Although the application of a neural bioelectronic device design to CMs acts as a proof of concept, it has become apparent that further optimization would be necessary for cardiac models and revisions of the bioelectronic mesh design would need to be carried out for the specific physiological characteristics of CMs. To enhance the robustness of the mesh bioelectronic device and optimize the mesh scaffold design specifically for CMs, we refined the selected ribbon widths (30–60μm), reducing the spacing between ribbons for better cell proximity, and increasing device thickness for enhanced stiffness (5pPa vs. 0.5pPa) and handling. These modifications have significantly improved cell-to-device interaction, promoting cell elongation and attachment. Future work will evaluate the effect of the new device geometry and stiffness on CMs calcium handling. These preliminary results indicate that our bioelectronic platform shows promise in creating cardiac tissue models for regenerative medicine, potentially offering a new avenue for cardiovascular disease therapy.

Conflict of Interest N/A

BS42 CAMKII AUGMENTS ROS AND CA2+ ENTRY PROCESSES IN FEMALE CARDIAC FIBROBLASTS IN HYPERGLYCAEMIC AND HYPERTENSIVE CONDITIONS

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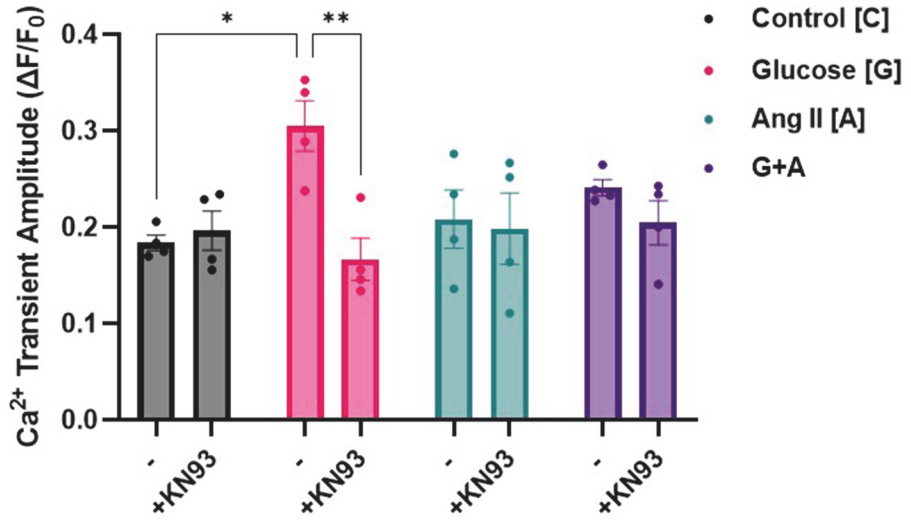
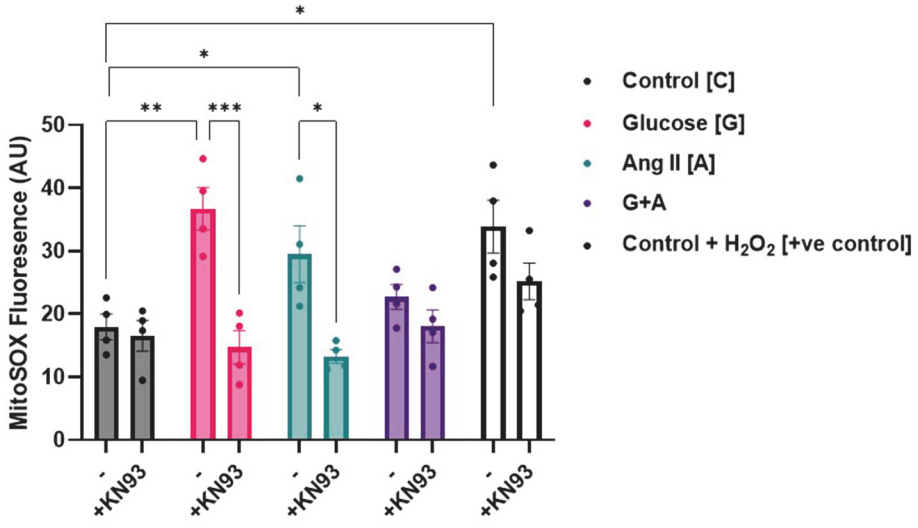
Introduction Heart Failure with preserved Ejection Fraction (HFpEF) affects over 50% of patients with HF, majority of whom are females with conditions such as hypertension, obesity and diabetes. Our in vivo animal models of these conditions show increased fibrosis and oxidized Calcium-calmodulin dependent protein kinase II (CaMKII) activity. At the cellular level, mitochondrial health is known to be implicated in HF, and our in vitro models have signified the impact of female cardiac fibroblasts (CFs) on altered calcium (Ca2+) signalling in cardiac myocytes (CMs), during co-culture. However,



Abstract BS42 Figure 1 Endothelin-1-induced calcium transient amplitude in female CFs. * p<0.05 and ** p<0.01



Abstract BS42 Figure 2 Mitochondrial superoxide production in female CFs. * p<0.05, ** p<0.01 and *** p<0.001

knowledge on the characteristics of CFs in HFpEF still remains obscure. Here, we investigated altered Ca2+ signalling, mitochondrial Reactive Oxygen Species (ROS) production, and the therapeutic potential of inhibiting CaMKII activity in CFs, during hyperglycaemia and hypertension.

Methods Adult human CFs (Promocell) sourced from both female and male donors were cultured under hyperglycaemic (22 mM Glucose), hypertensive (200nM Angiotensin II) or HFpEF-like (hyperglycaemic plus hypertensive) conditions, in the absence or presence of a CaMKII inhibitor (5μM KN93), for 48 hours. Following pathological conditioning of CFs, cells were loaded with Cal520AM calcium indicator or MitoSOX red mitochondrial superoxide indicator. Live cell fluorescence imaging was utilised to assess Ca2+ activity and mitochondrial ROS production in CFs.

Results Female CFs treated under hyperglycaemic conditions showed a greater Endothelin-1-induced Ca2+ transient amplitude (□F/F0) relative to control and hypertensive conditioning [Control: 0.184±0.008; Diabetes: 0.305±0.026; Hypertension: 0.208±0.030; HFpEF: 0.241±0.008, n=4passages]. Mitochondrial superoxide (AU) levels were elevated only in female CFs, in both hyperglycaemic and hypertensive conditions [Control: 18.0±2.0; Diabetes: 36.7±3.4; Hypertension: 29.5±4.5; HFpEF: 22.7±2.0, n=4passages]. These alterations in Ca2+ transient amplitude and superoxide production were impeded by the presence of KN93 [Ca2+ Transient Amplitude- Control: 0.197±0.020; Diabetes: 0.167±0.022; Hypertension: 0.199±0.037; HFpEF: 0.205±0.023, Mitochondrial superoxide- Control: 16.6±2.4; Diabetes: 14.7±2.6; Hypertension: 13.3±1.1; HFpEF: 18.1±2.6, [n=4passages].

Conclusions These results indicate CaMKII is important in mitochondrial oxidative stress in female CFs, in hyperglycaemic and hypertensive conditions. Further work is needed to investigate the importance of these processes in the development of fibrosis in HFpEF.

Conflict of Interest None

BS43 HYPERGLYCAEMIA INDUCES EPIGENETIC DYSREGULATION IN HUMAN ENDOTHELIAL COLONY-FORMING CELLS WHICH LIMITS THEIR VASOREPARATIVE AND THERAPEUTIC POTENTIAL

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Endothelial colony forming cells (ECFCs) are a defined progenitor subtype with established roles in vascular homeostasis and angiogenesis. Whilst ECFCs hold clear therapeutic potential for improved ischaemic cardiovascular disease (CVD) management, reduced pro-angiogenic capacity associated with diabetes and attenuated efficacy within the CVD microenvironment limit their translational potential. Given association between diabetes and ischaemic CVD pathogenesis, targeting of critical angiogenic-linked pathways remains a focus for both protecting and enhancing intrinsic diabetic ECFC vasoreparative function. Whilst DNA methylation (5 mC) regulates endothelial cell (EC) homeostasis and stress-induced dysfunction, understanding of its role in ECFC angiogenic response is limited. Alteration of maintenance regulators (DNMT1/UHRF1) with aberrant 5 mC is linked with numerous diseases including CVD. The aim of this study was therefore to define the specific influence of 5 mC on ECFC angiogenic dysfunction in both experimental and clinical diabetes. Treatment of healthy cord blood-derived ECFCs with 5'Aza-2-deoxycytidine (DNMT inhibitor; 1uM,72hrs) attenuated tube-forming capacity (Matrigel) in parallel with dysregulation of key pro-angiogenic proteins (Proteome Profiler®), confirming a specific role for ECFC 5 mC. Exposure of ECFCs to experimental diabetes (25 mmol/L D-glucose for 28 days) attenuated angiogenic capacity and increased DNMT1/UHRF1 expression versus L-glucose controls, whilst DNMT1 plasmid overexpression (OE) in healthy ECFCs led to reduced tube formation, specifically linking DNMT1 elevation with pathogenicity. In direct support of clinical relevance, ECFCs isolated from donors with gestational diabetes showed reduced angiogenic potential and increased DNMT1/UHRF1 expression, with TWIST meC screening highlighting intriguing gene-specific differential methylation, including hypermethylation with corresponding transcription changes linked to disrupted angiogenic function. Taken together, these data indicate a pivotal role for 5 mC in maintaining ECFC pro-angiogenic capacity in both health and disease, whilst highlighting exciting potential of selective epigenetic targeting (e.g. methylome editing via CRISPR/Cas9) to harness their intrinsic vasoreparative capacity for as an innovative approach ischaemic CVD management.

Conflict of Interest None

BS44 GENERATION OF CORONARY SMOOTH MUSCLE CELLS FROM HUMAN EMBRYONIC STEM CELLS FOR TRANSLATIONAL APPROACHES IN CARDIAC REGENERATION

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Introduction Smooth muscle cells (SMCs) are important for coronary artery formation, which is crucial for the blood supply of the heart. Dysfunction in coronary SMCs (cSMCs), seen in conditions like atherosclerosis, contributes to heart attacks. Generating these cells from heart multipotent progenitors in vitro offers promise for disease modeling and regenerative therapies, given the unique characteristics of heart-specific vessel cells.

Methods Previously, our laboratory developed a protocol for generating epicardial-derived SMCs from human embryonic stem cells (hESCs) using PDGF-B (P) and TGF-beta (T) signaling. We assessed coronary-specific marker expression using immunohistochemistry, RT-qPCR and a reporter cell line that we generated using CRISPR technology. We focused on PLA2G5, the most specific cSMC marker identified from our fetal heart atlas. Alongside PT conditions, we investigated the potential of RepSox, a small molecule that promoted SMC differentiation via NOTCH signaling, to potentially enhance cSMC marker expression.

Results Human embryonic stem cell (hESC)-derived SMCs were differentiated from epicardial cells using PT and RepSox conditions. Epicardial cell seeding density was optimised using