3D Bioprinting of Stem Cell Derived Tissues for Human Regenerative Medicine

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Introduction

Drug testing, medical transplantation and academic research all require human tissue that is often in short supply so a renewable alternative to human tissue is often desired. Immortalized cell lines from various tissues have been used in both, drug testing as well as various academic pursuits, such as disease modelling. Despite these cell lines represent an unlimited supply of cells, the intrinsic abnormalities and mutations they possess limit their use as a model of their relative tissue source. This in turn has pushed researchers to utilise endogenous stem cells as a source for renewable human tissue production. Embryonic, foetal and adult stem cells have been used to generate somatic cells from various tissue types that are more biomimetic than immortalized cell lines. However, stem cells in tissue reside within a niche, which when lost can cause spontaneous differentiation of the stem cells, or cell death[1]. In order to create artificial stem cell niches, researchers have looked to the field of tissue-engineering and recently, 3D bioprinting.

Tissue-engineering is the combination of biomaterials, cells and biochemical factors to support or replace endogenous tissues[2]. In the case of engineering stem-cells the aim is to reproduce a suitable niche for stem cell expansion and differentiation, and subsequent formation of artificial tissue. 3D bioprinting is a tissue-engineering technique which allow precise deposition of “bio-inks”, combinations of supporting biomaterials and desired cells[3], [4]. By combining multiple bio-inks, complex multi-cellular and multi-material structures can be formed that are analogous to human tissue for drug testing and medical transplantations[5][6][7][8]. 3D bioprinting has advanced rapidly since the first cell printing with a modified HP inkjet printer in 2005[9]. This review will cover the various methods of bioprinting, the development of “bio-inks”, as well as how bioprinting has already been applied to stem cell culture and the outcomes of such research. We will also cover the technical and regulatory limitations that 3D bioprinting currently face.

Bioprinting techniques

Bioprinting can be defined as robotically automated, layer by layer, additive biofabrication using both living cells and biomaterials, directed by a digital model.[3], [4]By layering deposited biological materials on top of each other, it is possible for a bioprinted structure’s architecture to be defined not only in 2D, as in patterned cells, but in 3D. This, along with the ability to deposit various biomaterials and cell types at once, gives the potential to generate complex, tissue-like structures from the ground up. There are various methods of bioprinting, the most prevalent ones are detailed in **Table A,** and visualized in **Figure 1.**

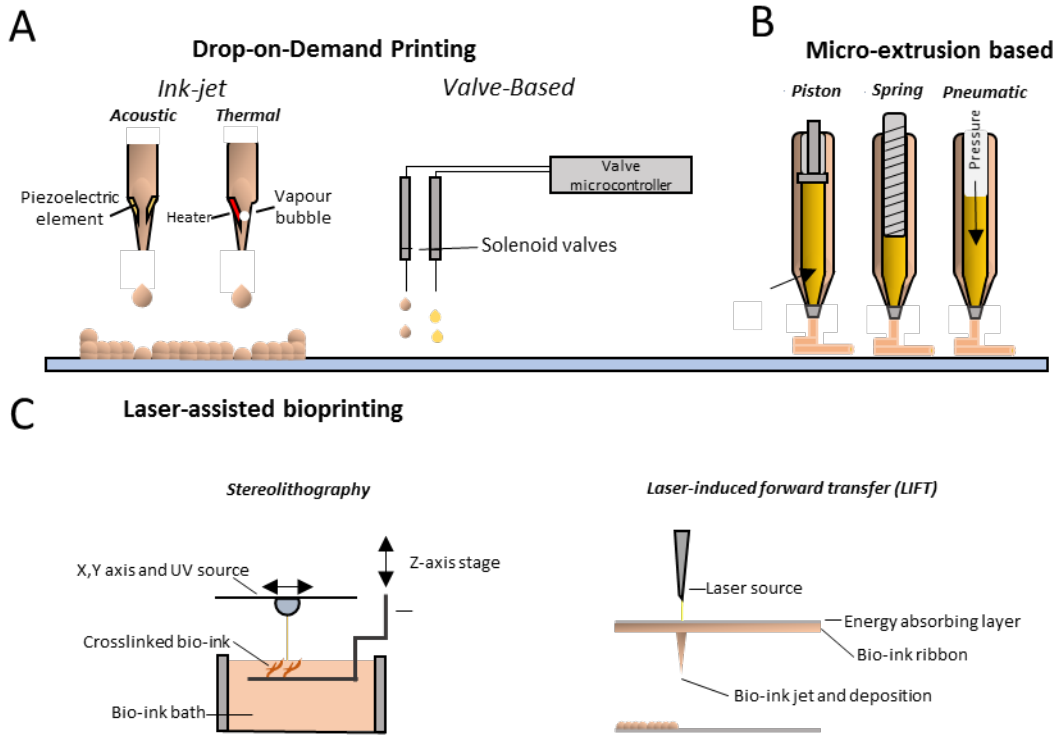

Figure 1. The three primary bioprinting methods. **A**: Drop-on-demand (DOD) bioprinting, comprised of: Ink-jet and valve-jet bioprinting. **B**: Micro-extrusion bioprinting. Pressure, spring, or piston forces the bio-ink to extrude through a nozzle. **C**: Laser-assisted bioprinting: Stereolithography and Laser-induced forward transfer (LIFT).

Drop-on-Demand Printing

Drop-on-demand (DoD) printing utilises controlled deposition of bio-ink droplets to generate artificial tissue in a high throughput manner. The two methods of DoD printing are ink-jet and valve-jet bioprinting.

In an inkjet printer, a droplet is dispensed by generating a discrete thermal, piezo-electric or electromagnetic effect. Indeed, some of the earliest bioprinting was done by utilising commercial ink-jet printers to pattern proteins [10], and then a patterned culture of hamster ovarian and motor-neuron cells[9]. The ability to deposit droplets in the range of picolitres means the resolution available to print live cells is very high and single cell printing is possible[11]. However, due to the potential for high shear and thermal stresses, cell viability is a concern, although while transient pores were visible following ink-jet printing of mammalian cells, these quickly sealed, and were actually used to successfully transfect cells with plasmids following printing[12]. Print nozzle clogging and need for low viscosity bio-inks also constrained the use for creating larger printed structures (in the scale of cm^3).

Valve-jet bioprinting controls dispensing through solenoid microvalves with bioinks driven by pneumatic pressure[13]. Very high spatial precision, small deposition volumes (in the range nanolitres), high cell viability [14], and high-throughput generation (1000 droplets per second) make valve-jet bioprinting very appealing for generating human tissue. But like ink-jet, valve-jet bioprinting is still a nozzle based printing technology where nozzle blockage can be an issue with high-viscosity and cell-density bio-inks.

Laser-assisted bioprinting

Laser-assisted bioprinting (LAB) encompasses two forms of bioprinting: laser-induced forward transfer forward-transfer (LIFT) technique, whereby a laser beam pushes a biological sample from a pool of bio-ink to the target substrate; and stereolithography which involves curing of a photo-sensitive polymer using a laser. Use of a laser allows very high resolution, with single cell placement being possible[15][11]. As there are no nozzles used in printing, clogging is not an issue, so high viscosity fluids can be printed. Cell viability is high in printed human stem cells [16]. Bearing perhaps the best resolution possible for *in situ* bioprinting, LIFT theoretically has the closest possibility of printing a completely biomimetic tissue structure. In reality, limited scalability makes this far from feasible for clinical translation. Like stereolithography, a large excess of bio-ink is required to print a structure and the technology is expensive. Constructing the bio-ink ribbon necessary for LIFT is also time-consuming and difficult, especially for larger structures.

Stereolithography is most often used in the production of acellular scaffolds. The capability of a laser-based system allows extremely high resolution, with printed structures having features in the nanometre range[17][18]. The need for excess materials to be present during the curation step represents a limitation for the bioprinting of large structures employing one-step stereolithographic printing. The presence of toxic photo-initiators and the resultant free radicals are also an ongoing concern for cell viability during and after printing[19].

Table A. Bioprinting techniques summary and examples of use.

Bioprinting technology	Summary of technology	Examples of tissue-structures produced	Benefits	Drawbacks	Average resolution
Ink-jet	Thermal, electromagnetic or piezo-electric force propels droplets of bio-ink to defined area.	Vascular[20] Skin[20] Osteochondral[21][22] Ovarian[23] Liver[24] Neural[25]	High resolution.	Potential for high cellular stress during deposition. Nozzle blockage common. Expensive technology.	20+µm.Picolitre (pL) deposition. Single/few cells possible per deposition.
Valve-based	Pressure based deposition of bio-ink droplet through valve.	Embryonic[26] Liver[27]	Gentler than ink-jet. High resolution available. High droplet deposition (1000/second)	Need for constant external pressure supply. Nozzle blockage common.	40+ µm. Nanolitre (nL) deposition. Single/few cells per deposition.
Stereolithography	Photocuring of photosensitive polymer by UV light produces solid structure from bath of acellular material or cellular bio-ink.	Vascular[28] Liver[29]	Very high resolution. High-throughput and fast printing possible using digital mirror device technology.	Excess bio-ink needed to create curing bath. UV radiation, photo-initiator and resultant free radicals are toxic to cells. Expensive technology	150+nm (higher with cell-laden ink). Specific cell deposition not possible.
Laser-assisted	Laser is fired to push cell from pool of bio-ink onto target surface.	Fibroblast[15] Vascular[11]	Highest resolution of bioprinting possible. Flexibility of printing material viscosity.	Excess bio-ink needed to create pool. Potential damage to cells. Expensive technology.	Single cell resolution possible

Extrusion	Deposition of materials through motor driven extruder.	Neural[30] Liver[31] Vascular[32][33] Pancreatic[34] Embryonic[35][36] Osteochondral[37]	Flexibility with printing material viscosity. Cell friendly printing process. Coaxial or multiple nozzles allow printing various bio-inks. Affordable technology.	Low resolution. Slow print times.	50µm. Cell aggregate or spheroid deposition only.

Extrusion-based bioprinting

Extrusion-based bioprinting is currently the most accessible and widespread bioprinting technique[38]. Originally developed for printing plastics as fused deposition modelling (FDM) technology, deposition of biomaterials is often through a motor driven or pneumatic extruder (Figure 1B). Unlike inkjet printing limited by low viscosity and LAB limited by scalability, extrusion based bioprinting can print large, scalable constructs with a wide range of viscosities.

However, extrusion is not without its own caveats. Resolution of printed products is lower than other forms of bioprinting, in the range of 30 to 100 micrometres for their finest details. Cells also experience shear stress while being deposited with highly viscous materials, therefore material selection is crucial for extrusion printing as the printed structure must maintain its shape while gelled. This means low viscosity bio-inks are often unusable, although this is being overcome by printing into baths [39], [47] .

Bio-inks

A bio-ink is the biological equivalent of ink for ink printers, but instead of dyes it employs biological materials to generate the 3D structures. Bio-inks are typically composed of structural supporting materials, live cells, and can also include bioactive molecules such as growth factors, either encapsulated or covalently tethered to the supporting material[40][41].

Hydrogels, high-water content polymers that can be cross-linked to form a gel [42], mimicking extracellular matrices (ECM), represent the main component in the bioink. Choice of material to form the basis of the bio-ink is crucial for successful printing and tissue formation. The desirable bioink should fulfil a range of properties including: 1) mechanical stiffness, 2) structural stability and biodegradability, 3) biocompatibility and tissue induction; and importantly for bioprinting 4) printability, all of which are summarised in **Figure 2**.

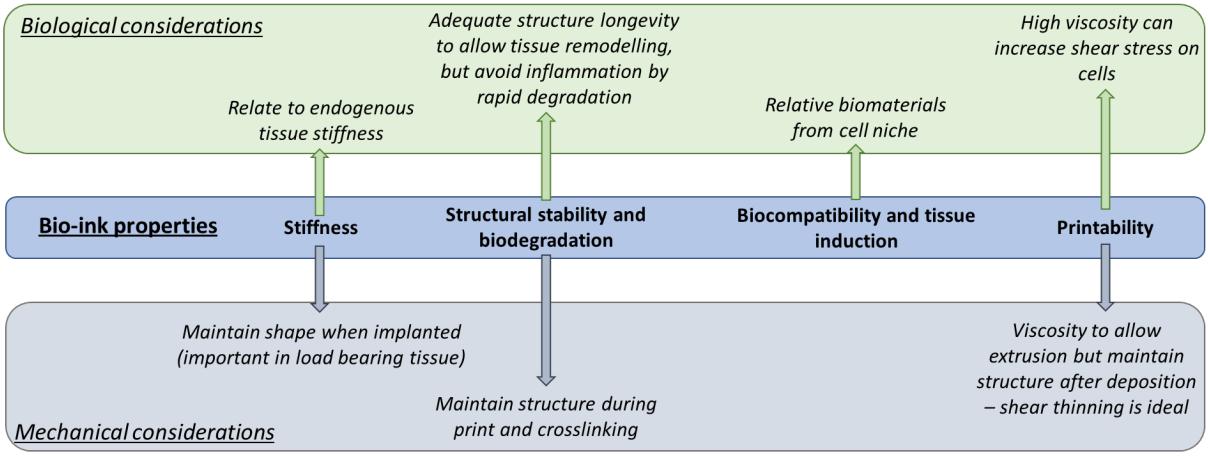

Figure 2. The primary properties of bio-ink material have biological and mechanical effects in the engineered tissue.

Mechanical stiffness: measured using the shear elastic modulus given in Pascals (Pa) or kilopascals (kPa), the stiffness of a desired tissue is a key biological characteristic often overlooked in *in vitro* cell culture. Tissue stiffness varies between tissues, from <1kPa for neuronal tissue to >100kPa in bone **(Figure 3)**[43]**.** When compared to the stiffness of common tissue culture plastics (1 GPa or 1,000,000kPa), it is unsurprising that *in vitro* culture alters cell biology, particularly of cells from low stiffness tissues such as liver, or brain. With a wealth of research showing the effects of increased tissue stiffness on tissues such as the liver[44][45], design of the bio-ink must be carefully tailored to match endogenous, healthy tissue. This can be achieved by material selection, modification and cross-linking parameters. **Table B** below details some of the commonly used materials in bio-inks. To alter stiffness, bio-inks can alter the molecular weights of its monomers, its polymer components or the crosslinking method used.

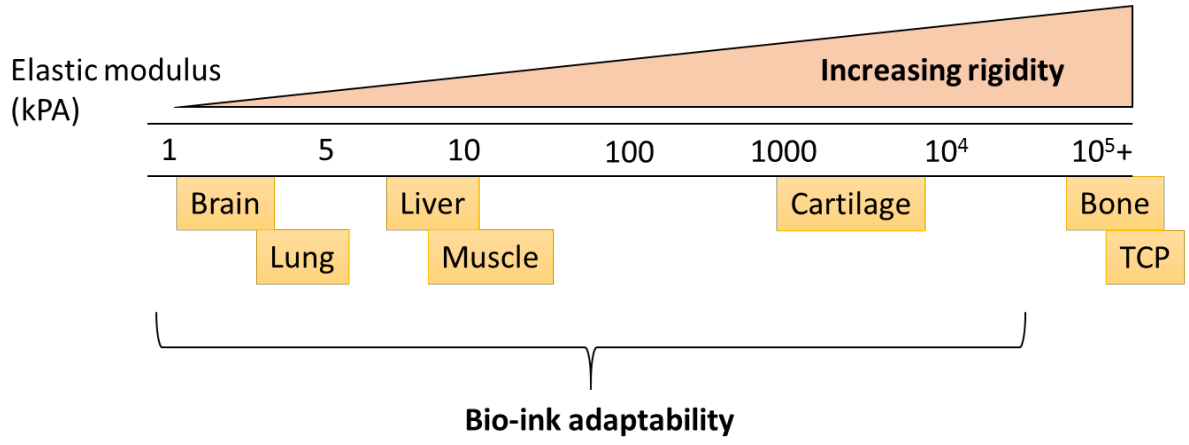

Figure 3. Different endogenous tissue types display varying rigidity. Brain, lung, liver and muscle for example all reside in relatively soft tissues, whereas common tissue culture plastic (TCP) is several orders of magnitude more rigid. This can lead to changes in cell viability, function and phenotype when culturing cells *in vitro*. However, the characteristics of bio-inks can be tuned for the bespoke need of the desired tissue.

Structural stability and biodegradability: The stability of the resulting structure is crucial for the printing process. When depositing low viscosity bio-inks, definition of the printed structure will suffer owing to spreading of the ink[46]. Structure resolution can also suffer as a result of imperfect crosslinking parameters: lack of crosslinking can cause spreading of the structure, whereas over-crosslinking can result in lamination and a failure of the entire structure to coalesce[47], [48]. Certain crosslinking methods, such as ionic with alginate, can also cause shrinkage which will affect the intended structure's shape[49]. Bearing this in mind, an ideal bio-ink would have sufficient viscosity to not spread upon deposition, and its crosslinking method be tightly controlled. The bio-ink must also have mechanical strength sufficient for initial printed layers to support subsequent deposition without collapse or impairment of the structure.

The ideal fate of most transplanted bioprinted tissue is the degradation of its scaffold, in tandem with integration of the encapsulated cells, and regeneration of the target tissue. Biodegradation properties of bio-inks are a key characteristic when considering implantation. There is a balance to strike between too rapid degradation, resulting in loss of cells and potential inflammation; and lack of degradation which can hamper tissue regeneration. Often biodegradability can be tuned by altering the properties of the bio-ink components and their crosslinking procedures.

Biocompatibility and tissue-induction: The biological activity of the bio-ink is a key aspect to make it suitable for cell viability and tissue formation. Certain bio-inks, such as alginate or poly ethylene glycol(PEG)-based hydrogels, are used as they are bio-inert and non-adherent. This simplifies the process as the bio-ink needs only conform to mechanical suitability, and the biological effect can be disregarded, or bio supportive molecules can be added to the ink. However other bio-inks make use of biologically active molecules such as gelatin, collagen, hyaluronic acid, and fibrin. This becomes a double-edged sword, as while in theory adherent and bio-active matrices should support cell attachment and viability, when constructing a stem-cell niche it is crucial to considering cell-matrix interactions. For example, collagen, while naturally adherent and a large part of endogenous extra-cellular matrix, is also largely associated with fibrosis of tissue[50]. Stem cell niches have specific ECM architecture, which likely needs to be mimicked to support stem cell culture and tissue development.

Printability: Printability of a bioink, or bioprintability, is determined by the rheological properties of the bioink, comprising its dispensability and ability to maintain structural integrity after bioprinting[46][51][52]. Successful and efficient deposition of the ink depends largely on its viscosity, as well as homogeneity of the solution. An ink that is too viscous will force high shear stresses upon the cells being deposited, and often lead to clogging of the printer nozzle and cell damage. Conversely, low viscosity inks will result in poor definition of the print due to flowing of the ink after printing. It can also lead to less homogeneous ink and nozzle clogging, as cells will likely sediment in the bio-ink chamber throughout the print. One of the most desirable characteristic for a bio-ink is that it displays shear thinning[53]. This is the behaviour of fluids where, under shear strain (i.e. while being pushed through the printer nozzle), the viscosity of the fluid will temporarily decrease. This allows easy printing with low shear stress on the cells, as well as maintaining the resolution of the printed product.

	Type	Crosslinker	Pros	Cons	References
Agarose	Natural	Thermoresponsive	Cheap, good printability	Nonadherent and bioinert	[54]
Alginate	Natural	Ionic, (Ca2+, Sr2+, Ba2+)	Good printability, tuneable characteristics, fast crosslinking	Difficult to control shrinking during crosslinking, bioinert	[55][56][57][58]
Chitosan	Natural	pH neutralization		pH changes required	[54]
Collagen	Natural	Thermoresponsive, pH	Biologically relevant, adherent, reasonable printability	pH changes required or cold bed for thermal gelling, characteristic of fibrosis, must be sourced from humans for clinical use	[59]
Fibrin	Natural	Enzymatic (thrombin)	Biologically relevant and adherent	Poor printability	[60][32]
Gelatin	Natural	Thermoresponsive, or UV if methacryloyl	Cheap, good printability, adherent and bioactive	Cold bed or UV exposure required to crosslink, must be sourced from humans for clinical use, poorly defined	[23][61]
Gellan Gum	Natural	Ionic (Ca2+)	Cheap, reasonable printability, tuneable with peptide motifs	Low mechanical properties, nonadherent and bioinert	[62], [63]
Hyaluronic Acid	Natural	Dependent on modification	Reasonable to print, biologically active and relevant	Low mechanical properties, must be human sourced for clinical use, crosslinker can be harmful (H2O2)	[64][28]
Pluronic	Synthetic	Thermoresponsive	Good printability, highly tunable viscosity, sacrificial	Mainly used for sacrificial inks, requires cold printing bed to maintain structure	[32]
Poly(ethylene glycol) (PEG)	Synthetic	UV exposure	Good printability, tuneable, well-defined polymer	Potentially harmful UV and photo-initiator exposure	[65]
Poly(caprolactone) (PCL)	Synthetic	Thermoresponsive (High temp)	Mechanically strong, bioinert	Not suitable for cell-printing due to high melting point	[32][66]

Table B. Characteristics of the most commonly used bio-ink constituents.

Developments in bioprinting stem cells

Bioprinting research has been important in the study of various tissues, including neural, liver, vascular, pancreatic, bone, cartilage, retinal, ovarian (**Table A**, and **Figure 4**) with the list growing rapidly. Stem-cell bioprinting, while still in its infancy, has also been developed immensely in recent years, delivering solutions for expanded and differentiating mesenchymal, neural and pluripotent stem cells.

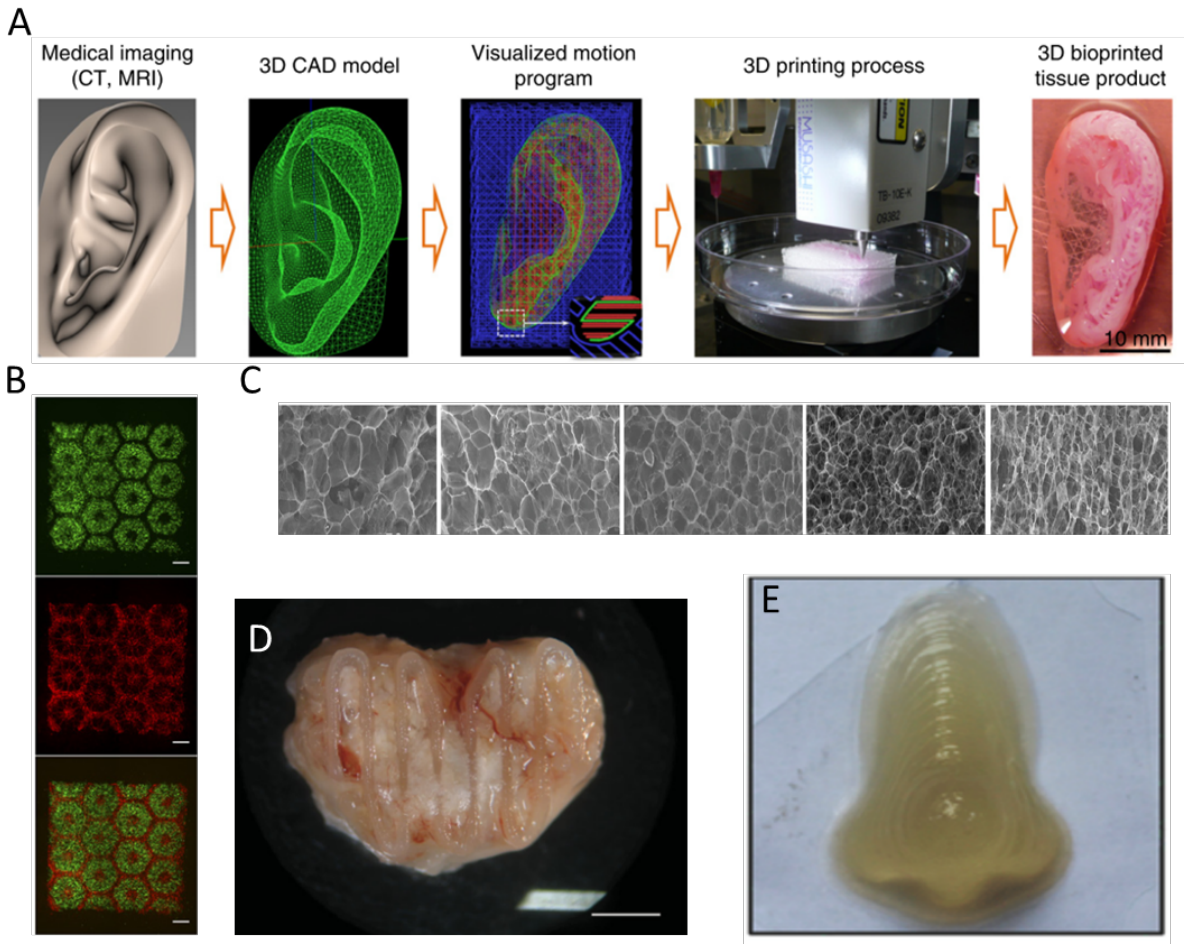

Figure 4. Examples of 3D bioprinting. **A:** Workflow of bioprinting large-scale human tissue from Kang *et al*[32]. Medical imaging is first used to generate a computer aided design (CAD) file that the bioprinter can then optimise for printing. The tissue was then printed in the integrated tissue-organ printer (ITOP) bioprinter to form the artificial tissue. **B**: Biomimetic patterning of stem-cell derived hepatocytes from Ma *et al*[61]. The hexagonal lobule of the liver was used as a template for printing iPSC-derived hepatocytes and supporting endothelial and mesenchymal cells. **C:** Fine tuning of bio-ink characteristics. By adding varying levels of carboxymethylcellulose, Gu *et al*[67] were able to control porosity in their bio-ink, optimising encapsulation of human neural stem cells. **D**: Post-implantation, anatomically designed bioprinted bone tissue from Daly *et al*[68]. A soft bio-ink with MSCs was reinforced with PCL fibres for mechanical stiffness prior to implantation. 12 weeks following implantation, the structure was shown to be vascularised. **E**: Bioprinting full-scale nose using hybrid bio-ink to optimise printability and porosity for cell viability[69]. This ink was used to encapsulate mesenchymal stem cells and differentiate them to functional chondrogenic and osteogenic cells.

Mesenchymal stem cell printing

To date, multipotent mesenchymal stromal cells (MSCs) are likely the most utilised stem cells in bioprinting, possibly owing to its ease of culture and expansion, multipotency, and resilience compared to other stem cell types. MSCs can be sourced from various tissues, including bone marrow and adipose tissue, and have been utilised to form various tissues[70][71][72].

Osteochondral engineering has seen the greatest contribution from hMSC bioprinting. Utilising the ability to print defined architecture of material and cells, Daly et al. [73] bioprinted a developmentally inspired template for bone growth, which allowed formation of a vascularized bone organ. The artificial tissue comprised MSCs, an alginate-based bio-ink, arginylglycylaspartic (RGD) acid peptide motifs for cell adherence, and supportive poly-caprolactone (PCL) struts to increase the compressive modulus of the structure, akin to endogenous bone. The artificial bone precursor tissue was then implanted, and 12 weeks later was shown to have successfully vascularized and

mineralized. Another study in bone engineering utilised ink-jet printing and bioactive ceramic nanoparticles to recapitulate the bone niche[74]. Encapsulated in a PEG-based bio-ink, MSCs were printed simultaneously with hydroxyapatite and bioactive glass nanoparticles. Hydroxyapatite-laden structures displayed the highest cell viability, as well as compressive modulus and collagen deposition, characteristic of bone development. Other works have shown deposition with acrylated peptides[65] and BMP2 tethered microfibres[75] can improve bone tissue generation from bioprinted MSCs. MSCs have also been used as examples to display the effects of shear stress on stem-cell viability during printing[76], precise cell patterning to study cell migration during vascularization[77], as well as cardiac differentiation controlled by focal adhesion[78]. Bio-ink derived from the endogenous, decellularized ECM of various tissues has been used to encapsulate MSCs, which produced tissue specific responses after encapsulation and printing[79].

Neural stem cells

Neurodegenerative diseases and challenging glioma tumours make bioprinted neural tissue from neural stem cells (NSCs) an attractive prospect for regenerative medicine and disease modelling. Encapsulation of neural stem cells seems to better mimic their endogenous phenotype when compared to culturing on tissue culture plastics (TCPs), likely due to the similarities in stiffness of soft hydrogels to neural tissue. One study showed that murine NSCs encapsulated in a water-based polyurethane gel showed increased expression of neurotrophic genes such as GDNF, BDNF and NGF compared to TCPs[80]. A zebrafish model of traumatic brain injury also showed significant recovery over untreated control when the bioprinted neural stem cells were implanted, giving hope to neural regeneration. In another example, human NSCs were encapsulated and printed in an agarose, alginate and carboxymethylcellulose-based bio-ink. Cells showed high viability post-printing, as well as being functionally active following a defined differentiation protocol[67]. Glioma tumours are challenging to treat owing to their malignancy, recurrence and resistance to drugs. However, this drug-resistance is not well recapitulated in standard culture conditions. To develop a more robust disease model and drug testing platform, researchers utilised a bio-ink that better mimicked the endogenous tumor micro-environment and embedded glioma stem cells therein[81]. Glioma cells maintained their phenotype and showed resistance to anti-cancer drugs, which they do not in standard 2D cultures, showing that bioprinted glioma tissue was a more robust glioma model to study the biology, and the drug treatment, of the cancer.

Pluripotent stem cells

Pluripotent stem cells (PSC) are cells of near unlimited potential owing to their pluripotent nature and theoretical unlimited self-renewal. Human embryonic stem cells (hESC) and the more recently described human induced pluripotent stem cells (hiPSC) represent the two mayor sources of pluripotent stem cells[82][83][84][85]. In such a light, they seem ideal candidates for generating renewable human tissue. Compared to MSCs, hESCs are more fragile and prone to spontaneous differentiation. To overcome this, a novel valve-based printing method was developed to print hESCs for the first time[86]. This technology was then used to print and differentiate hiPSC into hepatocytes[27]. Since then, the use of the Rho-kinase inhibitor Y-27632 to improve pluripotent cell viability[87], their use to form spheroids and organoids[88], and the possession of optimised printing and bio-ink parameters have allowed more diverse pluripotent cell printing. While still debatably in its infancy, bioprinting pluripotent cells has already been used to control the size of embryoid body formation[89], produce hepatocyte[90][61], neural[36] and pancreatic cells[34], as well as exploring their multilineage differentiation to all three germ lines[36][91].

Liver tissue is formed in characteristic hexagonal lobules, and bioprinting has been used to produce these shapes with hepatocytes and supporting cells in defined regions. By patterning matrix materials and then depositing different bio-inks, tissue-like architecture was achieved that supported hepatocytes matured from iPSCs[61]. Vascularizing artificial tissue is one of tissue engineering's largest hurdles, and pre-vascularized bioprinted liver tissue has been produced to combat this. By first differentiating both endothelial and hepatocytes from iPSCs, multi-fibred tissue structures were produced[92]. When transplanted, host vasculature also penetrated the structure, as seen by human albumin in the host animal. Treatment of degenerative diseases, such as diabetes, is an appealing translation of bioprinting research. As such, researchers have shown that by printing naïve ESCs in alginate and then differentiating them to pancreatic islet-like cells, they produced strong markers characteristic of pancreatic islet cells[34]. The encapsulation of these artificial islets also makes them amenable to transplantation, as the alginate shell would provide immune-protection from the host. Bioprinting of naïve pluripotent cells has also been shown with their directed differentiation towards neural lineages[36]. This resulted in population of mature, mixed phenotypes. Notably, functional GABAergic neurons were present, with supporting neuroglia.

Opportunities and future challenges

Bioprinted tissues have already found commercial success. The company Organovo is one of the few providing bioprinted human tissue for drug testing or research needs[93]. Both liver and kidney tissue are available, composed of parenchymal and supporting cells, in suitable bio-ink. These are not derived from pluripotent stem cells however. Censo Biotechnologies (formerly Roslin Cells) also offers bioprinting as part of their custom cell culture service[27].

Bioprinter platforms are gaining traction commercially including, among others, RegenHU, GemSIM and Cellink. The BioPen from the University of Wollongong introduces a handheld device for use during surgery for deposition of a chondrocyte-laden bioink to treat cartilage defects[37]. Cartilage is one of the favoured candidates for tissue engineering in general owing to its minimal vasculature.

To combat the issue of scale in bioprinting, the integrated tissue-organ printer (ITOP)[32] was constructed by researchers at the Wake Forest Institute to generate large, human sized tissue structures. The printer utilises multiple nozzles to deposit multiple types of bio-inks at once, to generate tissue. Some of these bio-inks are "sacrificial", so following printing these inks can be removed from the structure, generating micro-channels for nutrient delivery or vascularization. The design for the printed tissue is sourced from clinical imaging data.

Bioprinting has yet to breakthrough to clinical translation, likely due to regulatory issues, limitations of the size of structures and insufficient GMP infrastructure to support it. However, research has pushed bioprinting closer to the clinical stage, such as the Biopen and ITOP printer mentioned previously.

For drug-testing on truly biomimetic tissue, or transplantation of bioengineered organs to become a reality, significant hurdles must be overcome[5]. The source of the cells and materials used to construct the tissue must be both renewable and cost-effective. In the case of stem cells, renewability is less of an issue, but control of the cells becomes more important. Stem cells, particularly pluripotent stem cells, are fragile and prone to spontaneous differentiation, so well-defined printing and encapsulation methods must be in place for reproducibility. This is necessary to eliminate any spontaneous differentiation or cancer formation after implantation. Material selection for bio-ink composition is equally crucial. Not only must the material support the biological function in the tissue, ideally by mimicking endogenous stem-niche biomatrix, the material must be amenable

to the bioprinting process itself. Different bioprinting technologies are limited to material of specific viscosities: too low and the structure will lose resolution or not form; too high and the nozzle will clog and shear stress will destroy cells during deposition.

Tissue is a complex arrangement of cells and materials, and this is likely the crucial characteristic that is most often lost in *in vitro* cultures. Selection of cells and materials must be followed by their careful and precise positioning during fabrication to mimic endogenous tissue. Bioprinting is a "bottom-up" approach to biofabrication, where both material and cell deposition can be controlled in three dimensions. This gives bioprinting more flexibility in fabrication than other methods, but also has caveats dependent on the printing technology used. A balance must always be reached as higher resolution typically results in slower print times, poorer cell viability and increased price.

The format in which the cells are deposited also plays a role in tissue fabrication, with research showing various compositions of cells to be successfully bioprinted. Living cells have been successfully printed in suspension[27], encapsulated in hydrogel[94], preformed into spheroids before encapsulation[95], as well as formed into whole-cell fibres by bioprinting[96][97]. The malleability of this technique shows that it can be tailored to the formation of various, differently structured, tissue types.

After well researched design and proper technology choice, the next limiting factor of bioprinted tissue is size. As the desired construct becomes larger the subsequent printing time becomes longer, which can lead to decreased cell viability and structural integrity. As structures grow in size, diffusion of nutrients also becomes increasingly hampered. In endogenous tissue this is overcome through the vasculature supplying blood and nutrients throughout. The lack of this in large bioprinted tissue results in the development of necrotic regions that have received inadequate nutrition. Introducing or including an artificial vasculature is an area of avid research in bioprinting[32][98][28], and is seen in many ways as the "holy grail" needed to push construction of larger, organ-like structures.

A boon of stem-cell derived artificial tissue is the ability to generate autologous grafts from the patient's own stem cells. While in theory this has fantastic potential, it is met by the need for appropriate GMP grade handling facilities where a patient's own stem cells can be cultured, expanded, differentiated and bioprinted, all while under strictly defined conditions and free from contamination from other patients' cells. The infrastructure for such an endeavour is not yet in place, and, while in some ways the ultimate future of personalized medicine, it is still some way in the future.

Conclusions:

Bioprinting is an area of avid research, bringing together fields of cell biology, mechanical engineering, material science and many others. Stem cell bioprinting shows potential as a source for renewable human tissue. Various types of artificial tissues have been formed using bioprinted stem cells, from liver to brain. Printing adult stem cells seems to be closer to translation to the clinic or industry, as they are often more controllable and defined, whereas pluripotent stem cells have problems pertaining to viability and spontaneous differentiation.

At the moment, bioprinting is rapidly reaching a point where its main hurdles for translational is in the need for intrinsic vasculature in large bioprinted structures, and the controlled differentiation of stem cells *in situ* in 3D. If both can be tackled, production of allogenic and autologous tissue replacement moves from the realm of science fiction to reality.

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