modest pain and preserved movement), and 15 were healthy controls.

Paired and unpaired t-test showed significant different activations ($p < 0.05$) of these 4 muscles between the pathological and healthy joints in the same patient and between the experimental group and control group.

This study showed that a mechanism of muscular compensation is activated in patients suffering from rotator cuff tear involving the latissimus dorsi and the pectoralis major. It is therefore probable that in these patients, these muscles adapt in order to compensate for the pathological situation. We believe that these data are valuable in the surgical and rehabilitation planning of patients with massive rotator cuff tear.

(P 270) Myocardial Tissue Engineering: The *In Vitro* Generation of a Vascularized 3D Matrix

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Background: A major problem in generating 3D-artificial myocardial tissue is an efficient supply of oxygen and nutrients to cells *in vitro*. Here we report the generation of an *in vitro* vessel-bed based on biological vessel structures for vascularization of artificial tissue constructs.

Methods: Dezellularized segments of porcine small intestine with preserved pedicles were cut open along longitudinal axis and resulting patches were sutured to silicone cushions. Vessel structures were reseeded with 5×10^7 TAMRA labeled human cord blood derived endothelial cells (hCBEC) and cultivated in perfusion bioreactor for 4 or 14d. Fluorescence microscopy was performed to detect seeded cells in the construct. Oxygen saturation, pH, Glucose and Lactate were measured during cultivation.

Results: Immunohistochemistry against Laminin and Collagen IV revealed the intactness of the basal lamina in the vessel bed. Adherence of hCBEC to these proteins was shown by attachment experiments *in vitro*. Glucose reduction and Lactate accumulation were indicative for the metabolic activity of the seeded hCBEC. Monitoring of pH and oxygen saturation showed a slight shift in pH to 7.0 and a constant level of oxygen saturation of 99%, indicating proper culture conditions provided by the bioreactor. Over time an increasing colonisation of vessels by hCBEC was observed. Dil-acLDL assay revealed endothelial character of seeded hCBEC.

Conclusion: Our constructs may be a first step towards the generation of 3D-artificial vascularized myocardial tissue for surgical reconstruction. The use of these patches might serve for other 3D-artificial tissue constructions as well.

(P 271) Nanofiber and Polyester Culture Plates for a High Throughput Functional and Morphological Studies

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According to development of nanotechnology, biocompatible nanofibers have been studied with synthetic and natural polymers as wound dressings, drug delivery systems, vascular grafts, and scaffolds for tissue engineering. Adhesion of cells on surface is important fields of cell culture and tissue engineering. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHBV) is one of the most promising materials for tissue engineering. Composite solution of PHBV and the natural calf collagen peptide (PHCP) were dissolved in 2 wt% 2,2,2-trifluoroethanol(TFE) and the composite nanofibers were electrospun on polyethylene terephthalate(PET) film, which were carefully cut and moulded to fit precisely to each well sizes of 24, 48, 96 well culture plates and the round-cut nanomesh discs were located on the bottoms of the wells in a plate(nanomesh plates). In this study, nanomesh and type I collagen-coated PET disc plates (polyester plates) were also applied for the cell culture. The HCT116 colon cancer cells was spread on the both plates and cultured with anticancer drugs. The results were determined by MTT test, Calcein-AM assay, SEM, double IF, and immunocytochemistry. The nanomesh discs are exceptionally easy to freeze in a cryovial, to store in a LN^2 tank, and to reculture the cells. The nanomesh plates should be a good support for three-dimensional cell and tissue culture and engineering. The polyester plates were very convenient for morphological and functional tests of the cells. The 96 well nanomesh and polyester discs should be useful for a high throughput evaluation of different drug-treated cells or cell lines at the same time.

(P 272) Nanoscale Modification of Biological Polymer Surface via Vacuum Ultraviolet Irradiation

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The effect of the vacuum ultraviolet (VUV) treatment of the poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV) films onto their physicochemical and biological properties was studied. The air pressure during irradiation process was 0.02, 2.5 and 10 Torr. Surface topography was studied by means of AFM. Surface hydrophilicity degree was determined by its wetting contact angle value. XPS technique was used for surface chemistry analysis. Total internal reflection fluorescence method was applied for irreversible human serum albumin (HSA) adsorption study. Platelet adhesion was investigated using scanning electron microscope (SEM). NIH 3T3 mouse fibroblasts were cultured on all samples for 1, 3 and 6 days and observed by SEM. Topography of PHBV surface became smoother after VUV irradiation. RMS roughness of surface (Rq) reduced from 81 nm for the untreated PHBV to 22–40 nm for the VUV-treated one. The increase of pressure in the reactor chamber leads to contact angle decrease (82 degrees for untreated and 62 for modified samples). Noticeable changes were revealed in C-C, C-H

(from 25% to 52%) and C-O-C, C-OH (from 55% to 36%) groups containing. It was shown that VUV modification decreases amount of HSA adsorbed (from 63.4 to 30.9 ng/cm^2) and minimizes platelet activation (in 2.5 times) and adhesion (in 6 times). For VUV-treated samples fast cell attachment and growth was registered. Fibroblasts were well-spread and showed a polygonal cell shape. VUV-irradiation seems to be a possible modification to enhance hemo- and biocompatibility of the PHBV films. Such matrices may be recommended for *in vitro* cell culturing with subsequent transplantation.

(P 273) Nanostructured Polymeric Constructs as Blood Substitutes

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The present work is aimed at the development of biopolymeric nanoparticles to be used as blood substitutes. Developing alternative strategies to commonly used blood transfusion is essential because of the fewness of donor, the short time storage of blood samples and the presence of blood antigens. Two alternative polymeric matrices were used, the synthetic 2-methoxyethanol hemiesters of poly(maleic anhydryde-alt-butylvinylether) grafted with polyethylen glycol (PEG) VAM41-PEG and the natural derived polymer Alginate. Hemoglobin loaded nanoparticles based on VAM41-PEG were prepared by means of the co-precipitation technique. The process was performed under controlled conditions (N2 atmosphere, 4°C), in the presence of reducing agents (such as ascorbic acid and methylene blue).

The prepared nanoparticles have shown a spherical morphology and a diameter distribution of about 140 nm, detected by means of Scanning Electron Microscopy (SEM) and light scattering analysis, while the quantification of loaded haemoglobin, performed by means of Drabkin's assay, revealed a protein loading of about 50%. Zeta potential measurements confirmed PEG chains exposure on nanoparticles surface giving these structures stealth properties once *in-vivo*.

Alginate beads loaded with Hemoglobin were obtained via ionical gelation with a diameter of about 2 mm. UV-Vis analysis revealed that the protein maintain its functionality when loaded inside these structures.

Although further investigation aimed at testing alternative reducing agents, modifying the starting polymeric matrix and reducing Alginate beads size are currently ongoing, these results appear promising in order to produce polymeric nanoparticles to be used as blood substitutes.

(P 274) Neo-Artery *In Vivo* Re-Growth using a Tissue-Engineered Hyaluronan Based Scaffold

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The development of vascular tissue engineering has emerged as a promising technology in the design of an ideal, responsive, living conduit with properties similar to that of the native tissue.

The missing link in tissue engineered blood vessels is the elastin biosynthesis. Several biomaterials are currently used in this field but few of them support elastin biosyntheses in three dimensional arrangement. Aim of the present study was to test the ability of HYAFF 11™ biodegradable grafts to develop a neo-vessel of larger sizes in a porcine model, focusing on extracellular matrix remodelling and elastin biosynthesis.

HYAFF 11™ tubes were implanted in an end-to-end fashion in the common carotid artery. Grafts were analysed for patency using Duplex-scan every 15 days. Results were evaluated by histology and molecular biology to evaluate extracellular matrix and components.

All the animals survived the observation period without complications. Intimal hyperplasia and graft thrombosis were the cause of occlusion as demonstrated by histological examination. There were no signs of stenoses or aneurysms in the remaining grafts. At 5 months, the biomaterial was almost completely degraded and replaced by a neo-artery segment composed of mature smooth muscle cells, collagen and elastin fibres organized in layers, and completely covered on the luminal surface by endothelial cells.

Although patency rates seem to be inferior to those obtained in previous small animal studies, Hyaluronan-based grafts of larger size confirmed the ability to guide the development of a well functioning neo-artery, with the remarkable property of forming organized layers of elastin fibres.

(P 275) New Generation Bioactive Composites Promoting Growth of Adult Human Mesenchymal Stem Cells

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Bioactive materials are recognized in orthopedics, dentistry and oral surgery for their superior integration with bone tissue. The bioactive properties of certain glasses and glass-ceramics are attributed mostly to the spontaneous hydroxyl carbonate apatite (HCA) formation on their surface. Incorporation of bioactive glass into a metal, hydroxyapatite or polymer matrix offers several biological advantages such as a higher bioactivity and better integrity with a host tissue. Thanks to addition of bioactive glass it is also possible to control degradation kinetics of the resulting composites. We report here manufacturing procedures and biological properties of new hydroxyapatite (HA) and poly L-lactic-co-glycolic acid (PLGA) composites with bioactive glass. Starting compounds included bioactive glass of CaO-P_2O_5-SiO_2 system obtained by the sol-gel method, two synthetic and one natural hydroxyapatite powders, and PLGA. Composites HA/bioglass and PLGA/bioglass were obtained by sintering HA-bioglass powder mixture at 1100–1300°C, and by solvent casting of polymer-bioglass powder mixture followed by drying in air and vacuum, respectively. All composites showed homogenous HCA layers on their surfaces after contact with simulated body fluid (SBF). Bone marrow-derived adult human mesenchymal stem cells were seeded on the material surfaces at a density of 10000 cells/cm^2 and

cultured up to 7 days. A significant increase of live cell number was observed for bioactive glass-containing composites compared to starting compounds. We propose the new bioactive glass-based composites as promising biomaterials supporting growth of adult human mesenchymal stem cells.

(P 276) New Hydrophilic, Partially Degradable and Bioactive Cements (HDBC) to Improve Interface with Bone

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Acrylic bone cements aim to fix prosthesis to bone during hip arthroplasty. The commercial acrylic bone cements perform their function, however at the long term they fail due to aseptic loosening of two interfaces: prosthesis-cement and cement bone. To minimize these problems, the bone growth should be promoted on the surface and inside of the partially degradable bone cement.

In our work five different formulations were developed containing in the powder a biodegradable component such as modified corn starch with acrylic segments (methacrylated starch) as well cellulose acetate blended with corn starch (SCA). These components reacted with acrylic monomers (methylmethacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA)) to produce hydrophilic partially degradable bone cements by radical polymerization. Diverse molar ratios MMA/HEMA as well the amount of initiator/activator were employed in such cements.

The residual monomer content was studied by 1H NMR in these new formulations allowing the selection of the two with less amount of residual monomer for later degradation studies. Higher concentration of reducing sugars was found (0.42 mg/mL) in samples immersed in PBS supplemented with α-amylase counteracting with samples without enzyme (0.01 mg/mL) suggesting that enzymatic degradation had occurred. This result was confirmed by percentage of weight loss as well as morphologic analyses.

Our study revealed that bone cements performed with methacrylated starch seem to be promising due to less content of released monomer and good degradability properties, promoting at later stages bone growth by cellular adhesion and improvement of the interface with bone.

(P 277) New Pamam Dendrimers/Alginate Nanoparticles for Gene Delivery

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The basic idea in tissue engineering via gene delivery is to promote cell proliferation and differentiation inside a scaffold as well as the secretion of mitogens and/or morphogens from cells by gene transfection and expression. Efficient gene delivery vectors should thus be developed to accomplish this objective.

PAMAM (poly(amidoamine)) dendrimers with cationic termini have been used as vectors for gene delivery, being the results very promising. Nevertheless, several studies report concentration and generation dependent dendrimer toxicities. So, we hypothesized that a possible way to minimize this problem would be to combine the dendrimers with alginate, a natural anionic polymer that has found biomedical and biotechnology applications due to its biocompatibility and biodegradability.

The aim of this work was then to prepare and characterize a new family of nanoparticles based on PAMAM dendrimers and alginate able to act as vectors for gene delivery and presenting reduced cytotoxicity. Different generations of dendrimers, as well as alginates with different ranges of molecular weight were used. Nanoparticles varying in composition, size distribution and charge were obtained, characterized and their *in vitro* cytotoxicity was evaluated by the MTS assay using HEK 293T cells. Nanoparticles transfection efficiency was assessed using the same cell line and a plasmid containing the β-galactosidase gene.

(P 278) New Platelet-Rich Plasma Based Membrane with Improved Mechanical Properties

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Platelet-rich plasma (PRP) is used in a variety of surgical settings for soft tissue healing enhancement. It is used in orthopaedic surgery and especially in oral maxillofacial surgery where it accelerates autogenous bone graft healing. Once injected, or implanted, PRP is supposed to release locally growth factors for several days inducing tissue repair. PRP is currently available in liquid or gel form that has no, or poor mechanical properties and makes difficult the handling and the confinement of the growth factors at a specific location in open surgery. We investigated whether PRP mechanical properties could be improved. We obtained a PRP a disk-shaped that we named PR-fibrin matrix (PRFM). The PRFM obtained had a tear elastic modulus of $163 + 11$ and a tear load of $267 + 36$. PRFM maintained its mechanical properties during the period it was being tested (18 days). We also tested whether the PRFM could also be used as a scaffold for cell delivery. Results demonstrated that PRFM supported the culture and the delivery of mesenchymal stem cells (MSC). The improved mechanical properties of PRFM make the membrane easy to be handled. Furthermore, PRFM provides a solid scaffold for cell application that can contribute to bone and tissue regeneration.

(P 279) New Thermo-Responsive Hydrogels Based on Hyaluronic Acid/ Poly(N-Isopropylacrylamide) Semi-Interpenetrated Polymer Networks: Swelling Properties and Drug Release Studies

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Smart or stimuli-responsive hydrogels present the ability to change their volume or shape in response to an environmental signal. They have attracted great interest due to their potential application in biomedical areas such as drug delivery and tissue engineering. Among the family of temperature intelligent hydrogels, poly(N-isopropylacrylamide) (PNIPAAm) -based hydrogels show reversible volume phase transitions during heating/cooling across a certain temperature (~32°C), which corresponds to its lower critical solution temperature (LCST). These hydrogels significantly reduce their volume upon heating above the LCST and increase their volume upon cooling below the LCST. In this work semi-interpenetrating polymer networks (semi-IPNs) composed of PNIPAAM and hyaluronic acid (HA), which is a biodegradable, biocompatible, and natural pH-responsive polysaccharide, were prepared for pH/temperature–sensitive swelling and drug delivery studies. N,N′-methylenebisacrylamide was used to crosslink PNIPAAM. It was found that when the temperature increased the prepared HA/PNIPAAM hydrogels showed a faster deswelling than PNIPAAM hydrogels prepared with the same crosslinking degree. The reversibility of the pH- and temperature-dependent behaviour was also analysed for the developed semi-IPN hydrogels. Regarding the drug release studies, gentamicin was incorporated (as model drug) and the release profile was followed in the two hydrogels (with and without HA).

(P 280) NMR Spectroscopy—a Powerful Tool in Quality Control of Biosynthetic Mineralized Composite Materials

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Solid state nuclear magnetic resonance spectroscopy (SSNMR) is a powerful tool for characterizing biomaterials. Easily measured 13C NMR properties can give significant information about composition, from resonance frequencies, and short range structural order, from resonance frequency dispersion, with less ordered structures tending to show broader signals.

We are currently using SSNMR to study a variety of natural and *in vitro*-derived biominerals, such as from mineralizing cell lines (Saos-2 and MC3T3) and rat foetal calvarial cells. The primary aim of this work is to optimize biosynthesis of functional 3D scaffolds for biomineralization, while a secondary goal is the incorporation of NMR-active nuclei to aid assignment and structural characterization. The SSNMR is particularly useful in establishing whether collagen has been expressed, and if so whether it is possessed of the high degree of order which would be supposed necessary if it were to act as a scaffolding or templating structure for biomineral deposition. In particular the presence of a signal from the 3-carbon of hydroxyproline, at about 71 ppm, is a good diagnostic of collagen production, and its linewidth of structural ordering. Initial data show that the rat calvarial cells are producing bone-like biomineral within a collagenous matrix which however is not as ordered as that of bone itself. Under our culture conditions however the two cell lines are failing to produce significant collagen, or if they are it is highly disordered and therefore unlikely to be an effective scaffold.

(P 281) Non Invasive Longitudinal Tracking of Human Amniotic Fluid Stem Cells in the Mouse Heart

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Recently, stem cells have been isolated from human amniotic fluid (hAFS), and have potential applications for therapies. However, the clinical implementation of these therapies introduces the need for methods of noninvasive tracking of AFS cells. The purpose of this study was to evaluate a high resolution magnetic resonance imaging (MRI) technique for *in vivo* detection and tracking of superparamagnetic micron sized iron oxide particle (MPIO)-labeled AFS cells injected in the mouse heart. MRI images of mouse heart were acquired at set time-points during the 4 week course of this longitudinal study. At the end of the study, histological analysis was used to correlate cell localization with the MRI results. Introduction of MPIOs into hAFS had no significant effect upon cell proliferation and differentiation. Flow cytometry analysis indicated that hAFS cells remained labeled for up to 4 weeks. MRI of MPIO-labeled hAFS cells injected in agarose gels resulted in significant hypointense regions. Labeled hAFS cells injected into mouse hearts produced hypointense regions in the MR images that could be detected 24 hours and 7, 14, 21 and 28 days post injection. The co-localization of labeled cells within the hypointense regions was confirmed by histological analysis. These results indicate that MRI can be used successfully for noninvasive tracking of AFS cells injected in the mouse heart. The potential utility of this finding is that injected stem cells can be tracked *in vivo* in the heart and might serve as a noninvasive method to longitudinally monitor cell survivability, proliferation and integration into myocardial tissue.

(P 282) Non-Invasive Imaging of Collagen Type I Expression in a Transgenic Mouse Model

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Background: One strategy in bone tissue engineering is to combine cells, biomaterials and growth factors, and to implant these active scaffolds in animal models to evaluate bone formation. The current evaluation methods are time-consuming, invasive and difficult to quantify. Furthermore the dynamic nature of bone regeneration is ignored. *In vivo* bioluminescent imaging (BLI) may be a strategy to overcome these problems. In this study, a transgenic mouse model for collagen (I)-luciferase was evaluated under several bone remodeling processes to validate this reporter for future use in bone regeneration.

Results: Collagen (I)-luciferase mice emitted very strong light from the bony parts of the body, such as the vertebral column, paws, feet, jaw and joints. The light intensity was highest at young age and dropped dramatically in the first six months. Ovariectomy did not significantly change the luciferase signal. In contrast, in another bone remodeling model in which we created a Ø1 mm hole in one of the vertebra in the tail, we observed higher light intensity from that area compared with sham mice. The intensity peaked around 20 days after operation, and returned to the basal level after 8 weeks. We are currently investigating BLI of ectopic bone formation by implanting ceramic BCP particles with BMP2.

(P 283) Novel Biosensor Based Polymer for Blood Glucose Detection

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A novel polymeric nanofiber loaded with enzyme for the detection of blood glucose level has been developed and optimized. The new device can detect the glucose level down to small concentration with a very high accuracy.

(P 284) Novel Cell-Seeded, Tubular-Shaped and Highly Dense Collagen Gel for Tissue Engineering

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Collagen is known to be the most abundant protein in mammals. It gives structure, consistency and resistance to soft tissues. Many of these tissues are anatomically composed of tubes. We developed a new method to obtain a readily cell-seeded and tubular-shaped scaffold based on collagen gel. The resulting construct has an especially high collagen density and is characterized by very interesting mechanical properties for tissue engineering regarding for example burst pressure and water tightness. We showed that primary smooth muscle cells of human urinary tract origin seeded in this scaffold could proliferate during 14 days (end of the culture). These results support that the obtained scaffold is a significant step towards regenerative medicine of tubular anatomic structures such as blood and lymphatic vessels or urinary tract.

(P 285) Novel Collagen and Ceramic Based 3D Biomaterials for Tissue Engineering Applications

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The principle of Tissue Engineering is to build artificial replacements by means of vital components. In order to achieve a functional graft, applicable cells were seeded on three-dimensional scaffolds and expanded *in vitro*.

The common used cell culture techniques generate cell layers, but it is not possible to create a three dimensional, functional multilayer cell structure on the surface of a cell culture dish. Therefore, three dimensional scaffolds are necessary, which provide a specific environment and architecture for the formation of the tissue.

The scaffolds must accomplish several requirements. The most important attribute of the scaffold has to be its biocompatibility. Furthermore, the material composition and structural characteristics are very important. The surface constitution should promote the adhesion of the cells and a porous structure assures the supply with nutrition and the removal of metabolic waste of cells. In addition to the macroporous structure, microporosity is beneficial for capillary ingrowth and cell-matrix interactions. Moreover, the design of the scaffold should provide the neovascularisation from the surrounding tissue when it is implanted in to the patients defect.

Numerous materials have already been tested for their applicability, and both ceramics and natural and synthetic polymers are the most promising materials for Tissue Engineering. In this study, we characterized collagen- and ceramic based scaffold materials with regard to their three dimensional structure and biomechanical properties. Different applications including the dynamic cultivation on these cell seeded scaffolds in a novel rotating bed reactor are presented.

(P 286) Novel Conjugates of Dendrimers and Fatty Acids for Gene Delivery Applications

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Polycationic dendrimers, such as polyamidoamine (PAMAM) dendrimers with amino termini, have been studied as gene delivery vectors in unmodified and modified forms with a relative success. However, our previous experience revealed that mesenchymal stem cells (MSC), as well as osteoblastic cells (both cell lines and differentiated cells derived from bone marrow), which have particular importance in the scope of bone tissue engineering, are very difficult to transfect *in vitro* using PAMAM dendrimers.

In the present work, different generations of PAMAM dendrimers were coupled with fatty acids (lauric, miristic and palmitic acids) with the goal of improving their performance as DNA carriers. We explored the idea that the hydrophobic character of the fatty acid moieties could positively contribute for the transport of the vector/DNA complexes across the plasma membrane.

The conjugation of the fatty acids with the dendrimers was performed and the conjugates were characterized by NMR and MS

techniques. Their cytotoxity was assessed by using the MTS assay. The complexes formed between the conjugates and plasmid DNA were characterized in terms of charge and size, by means of the DNA retention assay, as well as zeta potential determination and particle size measurement. The transfection efficiency was evaluated in MSC cultures using a plasmid DNA containing the Green Fluorescent Protein reporter gene. A correlation between vectors structure and their performance is proposed.

The support of the FCT-Portuguese Foundation for Science and Technology through the J.L.Santos Ph.D. grant (SFRH/BD/19450/2004), the Project PTDC/SAU-BEB/71161/2006 and the NMR National Network (REDE/1517/RMN/2005) is also acknowledged.

(P 287) Novel Dexamethasone Releasing Multicomponent Implant

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Several inflammatory conditions are usually treated with corticosteroids. Earlier, we reported (Vapalahti *et al.* 2005) development of dexamethasone (DX) loaded fibers for treatment of inflammatory conditions. We also reported (Nikkola *et al.* 2005) the heat pressing technique for controlling the drug release from biodegradable polymer matrix. Thus, we assessed the development of multicomponent implant releasing dexamethasone to be used for bone fixation.

Two types of dexamethasone loaded poly(lactide-co-glycolide) fibers were manufactured by melt extrusion and fiber spinning. Some fibers were sterilized to obtain change in drug release properties. Different DX-fibers were then heat pressed to form one multicomponent rod. Half of the rods where sterilized. Drug release was measured form initial fibers and multicomponent rods using a UV/VIS spectrometer. Shear strength and changes in viscosity were also measured.

Drug release was commenced earlier from multicomponent rods than initial fibers. The drug release period of multicomponent rods and sterilized rods were 30–70 days and 23–57 days, respectively. The initial shear strength of multicomponent rod was 135 MPa and degreased 105Mpa during two weeks.

Results showed that heat pressing has effect on drug release and the release profiles were integrated from initial fiber drug release profiles. Even though the hear strength decreased 30 MPa during two weeks, it still retain over e.g. the shear strength of cortical bone. Thus, developed multicomponent implant could be developed further to be used in bone fixation treating late inflammatory reaction.

(P 288) Novel Hypoxia Mimicking Bioactive Materials for Tissue Engineering

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Next generation regenerative medicine concepts will depend on smart materials which activate ''self healing'' mechanisms. During endochondral bone formation or mature bone and cartilage regeneration, cells respond to changes in the oxygen pressure through a hypoxia-sensing pathway. This pathway then activates numerous genes necessary for osteo/chondrogenesis tissue regeneration, including progenitor cell recruitment, cell proliferation and differentiation. Here we propose that these fundamental processes involved in normal bone and cartilage development and repair can be leveraged for successful bone and cartilage tissue engineering strategies.

Here we report the development of new resorbable bioactive glasses (BG) as the delivery system for hypoxia stimulating ions. We achieve controlled ion release profiles that as assessed by ICP. The chemical composition of the BG was tailored to specific applications (cartilage or bone). Human osteoblasts osteosarcoma cells (SaOs2), were cultured with the dissolution products of different concentrations of BG formulated with and without hypoxia stimulating ions. Cell toxicity and proliferation assays were completed and hypoxia response was assessed by HIF-1α and VEGF gene expression.

The dissolution products of the hypoxia ion containing BG were shown to activate the hypoxia pathway and increased the production of VEGF. Moreover, a non-toxic level of hypoxia stimulating ions in the media was determined. These results demonstrate the potential of hypoxia mimicking materials for TE applications.

(P 289) Novel Nano-HA Composite Scaffold with Woodpile-Network Structure

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Bone is a highly complex material with hierarchical architecture and composition. To fulfil the needs of an ideal bone implant, we designed a 3D porous woodpile-network composite scaffold with nano-Hydroxyapatite (HA), micro-HA, poly (L-lactic acid) (PLLA) and chitosan via rapid prototyping technique and freeze-drying technique. The composite hybrid material integrated the osteoconductivity, biocompatibility and mechanical strength of three types of biomaterials. These scaffolds were examined with SEM, XRD, FTIR and compression test. The composite scaffolds had a ''wood-pile'' structure, where struts on the same layers were laying parallel, but each layer was turned 90° with respect to the previous one. The dimension of one strut and the distance between two struts were about 500 μm. Fine chitosan fibres filled the pores and tangled the strut, whereas it possesses hierarchical size of macro pores, micropores and film networks. Agglomeration of hydroxyapatite crystals was not evident. From the FT-IR spectroscopy, all three components were included in the peaks of the composite scaffold. All the three components did not be destroyed or inter-react chemically due to the tender method of processing. There is no difference detected in the XRD peaks position of the nano HA composite scaffold materials with the crystalline nanoHA and HA powders, but there is a broadening of the peaks suggesting a decrease in crystallinity of the HA component in the composite. The compressive strength and modulus increased much more than that of HA-chitosan composite, due to the addition of PLLA, nano HA and networks.

(P 290) Novel PHB/PCL Scaffolds Produced by Melt Base Technologies.

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On this work, the natural origin polymer Polyhydroxybutyrate (PHB) was melt blended with synthetic aliphatic polyester Poly-ε-caprolactone (PCL). The ratio of PHB/PCL was varied from 25% to 75% by weight. These blends were further injection moulded and characterized. The thermal properties of the developed blends were accessed by differential scanning calorimetry (DSC). The effect of the ratio PHB/PCL on the mechanical properties (tensile modulus and tensile strength) was determined by tensile tests.

After being characterized, the blends were compression molded with salt (salt particles size: 250–500 mm) having two different levels of salt content (60% and 80%) by weight. By leaching the salt particles it was possible to produce porous scaffolds with distinct morphologies. The relationship between scaffold morphology and mechanical properties was evaluated using scanning electron microscopy (SEM), micro-computed tomography (mCT), compression testing and differential scanning calorimetry (DSC). The produced scaffolds are characterized by having different morphologies depending on the amount of NaCl used. Specimens with higher porosity level have a less organized pore structure but increased interconnectivity of the pores. A decrease in the salt particle content used to create the porosity caused in general an increase in the mechanical properties of the foams. Cytotoxicity tests were also carried out using standard tests namely MTS test with a 24h extraction period, showing the low level of cytotoxicity of the materials developed.

(P 291) Novel Technique for HA Particle Coating Within 3D Polymeric Structures

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Poly(L-lactide) (PLLA) and poly(ε-caprolactone) (PCL) with average molecular weights of 200 kDa and 40 kDa, respectively, were synthesized by ring opening polymerization of the respective dimer/monomer. Polymer blends were prepared in three different weight ratios, 25/75, 50/50 and 75/25 w/w. After a set of mechanical tests and *in vitro* degradation studies for 6 months, 50/50 PLLA/PCL blend was found optimal by considering mechanical properties, degradation rate and ease of handling. Scaffolds prepared by salt leaching/sc-CO_2 treatment technique. The disk shaped scaffolds were highly porous with interconnected pores having in the range of 250–355 microns. The scaffolds divided into the following three groups: (i) the first group was coated with collagen (from rat tail); (ii) the second group was coated with hydroxapatite (HA) particles (particle size: 25–40 microns); and (iii) the third group was the original. SEM and micro-CT images clearly showed that the HA particles were very homogeneously distributed all over the scaffold matrix. The pore volume decreased from 94 to 82% after HA coating. These scaffolds were investigated for their biocompatibility by performing a short term static cell culture with MG63 osteoblastic cell line. Confocal microscopy images showed better cell attachment and growth with the scaffolds having HA particles.

(P 292) Numerical Analyses of the Polymeric Scaffolds for Bone Tissue Engineering

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The favourable scaffold for bone tissue engineering should be characterized, among others, by high mechanical strength and 3D open porosity, which guarantee biocompatibility and suitable environment for tissue regeneration. Numerical modeling could be used as a tool for preclinical evaluation and optimization of the scaffold.

The objective of this study was: 1) to generate and validate numerical (FEM) models of PCL scaffolds produced by Fused Deposition Modeling (FDM), and 2) to use these models to predict and evaluate mechanical performance of the scaffolds for bone tissue engineering. Two types of scaffolds were analysed. They differed in lay-down of fibers (0/90° and 0/60/120° lay-down pattern). The ultimate goal of study was to created optimal scaffold model for simulations of new bone formation.

The numerical models of the scaffolds were generated using ABAQUS. The generated mesh was optimized by SED criterium and finally consisted of about 50,000 finite elements. Modeled scaffolds were undergone simulations of compression loading. Results of simulations showed consistence with experimental data. Using validated models, analysis of octahedral shear strains considered as important factors affecting bone cell differentiation was carried out.

Results showed that, in case of scaffold fabricated by FDM, delamination of fibers is a significant threat. One of factors, which could be important for durability of filament joints is scaffold resistance for buckling. For example, scaffolds with 0/90° lay-down pattern have higher strength for buckling and higher stiffness (about 40%) than those with 0/60/120° lay-down pattern. Scaffolds with 0/90° lay-down pattern have also higher yield strength.

(P 293) Numerical Simulation and Analysis of Fluid Field in a Rotating Bioreactor on Three-Dimensional Fabrication of Tissue-Engineered Bones

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The two-dimensional flow field of rotating wall vessel bioreactor (RWVB) was simulated and calculated with Fluent software. The distributions of dynamic and total pressure, shear stress and velocity within the culture chamber were calculated when the engineered bone tissues with different size were installed in different radial positions in the vessel. The results showed that the dynamic pressure, velocity and shear stress around the surface of cell-scaffold construct at different locations in RWVB were changed periodically, which could result in the periodical stress stimulation for the cultured objects. Additionally, the shear stress in the bioreactor is relatively low and the fluid velocities are uniform. The inner culture environment is capable of fabricating engineered bone tissues. The above conclusions could be further confirmed by relative experimental results, such as the morphology of the harvested bone tissues observed under optical microscope and scanning electron microscope (SEM), alizarin red staining to visualize the newly formed bone, Acridine orange/ethidium bromide (AO/EB) double fluorescence staining used to analyze cell activity, etc. It is concluded that RWVB is an ideal culture system for tissue engineering applications.

(P 294) on the Accuracy of Techniques to Determine Cell Viability in 3D Tissues or Scaffolds

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Introduction: Cell viability is a key parameter for 3D tissue engineering. Several methods are commonly used, but their accuracy has not been evaluated. Here, we compare three methods, i) lactate dehydrogenase staining on cryosections (LDH), ii) Calcein AM / ethidium homodimer-1 (CaAM/EthH) staining and subsequent confocal laser scanning, and iii) digestion with trypsin and subsequent staining with Trypan Blue (TB) and counting with haemocytometer.

Material and Methods: Chondrocytes from bovine articular cartilage were expanded in monolayer under standard culturing conditions. Two cell populations, living and dead, were then generated in cell suspension. Dead cells were killed by acidic shock and enzyme activity was destroyed by heat treatment at 60°C. Five different cell mixtures with 0%, 25%, 50%, 75% and of 100% living cells were prepared, measured for cell viability with Trypan Blue (Premix) and seeded in fibrin carriers (7×10^6 cells/ml, $n = 5$ for each method). For cell counting, custom-written macros were used for LDH (KS400, Zeiss, Germany) and for CaAM/EthH (imageJ, NIH, USA) images.

Results and Conclusions: For assessment of cell viability percentage, only the CaAM/EthH method was not significantly different from the Premix (Fisher's PLSD, $p = 0.25$). However it was the least accurate for assessment of absolute number of living and dead cells. For absolute number of living cells, TB was the only method that was not significant different from Premix ($p = 0.24$). Similarly, for absolute number of dead cells, only the LDH method agreed with Premix values ($p = 0.37$).

(P 295) On the Dynamic Characterization of Collagen Gel Scaffolds for the Tissue Engineering of Intervertebral Disc (IVD)

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The tissue engineering method has potential for the regeneration of IVD and collagen gels show promise as scaffolds for this Nucleus Pulposus (NP) constructs. The mechanical properties and structure of such gels play an important role in the regulation of cell growth, ECM secretion and consequent tissue formation. There are, however many uncertainties in the gelling process for collagen gels and the resulting dynamic characteristics are still unclear. In this study different processing conditions including formulation and pH were investigated for a typical collagen. The viscoelastic performance, dynamic mechanical properties and micro-structure physical properties of the resulting gels were determined with the aim of developing an appropriate scaffold for IVD NP tissue engineering that could be consistently processed.

During the early stages of the sample conditioning process (i.e. ramping from 4°C to 37°C), viscous behaviours dominated. Subsequently the elastic properties quickly dominate the gel behaviour, at 37°C $G' > G''$. Further incubation results in steady increase of strength. CryoSEM examination showed that collagen formed fibres with a diameter of about 1.2 um and an inter-connected porous structure with average pore size of around 15 um. Examination of the fibres by TEM revealed the banding pattern typical of collagen indicating that it had not been denatured in the gelling process. DMA testing results indicated that property-consistent collagen gels could be only fabricated under controlled gelling conditions. Further work is necessary to determine the suitability of candidate gels for tissue culture using cell types appropriate for IVD NP.

(P 296) Optical Characterization of Fibrin and Fibrin-Agarose Corneal Constructs: the Coefficients of Absorption, Scattering and Extinction

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Introduction: Characterization of the optical properties of biomaterials used for construction of corneal substitutes by tissue engineering is very important in order to ensure the appropriate

functionality of the artificial corneas. As the main filter to visible and UV light, scattering (S), absorption (K) and extinction (S + K) coefficients of bioengineered human corneas should be comparable to normal native corneas.

Methods: Partial bioengineered human corneas were developed in the laboratory using fibrin-agarose scaffolds with human keratocytes immersed within. After 28 days in culture using specific culture media, the optical properties of the artificial corneal tissues were analyzed by determining the spectral radiance of these tissues using white and black backgrounds with a spectroradiometer PR-704 (Photoresearch) under geometry CIE 45°d illuminating conditions. Optical constants, including S, K and S + K coefficients, were calculated from the spectral reflectance data using Kubelka-Munk's ecuations within the visible spectrum (400–700 nm). The statistical analysis was computed by using SPSS 15.0 software.

Results and Conclusions: Our results revealed that fibrin-agarose corneal constructs showed high values of S and K, with a direct correlation between the incident wavelength used and the levels of S, K and S + K. Differences were particularly significant for the case of K.

Supported by PI-0132/2007 and P06-CTS-2191 from Junta de Andalucia.

(P 297) Optimization of a Human Osteoblastic-Endothelial Co-Culture System

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Angiogenesis, the formation of new capillaries from pre-existing vessels, is a tightly regulated process that is required for adequate bone growth and regeneration. Established research reports that fracture healing and ectopic bone formation can be disrupted by the direct or indirect inhibition of angiogenesis, while in other studies, the induction of vascular nutrition increases bone formation, in a significant way.

Several *in vitro* methodologies have been developed in order to further comprehend endothelial-osteoblastic cellular intercommunication that is supposed to combine soluble factors and junctional communications at the same time.

In this work, we aim to optimize the establishment and development of *in vitro* co-culture systems of osteoblastic-like MG63 cells and human umbilical vein endothelial cells (HUVECs).

HUVECs were isolated form fresh umbilical cords and expanded until desired confluence. Co-cultures were assayed after simultaneously culture of HUVECs (3rd subculture) and MG63 cells at 10:1, 5:1, 2:1 and 1:1 proportion, respectively.

Different proportions of cultured cells led to a distinct pattern regarding cellular organization. Scanning electron microscopy and confocal laser scanning microscopy (tracking staining of PECAM-1—a specific endothelial cell marker—and MG63-cell tracker stained cells) allowed to confirm that cellular interaction, endothelial cell viability and phenotypic expression are related to the initial cell proportion. In addition, cell proportion greatly determined the sustainability of the co-culture system.

This research offers the possibility to delineate optimized tissue engineering constructs for bone regeneration based on the interaction between osteoblastic and endothelial cells.

(P 298) Optimization of Chitosan-Based Composite and Bilayered Scaffolds Produced by Particles Aggregation for Osteochondral Tissue Engineering: Influence of Hydroxylapatite

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Osteochondral tissue engineering presents a challenge to the present research due to requirements' combination of both bone and cartilage tissue engineering. In the present study, bilayered chitosan scaffolds are proposed based in the optimization of polymeric and composite scaffolds. μ-CT was carried out for accurate morphometric characterization quantifying porosity, interconnectivity, ceramic content, particles and pores size. The results showed that the developed scaffolds are highly interconnected and present ideal pore size range, being morphometrically adequate for the proposed applications. DMA shown that scaffolds are mechanically stable in wet state under dynamic compression solicitation. The obtained elastic modulus at 1 Hz frequency was 4.21 ± 1.04 MPa, 7.98 ± 1.77 MPa and 6.26 ± 1.04 MPa for polymeric, composite and bilayered scaffolds, respectively. In composite scaffolds, surprisingly it was observed a cytotoxicity behaviour when unsintered hydroxylapatite was used. This study reports the investigation that was conducted to overcome and explain this behaviour. Cytotoxicity was evaluated by MTS with L929 fibroblast cell line for different conditions and ICP was carried out to confirm the influence of several elements. Sintered hydroxylapatite was further used showing no cytotoxicity. Bioactivity studies with simulated body fluid (SBF) and simulated synovial fluid (SSF) were conducted to assure that the polymeric component for chondrogenic part would not mineralized as confirmed by SEM, ICP and EDS for different immersion periods. It is concluded that chitosan-based bilayered scaffolds produced by particle aggregation could serve as alternative, biocompatible, and safe biodegradable scaffolds for osteochondral tissue engineering applications.

Acknowledgements: FCT (SFRH/BD/11155/2002) and EU funded projects HIPPOCRATES and EXPERTISSUES.

(P 299) Optimization of *In-Vitro* Expansion of Mesenchymal Stem Cells Isolated from Human Umbilical Cord

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Nowadays acute demand of human mesenchymal stem cells (hMSC) exists in the areas of regenerative medicine and cell based therapies. The extraction of hMSC from human bone marrow, the most commonly used source for these stem cells, is often less effective because of the low frequency of hMSC (~0.01%) in marrow aspirates. Investigations over the last years show that hMSC can be highly successfully isolated also from extra-embryonic tissues such as umbilical cord (UC), where the frequency of MSC is approx. 30 times higher.

We optimized culture conditions for extensive expansion of hMSC obtained from UC perivascular tissue or whole UC. Cells were seeded in culture flasks in different densities varying from 62.5 to 4000 cells/cm^2 and cultured in four slightly different alfaMEM containing 10% human serum and gentamycin. Cell growth and vitality, consumption of glucose, glutamine and essential amino acids as well as cell surface epitopes were analyzed by microscopic, fluorescencespectroscopic, HPLC and flow cytometric methods.

Cells were highly positive for typical stem cell surface markers CD73, CD90, CD105 and CD44. They exhibited high proliferative activity particularly supported by alfaMEM containing 2 mM L-glutamine or alfaMEM additionally supplemented with osteogenic agents (dexamethasone, ascorbate-2-phosphate, and beta-glycerophosphate). Cell progeny without intrinsic loss of viability was possible over 10 passages using initial cell seeding density of 4000 cells/cm^2. However cell seeding in lower densities (250, 500 cells/cm^2) on larger cultivation area allows to avoid frequent enzymatic disruption of cell-cell contacts and therefore displays a better expansion strategy.

(P 300) Optimization of Vitrification Protocols with CPA (D25) for Hematopoietic Stem/Progenitor Cells Derived from Human Umbilical Cord Blood

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Hematopoietic stem cells call for feasible cryopreservation methods before the clinical transplantation. In this work, according to the study on the vitrification characteristics of CPA (D25), the stepwise loading and removing processes were designed. Also, different equilibrium time and various concentrations of trehalose added in the elution process were investigated to weaken the osmotic and toxic injuries. Mononuclear cells (MNCs, rich in HSPCs) were cryopreserved using the vitrification protocol, and the conventional method (10%DMSO, 1°C/min cooling rate) was conducted as the control. The results showed that CPA (D25) possesses satisfactory vitrification ability when the cooling rate is higher than 30°C/min and with the increase of the warming rate, the de-vitrification time could be restrained effectively. The program of five-step addition with the interval of 90s and five-step elution with the interval of 60s respectively at each intermediate step attained the optimal HSPCs viability, around 83%, and 5% increase of cell viability was obtained by introducing 0.5M trehalose into the elution process. The recovery of MNCs after vitrification was 81.5%, higher than 74.7% of the conventional cooling method. The recovery of CD34+ cells for the vitrification sample was 52.6% compared with 51.2% of the control. It was concluded that CPA (D25) could achieve vitrification with the optimized loading and removing process at an affordable cooling rate, the cell viability and the recovery of CD34+ cells were clinical acceptable, higher than that of the conventional method, and this programmed vitrification protocol is much more convenient and economical.

(P 301) Optimum Ratio of Osteoblasts and HSCs Co-Cultured in Hypoxic Condition

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Evidence indicates that osteoblasts are crucial components of the haematopoietic microenvironments in adult bone marrow and can promote haematopoietic stem cells (HSCs) expansion both *in vivo* and *in vitro* culture. In this study, we constructed a novel hypoxic co-culture system and compared the expansion results under different quantity of human osteoblasts to obtain better HSCs expansion parameters *in vitro* culture. In the condition of 5% O_2, 20% CO_2, gelatin-alginate-chitosan (GAC) bio-microcapsules that encapsulated with osteoblasts were implanted to serum-free IMDM medium, and then cord blood mononuclear cells were inoculated to this culture system. The constituent ratio of osteoblasts and HSCs were respectively 1:2(group A), 1:1(group B), 2:1(group C), and non-osteoblasts group as control(group D). The initial HSCs density was 2×10^5 and the medium was half exchanged when the density was higher than 2×10^6. After 7 days culture, the number of total nucleated cells increased by 43.4 ± 3.3-fold in group A, by 61.6 ± 3.7-fold in group B, by 108.8 ± 4.2-fold in group C, and by 2.5 ± 0.51-fold in group D, compared with those before culture. The expansion folds of CD34+ cells were 51.7 ± 3.9, 104.2 ± 6.3, 171.6 ± 6.6, 0.77 ± 0.16, respectively. The expansion of BFU-E, which was similar among groups A, B, and C, was about 5 times. The percentage increasing of CFU-GM and CFU-GEMM of group C were obviously higher than that of group A and B. In hypoxic condition,osteoblasts can significantly stimulate HSCs proliferation and maintain cell growth *in vitro* for a longer time. The optimum ratio of osteoblasts-HSCs numbers were 2:1 for *ex vivo* co-culture.

(P 302) Origin of Cells in Scaffold Based Cartilage Defect Treatment

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Objectives: Partial thickness defects of articular cartilage are known not to heal spontaneously. The main reason is the low number of cells manifesting in the defect. The present study intends to determine the origin of the invading cells and how these cells participate in defect-filling in the presence of a scaffold.

Material and Methods: A collagen type I gel (CaReS®), collagen type I/III membrane (Biogide®), and a hyaluronan scaffold (Hyaff®) were implanted into the lateral facette of the femoropatellar joint of two horses. The repair tissue was taken 1.5 years after implantation and analysed by histochemical, immunohistochemical, ultrastructural and element analytical (EDAX) methods.

Results: Cell bearing repair tissue was found in all defects. It was largely fibrous but showed some differentiation at the interface regions. In the bordering cartilage, chondrocytes were found to form cell processes and penetrate the extracellular matrix. The matrix showed proteoglycan-depletion, especially around the chondrons. Indications suggest that also cells from the synovial space invaded the scaffold.

Conclusions: The present data imply that cells invading from the surrounding tissue are sufficient to fill a cartilage defect and form repair tissue if a scaffold is available for adhesion and distribution in the defect-area. Especially chondrocytes have a supportive effect for the integration of scaffold and repair tissue into the defect and intensify the contact between both. Based on our results we suggest that also in cell-loaded scaffold implants invading cells have a positive, supplementary effect on cartilage scaffold integration.

(P 303) Osteoblast Attachment Studied using Microtextured Thin Film Biomaterial Surfaces

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In this study, we investigated the effect of surface microtexturing with different thin films to adhesion and growth of Saos-2 cells. Microtextured surfaces were fabricated onto silicon wafers by using UV-lithography and physical vapour deposition of amorphous diamond, titanium and tantalum. The size of square or circular features were 5, 25 and 125 μm for diameters. The qualitative aspects and morphological details of the cellular attachment were studied using ESEM. Confocal laser scanning microscopy was used to study cell orientation and focal adhesion points. Specific qualitative studies show how the cells attach to the thin film features. Only few cells can be seen attach to the silicon. We can summarize the results from all tests as follows:1) Biocompatibility of these materials can be assessed as a pairwise comparison between the biomaterial and a reference material to construct a biocompatibility ranking series: Ti>AD>Ta>Si, 2) the size of the features affects the number of attachment cells to each feature, 3) the distance between features affects the dividedness or undividedness of the cell population, 4) the shape of the features direct the cells to grow closely around biocompatible features. UV-lithography with thin film techniques provides a convenient, effective and low-cost method for the formation different microenvironments for studying how the cells attach and adhere to a metallic implant and how we can guide cellular behaviour in various microdomains of medical devices. This functionalization allows development of intelligent implants, which in their versatile behaviour correspond to the complex requirements of the human body.

(P 304) Osteoblastic Behaviour of Serially-Passaged Adipose Tissue-Derived Mesenchymal Stem Cells

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Mesenchymal stem cells derived from adipose tissue have the potential to differentiate along different lineages, including the osteoblast phenotype. This characteristic makes them prospective useful for tissue engineering applications regarding the repair and regeneration of bone defects. The aim of this work is to evaluate the expansion and the osteogenic potential of serially-passaged adipose tissue-derived mesenchymal stem cells in different culture conditions. For comparison, stem cells derived from bone marrow were evaluated in parallel.

Stem cells derived from subcutaneous adipose tissue and bone marrow were obtained from 3-months-old Wistar rats and cultured in alpha-minimum essential medium containing 10% fetal bovine serum, antibiotics and 50 ug/ml ascorbic acid, both in the absence and in the presence of 10 nM dexamethasone. At 70–80% confluence, cells were subcultured in a combination of non-induced and osteogenic-induced conditions. Cultures were assessed for cell viability/proliferation, cell-cycle analysis and osteoblastic differentiation.

Results show that mesenchymal stem cells derived from rat adipose tissue presented a high proliferation rate over several passages. Dexamethasone, a classic osteoblastic inducer, caused a significant reduction in cell growth rate with a simultaneous increase in the expression of alkaline phosphatase. In mineralising-favouring conditions, i.e., cultures supplemented with Na-beta-glycerophosphate, mineralisation of the extracellular matrix was observed in selected conditions. Comparatively, mesenchymal stem cells derived from rat bone marrow presented a lower expansion potential but a higher expression of osteoblastic differentiation markers. In conclusion, the suitability of adipose tissue-derived stem cells regarding the osteogenic potential in bone regeneration approaches needs to be further investigated.

(P 305) Patient's Age as an Important Factor Affecting Number of Articular Cartilage Chondrocytes

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Background: Articular cartilage defects are common problem correlated with age. They are characterised by limited regenerative capacity of articular chondrocytes. Cultured autologous chondrocytes have been proposed as a source to repair of cartilage defects. The aim of this study was to check differences between number of isolated cells depending on patient's age.

Methods: Samples of articular cartilage ($n = 7$) were obtained from non-bearing surface of the joint from patients in different age. The first of examined groups were patients between 18–26 years old ($n = 3$). The second group were patients between 48–54 years old ($n = 3$). The last group consisted of one 73-year old patient. The samples were minced and digested with colleganase P and collagenase II at 37°C for 18 h to isolate chondrocytes. Total cell number was quantified using haemocytometer and cell viability was detrmined by trypan blue exclusion test.

Results: Number of isolated chondrocytes from young patients ($n = 3$) was $1{,}55 \times 10^5 \pm 3{,}3 \times 10^5$, from the second group was $1{,}67 \times 10^4 \pm 7{,}7 \times 10^4$. Cells obtained from 73-year old patient was remarkably lower—$2{,}0 \times 10^3$.

Conclusions: Age of the patient significantly affects on number of isolated living chondrocytes. Chondrocytes with age begin to lose their ability to maintain articular cartilage well. Further studies should be focused on graft function constructed from autologous chondrocytes obtain from patients in different ages.

(P 306) Performance of a Spinner Like Bioreactor for Dermal and Oral Connective Engineered Tissue

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The high cost and the lack of regulations for importing registered skin tissue-engineered products into Colombia limits their availability to low-income patients. In the case of oral mucosa, tissue–engineered replacements are not even commercially available yet. Therefore, the ability to generate reasonably priced skin and oral replacements would be desirable. To this end, our group constructed a spinner-like bioreactor for bank-level production of dermal and oral connective tissue equivalents.

The bioreactor was designed with a 2 L maximum capacity and a scaffold supporting system, consisting of seven removable Teflon dishes attached to four removable Teflon columns suspended from the bioreactor's lid. This removal system allows the amount of tissue equivalent produced to be controlled. A heat exchanger and CO_2 injection systems permit the bioreactor to operate automatically. To characterize the hydrodynamic properties of the bioreactor, the mixing time (MT) and the residence time distribution (RTD) were measured at different bioreactor configurations (number of dishes and columns), and at different stirring speeds (60, 80 and 100 r.p.m.). It was found that, irrespective of the configuration and the stirring speed used, it behaved as a well-mixed bioreactor. Histological analysis was performed on fibroblast-seeded collagen I scaffolds cultured in the bioreactor and sampled every other day, for two weeks. The results showed scaffold fibroblast adhesion and formation of artificial tissue with a more uniform distribution of cells, as compared with tissue obtained under static culturing conditions.

(P 307) Periodontal Regeneration of Maxilla Alveolar Bony Defect Repaired with BMP-2 Gene Engineered Autologus Bone Marrow Mesenchymal Stem Cells

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Introduction: The need of periodontal and alveolar bone regeneration was increased in modern clinical dental therapy. Recently, the application of bone morphogenetic proteins (BMPs) to the periodontal defect presented limited regenerative ability of such an approach with the establishment of abnormal periodontal relationship and complications such as ankylosis and root resorption. Short half life of rhBMP-2 and the need supraphysiological concentration also demonstrated the shortage of protein regeneration therapy. The purpose was to evaluate the regeneration of periodontal apparatus in critical maxilla alveolar bony defects by BMP-2 gene engineered autologus bone marrow mesenchymal stem cells (MSCs).

Materials and Methods: Twelve critical maxillary periodontal defects were surgically created over the premolar area combined with root denuded at least 2×1 cm^2 below cementoenamel junction (CEJ) in nine matured male miniature swine. Bilateral maxillary defects were randomly assigned into 2 groups: BMP-2 gene engineered MSCs/polymer (BMP-2 group), and MSCs/polymer as control. Histological examination was performed at the end of study to exam the microscopic healing and relationships between bone, periodontal ligaments, cementum, and tooth surfaces. Three-dimension computerized tomography was utilized to calculate the total volume of bone regenerated during experiments.

Results: Our data demonstrated the regeneration of periodontal apparatus was significant better in BMP-2 group. New cementum with Sharpey's fiber was observed on the instrumented denuded root surfaces in BMP-2 group. Larger amounts of bone were regenerated in BMP-2 group than control group after 24 weeks implantation. This approach is suitable for periodontal regeneration and may achieve healthy periodontal relationship.

(P 308) Phosphorylation Efficiency of Chitosan at Different Times and Temperatures for Bone Graft Substitute

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Research in bone implants has been focused to mimic the natural process of regeneration by using substitutes possessing characteristics of osteo-induction, integration, conduction and mechanical properties similar to that of natural bone.

Chitosan is a plausible alternative because it is non toxic, non antigenic, biocompatible, biodegradable and promotes normal cell behaviour.

Functionalized chitin and chitosan with phosphate groups has shown to promote the calcium ions binding, which may then induce the formation of a calcium phosphate layer promoting the osteoconduction of polymer-based implants.

Different authors have used Sakagushi (1981) method of phosphorylation at different temperatures (70 to 160°C) for 1 h for of reaction, without addressing why this time was selected.

Here we used 1 to 48 h of reaction at a constant temperature of 70°C. Phophorylation efficiency was evaluated by FT-IR showing maximum substitution of phosphate groups, as reveled by the appearance of P-O stretching bands at 1050 and 603 cm^{-1} from the PO_4 groups, at 3 hours. These results were confirmed by the use of polycationic titrations (pDADMAC).

These results can be explained by a saturation of the number of phosphate groups per surface area in the first 3 hours and/or by a change in the tridimensional structure of the chitosan molecules hiding the groups potentially capable to be functionalized with phosphate group after 3 h. Partially supported by FONDAP 11980002 and FONDECYT 1080185.

(P 309) PLA/PGA–Based Polyesters Filled with Calcium Carbonate May Serve as Cell Scaffolds with Controlled Resorption *In Vitro*

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The ideal tissue engineered product (TEP) should become fully replaced by host tissue; however, there are problems with controlling scaffold resorption after implantation. Thus, we are working on a TEP with scaffold resorption being highly advanced before implantation. This investigation is the first step toward this goal. 8 types of PLA/PGA–based polyesters were used both with and without a $CaCO_3$ filler (added to neutralise acidic hydrolysis products). The materials were investigated in MG63 osteoblast culture. Cell and material morphology was observed in optical and electron microscopes. Cell viability (XTT) was determined after 4 and 11 days. The biomaterials' resorption was assessed by Size Exclusion Chromatography (SEC).

As found, cell viability was diminished after 4 days on all materials but PGA/PLA. This is probably because of the overrun local lethal acids concentration due to the materials' resorption, shown to occur during the experiment (SEC results). After 11 days, cell viability drop (comparing to the control) was almost the same as earlier on samples with $CaCO_3$, but much higher on polymers alone. The alkalising benefit from $CaCO_3$ was not fully achieved probably because the filler was hidden under the material surface, additionally worsening the surface topography (SEM) favoured by cells. However, resorption was less advanced in samples with $CaCO_3$ (SEC), which probably positively influenced cell viability. The investigated materials are still not a perfect support for cells; however, the described system is promising for further research on resorbable scaffolds—base for tissue created *in vitro*.

The work supported by Grant 3T08E06930

(P 310) Platelet Lysate as Substitute for Fetal Calf Serum in the Culture of Stem Cells Derived from Adipose Tissue and Amniotic Membrane

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For cell expansion, supplementing culture media with fetal calf serum (FCS) is still the gold standard although FCS bears a potential risk for carrying diseases and eliciting immunereactions. Therefore, an alternative culture supplement without animal components would be advantageous. Thrombocytes are regularly substituted in trauma and oncologic patients with insufficient blood coagulation. They can be stored up to 5 days before they have to be discarded. Instead of wasting this precious raw material platelet lysate (PL) can be produced thereof containing growth factors at high concentrations.

Three batches of PL each consisting of 36 thrombocyte concentrates were produced and selected growth factors were determined by ELISA. PL was then used in comparison to FCS as supplement for expanding and differentiating human adipose-derived stem cells, human amniotic mesenchymal and epithelial cells into the adipogenic and osteogenic lineage. Cultures were characterized for their surface marker expression profile, proliferation capacity and their immunomodulatory properties. Adipogenic differentiation was demonstrated by Oilred O staining, osteogenic differentiation was detected by von Kossa and Alizarin Red staining, alkaline phosphatase activity and calcium measurement.

Cells cultured in presence of PL display a higher proliferation potential and *in vitro* life span compared to cells cultured under FCS conditions. PL preserved the differentiation capacity of the cells and did neither alter the surface marker expression profile nor the immunomodulatory properties of the cells investigated and should therefore be considered as substitute for FCS in cell culture for cell-based therapies.

Acknowledgments: HIPPOCRATES (NMP3-CT-2003-505758), Lorenz Boehler Fonds, EXPERTISSUES (NMP3-CT-2004-500283).

(P 311) PLGA Based BMP-2 Nanocapsules for Bone Tissue Engineering

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The objective of this study was to use poly(lactic acid-co-glycolic acid)-based nano/microcapsules in the encapsulation of BMP-2 for the purpose of its controlled release with the bone tissue engineering perspective. The structure, encapsulation efficiency, release kinetics and degradation behaviour of the PLGA particles were studied. The particles were then implanted s.c. in Wistar rats to study their biocompatibility and effect of BMP-2 on the peri-implant tissue. Five week old Wistar rats were used for the implantation of BMP-2 loaded and empty capsules, and analysis were done at 3, 10, 30 and 40 days.

On day 3, the BMP-2 loaded nanocapsules were found to be covered by a fibrin-rich peri-implant tissue. On day 10, the acute inflammatory response declined. The number of the macrophages and foreign body giant cells containing partly confluenced nanocapsule-particles increased. At the same time, extracellularly located particles could be observed. Together with clearly detectable

blood vessels in the margin of the capsules, multinucleated tartrate-resistant acid phosphatase (TRAP)-positive cells appeared adjacent to the biomaterial. On day 30, the increased vascularization of the biomaterial continued together with the decrease of the amount of the TRAP-positive cells. Until day 40, the particles had a clear round shape and were localized in direct contact to the multinucleated giant cells. No sign of activated fibroblasts and fibrosis were observed. The results of this preliminary data are encouraging and the PLGA nanocapsules appear to have a potential as a functional drug delivery system for various agents needed for successful tissue engineering applications.

(P 312) Poly-L-Lactic Acid/Hydroxyapatite Electrospun Nanocomposites Induce Chondrogenic Differentiation of Mesenchymal Stem Cell

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The use of mesenchymal stem cells (MSCs) for cartilage and bone tissue engineering needs to be supported by scaffolds that may release stimuli for modulate cell activity.

The objective of this study was to asses if MSC undergo differentiation when cultured upon a membrane of nanofibers of poly-L-lactic acid loaded with hydroxyapatite nanoparticles (PLLA/HAp).

The PLLA/HAp nanocomposite was prepared by electrospinning. Membranes microstructure was evaluated by SEM. MSCs were seeded on PLLA/HAp membranes by standard static seeding and cultured either in basal medium or Chondrogenic Differentiation Medium. Cell attachment and engraftment was assessed 3 days after seeding and MSC differentiation was evaluated by immunostaining for CD29, SOX-9 and Aggrecan under a confocal microscope after 14 days.

PLLA/HAp membrane obtained was composed by fibers (average diameter of 7 μm) with nano-dispersed hydroxyapatite aggregates (average diameter of 0.3 μm). 3 days after seeding, MSCs were well adhered on the PLLA/HAp fibers with a spindled shape. After 14 days of culture all MSCs were positive for SOX-9 in both basal and chondrogenic media groups. Aggrecan was present around the cells. MSCs were either CD29 positive or negative.

We demonstrated that PLLA/HAp nanocomposites are able to induce differentiation of MSCs in chondrocyte-like cells. Since HAp has osteoinductive properties, the chondrogenic phenotype acquired by the MSCs may be either stable or an intermediate stage toward enchondral ossification. The presence of CD29 and SOX-9 double positive cells indicate intermediate differentiation phases.

This nanocomposite could be a susceptible scaffold for bone or cartilage tissue engineering using undifferentiated MSCs.

(P 313) Polymeric Nanocomposites in Bone Tissue Regeneration

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Various aspects of medicine such as diagnostics, therapeutics, controlled drug or gene delivery systems and tissue engineering requires application of nanotechnology and specific nanodevices and nanomaterials. Ceramic nanoparticles such as a silica, wollastonite, carbon nanotubes are potential compatible components of biomaterials The unique combination of mechanical, physical and chemical properties of nanomaterials based on polymeric composites make them attractive not only as reinforcement constituent of various matrix (they have batter mechanical properties than pure polymers), but also as a physicochemical surface modification (surface free energy, nanoroughness, microstructures). The presence of nanoparticles in polymer matrix improve an interaction with proteins and subsequently with cells.

We evaluated adult human bone marrow-derived mesenchymal stem cell response to different polymers matrix (PSU, PL(L/DL)A, PCL) modified with $nSiO_2$, $nCaSiO_3$, CNT nanoparticles. Differing concentration of the silica (0,5% wt, 2% wt) in the polymer matrix increased the Young's modulus three fold compared to pure PL(L/DL)A (poly(L-lactid-co-D,L-lactid). Both ceramic particles and carbon nanotubes influenced surface parameters such as wettability. Nanocomposites with $nCaSiO_3$ appeared hydrophilic compared to pure polymer polycaprolactone (PCL). All nanopartices also increased the roughness of the composite surface in nano- and microscale.

Bone marrow-derived adult human mesenchymal stem cells proliferated significantly better on composites containing 0,5% and 1% single wall carbon nanotubes, compared to the pure polymer (polysulfone) sample. We suggest that all nanoadditives (mainly carbon nanotubes) into polymer coating impoves attachment and growth of adult human progenitor cells.

(P 314) Porous Polymeric Scaffolds Incorporating Ibuprofen Prepared in Supercritical CO_2 for Tissue Engineering of Bone and Cartilage

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The preparation of porous polymeric scaffolds for tissue engineering by using supercritical carbon dioxide ($SCCO_2$) technology is a clean process where the use of organic solvents is avoided. Porous systems are obtained by polymer saturation with CO_2 followed by depressurization that induces pores nucleation.The aim of this work is to obtain, in one step process using $SCCO_2$ technology, porous polymeric systems from samples of poly (methyl methacrylate)-poly (lactic acid) (PMMA-PLA) blends, that also have incorporated ibuprofen. The systems were characterized in order to find the appropriate conditions to obtain scaffolds with appropriate porosity and charge of ibuprofen. Different formulations with 10, 20 and 30%-wt of PLA were prepared by compression moulding and some of them included 5 or 10%-wt of ibuprofen. Samples were placed in the $SCCO_2$ reactor at different conditions of pressure (160–260 bars), temperature (40–60°C) and time, followed by fast depressurization of the system after desired time (2–41h). Porous samples were characterized by NMR spectroscopy, DSC, ESEM and

mercury picnometry. The results showed that samples exposed to high pressures (260 bar) exhibited porous interconnections, due to a better CO_2 diffusion through the polymer matrix, giving a porosity from 65 to 200 μm. When incorporating ibuprofen (soluble in SC-CO_2), drug retention was obtained by lowering the experimental time and using different supercritical conditions, that is at 200 bar and 60° C during 24h. Drug release and biocompatibility studies will be presented, and the results obtained showed a very interesting behaviour and good biocompatibility.

(P 315) Preparation of Small Diameter Vascular Grafts by Electrospinning of Biodegradable/Bioerodible Polymers

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Conventional vascular prostheses are functional for replacement of medium and large calibre vessels (diameter > 5 mm) while they fail when employed as small diameter grafts, because of thrombogenicity and compliance mismatch. Recently, tissue engineering has emerged as a promising approach to address the shortcomings of current therapies. Several opportunities for functional scaffolds production are arising due to the development of different techniques. In particular, electrospinning allows for the obtainment of non-woven fibrous structures which mimic the morphology of the extracellular matrix. The research project we are involved in, aims at the production of arterial small-diameter polymer grafts through electrospinning, as possible candidates in vascular tissue engineering. Commercial polymers and novel multiblock copolymers were electrospun and a roto-translating cylindrical collector for fibrous tubes production (4 mm diameter) was specifically designed. Using this high performance device, provided with a PLC-based controller and a software application for remote control, different directions and degrees of alignment of the electrospun fibers were achieved. Evaluation of the constructs, with regards to morphology, chemical and physical characteristics, mechanical properties, cytocompatibility and degradation rate is currently being performed. Future plans include cell response evaluation of the tissue engineered constructs cultured in a bioreactor designed to fulfil the specific needs for this type of application. Attention will be devoted to mechanical properties comparison among constructs with various degrees and directions of fibre alignment and to the different cell behaviour that fibre orientation may cause. The fibers will also be loaded with growth factors and drugs influencing cell behavior and host immune response.

(P 316) Preparation of Temperature-Responsive Matrices with Osteoinductive Behaviour Combining an Elastin-Like Polymer and Chitosan

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In this work two natural-based polymers were combined in order to produce new matrices intended to be used in drug delivery or as matrices for tissue engineering applications: chitosan, a poly (aminosaccharide) obtained by chitin's partial alkaline deacetylation and an elastin-like polymer, ELP, obtained by the bioproduction of genetically modified E. coli. This polymer was designed to be biocompatible and to contain an osteoinductive sequence, besides exhibiting a smart behaviour towards temperature.

The yield of the bioproduction process was assessed using electrophoresis, which was also used to assess the polymer purity upon the purification process. The general characterization of the ELP was done with Differential Scanning Calorimetry, to determine polymer Inverse Temperature Transition at different pH, and mass spectroscopy, to confirm the primary sequence of the recombinant protein.

The mechanical properties of the developed materials were tested using dynamic mechanical analysis. This technique permitted to assess the temperature dependence of their viscoelastic properties while the samples were immersed in simulated physiological solutions. *In vitro* citotoxicity and bioactivity of matrices were also tested.

(P 317) Preparation, Detection and Controlled Release of PLGA Microspheres-Based Scaffolds Embedded with BSA

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Biomaterial scaffolds play an important role in tissue engineering by providing physical and biochemical support for kinds of stem cells and progenitor cells. Bovine serum albumin (BSA) is a large globular protein with a good essential amino acid profile. It has been well characterized and the physical properties of this protein are well known, Isolated BSA has been reported to be a very functional protein. In this study, The microspheres with diameters of almost one hundrand micrometers were prepared and optimized by using bovine serum albumin (BSA) as the model protein and poly lacticoglycolic acid (PLGA) as the exoperidium materials. The protein microspheres were prepared by applying the multiple emulsion solvent volatilixation method, the protein content and the controlled release from the microspheres were measured with the method of Bicinchoninic acid (BCA). Moreover, the concentration of BSA, the rotation speed of stirrer, and the effect of the ratio of span 80 and tween 80 on the envelopped rate, particle diameter, carrying dosage and releasing amount of the microspheres were all investigated. The results showed that the optimal operated parameters consisted of the ppropriate suitable concentration of BSA, not so fast stirring speed and the ratio of span 80 and tween 80 were about 10:1. It is concluded that by regulating the above paramters, we could obtain the suitable microspheres with qualified sizes, higher carrying dosage and envelopped rate and excellent released functions.

(P 318) Pretreatment with Protocatechuic Acid Increases Neurons from Cultured Neural Stem/Progenitor Cells

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Neural stem/progenitor cells (NS/PCs) are capable of self-renewal and can generate both neurons and glia. Protocatechuic acid (PCA), a phenolic compound isolated from the kernels of Alpinia (A.) oxyphylla, plays crucial roles in the proliferation and neuroprotection of cultured NS/PCs in our previous study. However, whether PCA modulates the differentiation effects of NS/PCs needs to be elucidated. In this study, we examined the effects of PCA on neuronal differentiation of NS/PCs in the different culture manners. NS/PCs obtained from 13.5-day-old rat embryos were propagated as neurospheres and cultured under differential conditions with or without PCA over three passages. Such cells allowed to be immobilized, adhered and spread in three-dimensional (3D) type I collagen gels incorporating fibronectin (FN) or be adhered and grew into monolayer on the FN coated planar dishes. On both culture manners, the percentages of cells that adopted neuronal phenotypes in the PCA pretreated groups significantly increased, astrocytic percentages decreased and oligodendrocytic percentages remained constant compared with the non-treated control. The newborn neurons in the PCA group were morphologically more mature than in the control. Further, in 3D collagen gel, pretreatment with PCA increased the number of Tuj1-positive neurons 2.7 fold greater than in monolayer culture. In particular, the collagen-entrapped Tuj1-positive neurons formed functional synapses and neuronal circuits by confocal images. These results demonstrate that pretreatment with PCA effectively promotes the differentiation of neuronal phenotypes, suggesting that PCA has the new property of modulating the development of CNS stem cell.

(P 319) Processing of Polycaprolactone Filaments as Scaffold Materials for Tissue Engineering

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Polymers based on polycaprolactone (PCL) are extremely important due to the biodegradability of the material along with the biocompatibility. Our efforts have been to develop a textile structure by spinning PCL into monofilaments of required diameter and subsequently making it into a knitted structure. This method is helpful in designing the structures to a desired morphology and architecture. The spinning of PCL was carried out by melt spinning where PCL was brought to a temperature of 160C under nitrogen atmosphere. The filament thus obtained was subsequently drawn at various draw ratios so that the orientation of the chains takes place. This introduces the strength into the filament. The filament was further heat set to stabilize the structure. This is the stage where filaments were made into a knitted structure with required porosity. We have observed that the structure of the fiber is strongly dependent on the processing parameters. The spinning rate, the draw ratio and the heat set temperature influence the fibre structure, the crystalline structure and the surface morphology which in tern determines the degradability. The degradation of these knittings is being studied at various pH so that a correlation for the real time application may be established.

(P 320) Production of Recombinant Carbohydrate—Binding Modules Fused To RGD: Functional Studies using Bacterial Cellulose

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The attachment of cells to biomedical materials can be improved by using adhesion molecules, present in the extracellular matrix substances, such as fibronectin, vitronectin, or laminin. In many cases, Arg-Gly-Asp (RGD) was found to be the major functional amino acid sequence responsible for cellular adhesion. In the present study, a method for producing chimerics proteins, RGD-CBM, with functions similar to fibronectin, which contains a cellulose-binding module (CBM), was developed. The CBM used was from the cellulosoma of the bacteria Clostridium thermocellum. The genes encoding these CBM-containing chimeric proteins were cloned, and the protein expressed and purified. Bacterial cellulose (BC) secreted by Gluconacetobacter xylinus was produced. Polystyrene surfaces and bacterial cellulose sheets where ''coated'' with these RGD-containing proteins, and then used in adhesion/biocompatibility tests, using a mouse embryo fibroblasts culture. The results showed that the proteins containing the RGD or GRGDY sequence were able to improve the adhesion of the fibroblast on the polystyrene plate, furthermore proteins containing the RGD sequence were more effective than the proteins containing the GRGDY sequence. Preliminary adhesion studies of fibroblast cultures on cellulose sheets, functionalized with the recombinant proteins, showed positive effects on the adhesion and proliferation of the cells. The results demonstrated that the proteins containing the RGD sequence were able to increase significantly the adhesion of fibroblast to BC surfaces when compared with the controls (cellulose treated with the CBM or buffer). The results also demonstrated that the protein containing one RGD sequence have a stronger effect than the protein containing two RGDs.

(P 321) Proliferation and Differentiation of BMSCs on the PLGA/DBP Scaffolds

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Bone marrow stem cells(BMSCs) have the intrinsic ability to self-renew and differentiate into multiple functional cells. In this study,

we fabricated natural/synthetic hybrid poly(lactide-co-glycolide) (PLGA) scaffolds included demineralized bone particle(DBP) (PLGA/DBP). Schwann cells(SCs) and Olfactory Ensheathing cells(OECs) were seeded in PLGA/DBP scaffolds. We confirmed that effects of PLGA/DBP scaffolds seeded OEC and SC on proliferation and differentiation of BMSCs.

PLGA/DBP(20wt%) scaffolds were manufactured by solvent casting/salt leaching method. We designed control groups that BMSCs cultured with only PLGA and PLGA/DBP scaffold, and experimental groups that BMSCs cultured with PLGA/DBP scaffold seeded OECs, SCs or OECs and SCs(OECs/SCs). BMSCs viability was determined by using MTT assays. RT-PCR was used to examine the expression of BDNF, NSE and S-100 in BMSCs.

In MTT assays result, BMSCs viability was superior in PLGA/DBP scaffold than PLGA scaffold. Cell proliferation in PLGA/DBP scaffolds seeded OECs, SCs, and OECs/SCs, respectively was increased during culture period. BDNF, NSE and S-100 gene expressions were weakly detected in BMSCs cultured with PLGA/DBP scaffold seeded only one type cells, and were strongly expressed in the group of PLGA/DBP scaffold seeded OECs and SCs.

PLGA/DBP scaffolds enhanced proliferation of BMSCs. In addition, PLGA/DBP scaffolds seeded OECs and SCs promoted proliferation and differentiation into neural cell of BMSCs. This phenomenon was expected that the growth factor or cytokine in DBP and molecules secreted by OECs and SCs affect to proliferation and differentiation of BMSCs.

Acknowledgements: This research was supported by KMOHW (0405-BO01-0204-0006) and SCRC (SC3100).

(P 322) Promising Calcite-Based Tissue Engineered Product for Bone

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In our research synthetic calcite ($CaCO_3$) was tested as a candidate scaffold for tissue engineered product (TEP) (until now only natural $CaCO_3$ materials have been used). The aim was to confirm a biocompatibility of synthetic calcite and its competence to osteoinduction when seeded with human bone derived cells (HBDCs)—verified *in vivo* in SCID mice. The 5/5/5 mm calcite samples were precultured with HBDCs for 2 weeks in a bioreactor (Spinner Basket®) or pre-wet in a culture medium (control) before implantation. The mechanical properties of precultured scaffolds were compared to the control ones. The precultured and control samples were subcutaneously implanted in SCID mice contralaterally (one TEP and one control per mouse). The viability of cells (XTT) was assessed prior to implantation. Four or twelve weeks after implantation the explants were investigated histologically and by electron paramagnetic resonance spectroscopy (EPR). The synthetic calcite was well tolerated by HBDCs before implantation (XTT test). The precultured samples were mechanically stronger than the control. Well organized connective tissue, rich in blood vessels, with slight inflammation, was shown by histological observations of both precultured and control explants. After 12 weeks, bone tissue was found within the implants—only for scaffolds with HBDCs. EPR analysis supported the histological results–hydroxyapatite-characteristic spectra were found in HBDCs-seeded explants after longer observation. The experiment proved that synthetic calcite scaffold is a promising TEP-base and should be proposed for next steps of preclinical trials.

Work supported by the Polish Government (R13 01901) and Medical University of Warsaw (NZME/W2/07).

(P 323) Proteomic Anlysis of DJ-1 in Spastic Muscles: Implications for Spastic Nerve From in Muscle Generation

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This study was performed to see the characteristics of proteins which expressed in spastic cerebral palsy patients by proteomic analysis.

We studied 12 specimens from 6 patients with spastic cerebral palsy, 3 patients who were diagnosed as myelomeningocele and 3 orthopaedic patients without underlying disease as a control group. Specimens were obtained during orthopaedic procedures. We studied the extracted proteins which showed difference by 2-dimensional electrophoresis and outstanding 13 proteins was reevaluated by proteomics and the reverse transcriptional polymerase chain reaction(RT-PCR) was confirmed to clarify the relationship between gene and protein.

Among 13 proteins which we got from 2-dimensional electrophoresis, 6 proteins were increased in control group by spot histogram and 9 proteins were increased in patients group by spot histogram. The results of proteomic analysis for 13 proteins showed that the expression of DJ-1 related to the cerebral palsy and further study with MALDI-TOF analysis was done.

This study shows that in the cerebral palsy patients, strong expression of DJ-1 has relation to cerebral palsy not only degenerative neuropathologic condition as Parkinson's disease. In this study, we show the possibility of connections between the cerebral palsy and DJ-1 protein for the first time, although multiple mechanisms act on it.

(P 324) Psoriatic Skin Model: Evaluation of Percutaneous Absorption

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Introduction: Psoriasis is a complex dermatosis for which no cure has yet been found. Disease incidence is estimated at 2% of the world's population. As it is impossible at present to test anti-psoriatic drugs on many pathological samples (ethical, availability, interpersonal variations problems), drug formulations offered to the psoriatic population are not well suited to their actual situation.

Purpose: The main objective of this research was to assess skin penetration of drug capacities with a new psoriatic skin substitute whose structure is very similar to *in vivo* pathologic conjonctive tissue.

Methods: Healthy and psoriatic skin substitutes were produced using the self-assembly method, and their functionality was tested by percutaneous absorption. Drugs with different physicochemical properties were chosen based on previous studies: benzoic acid, caffeine and hydrocortisone.

Results: An efficient barrier function against all the 3 different drugs tested was observed for normal skin substitutes when compared to mouse skin. In fact, it was interesting to demonstrate that the self-assembly method allows a more efficient stratum corneum than mouse skin. It is known that *in vivo* psoriatic skin compared to *in vivo* normal human skin shows a less efficient barrier function. It appeared that psoriatic substitutes compared to normal substitutes show similar results.

Conclusion: Being a non-invasive method for tissues, the passage of substances through the skin will allow a better understanding of the anti-psoriatic activity of tested molecules. We believe that our psoriatic skin substitute is an interesting novelty for the percutaneous absorption field of research.

(P 325) Quantification of Fluid Flow Inside Porous Scaffolds by Means of an Experimental and Computational Analysis

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As part of a bone tissue engineering therapy cell-seeded scaffolds can be cultured in perfusion bioreactors prior to implantation. Fluid flow mediated mechanical stimuli inside the scaffold appear to affect the *in vitro* proliferation and osteogenic differentiation. This study focuses on the flow characterization and quantification through a regular scaffold by means of experimental and computational analysis.

Experiments were carried out in a perfusion bioreactor designed for live imaging. Distilled water with microparticles was perfused at a flow rate of 0.018ml/min through the titanium scaffold ($20 \times 6 \times 0.5$ mm) with regular struts (0.5×0.5 mm) and channels of 0.3 mm wide inclined at 45° to the main flow direction. Images were captured at 10 fps with a CCD (Charge Coupled Device) camera mounted on a stereomicroscope. For every two subsequent images a vector field of 24 by 24 grid points was calculated with a multi-scale Lucas-Kanade optical flow algorithm.

A computational fluid dynamic (CFD) model was developed to predict the velocity profile and the wall shear stress (WSS). The mesh presented 1,845,916 tetrahedral elements with an average edge length of 25 μm .

Both experimental measurements and CFD model calculations led to a similar flow field, with a peak velocity of 0.4 mm/s. The model predicted an average WSS of 3.4 mPa.

The combination of experimental and computational techniques enables the flow characterisation inside a scaffold, which is an important step towards a better understanding of the importance of the hydrodynamic environment for bone engineering.

(P 326) Quantifying and Overcoming Oxidative Stress in Cell Cultures

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In biological systems, oxidative stress is defined as the state in which the spontaneous formation of reactive oxygen species (ROS) is greater than the cellular capacity to detoxify the reactive intermediates or easily repair the damage. Physiochemical parameters such as oxygen concentration, cell density, medium protein content as well as protein composition influence the culture system's capacity to both generate and to effectively eliminate ROS. While these physiochemical parameters have all been demonstrated independently to influence the oxidative stress of the culture environment, no literature exists which defines the relative contribution each of these physiochemical parameters have on the final oxidative load in cell culture. Understanding the specific contribution that each of these physiochemical parameters plays is essential in the optimization of bioprocesses, such as the expansion of stem cells for cellular therapies, where cost and regulatory drivers prohibit the use of animal derived proteins transferring significant oxidative load onto the cell mass. Here we introduce a strategy to quantify the contributions made by cell number, medium protein content and percent oxygen make towards conditioning the culture environment and specifically, mitigating oxidative stress.

Dihydrorhodamine 123 (DHR123) reacts with ROS resulting in the generation of the fluorescent compound rhodamine 123. The environmental oxidative load alleviated by varying each of the physiochemical factors independently, and in combination is quantified by measuring the relative fluorescence of oxidized DHR123. We identify key relationships between physiochemical parameters and suggest strategies for minimizing medium protein content while simultaneously mitigating oxidative stress.

(P 327) Quantitative Assay of Stem Cells Loaded on Porous Scaffolds using Bioluminescence

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In stem cell-based tissue engineering strategies, the capacity to monitor *in vitro* and *in vivo* proliferation of cells seeded on porous

scaffolds is of great importance to scaffold design. We used bioluminescence technology for quantitative cell assay inside scaffolds using cells tagged with the luciferase gene reporter.

A clonal cell line exhibiting high luciferase expression was selected from murine mesenchymal stem cells genetically modified using lentiviral vectors encoding luciferase. Two materials were tested: AN69 hydrogel and porous coral scaffold. *In vitro* quantitative evaluation of luminescence from cells loaded scaffolds was performed in cellular destructive (measurement of light with a luminometer after cell lysis) and non-destructive (using a bioluminescence imaging system) conditions. *In vivo* quantitative evaluation of cells adhered onto materials was accomplished in non-destructive conditions after subcutaneous implantation of implants in mice.

In cellular destructive conditions, light emission from either cells or cells adhered onto scaffolds indicated a linear relationship with the cell number, despite attenuation of the transmitted light photons due to the presence of the material. Based on the ratio of slope values of standards plots, coefficient of transmission particular to each material scaffold was calculated as 0.27 ± 0.05 for AN69 hydrogels and 0.35 ± 0.03 for coral. In non-destructive conditions both *in vitro* and *in vivo*, analysis of luminescent images also provided evidence of correlation between the emitted photon flux and the number of cells adhering onto the materials. Bioluminescent imaging enabled non-invasive monitoring of the fate of cells present in each scaffold in living mice for 28 days post-implantation.

(P 328) Quantitative Evaluation of Adipose Tissue Engineering in Scid Mice

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Introduction: Adipose tissue has strong significance in plastic and reconstructive surgery (body-contour, mobility of tissue layers). The aim of our present study was to evaluate the feasibility of adipose tissue engineering in a SCID-mouse model.

Methods: Adipose tissue stromal cells (ATSC) were harvested from human adipose tissue obtained during elective surgery. Differentiation was induced with two cycles of IBMX administration. Adipose tissue equivalents were generated *in vitro* and transplanted into NOD-SCID-mice ($n = 90$) with and without co-transplantation of endothelial cells (HUVEC). Histomorphometric evaluation of adipose tissue formation was performed on whole sections imaged by a ZEISS MIRAX SCAN slide scanner. The resulting images were segmented into regions of different tissue types and evaluated quantitatively by means of image processing.

Results: After explantation of SCID mice 10 days, 4 weeks, 6 months postoperatively, mature adipose tissue could be observed. Transplanted cells were S-100 positive (adipocyte-typic). Thickness of the implants diminished from 10 days to 4 weeks and remained almost constant until 6 months with completion of adipocytic differentiation. There was no significant advantage for endothelial co-transplantation and small sized implants showed significant better differentiation than larger sized implants (ca. 10 mm).

Conclusion: Differentiation of three-dimensional adipose tissue constructs is feasible and reproducible *in vitro* and *in vivo*, however reliable solutions for microvascular network formation still have to be found.

(P 329) Raman Monitoring of Cartilage Cell Differentiation and Proliferation

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Raman spectroscopy is a noninvasive, label free *in vitro* technique. Combining cartilage tissue engineering techniques with Raman analysis can provide the means to collect data on a wide spectrum of nutrient, extracellular matrix, and cellular components of tissue engineered samples. We studied how to use specific Raman markers for the visualization of cartilage cell differentiation using primary chondrocytes and pellet culture system.

In our study, Raman analysis was carried out by a confocal Raman microspectroscopy setup. Primary chondrocytes were isolated from calf articular cartilage, cultured in centrifuge tubes in chondrocyte medium and centrifuged to form a pellet. Each experimental group - 3 days, 1 week and 2 weeks cultured samples-were further processed for histology and Raman analysis. In addition different components of the extracellular matrix were measured to identify the Raman peaks of the pellets.

Sulfated glycosaminoglycan was visualized with Safranin O staining. We detected extracellular matrix formation in the chondrocyte pellets after 1 week culturing. Comparing the Raman spectrums of the chondrocyte pellets cultured for 1 week and for 2 weeks with the spectrums of the components of the extracellular matrix, the Raman peak at 878 cm^{-1} (wavelength shift) can identified as the peak of the collagen type II and the peaks at 1043, 1081, 1129, 1209, 1243 and 1278 cm^{-1} as the peaks of chondroitin sulfate.

We were able to collect Raman data of different components present in the extracellular matrix of chondrocyte pellet culture samples and visualize chondrocyte differentiation.

(P 330) Rapid, Non-Viral Gene Transfection using a Magnetic Nanoparticle-Based Halbach Array System

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With the sequencing of the human genome and the advent of gene therapy has come the need to develop effective delivery and transfection agents. These agents must be able to target therapeutic and reporter genes to the relevant cells and organs both *in vitro* for basic investigations as well as *in vivo* for therapeutic applications. Recent safety concerns over the use of viral vectors has begun to

shift the emphasis toward the development of non-viral delivery agents, primarily cationic lipids. At present, this is generally accomplished through lipid-mediated transfer or electroporation. However, these techniques suffer from significant drawbacks such as: (i) low levels of transfection in primary cells and some cell lines (ii) their inability to effectively transfect tissue explants (iii) detrimental effects on cell viability (primarily with electroporation) and (iv) difficulty in translating to *in vivo* (clinical) applications.

We have developed a novel gene transfection system based on attaching DNA to magnetic nanoparticles. High-gradient Halbach-type magnet arrays direct the particle/gene complex to cells *in vitro* resulting in significantly faster transfection times and higher transfection efficiencies (up to 100× at short exposure times) in comparison to the best cationic lipid-based agents available. It also improves speed and efficiency in comparison to commercially available magnetofection systems.

(P 331) Re-Endothelialization of Decellularized Pulmonary Valve Scaffold Under Simulated Physiological Dynamic Conditions *In-Vitro*

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Objective: Optimization of techniques for serial production of viable biological heart valves is of central interest in bioengineering. The aim of study was to generate decellularized heart valves with preserved ultra-structure and to repopulate those with endothelial cells (EC) under simulated physiological conditions.

Methods: Ovine pulmonary valve conduits ($n = 16$) were decellularized in detergents (Sodium-deoxycholate/SDS) followed by 8 wash cycles in PBS (12 h each). Viability of EC cultures exposed to washing solution served to prove efficiency of washing. Luminal surfaces of decellularized grafts ($n = 11$) were seeded with ovine jugular vein EC (1.2×10^7 cells) in special bioreactors. After rolling culture for 48h, pulsatile circulation was started with a flow of 0.1 L/min (system mean pressure: 25 ± 4 mmHg). The flow rate was gradually increased with 0.3 L/day to a final rate of 2.0 L/min (cycle rate: 60 beats/min), while pH, pO_2, pCO_2, lactate and glucose in the medium were maintained at constant physiological levels.

Results: Resulting scaffolds were cell-free with preserved architectonic of extracellular matrix. Biomechanical tests demonstrated comparable properties to native tissue ($n = 5$). Histological examination, after 7d of dynamic cultivation, revealed a cells monolayer covering the inner valve surface. Metabolic assays demonstrated high activity of these cells, which express immunohistochemically vWF/eNOS, indicating an endothelial origin. Electron microscopy revealed their, typical endothelial, cobble-stone arrangement.

Conclusion: Complete and flow-resistant re-endothelialization of detergent decellularized matrix can be achieved in a pulsatile bioreactor system under conditions simulating physiological environment. Permanent monitoring of biotechnological processes and continuous adjustment to physiological parameters is essential for cardiac valve engineering.

(P 332) Real Time Monitoring of Scaffold Degradation Under Culture Conditions

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The understanding of scaffold degradation in pre and post implantation stages can only be fully accomplished under real time *in situ* evaluation. Therefore, the development of real time monitoring techniques for scaffold degradation is crucial for Tissue Engineering. Our group has reported for the first time a consistent strategy to achieve that goal [1]. We have proposed a fluorescence based model in which a fluorescent probe is covalently bound to a polymer used in scaffold manufacturing. We have shown that the fluorescence intensity (FI) of the scaffolds can be used as an indicator of the extent of scaffold degradation, and that it can be measured in a real time mode.

This work aimed to confirm if this model could be applicable to complex systems in which new extracellular matrix is being formed as the scaffold degrades. The FI of chitosan scaffolds labelled with TRITC was quantified by confocal microscopy before and after one week of culture with MG63 osteoblast like cells. Cell morphology, viability and production of extracellular matrix were assessed. The results showed that after one week of culture cell viability was high and cell morphology was typical from this cell line. During that period an abundant amount of collagen was produced. No statistical difference was found on scaffold FI before and after cell culture, showing that the model can be applied under culture conditions.

[1]Cunha-Reis C, Bagnaninchi PO, El Haj AJ, Yang Y. Exploring fluorescence as a tool for real-time monitoring of scaffolds degradation. Tissue Engineering 2007 Jul;13(7):1694–1694.

(P 333) Recombinant Erythropoietin Application in Complex Therapy of Chronic Heart Failure (CHF)

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Background: CHF becomes widespread syndrome in many patients. It is related with the ageing and rise of the morbidity of cardio-vascular system among this category of people. Modern methods of CHF treatment doesn't ensure the clinical success in all cases, often are contradictive because of the accompanied pathology.

Materials and methods: We used recombinant erythropoietin-ß in complex therapy of CHF II-III functional class under NYHA in 17 males, average age 71 ± 3,2. The cause of CHF in all patients was integration of IHD and hypertonic disease; all the patients conducted myocardium infarction more than 6 months before the research. Seven patients suffered from the constant form of atria fibrillation. Basic therapy of CHF included ß-adrenal blockers, ACE inhibitors, desaggregants, diuretics and anticoagulants instead of desaggregants if necessary. Nine patients additionally got recombinant erythropoietin-ß in the dose of 2000 IU twice with an interval of 2 days. Laboratory and instrumental tests were done before the research and 2 weeks after first injection of erythropoietin.

Results: in patients after erythropoietin treatment no arterial pressure rise was observed. Clinical data were approximately similar to those in the group without erythropoietin treatment. No positive dynamic of creatinin and transaminases of blood were investigated. The decrease of the level of Na-uretic peptide (22,8 % lower) and improvement of global (systolic-diastolic) function of the left ventricle under results by Tei-index (24,1 % decrease) were found.

Conclusions: the obtained data demonstrate safety and effectiveness of recombinant erythropoietin-ß in complex therapy of CHF of ischemic genesis in aged males.

(P 334) Recombinant Human Collagen as a Bioactive Element in Scaffolds for Tissue Engineering

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Scaffolds are made from naturally derived as well as from synthetic polymers. Synthetic polymers have the advantages of being manufactured with high reproducibility, controlled degradation rate and mechanical properties. But they lack cell-recognition signals that confer bioactivity to the scaffold. An attractive concept is to combine synthetic materials with natural cell-recognition sites present in ECM proteins like collagen. We will create a novel bioactive scaffold by combining a knitted fabric of poly(lactic acid-co-epsilon-caprolactone) (PLA-CL) polymer with a layer of recombinant human collagen produced and deposited onto the polymer by Chinese hamster ovary (CHO)-derived cell line.

We established CHO-derived cell lines that stably express recombinant human collagen I (chain alpha 1 and alpha 2) and IV (chain alpha 2). Sirius Red assay and western blot showed that our cell lines secreted recombinant human collagen into the cell culture medium. These cell lines will be seeded onto PLA-CL scaffolds to deposit a layer of recombinant collagen. CHO cells will be removed by osmotic shock assuring a soft cell removal without detaching the recombinant collagen present within the polymer. Human bladder smooth muscle cells will be reseeded onto the new bioactive scaffold and an *in vitro* analysis of their attachment and growth will be performed. These experiments will establish the proof of principal that a scaffold made of a synthetic polymer and recombinant human collagen can be used to grow patient cells, paving the way to the generation of a novel scaffold for tissue engineering with good mechanical strength and cell recognition sites.

(P 335) Reconstruction of Bone Defect in Canine Femoral Head By Structural Biphasic Calcium Phosphate Scaffolding and Marrow-Derived Mesenchymal Stem Cells: an Experimental Study

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Osteonecrosis (ON) or avascular necrosis (AVN) of the femoral head is a debilitating and painful condition characterized by progressive joint degeneration in adults. We fabricated biomimetic biphasic calcium phosphate (BCP) for tissue engineering in repair of ON or AVN and observed its reconstructive result in canine femoral heads with ON. BCP ceramic scaffolds mimicking a trabecular structure were fabricated by a 3-D gel-lamination technique according to 2-D images of trabecular specimens from the femoral head. Then, the 3-D structure of the scaffolds was scanned and reconstructed with use of micro-computed tomography (micro-CT) for 3-D characterization of patterns of the material structure related to trabecular parameters, including bone volume fraction (BVF); bone surface/bone volume (BS/BV); trabecular thickness (Tb.Th), number (Tb.N), spacing (Tb.Sp) and pattern factor (Tb.Pf); and structure model index (SMI). After coating the biomimetic porous BCP with marrow stromal cells (MSCs) as seed cells *in vitro*, tissue-engineered bones were implanted into the bony defects of canine femoral heads by use of a trapdoor procedure. Femoral heads in a control group were packed with autograft bone chips. Control and BCP-scaffold femoral heads did not differ in micro-CT data, except for a more plate-like ''trabecular'' pattern found in BCP-scaffold heads. After 30 weeks, the contour and integrity of the BCP-scaffold femoral heads was basically maintained and showed trabecular bone formation and integration. However, control femoral heads showed collapsed contours and osteoarthritic changes. Biomimetic porous BCP scaffolds coated with MSCs might be effective in repair of bony defects in femoral heads with ON.

(P 336) Regeneration of Abdominal Wall Musculofascial Defect by a Human Acellular Collagen Matrix.

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Purpose: This work studied the reconstruction of abdominal wall defect by a ''human acellular collagen matrix''.

Methods: Fascia lata from human tissue donors (63 ± 25 yrs old, $n = 4$) were chemically/physically treated to reduce the immunogenicity and risk of conventional and non-conventional agents transmission. Abdominal wall defect was cured, on 40 rats, by implantation of (i) polypropylene (Pro)/ (ii) polyester (Mers) meshes and (iii) human acellular collagen matrix with 2 orientations: fibres in parrallel (FLL) or perpendicular (FLT) to native rats abdominal wall. Hernia recurrence, adhesions and histology for inflammation (lymphocyte, CD3/macrophages, CD68) and remodelling (Masson's Trichrom and dystrophin staining) were assessed at 4 and 8 weeks post-implantation. Two large abdominal eventrations were cured by human acellular matrix in human patients.

Results: A higher hernia recurrence rate was observed for rats transplanted with FLL in comparison to FLT/Pro/Mers after 4 and 8 weeks post-implantation. A lower intestinal adhesions rate was obtained for FLL/FLT than Pro/Mers meshes (50% of adhesion coverage area at 8 weeks).

Conclusion: Human acellular collagen matrix, placed in FLT position, can induced an abdominal wall reconstitution without adhesions and hernia recurrence.

(P 337) Regeneration of an Artery by using of the De-Elastinized Aorta

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Although an artificial blood vessel is in general use, the development of regenerative vascular grafts is strongly desired especially for the pediatric patients. In this study, regenerative collagenic vascular graft was developed from porcine aorta by removing cells and structural proteins except collagen from the tissue.

Porcine aorta was isolated from the Clawn miniature pig (Japan Farm, Co. Ltd.). The tissue was placed in a vacuum oven at 120°C to cross-link collagen fibers. Elastin fibers were then taken away from the tissue by enzymatic digestion using elastase solution at 37°C for 3 days with gentle stir. The tissue was incubated in 80% ethanol solution for 3 days at 37°C to remove phospholipids from the inside. The vascular grafts made of miniature pig descending aorta were transplanted allogenically.

There was no thrombus on the intimal surface and aneurysm formation even after 6 months of the implantation. A large amount of the cell migration into the graft was observed. These cells were identified immunohistologically as smooth muscle cells and fibroblasts. Calcific depositions in the graft were not observed after 3 months of the implantation. A few of depositions was seen after 6 months. However that was not severe calcification. Currently long-term implantation experiments are in progress.

The processed graft may have better ability to promote cell infiltration and tissue remodeling as compared with only decellularized tissues. We conclude that the collagenic vascular graft developed in this study may be adapted to the vascular tissue regeneration.

(P 338) Regeneration of Articular Cartilage using Mesenchymal Stem Cells to Reconstruct Irreparable Chronic Focal Cartilage Defects in a Large Animal Model

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Introduction: The aim of this project is to investigate the reconstruction of a focal osteochondral defect of a knee joint in sheep by using autologous, bone marrow-derived mesenchymal stem cells (MSCs). The issue of whether chondrogenic pre-differentiation of the MSCs *in vitro* bears an influence on the regeneration *in vivo* is also to be addressed.

Methods: An osteochondral incision (Ø 7 mm) was made in an initial operation on the sheep knee and bone marrow aspirate was removed from the iliac crest of 9 Merino sheep. The isolated and expanded MSCs were cultured with 0.5 mio cells/ml in a collagen-I-matrix. One part of the MSC gels was pre-differentiated with chondrogenic medium + 10 ng/ml TGF-beta3, whilst the other part of the gels was not pre-differentiated. The implantation of the constructs then followed. The knee joints were explanted and investigated histologically after 6 months.

Results: Gene expression, immunohistology and measurements of sulfated glycosaminoglycans enabled the successful demonstration of a chondrogenic *in-vitro*-differentiation. In contrast to the unseeded control gels, the stem cell-seeded implants showed *in vivo* good structural bonding with a smooth surface and hyaline type matrix. The chondrogenic pre-differentiated stem cell gels showed the best results and yielded an ICRS Visual Histological Score of 14.25 and an O'Driscoll Score of 17.38.

Conclusion: The collagen gel implants based on autologous MSCs lead to a partial hyaline type structure of the regeneration matrix after 6 months. Despite its high variability the procedure is a promising development in the area of biological cartilage regeneration.

(P 339) Regeneration of Dentin/Pulp-Like Tissue using a Dental Pulp Stem Cell/ Poly (Lactic-Co-Glycolic) Acid Scaffold Construct in Rabbits

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Introduction: Regenerative dental procedures are currently being explored by numerous researchers with more expanding focus on functional regeneration and biomimicking of natural biological events. The objective of the present study was to evaluate the ability of tissue engineering to regenerate normal healthy dentin/pulp tissue in *ex-vivo* sites, when transplanting cell/scaffold constructs subcutaneously in the dorsal surface in the rabbit model.

Materials and Methods: Biodegradable synthetic 50/50 poly (lactic-co-glycolic acid) scaffolds were fabricated using the solvent casting/particulate leaching technique to form scaffolds with different groups of porosity. Dental pulp stem cells were isolated from healthy rabbit teeth and characterized using Confocal laser microscopy. These cells were used for seeding the scaffolds from each group. Seeded scaffolds were analyzed using phase contrast microscopy and scanning electron microscopy and implanted subcutaneously in the dorsal surface of each rabbit and evaluated up to six weeks post-transplantation.

Results & Conclusions: The scaffolds prepared showed good porosity characteristics and supported the attachment, proliferation, migration, and differentiation of the dental pulp stem cells. Dental pulp stem cells maintained their ability to differentiate in culture flasks as well as on seeded scaffolds to form organized structures even without the addition of differentiation induction components. When cell/scaffold constructs were transplanted *in-vivo*, they supported angiogenesis and gave rise to a variety of newly formed tissues that ranged from osteodentin-like tissues to more tubular bi-layered structures as well as the presence of some complex tissues similar to the configuration of the dentin-pulp complex in a natural tooth.

(P 340) Regeneration of Tympanic Membrane Perforation using BFGF Releasing Alginate/PCL Patch

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Damage of tympanic membrane (ear drum) is one of common diseases in otorhinolaryngology fields, which is frequently arisen as a result of either otitis media or trauma. In recent years, basic fibroblast growth factor (bFGF) which can enhance fibroblast growth and angiogenesis has been employed into the various patches to improve restoration rate of damaged tympanic membranes. However, daily administration of growth factor has been considered as a critical limitation. In this study, we fabricated a hydrophilized polycaprolactone (PCL) nanofiber patch by electrospinning and the following oxygen plasma treatment. The prepared patch was impregnated with bFGF/alginate/$CaSO_4$ mixture solution to provide the crosslinking of alginate and sustained release of bFGF. From the bFGF release experiment, we observed that the bFGF is continuously released from the alginate/PCL patch, up to 700 ng for 21 days. To compare tympanic membrane regeneration through the various patches (PCL, alginate/PCL, and bFGF/alginate/PCL), *in vivo* animal study was also conducted using a guinea pig (acute damaged tympanic membrane model). The bFGF/alginate/PCL patch showed faster tympanic membrane regeneration than the other groups, probably due to the continuous release of the bFGF. From the results, we conclude that the bFGF/alginate/PCL patch can be a good candidate as an effective tympanic membrane regeneration patch.

(P 341) Repair of Large Full-Thickness Articular Cartilage Defects using Novel Decellularized Artilage Scaffolds and Adipose-Derived Stem Cells in a Rabbit Model

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The purpose of this study was to develop a natural, decellularized cartilage scaffold, and evaluate the use of a autologous diposederived stem cells (ADSCs) as a cell source for cartilage defect repair in rabbits. ADSCs were isolated and induced with defined medium (including TGF-β1)and placed in decellularized scaffold. An articular cartilage defect was created on the patellar groove of the femur, and the defect was filled with the ADSCs/scaffolds constructs (Group A), RCCS bioreactor-based ADSCs/scaffolds constructs Group B), scaffold alone (Group C), or empty (Group D). 12, 24, 48 weeks after the operation, the histological analyses showed that the defects were filled with reparative hyaline cartilage in Group A and Group B. Quantitative histologic analysis revealed that an average score of bioreactor-based Group (Group B) is higher than non-bioreactor Group A.

(P 342) Reporter-Vectors for Chondrogenic Differentiation Status

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2D cultivated chondrocytes dedifferentiate towards a fibroblastic phenotype. With the proceeding dedifferentiation, collagen type I takes over the chondrocyte specific collagen type II expression. To follow the state of dedifferentiation, we now introduce a combination of collagen I and collagen II reporter plasmids. The collagen I plasmid contains the red fluorescent protein dsRed driven by a collagen I alpha 1—enhancer/promoter combination. The collagen II reporter contains a CMV-enhancer element for signal amplification linked to a collagen II-promoter fragment containing chondrocyte specific cis-acting activator and repressor recognition sequences. In contrast to the collagen I reporter, the collagen II plasmid includes the fluorescent protein EYFP. Therefore both markers can be detected separately. Furthermore, the differentiation kinetics can be monitored for both directions, de- and redifferentiation. We achieved the design of a reporter-vector system simultaneously indicating collagen expression-levels of transfected cells by cloning of the two reporter-cassettes into one plasmid. Initial transfection of porcine BMSC with the separate reporter-vector systems confirmed the functionality of the designed chimeric transcription activating elements by fluorescence microscopy. The collagen-expression specific response of the designed reporter-vector systems is still under investigation in human adipose derived stem cells. The described systems will be suitable for investigating collagen I and collagen II expression of human mesenchymal stem cells and articular chondrocytes *in vitro*.

This work was supported by the European projects
Hippocrates (NMP3-CT-2003-505758) and Expertissues (NMP-CT-2004-500283)

(P 343) Rheological Analysis of Biomaterials Used for the Development of an Artificial Substitute of the Corneal Stroma

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Introduction: New biomaterials have been recently used for the construction of artificial corneas by tissue engineering. Evaluation of the mechanical properties of these biomaterials is necessary before the artificial corneas can be used for clinical purposes. In this work, we have analyzed the physical properties of three different kinds of corneal substitutes developed by tissue engineering.

Materials and Methods: Primary cultures of human corneal keratocytes were established from small biopsies of the scleral limbus, and partial human cornea substitutes were developed by using artificial stromas made of agarose, collagen or fibrin with human keratocytes imersed within. Viscosimetric and oscillometric measures were obtained by using a Bohlin CS10 rheometer in a plate-plate configuration. The yield stress point of each biomaterial and the biomechanical properties of the different corneal substitutes were determined.

Results and Discussion: Rheological evaluation of the different biomaterials demonstrated that the yield stress points of fibrin (0.5698 Pa) and agarose (1.0206 Pa) were lower than that of collagen (10.4210). Low shear viscosity was similar for fibrin and agarose ($\eta_0 < 10^{-4} s^{-1}$), whereas collagen hydrogels showed a h_0 value of 3.8357 s^{-1}. Our analysis revealed that the rheological properties of the different corneal substitutes were strongly dependent on the biomaterial used as scaffold. In general, collagen hydrogels were more viscous and sustained higher levels of yield stress. All biomaterials evaluated in this work showed a viscoelastic behavior and could, therefore, be used for the construction of artificial corneas by tissue engineering.

Supported by PI-0132/2007 from Junta de Andalucia.

(P 344) Role of NEU3 Sialidases Into the Muscle Differentiation Process

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Introduction: Sialidases influence cellular activity by removing terminal sialic acid from glycoproteins and glycolipids. Four genetically-distinct sialidases (Neu1-4) have been identified in mammalian cells. The intricate mechanisms regulating myoblast differentiation are still under investigation. Along this line, a few reports suggest the involvement of sialidases in muscle differentiation. However, while down-regulation of Neu2 (the cytosolic sialidase) has been shown to inhibit myotube formation [1], nothing has been reported about the role of Neu3 (the plasma-membrane sialidase) in this process.

Methods: The role of Neu3 sialidase has been studied in muscle differentiation by stably overexpressing it in C_2C_2 cells. The effect of Neu3 overexpression was studied by looking at muscle differentiation markers and ganglioside pattern.

Gangliosides are major regulatory molecules present in the cell membrane and are potential substrates for both Neu1 (the lysosomal sialidase) and Neu3.

Results: The main ganglioside in this cell is GM3 and the overexpression of sialidase caused a decrease in GM3 content. Neu3 overexpressing cells also formed hyperthrophic myotubes and showed a delay in proliferation towards terminal differentiation.

Conclusion: Since the overexpression of Neu3 in C_2C1_2 cells caused an increase in GM3 levels sialidases may influence cellular pathways by modulating the GM3 content.

[1]Fanzani, A., Giuliani, R., Colombo, F., Zizioli, D., Presta, M., Preti, A., Marchesini, S. (2003) Overexpression of cytosolic sialidase Neu2 induces myoblast differentiation in C2C12 cells. FEBS Lett 547, 183–8.

(P 345) Scaffold Properties and Interaction with Cells Investigated By Magnetic Resonance Methods

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Many properties of scaffolds have to be considered to ensure their suitability for tissue engineering approaches. The majority is usually investigated by techniques which are associated with a destruction of the sample. The non-invasive analytical tools of magnetic resonance imaging (MRI) and electron paramagnetic resonance spectroscopy (EPR) are used to avoid scaffold damages.

Scaffolds composed of hydroxyapatite, collagen and chitosan have been produced and some crucial properties have been characterized using these techniques. For example, the pore size is typically measured microscopically in dry state. During incubation in culture medium or body fluids, some scaffold materials are in a swollen state. So the actual space for diffusion processes can differ between scaffolds of the same measured pore size but different materials. This issue has been visualized non-invasively by MRI and the Gd-DTPA contrast agent. Additionally, the channels for mass transfer can be narrowed by cells growing on the surface. The observed hindered diffusion leads to insufficient supply of oxygen and nutrients inside the scaffold, delimiting the growth of cells to the surface. Concerning this matter, also the pH of the aqueous medium inside the scaffold is important, especially in the face of acidic degradation products arising from common scaffold materials. Information about pH and oxygen concentration inside the scaffold has been obtained non-invasively by EPR.

Furthermore, the seeding of magnetite loaded osteoblasts on the scaffolds has been followed by the new method of benchtop-MRI. The results have been compared with histological cuttings stained by Prussian blue and nuclear fast red.

(P 346) Scaffolds Designed and Fabricated with Elastic Biomaterials Applying CAD-CAM Technique

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Rapid prototyping (RP) technique is increasingly recognized to be an efficient process to develop scaffolds for tissue engineering applications. Scaffolds are fabricated according to user-defined architecture and customized shape through layer-by-layer deposition of material utilizing profound advantages of CAD-CAM techniques. An in-house built desktop robot based rapid prototyping (DRBRP) system was employed to manufacture scaffolds using two polymers (PCL & PCL-PEG) and three architectural patterns. Results from ultra pycnometer, micro-CT and SEM were used to evaluate the scaffold morphology that evidenced well-customized scaffold structure in terms of pore shape, size and interconnectivity. The *in vitro* cell culture study using rabbit smooth muscle cells, demonstrated bioactivity of both PCL and PCL-PEG scaffolds for cell attachment and proliferation. However, the PCL-PEG copolymer scaffolds showed overall better performance than the PCL homopolymer scaffold that was reflected by the DNA quantification assay. The reported results demonstrate an encouraging suitability of the developed scaffolds for tissue engineering applications.

(P 347) Self-Assembling Peptide Hydrogels: Directing Cell Behaviour By Chemical Composition.

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Over recent years, there has been a growing interest in the design of self-assembly systems using aromatic short peptide derivatives for biomedical applications including 3D cell culture. We previously demonstrated that these peptides derivatives self-assemble into stiff nano structural hydrogels with tunable properties(Jayawarna V., *et al.*, Adv.Mater.18(2006).

These materials have already shown to be successful in cell culture of chondrocytes, they have not been tested for other cell types. Initial testing of the Fmoc-Phe-Phe-OH with skin cells such as human dermal fibroblasts and Mouse 3T3 cells highlighted some limitations and some development areas in terms of long term gel performance, stability and applications. The objective of this paper therefore is to investigate the design and the development potential of a series of peptide hydrogel systems of different chemical compositions were achieved through mixing Fmoc-Phe-Phe-OH with positively changed (Lysine), uncharged/polar (Serine) and negatively charged (Glutamic acid) Fmoc amino acids. The propensity of these hydrogel systems to promote proliferation, survival and proliferation of chondrocytes, 3T3 and HDF cells have also been tested. In addition methods were developed to form gels rapidly upon expose to culture media for both 2D and 3D cell culture applications.

Hydrogels were characterised using FTIR and TEM. The results suggest that these peptide systems undergo spontaneous assembly into nanofibre scaffolds by mainly adopting an antiparallel β-sheet conformation. Both 2D and 3D cell culture analysis shows that these new hydrogels give rise to improved culturing of different cell types with respect to the Fmoc-Phe-Phe-OH hydrogel. Specifically, Fmoc-Phe-Phe-OH+Fmoc-Serine is

(P 348) Si and Ti-Containing Glasses as Bone Cement Fillers: a Bioactivity and Biocompatibility Study

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The main condition for a synthetic material to form a stable bond with the bone is the precipitation of an apatite layer on its surface, assumed to be responsible for its bioactivity[1].It is well known that some Si-based glasses exhibit high level of bioactivity but it has also been reported that Ti-containing glasses can induce the surface precipitation of calcium-phosphates.In both cases the formation of OH groups in physiological medium is the key of the observed behaviour[2]. In the present study, PMMA- co-EHA composites filled with glass particles of the Ca-P-Ti (CTi) and Ca-P-Mg-Si (CSi) systems were developed and compared in terms of their *in vitro* behaviour both in accellular and in cellular media.Accellular tests were carried out in SBF, for periods up to 21 days at 37 °C.Surface modifications were followed by SEM-EDS and FTIR, and ICP was used to assess the variation of ionic concentration in the fluid. After 3 days of soaking, both composites showed the presence of Ca-P precipitates with average Ca/P atomic ratio of 1.53 in the layer covering CSi and 1.06 in CTi.Compatibility evaluation was performed with MG63 osteoblast-like cells up to 21 days and was followed by confocal microscopy.The observed behaviour of CSi and CTi composites was explained in terms of the differences found in accelular media. The results showed that the glass composition was decisive to explain the *in vitro* behaviour of the composites, being SiO_2-based glass much more effective than the TiO_2-based glass.

[1]H.Takadama,*et al.* J.Am.Ceram.Soc.2002:85;1933–1936.
[2]T.Kokubo, Acta Mater. 1998:46;2519–2527.

(P 349) Silica Based Nanomaterials: Characterisation and *In Vitro* Studies using Osteoprogenitor Cells

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This work studies the potential of mesostructured powder materials based on the organised SBA-15 type silica in tissue engineering applications. A bare SBA-15 silica material and a SBA-15 based composite containing HA nanoparticles have been investigated. The first part of this paper is focussed in the characterisation of the microstructure and the textural properties of the materials with special emphasis on the influence of conventional sterilisation treatments on their final properties. The second part of the paper reports *in vitro* cell biocompatibility studies. Cell culture studies were carried out using the mouse osteoblastic cell line MC3T3-E1 as well as adult bone marrow mesenchymal stromal cells from rat and human species. The three cell-types were shown to attach to the materials and to form three-dimensional (3D) cell-material aggregates. These initial small aggregates were observed to coalesce and growth in size with culture time by the joining of smaller

units. Preliminary results also indicate that the HA-SBA-15 material can support the expression of an early bone differentiation marker. In summary, our results show that both SBA-15 and HA-SBA-15 materials can be easily sterilised with conventional techniques preserving their textural properties, and that they support cell attachment and proliferation.

(P 350) Spinal Injury Repair: Novel Injectable Fibronectin Scaffolds

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Spinal cord degeneration following crush injury presents major clinical problems. Repair strategies involving surgical intervention risk further damage to remaining functional neurones and formation of fibrous scar tissue.

A biomaterial scaffold that could be injected as a liquid and solidify *in situ* offers a minimally invasive alternative to surgery. It could also act as a carrier for transport of cells and bioactive molecules to the injury site.

Previous studies have shown that fibronectin materials implanted into spinal injury sites had a positive effect on neuronal regeneration. This study describes materials produced from highly concentrated viscous solutions of fibronectin (FN) that were stimulated to form a gel within seconds on contact with metal ions.

Fibronectin pellets were dissolved in 6M urea and a range of metal salts were added including copper, zinc, silver, magnesium, calcium and manganese at different concentrations (1uM-100 mM). Gel formation was measured by the change in turbidity at 450 nm.

The addition of copper, zinc or silver to the fibronectin solution initiated gel formation within seconds and the rate of polymerisation was dependent on metal ion concentration (100uM-100 mM). Polymerisation did not occur in the presence of calcium, magnesium or manganese salts. Analysis of the ultrastructure of the gels by electron microscopy revealed variations in the porosity, fibre diameter and general architecture of ZnFN, AgFN and AgFN gels.

In conclusion, fibronectin gels are important injectables for promoting spinal regeneration and zinc and silver produce rapid aggregation.

We are grateful for funding from the BBSRC (BB/C513893/1)

(P 351) Study of Novel Nanofiber Mats Obtained By Electrospinning Ternary Blends Containing Chitosan, Cellulose Acetate and Poly(Ethylene Oxide)

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The aim of our work is the production of biodegradable polymeric scaffolds to be used in skin regeneration. We produced nonwoven highly porous membranes electrospinning solutions containing chitosan, cellulose acetate (CA) and poly(ethylene oxide) (PEO) in 85 w/w% aqueous acetic acid. The rationale behind this novel ternary blend approach is: the bioactivity and wound healing properties of chitosan, which is, however, difficult to electrospin; the easy processability of PEO, which has the handicap of being water soluble; the biocompatibility and water insolubility of CA, which helps preserve the structural integrity of the scaffolds. The rheologic and electrochemical properties of the solution determine its electrospinnability and, together with the processing conditions, the morphology of the fibers. We considered six solutions with a total polymer concentration of 6 w/w%, equal parts of PEO and CA and chitosan concentrations ranging from 0 to 5 w/w%. Shear viscosities, surface tensions and conductivities of the solutions were measured. For the blend containing 3 w/w% chitosan the influence of solution feed rate, applied high voltage and tip to collector distance on fiber morphology was studied. The same processing parameters were used for the 5 chitosan-containning blends in order to evaluate the influence of composition on morphology, mechanical properties (tensile tests) and structural stability of the scaffolds in phosphate buffer saline (PBS) solutions. Morphological studies were based on SEM images which revealed fiber diameters decreasing with chitosan content. Young's modulus and ultimate tensile strength increased with chitosan content. Structural stability improves with chitosan content.

(P 352) Surface Activated Microparticulate for Bone Tissue Engineering

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Spherical microparticulates are useful in delivery of proteins and cells. Many polymeric materials, however, are hydrophobic and lack of cell binding sites, thus the potential for cell delivery and tissue engineering scaffold has been limited. Herein, the surface of degradable polymeric (PL-DLA) microspheres was activated by immobilizing lab-designed fibronectin (FN) residue for use as bone tissue engineering matrix.

Bone marrow derived stem cells(BMSCs)were isolated from adult rat and cultivated upon the microspherical scaffold with varying cell densities. BMSCs were shown to adhere well and populate actively on the FN-activated microparticulates, even forming a thick cell layer at high loading density (>300000), whereas poor cell adhesion and growth were noticed on the FN-free microspheres. The cell sheet exhibited differentiation potential into bone forming cells with significant level of alkaline phosphatase expression. Moreover, the cells at day 14 started to form discrete mineralized nodules and the nodule formation was significantly enhanced at day 28. BMSCs loaded at low density (<50000), however, showed low level of mineral formation even at day 28. Based on the current study, the FN peptide was useful to direct BMSCs adhesion and population onto spherical scaffold. The FN-activated polymeric microsphere is considered useful in stem cell delivery and tissue engineering.

(P 353) Surface Characterization of Biologic Scaffolds Composed of Extracellular Matrix

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Biologic scaffolds composed of extracellular matrix (ECM) have been shown to modulate the mammalian host tissue response from scar tissue formation to a more constructive remodeling response. The mechanisms underlying this altered response are poorly understood. As the surface of an ECM scaffold is the first site of host-scaffold ''interaction'', it likely plays a significant role in determining both the initial host response and downstream tissue-remodeling events.

The present study investigated the surface properties of ECM scaffolds derived from porcine small intestine (SIS-ECM), urinary bladder (UBM-ECM), and liver (L-ECM). Immunohistochemistry, electron microscopy, and time of flight secondary ion mass spectroscopy (ToF-SIMS) were used to examine surface characteristics. Cell culture studies were performed to determine the effects of the differences in ECM surface properties upon growth patterns of NIH 3T3 fibroblasts, human microvascular endothelial cells, and hepatic sinusoidal endothelial cells.

The immunohistochemical and SEM results demonstrated that surface composition and ultrastructure of an ECM scaffold are dependent on the organ from which it is derived. SIS-ECM and UBM-ECM, but not L-ECM scaffolds display a distinct ''sidedness'' (abluminal/luminal). ToF-SIMS was used to further elucidate differences in scaffold surface characteristics. Cell culture studies showed that differences in scaffold ultrastructure, composition, and ''sidedness'' have an effect upon growth patterns and maintenance of phenotype *in vitro*.

We conclude that the tissue source of a biologic scaffold composed of ECM has a distinct effect upon both the surface characteristics and the host-scaffold interaction associated with the scaffold, which in turn likely affect downstream remodeling events.

(P 354) Surface Functionalization of Starch/Polycaprolactone Fiber Meshes for Bone Guide Regeneration

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Surface design of biomedical devices is crucial for their acceptation or rejection by the body. One of the most versatile and effective tools to tailor surface chemistry and properties of solids is polymer grafting. This work describes the modification of starch/polycaprolactone scaffolds by surface grafting of sulfonic and phosphonic groups and the effect of those groups on osteoblast-like cells behaviour on a preliminary base.

Scaffolds with porosity of about 68% were obtained from a blend of starch with polycaprolactone (30/70wt-%) by a fiber-bonding process. Oxygen plasma treatment was applied on the produced scaffolds in order to create highly reactive, free radicals on their surface. The pre-activated scaffolds were subsequently immersed in monomer (vinyl phosphonic acid (VPA) or vinyl sulfonic acid (VSA) solution. The successful grafting was confirmed by X-Ray Photoelectron Spectroscopy. Scanning Electron Microscopy was used to evaluate the possible effect of the plasma activation and grafting process on the surface morphology. It was demonstrated that none of the applied processes affect the scaffold/fiber texture.

Direct contact assays with osteoblastic cells (SaOs-2) were performed in order to evaluate the effect of the modification on their behavior. Cells were seeded onto the materials and incubated for 2 weeks. Cells viability, morphology and proliferation were also evaluated after different culture periods. The performed tests revealed that the presence of sulfonic and phosphonic groups increased significantly the proliferation and viability of SaOs-2 compared to untreated scaffolds. From both studied monomers, the scaffolds modified via VPA grafting performed better than the VSA grafted ones.

(P 355) Synthesis and *In Vivo* Characterization of Injectable and Biodegradable Polyurethane Scaffolds in a Wound Healing Model

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Limited availability of autograft tissue for burns and wound healing has generated a need for improved synthetic biomaterials. Polyurethane (PUR) scaffolds exhibit beneficial properties for wound healing, as they support cellular proliferation and new tissue formation both *in vitro* and *in vivo*. We synthesized injectable, porous, PUR scaffolds from lysine triisocyanate (LTI) and hexamethylene diisocyanate trimer (HDIt) with polyester triols of two different compositions and mass and containing 0, 30, and 50% MW-600 poly(ethylene glycol). We evaluated the effects of these compositional variables on PUR physical, mechanical, and biological properties. Foams showed regular pore structures, with an average porosity of 92 vol-%. The polyol composition and PEG content affected the mechanical properties of the foam, while the isocyanate and PEG contents influenced their *in vivo* behavior. LTI foams degraded faster than HDIt foams *in vivo*, probably due to enzymatic degradation of lysines in LTI, although this difference was minimized with PEG plus HDIt. These tunable differences in the physical and mechanical characteristics of scaffolds can be applied to both bone fracture and soft tissue wound healing applications. Wound healing experiments with pre-formed PUR scaffolds showed that biodegradation occurred over 2–3 weeks while supporting cellular infiltration and connective tissue formation with minimal inflammation. Infiltration and organization could be augmented by sustained release of morphogens from

PUR. The injectable aspect may be particularly favorable for obtaining close conformation of the PUR scaffold to complex wound cavities. Supported by NIBIB/NIA/NIAMS, the Department of Defense, and the Department of Veterans Affairs.

(P 356) The Angiogenic Potential of Three-Dimensional Macroporous Ceramic Scaffolds using the Chorioallantoic Membrane Assay (CAM)

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When designing biomaterials for tissue engineering, namely for repair of critical size defects, there is the need to accelerate the remodelling process. A well vascularised network is essential for nutrient transport and cell delivery, thus contributing for bone formation. Angiogenesis, the process responsible for the growth of new blood vessels from pre-existing ones, should be considered as an important parameter. One of the most well accepted models, the chick embryo chorioallantoic membrane assay (CAM), was used to evaluate the angiogenic potential of 3D macroporous scaffolds based on hydroxyapatite and collagen type I.

The scaffolds were produced using the replication method. Polyurethane sponges were used as a template and impregnated with a ceramic slurry composed by hydroxyapatite (HA), water and surfactant. After sintering, collagen was incorporated. To increase the resistance, collagen was crosslinked by EDC/NHS conjugation method and heparin was added. Fertilized eggs were incubated and after 3 days, 3 ml of albumin were removed from the obtuse end of the egg. A window was cut, being the egg viability determined. The window was sealed and the eggs placed in the incubator. Scaffolds (loaded with 25 ng of VEGF) and controls were placed on the CAM on day 7, sealed and returned to the incubator. Images were captured on day 10 and blood vessels were counted. The VEGF loaded scaffolds performed better than the scaffolds alone, as expected. The later are characterized by a non-organized blood vessel network whereas VEGF loaded possess a structured and similar network to the control samples.

(P 357) The Bioengineered Renal Tubule

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Introduction: Bioengineered renal tubules can be used to support endocrine and metabolic renal functions in chronic kidney patients. However, proliferating renal tubule epithelial cells rapidly loose their differentiated phenotype during culture. Our goal is to maintain highly differentiated primary human tubule epithelial cells (PTECs) by culturing the cells on a synthetic scaffold under perfusion conditions. We evaluated the viability and phenotype of PTECs that were cultured on microporous polycarbonate filters and on electrospun supramolecular ureido-pyrimidinone (UPy)-modified polycaprolactone meshes in a commercial perfusion culture system for 7 days.

Results and Discussion: Although cells remained viable on both materials throughout the culture period, PTECs that were cultured on the electrospun meshes had a more differentiated phenotype with maintained gene expression of SGLT-1, PEPT-1, OAT-3, NaPi and AQP-1 genes. Perfusion culture did not modulate the gene transcription profile of these cells. However, perfusion culture improved the integrity of the monolayer on electrospun supramolecular polycaprolactone material as demonstrated by electron microscopy. In these samples also enhanced activity of the brush border enzyme alanine aminopeptidase was seen, whereas the activity of other brush border enzymes remained low.

Conclusion: We conclude that electrospun supramolecular UPy-modified polycaprolactone meshes are very suitable substrates for renal epithelial cell monolayers and that perfusion culture can further improve the quality of the monolayer. Work is in progress to investigate the effect of long-term perfusion culture, as well as the use of bioactive culture substrates, on renal epithelial cell viability and function.

(P 358) The Effect of BMP7 Overexpression in Dedifferentiating Human Chondrocytes

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Purpose: The state of the art in cartilage tissue engineering techniques is the autologous chondrocyte transplantation. This method employs cultivation of chondrocytes with a continuous dedifferentiation to fibroblastic phenotype. Evidence of decreased but potentially important BMP7 expression in cultivated chondrocytes led us to overexpress BMP7 by transfection.

Material and Methods: Human chondrocytes were isolated from femur heads, obtained from total joint replacement, and cultivated in a 2D environment. For BMP7 overexpression a construct with a CMV promoter and the BMP7-cDNA was cloned. The isolated cells were transfected by magnetofection with the plasmid at day 1 and compared to an untransfected control group. The ratio of Collagen Type II and Type I was measured by quantitative PCR as a differentiation index.

As further controls cells were transfected with plasmids over-expressing GFP or DSRed, with the empty vector, and the reagent without a plasmid.

Results: The BMP7 construct was shown to be transfected with approximately 30% efficiency and low toxicity. The translation to the protein was shown by Western Blot and quantified by ELISA. The group of transfected cells showed a significantly higher Col II/I index, implicating a more chondrogenic phenotype, compared to the control group in a dose dependent manner. The proliferation rate was slightly reduced compared to the controls. The unspecific plasmids had a minimal influence on the index as well. In conclusion, BMP7 transfection improved differentiation without much effect on proliferation.

This work was supported by the European projects
Hippocrates (NMP3-CT-2003-505758) and Expertissues (NMP-CT-2004-500283)

(P 359) The Effect of Carbon Nanotubes on Mesenchymal Stem Cells in a 3D Environment

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Carbon nanotubes (CNTs) offer the potential to increase the intrinsic mechanical properties of biological scaffolds used in tissue engineering. Little is known as to the effect of CNTs on cells that may be used to seed such scaffolds. This study analyses the suitability of CNTs in tissue engineering using mesenchymal stem cells (MSCs).

Collagen gels with or without 2% CNT solution were seeded with hTert4 MSCs. After 7 and 14 days, the cell viability was measured using the MTT assay (Promega). The effect of mechanical stretch on MSC morphology while seeded in a collagen gel containing 2% CNT solution was examined using the Flexercell Tissue Train Culture system.

Viability of cells in collagen gels with and without CNTs was comparable after 7 days (0.68 ± 0.04 in the absence of CNTs, 0.67 ± 0.04 in the presence of CNTs; values represent optical density). However, after 14 days the number of viable cells in collagen gels with CNTs was reduced to 44.9% (1.30 ± 0.15 in the absence of CNTs, 0.59 ± 0.06 in the presence of CNTs; $p < 0.05$). Both the stretched and non-stretched MSCs with or without CNTs adopted a spindle shaped morphology. There was little difference in cell morphology between the stretched and non-stretched, or between those with and without CNTs. However the CNTs were seen to align along the extensions of the cells.

These findings demonstrate that CNTs have no detrimental effects on MSCs and that further work is required to develop this novel and exciting strategy to enhance the integrity of tissue engineered constructs.

(P 360) The Effect of Chemotherapeutic Agents on the Proliferation and Osteogenesis of Human Bone Marrow Stromal Cells

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The administration of chemotherapeutic agents during cancer therapy may lead to osteopathy and the failure of endoprosthetic replacements. This study investigated the effects of chemotherapeutic agents specific to osteosarcoma on human bone marrow stromal cell (hBMSC) proliferation *in vitro*.

HBMSCs were exposed to individual chemotherapeutic agents at a range of doses, after which cell proliferation was measured. These results were compared to primary hBMSCs pre-cultured with osteogenic supplements (OG) and a human osteosarcoma (HOS) cell line. Cell proliferation was also measured after HBMSCs and hBMSCs pre-cultured with OG were exposed to three agents at their clinical doses, either individually or in combination.

A significant dose-dependent effect upon cell proliferation of hBMSCs, hBMSCs with OG and HOS cells was observed with cisplatin (CDP) and doxorubicin (ADM), with maximal doses leading to a significant reduction in proliferation ($p < 0.05$). No dose-dependent effects were found when cells were exposed to methotrexate (MTX). When cells were exposed to a combination of MTX, ADM and CDP at clinical doses, a synergistic effect on cell proliferation was not observed.

In summary, individual chemotherapeutic agents significantly altered the cell proliferation of hBMSCs *in vitro*. Interestingly, the use of combined chemotherapy on cells did not amplify the observed effects of individual agents. These results suggest that osteopathy and osteopenia in patients receiving chemotherapy may be related to a reduction of the osteoblast precursor cell pool.

(P 361) The Effect of Human Knee Meniscus Preservation on Viability of Isolated Chondrocytes

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Background: The meniscus plays an important role in the knee joint stability. Meniscal fibrocartilage, like articular hyaline cartilage, has limited healing capabilities. Tissue engineering could be an alternative and promising option for the treatment of meniscus defects. The aim of this study was to check how the time of meniscus preservation influences on cell viability within meniscus tissue.

Methods: Human menisci were harvested 24-hours postmortem ($n = 6$). To check how the time of meniscus preservation influences on cell viability, we performed 2 experiments.

In first experiment half of each sample was immediately enzymatically digested for 12h with collagenase II to obtain a single cell suspension. The second experiment considered viability of isolated chondrocytes from the second half of meniscus after 14-day preservation in CO_2 incubator in 37°C. Cells from both experimental groups were counted and viability checked using trypan blue exclusion test.

Results: No bacterial or fungal contaminations were found after 2 weeks of incubation. Number of cells isolated immediately and after 14 days were comparable. Number of viable isolated chondrocytes after 1 day postmortem and 14-day storage were $3.5\times10^4 \pm 8.0\times10^4$ and $2.7\times10^4 \pm 6.0\times10^4$, respectively.

Conclusion: Two weeks of preservation of the menisci does not influence on human chondrocyte viability. It seems that meniscus after 2 week of preservation in CO_2 incubator can serve as a whole graft in surgery or as a donor tissue for isolation the chondrocytes for tissue engineering purposes.

(P 362) The Effect of Hypoxia on Cultured Mucosal Substitutes

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Cultured mucosal substitutes (CMS) have been developed for clinical use to replace keratinizing skin. Upon transplantation of CMS, a period of hypoxia occurs as vasculature invades the avascular dermis. This makes the CMS fragile and susceptible to e.g. infection.

The effect of hypoxia on epidermal morphology, proliferation and differentiation was studied using reconstructed mucosal substitutes as a three-dimensional model. Hypoxia was shown using a pimonidazole staining after 24 up to 72 hours in hypoxic culture using 1.5% oxygen. CMS were stained for the differentiation marker cytokeratin 10 (K10) and the integrin subunit β1. Epidermal proliferation was examined by a Ki-67 staining. Finally, the secretion of VEGF was examined by ELISA

The results show that hypoxia disturbed epidermal architecture and decreased β1 integrin expression in basal keratinocytes, while the differentiation program (K10 expression) was altered as well. In addition, mitotic indices decreased while apoptotic cells were not observed after hypoxic treatment. Finally, a transient upregulation of VEGF secretion was observed the first 48 hours.

In conclusion hypoxia delays differentiation, reduces proliferation, and decreases metabolic activitity.

(P 363) The Effect of MMPs and Pathogenic Micro-Organisms on Porcine Collagen Biomaterials

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Introduction: Tissue repair is a complex process which may be compromised by infection. Biomaterials such as Permacol® (crosslinked acellular dermal collagen) are used to facilitate the natural wound healing process. Permacol® is cross-linked for durability and performance in complicated wounds. Cross-linking increases resistance to proteolytic enzymes released during infection which degrade components of the ECM such as collagen and elastin. Permacol® was incubated with micro-organisms commonly isolated from infected wounds to assess potential performance in infected sites. Permacol® and process intermediates were tested for resistance to neutrophils elastase and MMP-8 released as a result of infection.

Methods: Permacol® was incubated with Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli or Candida albicans. Samples were taken at different time-points and CFU counted. Permacol®, non-crosslinked acellular collagen and untreated collagen were digested with neutrophils elastase and MMP-8. Histopathology and percentage of dry-weight loss were calculated for all samples.

Results: Permacol® had no significant influence on the micro-organisms growth. High levels of micro-organisms were observed coating the Permacol® but cellular infiltration was minimal. Following enzymatic digestion percentage of dry-weight loss increased with incubation time in all matrices tested. Permacol® showed higher resistance to enzyme digestion compared to the other matrices. In all experiments collagen was naturally birefringent and non-degraded before and after treatments.

Discussion: Permacol® was not degraded by any of the micro-organisms tested suggesting resistance to bacterial proteases. Non-crosslinked matrices were more vulnerable to enzymatic digestion, therefore these data suggest that crosslinking of porcine collagen materials confers resistance to neutrophils elastase and MMP-8.

(P 364) The Effect of PMA, IFN-g and PHA-P on Macrophages and T-Lymphocytes Activation Evaluated by FACS

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Materials are not biologically inert and may induce immune responses, both nonspecific and specific, contributing to the failure of the implant or treatment. The potential for host specific immune responses has been focus of attention as a result of the advances made in the incorporation of proteins and cells in medical therapies. The nature of this reaction depends on the type, size, concentration and duration of material exposure and the surface characteristics of the implant. Immediately cells of the immune system accumulate at the site of implantation and a sequence of events targeted against the material take place. In the absence of infection Macrophages and T-lymphocytes are the most persistent in the material tissue interface and surrounding tissue. Upon activation, cells secrete many inflammatory cytokines including, IL-1, IL-2, IL-4, INF-gamma and TNF-alpha, ICAM-1 and the expression of major histocompatibility complex (MHC-I and MHC-II), CD4 and CD8, molecules implicated in specific immune responses.

The aim of this research was to determine the activation of macrophages and lymphocytes to find out the critical stimuli that contribute to implant failure by inducing an immune response. Different stimulatory agents have been applied: PMA, INF-gamma and PHA-P, to establish the elements of these cells material interactions that can be objectively determined *in vitro*. The expression of activation markers on the cell surface was determined by FACS. On the basis of these results and due to their different mechanisms of activation all agents can be used as factors to study the immune response of biomaterials.

(P 365) The Effect of Pore Size on Osteoblast Activity in Collagen-Glycosaminoglycan Scaffolds

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The mean pore size is an essential aspect of scaffolds for tissue-engineering. Previous studies (1) investigating tissue regeneration on collagen-glycosaminoglycan (CG) scaffolds hypothesised that there is a critical range of pore sizes for optimal cellular activity. A more recent study showed that pore size and specific surface area significantly affected osteoblast attachment, indicating there may be different optimal pore sizes for different cell types (2). This study aimed to determine the optimal pore size for osteoblast attachment and proliferation using a range of CG scaffolds produced in our laboratory (3).

The freeze-drying fabrication process of the CG scaffolds was varied to produce scaffolds with mean pore sizes ranging from 21–183 μm (3). These scaffolds were seeded with osteoblasts and assessed 24 h, 48 h and 7 days post-seeding for cellular activity (alamarBlueTM), cell density (Hoechst 33258) and cell distribution (routine histology).

Results show that pore size has a significant effect on cellular activity within a construct and the optimal pore size for osteoblasts appears to be approximately 150 μm. Cell number decreases above and below this level indicating that there is a critical range for optimal cell activity. Future work will expand this study to compare optimal pore size for both osteoblasts and mesenchymal stem cells.

Acknowledgements: Science Foundation Ireland & Integra Life Sciences

[1]Yannas IV, (*et al*), Clin Mater 1992; 9(3–4): 179.
[2]O'Brien FJ, (*et al*), Biomaterials 2005; 26: 433–41.
[3]Haugh MG, (*et al*), 2007; 4th International Conference on Lyophilisation and Freeze Drying, Dublin,

(P 366) The Effect of Shear Stress on Cell Docking Inside Microfluidic Systems: Computational and Experimental Analysis

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Microfluidic devices are enabling platforms that can be useful to control and test the cellular microenvironment to study high-throughput drug screening and cell biology. The aim of this study is to correlate computationally predicted shear stress at the base of microwells inside microfluidic devices with experimental cell docking within the microwells. Briefly, microchannels with 20 cylindrical microwells, 150 μm in diameter and either 20 or 80 μm deep were studied. NIH 3T3 mouse fibroblasts were seeded and medium was infused at an average inlet velocity of 2.31×10^{-4}m/s. Computational models of microchannels were built by means of finite-element code Comsol Multiphysics, with and without modelling the cells presence (15 μm cell diameter). Flow within the 80μm deep microwells was found to be subject to major recirculation areas near the well base, where the wall shear stresses were low.

(P 367) The Effect on the Manufacturing Method of PLGA Scaffold for the Osteogenic Differentiation of Bone Marrow Stem Cells

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A key component in tissue engineering for bone regeneration is the scaffold that serves as a template for cell interaction and the formation of bone-extracellular matrix to provide structural support to the newly formed tissue. In this study, three types of PLGA scaffolds were prepared and confirmed effect on method of fabrication for bone tissue engineering applications.

We fabricated prepared scaffolds by solvent casting/salt-leaching, ice particle-leaching and gas-forming/salt-leaching method. Proliferation of BMSCs in PLGA scaffolds manufactured by various methods was analyzed using MTT assay. Also, we have characterized the osteogenic potential of BMSCs in scaffolds by ALP, RT-PCR and histology for osteogenic phenotype.

The biological activity and ALP activity of BMSCs in the scaffolds was the highest in the scaffolds using solvent casting/salt-leaching method. In RT-PCR results, specific gene, ALP and osteocalcin, related with osteoblast expressed in the all type of scaffolds. BMSCs in scaffolds were stimulated by osteogenic medium and characterized by Masson's trichrome and von Kossa staining. We could observe collagen and calcification over broad region in solvent casting/salt-leaching scaffolds.

We fabricated three types of scaffolds by mean of the manufacturing method for osteogenesis confirmation. Results from this preliminary work showed that solvent casting/salt-leaching method provided highly porous structure with good interconnections between each pore, which can support the structure of cell proliferation and differentiation. We concluded the manufacture method of scaffold affect to differentiation of BMSCs and solvent casting/salt-leaching is useful for osteogenesis of BMSCs.

Acknowledgements: This research was supported by KMOHW (0405-BO01-0204-0006) and SCRC (SC3100).

(P 368) The Effects of Double-Layered Electrospun Poly (P-Dioxanone)-Hybrid-Poly (Lactide-Co-Glycolide) Nerve Conduit and Adhesion Molecule on Peripheral Nerve Regeneration in Rats

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The effects of a double-layered electrospun PDS-hybrid-PLGA nerve conduit and a heparin binding peptide (Hep12) on regenerating nerve in PC 12 cells and the rat sciatic nerve were investigated.

Materials and Methods: 1. Fabrication of electrospun nerve conduit All samples were made with 10/90 poly (lactide-co-glycolide) (PLGA), poly (p-dioxanone) (PDS), and poly (p-dioxanone)-hybrid-poly (lactide-co-glycolide)(PDS-hybrid-PLGA) using electrospinning technique. 2. *In vitro* adhesion of PC12 cells on different type of nerve conduits and a heparin binding peptide-12 (Hep12). 3. *In vivo* implantation of different type of nerve conduits and a heparin binding peptide-12 in rat sciatic nerve. After the sciatic functional index (SFI) was calculated at the end of 8 and 12 weeks, the innervated tissues of sciatic nerve were examined using light microscope and TEM. The axonal density and thickness of myelinated nerve were measured using image analyzer. The results obtained from the samples were analyzed by Krusal-Wallis with Dunn's post test.

Results: 1. Nerve conduit with 1–8 μm sized pore was biodegradable intercommunicated multi-porous structure and double-layered structure reinforced the mechanical properties for nerve conduit. 2. PDS-hybrid-PLGA nerve conduits coated with Hep12 improved PC12 cell attachment and infiltration. 3. Walking-track analysis showed functional recovery in PDS-hybrid-PLGA/Hep12 superior to autograft in 12 weeks 4. In 8, 12 weeks, PLGA/Hep12 and PDS-hybrid-PLGA/Hep12 shows the similar thickness to autograft and PLGA/Hep12 and PDS-hybrid-PLGA/Hep12 in 8, 12 weeks show the similar axonal density to autograft.

Conclusion: Double-layered electrospun nerve conduit combined with adhesion peptide has great potential in peripheral nerve regeneration without growth.

(P 369) The Effects of Granulocyte-Colony Stimulating Factor (G-CSF) on the Isolation of Peripheral Blood-Derived Stromal-Like Cells

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Hematopoietic stem cell (HSC) mobilization can be induced using granulocyte colony stimulating factors (G-CSF). Direct interactions between HSCs and bone marrow stromal cells (BMSCs) exist in the bone marrow stroma, which may be disrupted after G-CSF administration. We thus hypothesised that G-CSF would increase the mobilization of peripheral blood stromal-like cells, which could be isolated, expanded, and characterised similarly to BMSCs. Six sheep were given 5 μg/kg Neupogen? (G-CSF) for five days. Blood samples were taken before G-CSF was given, and 4, 12, 24, and 2 weeks after the last G-CSF dose, for cell culture and hematology. Fibroblastic colony forming units (CFU-F) were counted after 7 and 14 days in culture. FACS analysis and cell differentiation was also performed. No CFU-F formation was observed in blood samples taken pre-treatment. After G-CSF treatment, CFU-Fs were observed in blood samples taken 4, 12, and 336 hours post-G-CSF. Expanded cells were fibroblastic in morphology, and positive for CD14, CD54, and CD164, and negative for CD45, CD73, CD105, and CD106. Upon treatment with osteogenic, adipogenic, or chondrogenic supplements, cells were positive for Von Kossa, Oil Red 'O' stain, or Alcian Blue stain, respectively. PBSCs can be isolated after G-CSF administration and may be an important source of cells with multipotent characteristics.

(P 370) The Experimental Study on Constructing the Tissue Engineered Myocardium-Like Tissue *In Vitro* with Bone Mesenchymal Stem Cells of Infant

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In this paper, we report on engineering 3-D myocardium¨Clike tissue constructs *in vitro* with bone mesenchymal stem cells (BMSCs) of infant. Bone marrow was obtained from the sternal marrow cavum outflow of infant with congenital heart disease(CHD) undergoing cardiac operation. BMSCs were obtained by density gradient centrifugation. The Changes of their shapes, anchoring rate, clone formation rate, the surface markers and ultrastructure were observed. The cells during the second passage period were induced in dulbecco's minimum essential medium (DMEM) with 10umol/L 5-Azacytidine(5-Aza) for 24 hours. After having been cultured for two weeks *in vitro*, phenotype and morphology of induced cells were examined by immunocytochemistry and transmission electron microscope(TEM). When the induced BMSCS had been cultured into filled, the cells were planted in the scaffold of poly-lactic-co-glycolic acid(PLGA) in the density of 5.5×10^6cells/cm^2. The cell-scaffold complex had been cultured in the shake cultivation for 1 week, then the complex had been planted in the dorse of the nude mouse. When the experiment had been finished, the histology, immunology, real time PCR and so on were done. The data suggested that BMSCs of infant with congenital heart disease have the property of the stable growth and the rapid proliferation. Results of mmunocytochemistry showed that BMSCs induced by 5-Aza after two weeks expressed cardiac related protein, such as α-actin, Desmin, cTni and Cx-43.The transparent myofilaments could be observed in the induced cells by TEM. The engineered myocardium¨Clike tissue constructed by BMSCs of infant and the scaffold of PLGA had the auto-myocardial property.

(P 371) The First Trial Use of Tissue-Engineering Design on Basis of Autogenous Multipotential Stromal Cells of Adipose Tissue for Sinus-Lifting and Compensate for a Deficiency Tissues Alveolar Septum

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The cells technology allows to receive natural equivalents of bone tissue—tissue-engineering design on basis of multipotential stromal cells (MMSC).The marrow or adipose tissue can be used as a source of MMSC. Cell culture MMSC was received and described according with the passport of the culture of standard proceeding. Demineralized bone matrix in the form of blocks or chips was used as a scaffold. The selection of 3D structure was determined according to clinic problem. Patient 1. Transplantation construction was executed in the area of AS after ablation ''BioOss''. The improvement of bone tissue and increase of width AS was detected after 1 and 3 months. Patient 2. The extraction of 14,15 at the inspection perforation of the bottom maxillary of sinuse, the absence of bone wall from the vestibular surface. On the spiral computer tomography (SCT) it was revealed, that the thickness and the width of AS amount 0.61 cm. and 0.59 cm. agreeably. The sinus lift operation with transplantation construction was made in area bottom sinus as well as increase of width of the alveolar septum. After 3 months treatment it was determined by SCT that the height of the alveolar septum amount 2.4 cm. and width 1.0 cm. Patient 3. The transplantation construction in alveolar socket, extraction 18 at operation instant. The complete regeneration of the cortical plate in the area of extraction of 18 was revealed after 3 months by SCT. After histological study of tissue revealed by reclaim every patients was detected the forming new bone tissue.

(P 372) The Immune Suppressive Effect of Human Adipose Derived Stem Cells

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Adult stem cells are differentiated into multiple lineages and have less ethical controversies than embryonic stem cells. This character makes bone marrow derived stem cells (BMSCs) used in therapeutic cell sources. However researches with BMSCs have patient's pain and low number of cells. In this study, we focused on adipose derived stem cells (ADSCs) for the alternative source of BMSCs in cell therapy and verified the immune suppressive effect of ADSCs for applying to cell transplantation. We obtained adipose tissue from donors, isolated and cultured ADSCs. We have examined phenotype of the human ADSCs by the FACS analysis and differentiated into multi-lineages. Immune suppressive properties of ADSCs were analyzed by mixed lymphocyte culture and lymphocyte proliferation response to non specific stimulators. The immune suppressive effect of ADSCs was compared with BMSCs. Both ADSCs and BMSCs were obtained from 3 different donors and the mixture was applied.

The results showed that cultured human ADSCs have mesenchymal surface markers and differentiation potency. ADSCs did not arise *in vitro* immune response to the lymphocyte stimulation, moreover suppressed the proliferation in mixed lymphocyte reaction. ADSCs also suppressed lymphocyte proliferation to the non specific stimulator such as PHA, Con A and IL-2. The suppressive effect of ADSCs mixture from multi-donors was stronger than ADSCs from single donor. These findings support that ADSCs have the similar properties of BMSCs and immune suppressive properties. Therefore ADSCs could be an alternative cell source of BMSCs for using allogeneic stem cell therapy.

(P 373) The Implementation of Control Cells in Experiments on the Immunomodulatory Properties of Mesenchymal Stem Cells– a Technical Note

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It is widely recognized that mesenchymal stem cells (MSCs) from various sources such as bone marrow, adipose tissue or amnion possess suppressive activity on peripheral blood mononuclear cell (PBMC) proliferation *in vitro*.

The purpose of this work was to select suitable primary human cells which can be used as negative control for immunomodulatory experiments and to ensure that the effect of MSCs on PBMCs is a specific property of stem cells.

Human fibroblasts and human epithelial kidney cells (HEKCs) among others were tested in parallel to adipose-derived stem cells (ASCs) in a 6 day co-culture with stimulated and unstimulated PBMCs. PBMC proliferation was detected by two different methods, namely BrdU ELISA and [3H]-thymidine incorporation assay. Furthermore a cytotoxicity assay was performed by co-culturing PBMCs with ASCs or HEKCs as targets.

Both evaluated detection methods using either BrdU or [3H]-thymidine showed similar results in immunomodulatory experiments. It has been found that fibroblasts possess the potential to suppress proliferation of stimulated PBMCs, which is in consistency with the literature. Surprisingly suppression of PBMC proliferation was also detected to a certain degree when stimulating them in the presence of HEKCs. In contrast to ASCs, HEKCs were able to elicit an alloreaction in co-culture with PBMCs. A functional cytotoxicity assay revealed that HEKCs are lysed by cytotoxic cells whereas ASCs are mostly unaffected.

Overall, these findings provide evidence that HEKCs can be applied as control cells in functional assays but not in co-culture experiments measuring proliferation through BrdU or [3H]-thymidine.

Acknowledgments: EXPERTISSUES (NMP3-CT-2004-500283)

(P 374) The Importance of Multislice CT-Exam in the 3D Evaluation of Human *In Vivo* Metallic Implants

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The study points out the importance of Multislice CT-scan exam in the evaluation of the metallic human *in vivo* implants materials with a special value in the diagnostic assessment of pathology associated with their use.

We retrospectively reviewed the Multislice CT-scan imaging studies of 122 patients with metallic implants examined between January 2007 and December 2007 at The Imagistic Diagnostic Centre Neuromed Timisoara using a 64 Somatom Sensation.

The 64 Multislice CT-scan exams allow the visualization of the integrity of the metallic prosthetic devices and osteosynthesis materials, as well as their disfunctionalities, without being obstructed by metallic artifacts (major inconvenient in classic CT). Also, a special interest was to detect the subtle local pathology associated with their use.

We evaluated the permeability and side effects of intraventricular shunts for hydrocephalia in 17%. The good location and efficiency of arterial aneurisms vascular therapeutically clips were checked in 10%. Dental implants with regional tissular involvement were assessed in 10%. Cardiac metallic valves prosthesis, coronary and peripheracally endolumenal arteries stents with their specific morphology, functionality parameters and local tissular side effects were revealed in 42%. The topography, anatomic alignment and local side effects of osteosynthesis materials were evaluated in 18%.

Hip metallic prosthetic devices integrity and regional secondary reactive and pathologically deviations were detected in 3%.

The Multislice CT-scan examination represents the election method in current practice for the 3D evaluation of the metallic human *in vivo* implants materials with special abilities in the detection and characterization of the local tissular side effects.

(P 375) The Interaction Between Nanofibers Mesh Covered Scaffolds and Chondrocytes

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The replacement of cartilage tissue or nonunion bone defects caused by injury, infection or resection, became one of the most significant problems for todays tissue engineering. Scaffolds covered with nanofibers seem to be a promising solution for these problems. One of the best methods for making nanofibers is electrospinning. Electrospinning is a fabrication process that uses an electric field to control the deposition of polymer fibers onto a target.

The aim of the study was to investigate the influence of nanofibers made by electrospinning method, covering the 3D microporous structures (scaffolds) on the colonization and differentiation of chondrocytes cells. Scaffolds were made of microfibers with use of rapid prototyping method.

Various parameters of electrospinning process (applied voltage, solution flow, thickness of needle) and different types of polymers (PLLA, PDLLA), solution contents were studied to fabricate nanofibers. The choosing of optimum parameters allowed for obtaining fibers with diameters of tens to hundreds nanometers. The selected nanofibers were directly electrospun on the scaffolds. The nanofibers meshes with different density were generated on the scaffold surfaces. The influence of fibers diameter as well as fiber mesh density on cellular behavior was investigated. Mesenchymal stromal cells isolated from lipoaspirate differentiated in 3–4 weeks to collagen type II and aggrecan producing chondrocytes/cartilage when grown as pellets on these nanofibers, driven by transforming growth factor-beta3. Thus, nanofibers can be used as a growth platform for such cells during chondrogenesis.

(P 376) The Migration Behaviour of Chondrocytes is Affected by the Three Dimensional Environment. Implications for One Stage Cartilage Repair Procedures.

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Introduction: Materials with good biomimetic properties may facilitate the initial fases of regeneration. Migration is a complex process that is important during development and tissue repair. The aim of this study was to evaluate the biomimetic properties of different materials.

Methods: Cartilage from young pigs was obtained after euthanasia. The cartilage was minced using a scalpel. Similar amount of cartilage was combined with Hyaff 11 scaffolds (Hyaff 1112, 37mg $\pm$ 2,41)without coating or gel coated with Puramatrix ™ or Cartipatch™. The constructs were cultured with proliferative media for 21 days. After this period the media was changed to a chondrocyte differentiation. The number of migrated chondrocytes was determined by measuring the amount of DNA in the different groups. The chonrocyte differentiation was assessed with the quantification of glycosaminoglycans (GAG) and the presence of collagen type II.

Results: Cells migrated and populated the constructs in all groups. Puramatrix had a significantly higher number of cells after 33 days ($p = 0.0143$). All the groups evidenced collagen production. Collagen type II was only present in the puramatrix group. The amount of GAG was significantly higher in the puramatrix group. ($p = 0.00270$).

Conclusion: The different three dimensional and chemical properties of materials affect important processes that are involved in tissue repair. Puramatrix evidenced to elicit better migration and differentiation capacity in pig chondrocytes

(P 377) The Morphology of ß-TCP- Based Biomaterials Influences Their Bioactivity *In Vivo*

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Introduction: This study was undertaken to analyse the bioactivity of ß-tricalcium phosphate (TCP)-based biomaterials of different granulate shape and size by characterizing the cells involved in their degradation.

Materials and Methods: Using a modified subcutaneous implantation model of Hafemann *et al.* 2007, 120 female 5-week-old Wistar rats were randomly divided into 6 groups of 20 animals each. The

animals received polygonal splint granulates of different sizes as follows: Group 1) 500–1000 μm, 2) 150–500 μm and 3) 63–250 μm. Alternatively, the animals received spherical granulates of different sizes as follows: 4) 500–1000 μm and 5) 50–150 μm). Group 6 was a sham group (i.e., operation without biomaterial implantation). All biomaterials were kindly provided by Curasan AG, Germany. The biomaterials were explanted for further histological, histochemical and immunohistochemical analysis at 3, 10, 15, 30, or 60 days after implantation (4 animals per indicated time point).

Results: All ß-TCP based biomaterials showed a distinctive vascularisation and degradation 60 days after implantation. The inflammatory response, however, depended on shape and size of the biomaterials. Already at day 10 after implantation polygonal splint granulates of medium size showed the highest presence of TRAP-positive osteoclast-like cells, while few (group 4) or no (group 5) osteoclasts were detectable around spherical granulates at the same time point.

Conclusion: This *in vivo* study shows that not only the composition of a biomaterial, i.e. β-TCP, but its shape and size may have a profound influence on its bioactivity and on its potential to stimulate the local/peripheral pluripotent cells to differentiate to osteoclasts.

(P 378) The Presence of Chitosan at the Surface Influences the Interaction of Biomolecules and Cells with Chitosan-Poly (Bultylene Succinate) Blend

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Chitosan blends with synthetic biodegradable biomaterials have been proposed for various biomedical applications. However, there are still missing details about the main surface characteristics that may benefit from the blending of these two types of materials. Hence, this work aims at characterising the surface properties of those materials and at illustrating how these properties outline the interaction with proteins involved in cell adhesion and consequently with osteoblast-like cells. Etching by polishing or by plasma was performed to compare the composition of the surface with that of the bulk. The characterization of the unmodified and modified surfaces was carried out by optical microscopy, scanning electron microscopy, Fourier Transform Infrared spectroscopy, X-Ray photoelectron spectroscopy, contact angle measurements and surface energy calculations. The adsorption of human serum albumin and human plasma fibronectin onto the different surfaces was quantified by coupling an indirect method with a colorimetric assay. A preferential adsorption of albumin over fibronectin was registered. Furthermore, the presence of chitosan at the surface of the materials enhanced the protein adsorption. The *in-vitro* biological performance of the studied materials was further investigated by a direct contact assay with osteoblast-like cells (SaOs-2). The cell culture results revealed a positive influence of chitosan over SaOs-2 morphology and activity, while a higher proliferation rate was promoted by the synthetic component. This work further confirmed the interest of combining synthetic and natural polymers, showing a distinctive advantage of using chitosan, within this strategy, to tailor the surface properties of a biomaterial thus improving its biological behaviour.

(P 379) The Use of Bone Tissue Engineered Grafts for 3D Composition in Stimulation of Osteogenesis in Rat Mandible

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Congenital anomalies, trauma, and diseases affect bone structure and mineral density and can lead to the lost of bone tissue. Properties of stem cells together with tissue engineering approaches can be used to overcome bone degeneration and its loss in dentistry, plastic surgery and orthopedics.

We have investigated the efficacy of β-tricalcium phosphate (ChronOs™, Synthes GmbH, Switzerland) together with stromal cells (SC) from adipose tissue on osteogenesis after osteoabrasion on rat mandibles. All grafts were autologous. There were 24 animals in experimental group and 12 animals in control group. Experimental animals were grafted either with a graft of β-tricalcium phosphate with SVC or with β-tricalcium phosphate only. Animals were sacrificed on 7, 21, 40 and 120 days after osteoabrasion and simultaneous grafting of mandible.

The highly porous matrices support the induction of osteogenic differentiation of stem cells as well as progenitors from periosteum, confirmed by positive staining with monoclonal antibodies to osteocalcin and Von Kossa staining. Stromal cells also posses angiogenic potential and stimulate vasculogenesis in a graft, though, newly formed tubules mostly originate from host vessels. These structures are positively stained for CD 31 and von Willebrandt factor. Were found regions of osteogenesis on terms of 21–120 days and strong vasculature of grafts, seeded with SCs.

We assume, that the scaffold create and maintain a space that facilitates progenitor cell survival, proliferation and stimulates osteogenic differentiation of host progenitor cells. SVC stimulate the formation of new vascular net, that provides newly-formed bone with nutrition and oxygen supply.

(P 380) The Use of Carbon Textile Matrixes for Study of Implanted Cell Localization

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Objective: There are two approaches for the introduction of the eukaryotic cells that are widely used to solve the practical prob-

lems of the regenerative medicine, namely in suspension or attached to the scaffold. During the formation of future tissue, the attachment is achieved by growing of the cells into 3D matrix. And in that case one can compare cells not in free (in direct introduction) and attached form, but free and those that has formed the precursor of the tissue.The aim of our work was to investigate the fate of the cells, which are introduced in suspension or in attached to two-dimensional matrix form into the lesion focus of organism of experimental animals. Cell delivery systems include nonbiodegradable matrix having different chemical composition physically associated with viable cells. The material has been introduced into abdominal cavity in order to avoid quick active washing out and in the same time to exclude the ''forced'' attachment, as it takes place during introduction into dense tissues, where the introduced cells are actively pressed to the resident cells. Material and methods: For cell immobilization we have used nonbiodegradable matrixes: carbon textile, cellulose membrane (pore size up to 20 μm) and silicon polymers. Matrixes-carriers for the transplanted cell culture were made in the form of sponges, gauzes, globules and fibers. The murine cell sublines of two different morphological species: epithelial-like and fibroblast-like, were used. After cultivation immobilized-cell matrixes were implanted intraperitonealy into male mice of C57BL/6J line.. The morphology and functional state analysis of

(P 381) The Validation of a Novel Compression Bioreactor to Ensure the Accurate Application of Loading Regimes

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Bioreactors enable the application of reproducible and accurate mechanical loading regimes to cell-seeded constructs (Démarteau *et al.* 2003). One of the most important aspects of bioreactor design to ensure that the application of the required forces is accurate and reproducible is the validation stage. A novel multi-construct rotating bioreactor was designed to investigate the effects of compression on cell-seeded PLLA scaffolds *in vitro*. This bioreactor underwent extensive validation to determine the amount of error in the system and therefore the actual amount of compression being applied to the cell-seeded constructs. This included characterisation of the base of the bioreactor chamber, confirmation of contact between the constructs and the loading plate, analysis of the movement of the loading plate and determination of the average amount of strain being applied, at different positions, across the surface of the loading plate. The scaffolds were also mechanically tested to ensure they had suitable mechanical properties to support the application of the required loading regimes. The validation of this bioreactor demonstrated that it was capable of accurately compressing numerous constructs. This bioreactor was then used to apply 2% compression to PLLA scaffolds seeded with mesenchymal stem cells which induced the chondrogenic differentiation of these cells. These experiments produced reproducible quantitative PCR and biochemical assay data from each of the cell-seeded constructs ($n = 5$) which shows the importance of the validation stage of bioreactor design in generating high quality data.

(P 382) Thermal, Mechanical and Biological Properties of PLA Fibers

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Multifilament (filament diameter 18 μm) and monofilament (diameter 150 μm) PLA yarns used as reinforcing components of tissue engineering (TE) scaffolds displayed different mechanical and thermal behavior according to their sourcing.

Melting of multifilament PLA fibers in the temperature interval 142–175°C with peak at 162°C correlated with lower biodegradation rate and higher mechanical properties after aging in saline compared to PLA monofilament fibers melting in the temperature interval 91–157°C with the peak at 150°C.

Distinct thermodynamic and mechanical properties were observed for multifilament and monofilament PLA fibers. Moderate decrease of melting enthalpy for multifilament fibers and drastic decrease of melting enthalpy for monofilament fibers was observed in DSC profile in second heating. Crystallization peak was absent in DSC profile for monofilament fibers and clearly detected for multifilament fibers in second heating. Crystallization peak for monofilament fibers appears on the second scan of DSC profile and melting enthalpy increased during aging from month 3 to month 6 from 4.9 J/g to 20.1 J/g due to intensification of chemicrystallization process. Sharp decrease of mechanical properties for monofilament fibers and slight changes for multifilament fibers were observed after 3 months storage in saline.

Animal tests (full thickness abdominal wall defect in rats) confirmed fast degrading properties of PLA monofilaments, with lower melting temperatures and lower crystallinity.

The selection and combination of PLA fibers for TE scaffolds have a major impact for successful clinical indications.

(P 383) Three-Dimensional System (Bioreactor-Biomaterial) to Engineer Ligament Tissue

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A simple and compact bioreactor system has been planned and developed to engineer ligament grafts, under monitored biomechanical stimuli. Partial or complete damage to ligaments is frequent especially in young/sportive people; the possibility to regenerate/substitute damaged ligaments becomes thus crucial. Therefore, we have developed a 3D bioreactor system able to culture cells onto biomimetic substrates under controlled biomechanical conditions.

Gelatin-based fibers with proper bulk properties have been developed and tested as ligament substitutes. Scaffolds were placed

within a cylindric culture chamber, clumped at the extremity, and exposed to torsion/strain, through a stepper-motor connected to the chamber, reproducing the physiological ligament stresses. Adult stem and/or skin-fibroblast cells were seeded onto the scaffolds and cultured within the bioreactor under different experimental conditions (w/o AA, w/o TGF-beta). Cells proliferation and matrix deposition has been evaluated.

The 3D bioreactor system has been validated in terms of sterility, experimental reproducibility and ease to use. The developed bioreactor is based on the concept of controlled alternating torsions of scaffolds embedded in cell suspension/culture medium. Gelatin based grafts with different compositions and architectures have been developed and tested in terms of cells adhesion, proliferation and matrix production under different chemical-physical stimuli conditions.

In conclusion, we have developed and validated a simple (bioreactor-biomaterial) system to engineer ligament grafts, for a clinical use. Particular attention was focused on the production of a simple, efficient and low-cost 3D system.

(P 384) Tibial Defect Reconstruction using Hydroxyapatite Scaffold Seeded with Expanded Stem Cells

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Introduction: Critical long bone defects can not heal spontaneously and a replacement technique is needed.

Material and Method: A 6 cm. tibial defect of a 24 years old male, result of a complicated open fracture, had to be reconstructed. Because of the comminution of the homolateral fibula and the refuse of the patient to harvest the contra lateral fibula for a pediculated bone graft, another reconstruction method was needed. Classic reconstruction methods (calcium sulfate pellets, autologus bone marrow and iliac crest bone graft) failed.

A tissue engineering approach was then used by obtaining a three dimensional construct made of a porous hydroxyapatite ceramic scaffold, seeded with culture expanded stem cells isolated from the patients own bone marrow. The resulting living structure was implanted in the diaphyseal defect. By plating the fibula using long fibulo-tibial screws, internal bone stabilization was secured.

Results: The surgical wound healed without complications. The patient was monitored by x-rays and periodical clinical examination. Callus formation was observed at the bone-scaffold interface at 80 days after the operation when the patient was allowed to a partial limb bearing. Now, at 120 postoperative days, using a cane, he is walking without having pain and on x-ray examination periimplant bone formation is detectable.

Discussion: Although the described defect can not be considered healed yet, the clinical and radiological evolution of the lesion is promising. The use of expanded autologus stem cells seeded on porous ceramic scaffolds could represent a progress in critical long bone defects reconstruction.

(P 385) Tissue Engineered Bowel using Biologically Derived De-Cellularised Scaffold.

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Short bowel syndrome is characterised by a severe reduction in the amount of functional intestine available as an absorptive surface. Attempts to lengthen the intestine by interposition of artificial tubular scaffolds juxtaposed between healthy tissue has shown limited success mainly because while neo-intestine can be induced to grow, it rarely survives for long and therefore does not achieve absorptive status.

Using a completely novel approach, lengths of adult rat colon were harvested, de-cellularised such that they retained all the normal features of bowel except for the cells (therefore totally non-immunogenic) and cross linked for stability, implanted into Lewis rats followed by seeding with crypt cells. Using this methodology it has been possible to produce neo-intestine mucosa consisting of crypts, goblets cells, active enteroendocrine Paneth cells (a marker of functioning mucosa which has not been successfully achieved previously) and, uniquely, sub-mucosa, all of which is appropriately vascularised. In order to strengthen the de-cellularised bowel which is less robust than normal bowel until it has re-cellularised with the host cells, attempts are currently underway to temporarily strengthen the scaffold using poly 3-hydroxybutyrate (P3HB), a biological polymer which is known to be non-toxic and biocompatible.

A significant advantage of this approach is that the associated blood vessels can also be de-cellularised while still attached to the intestine. They can then be reconnected when the bowel is implanted and we have evidence that these vessels will re-cellularise appropriately to form functioning blood supply such that the implanted intestine is immediately re-vascularised.

(P 386) Tissue Engineering and Fetal Therapy: the Moral, Social, and Political Implications

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The clinical application of scientific knowledge is not morally neutral. This is especially true when advanced therapy medicinal products (e.g., tissue-engineered products TEPs) and innovative therapies (e.g., fetal therapy FT) are at stake.

Our task within EuroSTEc is to analyze the moral-legal implications of FT with use of tissue-engineered products.

In this context, we assume, as fundamental premise, that the clinical use of soft tissue engineering for prenatal surgical correction of congenital defects is neither immediately justified nor straightforwardly desirable.

Apart from legal, political and market concerns about safety, production, availability, and commercialization, the availability of tissue engineered products and its use in FT for correction of birth defects raises unique ethical questions:

What are the necessary and sufficient conditions for the responsible application of TEPs in FT? Do women have a special duty to have surgery on behalf of their fetuses? Should research on innovative therapies for relatively rare conditions be a funding priority? Or should resources be committed to research and treat-

ment of diseases affecting a larger number of patients? In which way does tissue engineering for prenatal correction of congenital defects influences citizen's views about the body and its boundaries?

Few has been written about the ethical, social, and political impact of this new—in the minds of most people, still part of a science fiction book—medical possibility.

We offer an overview of the complex moral issues researchers, legislators and politicians will have to keep in mind when trying to regulate TEPs application in FT

(P 387) Tissue Engineering for Diaphragmatic Replacement with Collagen Matrices. Prelimenary Result

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Introduction: Diaphragmatic reconstruction with tissue engineering is a viable and potentially improved alternative for diaphragmatic replacement in the neonatal period as was shown in previous studies.

The aim of this pilot experiment was to study the feasability of reconstructive surgery with tissue engineering of the diaphragma with special interest in the ingrowth of muscle cells in the grafts in a rat model.

Materials and methods: For this pilot we used 10 Wistar rats. Under anaesthesia the right hemidiaphragm was exposed and 1/3 was resected. In the defect a collagen matrix, size of approximately 15 mm $\times$ 20 mm, was implanted.

Four weeks after matrix implementation the rats were harvested.

Results: Surgery was performed on 10 rats (60% overall survival rate).

Histological examination showed high cell density and organization in the implants. Cell alignment in comparison with the collagen fibres within the construct was seen. The grafts showed fine muscle cells ingrowth from the margins of the grafts.

Discussion: We succeeded in the development of a rat model for reconstructive surgery of the diaphragmatic hernia with tissue engineering. Moreover, we observed ingrowth of muscle cells into the scaffolds.

In future studies we will study the effect of growth factors and GAGs on the speed of regeneration of the diaphragm. Finding a scaffold with the least side effects (ie reherniation), allowing the fastest muscle cells and nerves ingrowth and uncompromised function of the diaphragm is crucial. This study is part of the Eurostec program, a soft tissue engineering program for congenital anomalies.

(P 388) Tissue Engineering of Functional Salivary Gland for Replacement

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Objectives: The treatment of conditions that lead to salivary hypofunction, such as radiation induced xerostomia, is currently limited to the administration of saliva substitutes and sialogogues. The transient effect of these medications necessitates frequent administration and systemic side effects may be intolerable. The creation of implantable, functional salivary gland tissue from autologous glandular cells would provide a physiologic solution to this problem. We investigated the feasibility of engineering such tissue *in vivo*.

Methods: Primary human submandibular gland cells were grown, expanded and seeded on biodegradable polymer scaffolds. A total of 48 scaffolds with cells and 16 control polymers without cells were implanted subcutaneously in athymic mice. The implants were retrieved 2, 4 and 8 weeks after the implantation for phenotypic and functional analyses.

Results: Human salivary gland epithelial cells retained their phenotypic and functional characteristics at all culture stages. Histologically, formation of acinar gland-like structures was observed within the engineered tissue by 4 weeks after implantation. Immunocytochemical and Western blot analyses of the implanted tissues demonstrated the expression of human α-amylase, cytokeratins AE1/AE3, and aquaporin-5. RT-PCR analyses confirmed the expression of human α-amylase mRNA. The retrieved tissues demonstrated the production of human α-amylase over time using a biochemical amylase detection system.

Conclusions: Primary human salivary glandular cells seeded on polymers are able to form functional tissues *in vivo*. The engineered tissues, composed of glandular epithelial cells, produce amylase and possess water channel proteins. This autologous cell-based system may provide a new treatment modality for patients suffering from inadequate salivary secretion.

(P 389) Tissue Engineering the Tumor Microenvironment: Synergy Between Biophysical and Biochemical Cues in Tumor Invasion

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Stromal fibroblasts can regulate the biophysical tumor microenvironment through matrix contraction, matrix remodeling, and altered interstitial flow, which redistributes biochemical gradients and has been implicated in breast cancer metastasis. Fibroblasts also communicate with the tumor via biochemical signals to promote cell growth and migration. To examine the interplay between the biophysical environment, fibroblasts, and the tumor in metastasis and invasion, we developed a novel *in vitro* model of the tumor microenvironment, incorporating breast cancer cells, fibroblasts, a 3-D collagen matrix, and interstitial flow. We have shown that interstitial flow and tumor-fibroblast interactions synergistically promote matrix remodeling of the tumor microenvironment. Confocal reflectance microscopy revealed marked changes in the collagen matrix density and morphology. The apparent permeability of the matrix increased 6.5-fold with interstitial flow and co-cultured tumor cells and fibroblasts, while flow alone (with a single cell type) only resulted in a 1.8 to 2.2-fold increase. This suggests that the biophysical environment plays a crucial role in modulating the effects of biochemical crosstalk between tumor cells and fibroblasts, leading to marked changes

in the structural and functional properties of the ECM. These differences may lead to a more tumor-permissive matrix, by facilitating migration, providing pro-invasive signals, and increasing interstitial flow to drive autologous chemotaxis by tumor cells. The implications of this work are two-fold: (1) we have established a novel model for the human cancer microenvironment, and (2) we have demonstrated that interstitial flow plays an important role in tumor microenvironment, which in turn governs cancer invasion.

(P 390) Tissue Engineering using Electrospun Functionalizable Scaffolds

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Electrospinning is a flexible and effective technique for the fabrication of polymer nanofibers. Various polymers have been successfully electrospun into ultrafine fibers in recent years: mostly in solvent solution and some in melt form. There is strong medical interest in the generation of scaffold materials for tissue regeneration and 2D and 3D cell culturing. Electrospun nanofibrous materials could act as three dimensional scaffolds for the directed growth of cells, e. g. in nerve regeneration and nerve guiding. Such material can be produced by electrospinning in the form of aligned nanofibers. The key property that differentiates the envisioned material from existing systems is that it mimics the extracellular matrix (ECM) and offers a simple way to control structural alignment and promote target cell binding. The native ECM provides more than just a mechanical support for cells, it also serves as a substrate to display specific ligands and factors that control cell adhesion, migration and regulate cell proliferation and function 4. This requires the introduction of biological guidance cues for the controlled growth of cells on aligned electrospun nanofibers. Electrospun functionalizable materials for medical applications will be further investigated as ECM mimicking scaffold substrates for nerve guiding and regeneration.

(P 391) Towards a Functional Multi–Cell Co-Culture Model Starting From a Single Human Progenitor Cell Source

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The scope of this project is to generate a 3D osteoblast-osteoclast-endothelial cell co-culture system, as an *in vitro* model, studying the effect of specific biochemical and biomechanical factors on bone remodelling process. Here, we investigated the feasibility to derive the three cell lineages starting from a single source of human progenitor cells, in monolayer cultures.

Human bone marrow(BM) and adipose tissue(AT) cells were cultured in presence of different cytokines and oxygen percentages for up to 4 weeks. Cell phenotypes were assessed by cytofluorimetric and Tartrate Resistant Acid Phosphatase (TRAP) assays.

We first aimed at maintaining the endothelial and mesenchymal populations and at generating an increased number of osteoclastic cells, by expanding the fraction of monocytic cells with macrophage colony stimulating factor and subsequently inducing their differentiation with the receptor activator for NKκB ligand. A co-culture of endothelial lineage cells (5.7% of CD34/CD31+) with mesenchymal cells (55.7% of CD105+) and pre-osteoclasts (3.7% of CD14/Vitronectin+) was only obtained from AT, whereas BM yielded a lower fraction of per-osteoclasts and absence of endothelial cells. Medium supplementation with FGF-2 supported mesenchymal cell proliferation, but eliminated the presence of TRAP+, CD14/Vitronectin+ cells. Lowered oxygen levels, reduced only the endothelial-lineage cells to 1.7%.

The study indicates that AT progenitor cells can be manipulated appropriately to establish a co-culture of mesenchymal, osteoclastic and endothelial lineage cells. Ongoing experiments will investigate whether osteoblastic differentiation of mesenchymal cells and their production of paracrine signals are able to replace the soluble factors used to stimulate osteoclastic differentiation of monocytic precursors.

(P 392) Towards Functionalisation of Collagenous Nano- and Micro- Fibrous Structures

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Type I collagen accounts for up to 70–90% of the collagen found in the body and is present in the form of elongated fibres in tissues such as nerves, skin, tendon and cornea. *In vitro*, under appropriate conditions, extracted forms of collagen spontaneously self-assemble to form fibres that are indistinguishable from those occurring *in vivo*. However, the natural cross-linking pathway (lysyl oxidase mediated) does not occur *in vitro* and consequently such structures lack sufficient strength. Although a number of different chemical cross-linking approaches have been introduced, none of them provides a potential for additional functionalisation. Towards this goal in this study a PAMAM dendrimeric system was investigated to develop a multi-functional cross-linking method. The influence of the novel dendrimeric cross-linking approach was evaluated through thermal, mechanical and neuronal cellular viability analysis of the produced fibrous collagen scaffolds. Differential Scanning Calorimetry investigation indicated an elevated denaturation temperature over non-cross-linked or mild cross-linked (water soluble carbodiimide) scaffolds ($p < 0.05$). Similarly, extension to failure mechanical testing made apparent that the produced scaffolds were characterised by increased tensile strength over their non-cross-linked counterparts ($p < 0.05$). Biological evaluation of the cross-linked scaffolds exhibited superior neuronal cell cytocompatibility over the one obtained from conventional cross-linking approaches (water soluble carbodiimide). Neuronal cells differentiated and exhibited normal cellular morphology as evidenced by SEM and Rhodamine-Phalloidin staining. In addition, neuronal cells aligned in the direction of the oriented fibres. These results demonstrate the feasibility of dendrimeric systems as a novel cross-linking approach to stabilise and consequently functionalise fibrous collagenous structures.

(P 393) Towards Improved Neovascularization–Endothelial Progenitor Cells and PLA/Bioactive Glass Composite with Angiogenic-Inducing Properties

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Vascularization is crucial process for the success of tissue engineered constructs. Without a proper vascular network, implants, both *in vivo* and *in vitro*, suffer from hypoxia, lack of nutrients and accumulation of waste products that ultimately result in cell death. This problem can be circumvented by promoting enhanced blood vessel ingrowth into the implant. To this end, we created an *in vitro* system using Endothelial Progenitor Cells (EPCs) and a PLA/calcium phosphate glass composite (PLA/G5) developed in our group for bone regeneration (Charles-Harris *et al*, 2005). These scaffolds show a high porosity (95%) and a pore size larger than 500 um with very open interconnected porosity. We expected G5 particles to introduce bioactivity in the composite, improving bone and vascular tissue regeneration. EPCs are stem cells that can be isolated from bone marrow. They show expression of several endothelial and stem cell markers (CD31, CD34, VEGFR-2, vWF) and have strong vasculogenic properties. Our results showed that proliferation-measured by the WST-1 method was increased compared to controls. Acridine Orange staining for live/dead cells showed good viability, with no observable apoptotic or necrotic cells. After 3 days, cells assembled into long tube-like structures with sprouts delving deeper into the material. We decided to test its possible angiogenic properties by comparing cells seeded in PLA/G5 and in angiogenic conditions. Tubule density quantification results demonstrated a strong induction of angiogenesis on PLA/G5 discs. Our next steps are to establish co-cultures of EPCs-MSCs for bone and vessel regeneration and measuring differentiation by qRT-PCR.

(P 394) Towards *In Situ* Tissue Repair in Osteoarthritis: Differentiation and Migration Potential of Patient-Derived Mesenchymal Stem Cells

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In situ cartilage tissue engineering represents an alternative approach to treat degenerated articular cartilage in osteoarthritis. The concept aims to recruit mesenchymal stem cells from bone marrow by means of chemotactic agents to the defect padded with biomaterials. Present there cells are induced to proliferate and differentiate to restore articular surface. It was the aim of this study to investigate the differentiation potential as well as the chemokine guided migration potential of human mesenchymal stem cells derived from patients with osteoarthritis.

Cells were isolated from femoral heads derived from patients undergoing total hip athroplasty, expanded, checked for standard surface marker profile and differentiated towards osteogenic, chondrogenic and adipogenic lineage using standard differentiation assays. A 96-well plate chemotaxis assay was used to investigate the migratory response to a set of chemokines. Finally gene expression of the isolated cells during chondrogenic differentiation in fibrin-PGLA biomaterial constructs was analyzed using microarrays.

FACS analysis revealed a typical mesenchymal stem cell surface marker profile (CD44+, CD73+, CD90+, CD105+, CD166+, CD14-, CD34-, CD45-). In multilineage differentiation assays histochemistry confirmed chondrogenic, adipogenic and osteogenic potential of the isolated cell populations by formation of collagen type II, lipid vacuoles and calcium deposits, respectively. Chemotaxis experiments could demonstrate migratory response to CCL25. Cells maintained in 3D culture showed proper viability over six weeks, expression profiling supported chondrogenic potential as typical markers were clearly up-regulated (COL2A1, COMP, ACAN, etc.).

To conclude, human mesenchymal stem cells from patients with OA showed feasibility for *in situ* tissue engineering.

(P 395) Transport of Small Anionic and Neutral Solutes Through Chitosan Membranes: Dependence on Crosslinking and Chelation of Divalent Cations

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Chitosan membranes were prepared by solvent casting and crosslinked with glutaraldehyde at several ratios under homogenous conditions. The crosslinking degree varying from 0 to 20% is defined as the ratio between the total aldehyde groups and the amine groups of chitosan. Permeability experiments were conducted using a side-by-side diffusion cell to determine the flux of small molecules of similar size, but holding different chemical moieties, either ionised (benzoic acid, salicylic acid and phthalic acid) or neutral (2-phenylethanol) at physiological pH. The permeability of the different model molecules revealed to be dependent on the affinity of those structurally similar molecules to chitosan, i.e., related to the partition coefficient determined in an independent experiment. The permeability of the salicylate anion was enhanced by the presence of metal cations commonly present in biological fluids, such as calcium and magnesium, but remained unchanged

for the neutral 2-phenylethanol. This effect was explained by the chelation of metal cations on the amine groups of chitosan, which increased the partition coefficient. The crosslinking degree was also correlated with the permeability and partition coefficient. The change in the permeation properties of chitosan to anionic solutes in the presence of these metallic cations is an important result and should be taken into consideration in the *in vitro* predictions of the drug release from chitosan based controlled drug release systems.

(P 396) Treatment of Diabetic Foot Trophic Lesions using a Tissue-Engineered Dermal Graft

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Introduction: Treatment of diabetic foot ulcers includes necrotic tissue debridement, infection control, mechanic discharge and whenever possible, revascularization of the lesion. Nevertheless there are not many therapies that improve tissue regeneration and wound healing. The use of tissue-enginneered constructs could help to regenerate these lesions.

Aim of Study: The aim of this study is to assess the effects of the transplant of an artificial, tissue-engineered dermis in the healing of surgical injuries of diabetic foot.

M&M: Artificial Dermis: Fibroblasts were obtained from skin biopsies of tissue donors from our bank. The artificial dermis was made by mixing cultured fibroblasts and donors plasma. This mixture was clotted by adding $CaCl_2$.

Patients: 15 diabetic patients (bearing open injuries that did not ameliorate for the past 4 weeks) were included in the study. One dermal graft was transplanted on the wound bed once a week until wound healing or, if adverse events appeared, suppression of the treatment.

Results: 15 patients were treated. Wound healing was achieved in 80% cases. The mean period of wound closure was 7 weeks. The mean hospitalisation period was 2.5 weeks. Neither reject signs nor secondary complications were observed.

Conclusion: The treatment of post-surgical wounds in the diabetic foot using an artificial dermis is a reliable and secure procedure.

(P 397) Umbilical Cord Derived Progenitor Cells as a Potential Source for Autologous Cell Harvesting

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Umbilical cords have been identified as a potential niche for harvesting numerous multipotential progenitor cells. Contemporary isolation protocols generate heterogeneous cell populations constituting numerous stem and progenitor cell populations each exhibiting shifting degrees of multipotentiality. Homogeneously isolating each subpopulation and gauging therapeutic potential may highlight umbilical cord as a potential cell source for clinical applications. Furthermore, utilisation of autologous cells overcomes immune compromises associated with tissue engineering.

Wharton's jelly was circumspectly dissected from washed cords and plated into 6 well plates with basal media. Undifferentiated confluent cells were removed and analysed by flow cytometry. Cells were cultured in media known to cause lineage differentiation along adipogenic, chondrogenic, osteogenic and neurogenic pathways. Phenotyping by immunostaining, histological staining and western blotting for proteins associated with the desired lineage was carried out at 7, 14, 21 and 28 days.

Undifferentiated cells expressed the MSC markers CD29, CD90, CD105, CD146 and CD166 as observed by flow cytometry. Expression of the ES markers CD9, CD30, CD56 and CD135 suggest the presence of cells with increased plasticity. Further analysis of the undifferentiated population demonstrated expression of STRO-1 and the stem cell proliferation marker Nucleostemin within subsets of the population. Cells introduced into adipogenic, chondrogenic, osteogenic and neurogenic differentiation medias exhibited differentiation towards the desired lineage, as confirmed by morphological examination, immunostaining and western blotting.

It is therefore concluded that umbilical cord tissue is a good source of progenitor cells with multipotentiality.

(P 398) Umbilical Cord-Derived Stem Cell as a Hepatic Cell Source for Regenerative Medicine

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There are some intrahepatic liver stem cells, such as oval cells in relation to liver regeneration. However, recently it has been reported that extrahepatic liver stem cells exist in bone marrow and contribute to liver regeneration. Many researchers have investigated which cell can differentiate into hepatocyte and contribute to liver. Until now their potential is yet controversial. Besides HSC, there are many origins of MSC, bone marrow (BM), adipose tissue (AT), cord blood, and cord Wharton's jelly (UC).

Moreover, it is reported that BM-, AT-, UC-MSCs can differentiate into hepatocyte *in vitro* or after *in vivo* transplantation. We have focused on UC-MSC as a stem cell source, because UC-MSC can be easily isolated and cultured from generally discarded cord tissue without any ethical problem.

So we confirmed whether the isolated cell from cord by our protocol is one of MSCs or not using FACS and differentiation analysis. After that, we checked which condition can improve the differentiation of UC-MSC into hepatocyte. In addition to *in vitro* experiment, we transplanted UC-MSCs into rats with liver failure induced by bile duct ligation, carbon tetrachloride intoxification.

As results, UC-MSCs could differentiate into hepatocytes expressing not only hepatocyte lineage markers (albumin, CK8, CK18) but also liver-specific functions (albumin and urea syntheses, cytochrome P450 activities) *in vitro*. In conclusion, UC-MSCs will be a good stem cell source not only for hepatic cell therapy but also for bioartificial liver system in the field of regenerative medicine.

(P 399) Use of Human Synovial Fluid-Derived Stem Cells for Cartilage Tissue Engineering

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Introduction: Mesenchymal stem cells (MSCs) are a potentially useful for cartilage tissue engineering due to their proliferative capacity and chondrogenic potential under appropriate differentiation signals. We recently described the presence of MSCs in human and bovine synovial fluids (SF) and shown that bovine SF-MSCs could form a cartilage tissue with hyaline characteristics[1]. The aim of this study was to characterize MSCs in human synovial fluid and investigate whether they could form a hyaline-like cartilage on polyglycolic acid (PGA) scaffolds.

Methods: SF and matched articular cartilage were obtained from patients undergoing joint replacement. SF-MSCs were isolated, grown for at least 24 population doublings, characterized for multipotential differentiation in classical *in vitro* assays or seeded directly onto PGA scaffolds. Chondrocytes were isolated from macroscopically normal, articular cartilage and used as a comparator. Constructs were cultured in classical chondrogenic medium and after incubation, they were mounted in OCT, and frozen sections taken for analysis of the extracellular matrix for collagen types I and II, proteoglycan and alkaline phosphatase.

Results and Conclusions: SF-derived MSCs readily seeded (96%) onto the PGA scaffolds. Immunohistochemical and histochemical localisation of matrix components indicated all constructs produced extracellular matrix with hyaline characteristics which was positive for deposition of collagen II and proteoglycan. We suggest that synovial fluid represents a potentially attractive source of chondrogenic cells which may have utility for cartilage repair therapies in trauma and arthritis.

Acknowledgements: Presenting author funded by EXPERTISSUES [NOE:FP6/500283].

[1]Crawford A *et al* Tissue Engineering 2007;13:1745.

(P 400) Use of Pancreas-Derived Stem Cells to Improve Vascularization in Skin Tissue Engineering

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Clinical success in skin tissue engineering requires improvements in the vascularization capacity of scaffolds for dermal regeneration (SDR). Several efforts have been done in this field including cellular and acellular technologies. In this work we combined the use of GFP+ Pancreas-derived stem cells (PSCs) with a commercially available SDR (Integra matrix, IM). 1 million cells were seeded and cultured in a 15 mm SDR. Cell viability, proliferation and cytokine release from matrices containing cells was assayed *in vitro*. *In vivo*, scaffolds containing cells were used to induce dermal regeneration in a 15 mm full skin defect model. After three weeks of *in vivo* regeneration tissues were harvested and vascularization analyses were performed. Results showed that after seeding, PSCs can survive and proliferate in IM, releasing significant amounts of cytokines. *In vivo*, the presence of PSCs significantly enhance the vascularization levels compared than empty scaffolds ($p < 0.05$). Presence of PSCs in the tissue in regeneration was confirmed by detection of GFP+ cells in the wound area. These results suggest that combined use of PdSC and SDR could be a rational way to improve vascularization in scaffold-dependent dermal regeneration. However, further studies must be performed in order to analyze the cellular and molecular mechanisms involved in the contribution of PSCs and its possible physiological role in wound healing and tissue regeneration.

(P 401) USING Cells as Micro Factories for ECM Polymer Hybrid Material Production

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Scaffold design should aim at creating structures which can guide cells into forming new, functional tissue. This puts high demands on the scaffold, which should ideally act as a temporary support that degrades as new tissue is formed. By combining the possibilities of tailoring polymers into desired shape and mechanics with the ability of the natural ECM to remodel, self organize and store growth factors, hybrid scaffolds enjoying the advantages of both materials could be produced.

In this study, the concept of *in situ* deposition of ECM by cells onto compliant, knitted polymer supports creating *in vitro* produced ECM polymer hybrid materials for tissue engineering was demonstrated. Specifically, human dermal fibroblasts were grown on a compliant poly(ethyleneterephtalate) (PET) support.

Fibroblasts are cells responsible for synthesis and maintaining the ECM of many tissues. When exposed to forces, fibroblasts are known to respond with expression and remodeling of ECM proteins, in particular collagen type I. To increase the collagen deposition on the polymer support, the fibroblasts were cultured in a bioreactor with a pulsating flow and the deposited collagen was compared to static culture conditions.

By culturing the cells under dynamic conditions a 6 fold increase in collagen deposition could be detected compared to static culture. After two weeks of dynamic culture, scanning electron micrographs showed bundles of ECM fibers bridging between polymer filaments and anchoring cells to the scaffold. Histology of the so formed hybrid materials showed an even distribution of cells in the scaffold, surrounded by matrix-like structure.

(P 402) Validation of Mechanical Test Rig to Determine the Crush Strength of CaP Ceramics

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New research is moving towards the development of algae derived CaP ceramics, as it provides a replenishable source material. The processing techniques used in the conversion process of algae to CaP ceramics can compromise the mechanical integrity of the material. However, there are currently no standard tests available or comparative data in the literature to quantity the change in mechanical properties of the resultant CaP bone filler. Some studies in the literature have applied ASTM C1161 test standard to determine the flexural strength and modulus of coral derived CaP ceramics. However, the granular sizes of the alga after heat treatment are too small to apply this technique. The aim of this study was to establish a protocol to quantify the mechanical properties of porous granular materials. Commercially available CaP filler material was used to validate the mechanical test rig designed. The rig was attached to a Universal Test Machine (Lloyds EZL6000K) compression tester. The compression test results were compared to the change in specific surface area measured by gas porosimetry. After the rig was validated, it was used to quantify the change in mechanical strength of algae after different heat treatments. It found that a decrease in mechanical strength was directly proportional to an increase in heat treatment. The study concluded that the mechanical test rig was a reproducible miniature specimen test method that can be used to quantify the mechanical properties of any CaP ceramics with no size limitations.

(P 403) Variations in Essential Ion Concentration Across Batches of Foetal Calf Serum May Influence the Expression of Key Adhesion and Regulatory Molecules of Human Umbilical Vein Endothelial Cells.

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Contemporary *in vitro* cell culture techniques rely heavily on the inclusion of foetal calf serum (FCS). The composition of this undefined mixture of proteins, growth factors and other endogenous regulatory factors fluctuate dramatically, depending on coarse physiological factors of the donor animal. In this study the variation in elemental composition of FCS across donors has been considered and it hypothesised that deviations in elemental composition of sera could influence cell fate and function at a similar magnitude to protein composition.

Elemental composition of FCS from five donors was quantified using inductively coupled plasma mass spectrometry (ICP-MS). Primary human umbilical vein endothelial cells (HUVECs) were systematically cultured using DMEM basal media with 20% foetal calf serum from each of the five donors until confluencey was reached. Expression of surface markers was characterised using immunohistochemistry in conjunction with flow cytometry.

Throughout the five sera significant differences in the elemental composition encompassing a wide range of regulatory ions was identified. In addition to this, across the five donors, statistical variation was observed in a number of molecules critical for cellular adhesion including PECAM-1, ICAM-1 and VCAM-1 and also Von Willeband factor, an indicator of endothelial cell phenotype.

The efficiency of a multitude of enzymes and other key cell signalling pathways is mediated by cofactorial ions within the cell. Elemental analysis of FCS revealed substantial differences in the concentrations of several essential regulatory ions. These sera also demonstrated the capacity to influence cellular phenotype, adhesion and behaviour.

(P 404) Vascular Development in Microtissues using the Hedgehog Signalling

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Introduction: In *in vitro*-generated tissues, the development of a vascular system is crucial to rapidly enhance perfusion and survival after implantation [1] and promote pattern formation and tissue development [2]. The hedgehog signalling was lately shown to be essential for endothelial tube formation during embryonic vasculogenesis [3]. We are investigating the use of this signalling pathway in promoting vascular development in 3D microtissues.

Results and discussion: The co-culture of human mesenchymal progenitor cells (hMPC) and human endothelial cells (hUVEC) results in the formation of a primitive vascular network [4]. Sonic hedgehog, a morphogen from the hedgehog family, induced further *in vitro* vascular development including the formation of lumens and tubes in a dose dependent manner. The cellular mechanism is under current investigation and suggests that both hMPC and hUVEC are stimulated and promote tubulogenesis.

Conclusion: This study shows the possibility to use the hedgehog signalling to promote vascular development *in vitro*. The prevascularized microtissues should prove useful to investigate tubulogenesis and as blocks to built vascularized tissues in a bottom-up approach.

[1]Levenberg. Engineering vascularized skeletal muscle tissue. Nature biotechnology 2005.
[2]Red-Horse. Endothelium microenvironment interactions in the developing embryo and in the adult. Developmental cell 2007.
[3]Vokes. Hedgehog signaling is essential for endothelial tube formation during vasculogenesis. Development (Cambridge, England) 2004.
[4]Rouwkema J, de Boer J, Van Blitterswijk CA. Endothelial cells assemble into a 3-dimensional prevascular network in a bone tissue engineering construct. Tissue engineering 2006 Sep;12(9):2685–2693.

(P 405) *In Vitro* and *In Vivo* Effect of Platelet Lysate on Osteogenic and Chondrogenic Differentiation of Human Bone Marrow Stromal Cells

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The heterogeneous population of cells residing in bone marrow, Bone Marrow Stromal Cells, has been a source of osteoprogenitor cells for bone tissue engineering. This process implies the inter-

action between the cells and growth factors, normally derived from activated blood platelets, in the healing microenvironment. Since, by its turn, the platelet rich plasma offers a concentrate of these ''favorable'' growth factors.In this study we evaluated the effects of Platelet Lysate (PL; the growth factor concentrate derived from PRP) on the proliferation and the differentiation of human BMSC. The effect of Pl on BMSC proliferation was investigated taking advantage of the MTT assay and counting the number of cell doublings. BMSC were cultured in presence or absence of PL with and without the supplement of 10% Fetal Calf Serum (FCS) in the culture medium. The highest level of proliferation was observed in presence of PL without FCS supplement and the induction was dose dependent. In this culture condition we observed a high level of expression of mesenchymal and osteogenic markers. The PL treatment resulted in an increased calcium deposition when cultured cells in an osteogenic medium in vitro and in a bone tissue formation when seeded on a porous ceramic scaffold and subcutaneously implanted in an immunodeficient mouse. Interestingly, the cells differentiated in chondrocytes when cultured in a three-dimensional system under chondrogenic conditions. We conclude that the PL from human source can substitute the serum from animal source to support the culture of hBMSC before *in vivo* implantation for tissue engineering.

(P 406) Measurement of Strain Produced by a Pulsatile Flow Bioreactor

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Pulsatile bioreactors are used in tissue engineering to simulate a vascular environment for cells grown *in vitro*. We characterised fluid flow and quantified strain on a tube where cylindrical scaffolds are mounted. Strain was quantified by measuring tube wall displacement using a travelling microscope. The tube was pulsed using a 3-roller peristaltic pump and pulsing measured with 1, 2 or 4 bioreactors. Flow through the system was either unconstrained or constrained by tying off the outflow.

Initially strain was measured for an unconstrained tube. Strain increased from 0.64% (120 rpm) to 2.16% (330 rpm), though such unconstrained measurements were difficult as total strain was so small. When the tube outflow was constricted to maximise back pressure, gross movement of the whole tube was reduced and this was replaced by pulsing of the tube walls in a sine wave pattern of variable amplitude. The constrained system strain was inversely proportional to frequency, decreasing from 50.5% (60 rpm) to 40% (240 rpm). Strain was measured for both 2 and 4 bioreactors in parallel. For these configurations the inverse strain:frequency relationship remained but the strain for each comparable frequency decreased with increasing bioreactor number (i.e. 1, 2, 4 bioreactors: 50.5%, 12.6%, 12.1% strain respectively). The results show that greater strains were generated by using constrained flow to enhance back pressure rather than the conventional free-flow configuration. Moreover the whole-tube displacement observed when unconstrained, may give the wrong impression of straining the scaffolds, though this will mix the medium.

Framework 6 EU funding: 3G-SCAFF

(P 407) Poly(trimethylene carbonate)-Based Porous Tubular Structures for Tissue Engineering of Small Diameter Blood Vessels

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For tissue engineering of small-diameter blood vessels, biodegradable, flexible and elastic porous tubular structures are most suited. In this study, we prepared crosslinked porous tubular structures from poly(trimethylene carbonate) (PTMC), in which smooth muscle cells (SMCs) were seeded and cultured in a pulsatile bioreactor mimicking the physiological conditions.

PTMC was synthesized and porous tubular structures were prepared by dipping coating, cross-linking by γ-irradiation, and leaching. SMCs were seeded into the porous structures by perfusion and then the constructs were cultured in a pulsatile bioreactor system. The morphologies, mechnical properties were analyzed and SMCs attachment and proliferation were evaluated by histology studies and CyQuant.

Flexible tubular structures were obtained by dip coating with 3 mm inner diameter and 1mm wall thickness. The porosity of the structures in wet state reached 85 vol% and the pore sizes were 60-150 μm. PTMC tubular structures showed comparable tensile strength and higher elongation compared with natural blood vessels.A pulsatile bioreactor system mimicking the conditions *in vivo* (dynamic pressure 70 mmHg, 75 beats/min) was successfully built. Experiements showed 7-day dilation was $< 10\%$ and variation of diameter at each pulse was $< 1\%$. SMCs were homogeneously seeded in the porous scaffolds by perfusion. SMCs proliferate well to form confluent cell layer during a time period of up to 14 days, leading to constructs with even better mechanical performance.

PTMC Porous tubular structures were prepared with good microstructures, elasticity and biocompatibility. SMCs were seeded and proliferated well in pulsatile bioreactor system and significant improvement of mechnical strength was observed.

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2. M Centola, A Rainer, C Spadaccio, S De Porcellinis, J A Genovese, M Trombetta. 2010. Combining electrospinning and fused deposition modeling for the fabrication of a hybrid vascular graft. *Biofabrication* **2**:1, 014102. [CrossRef]