less prevalent in PROTEA, while atrioventricular septal defects (PR: 2.2, 95%CI: 1.90-2.61), pulmonary stenosis, aortic stenosis, tetralogy of Fallot and double outlet right ventricle were significantly more prevalent. CMA analysis of 89 patients identified likely disease-causing copy number variants in five patients (5.4%), while a further 4.5% had variants of uncertain clinical significance. Using exome sequencing on 95 patients, pathogenic or likely pathogenic mutations were identified in 15 patients (15.8%); 65.3% of the sequenced cohort had variants of uncertain significance in established CHD genes.

Conclusions Preliminary analysis of the PROTEA cohort indicates that the prevalence of CHD subtypes is largely in accordance with international data, although mild lesions had a lower prevalence, and certain severe phenotypes were more prevalent. Genetic analysis of these patients demonstrates that disease-causing variants can be identified using CMA and exome sequencing, and will yield results in the expected range from international studies. Together these findings illustrate the feasibility of conducting epidemiological and genomic research amongst CHD patients in sub-Saharan Africa.

Conflict of Interest None

BS27

HARNESSING THE POWER OF PALMITOYLATION TO TUNE NCX1 PHYSIOLOGY

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Introduction The cardiac Na⁺/Ca²⁺ Exchanger (NCX1) is a primary regulator of Ca²⁺ handling in cardiac tissue by mediating electrogenic exchange of Na⁺ for Ca²⁺. In cardiac muscle NCX1 functions primarily in the forward mode that facilitates Ca²⁺ extrusion from cytosol. NCX1 is palmitoylated at a single cysteine at position 739 within its large regulatory intracellular loop. Palmitoylation is critical for proper inactivation of NCX1, which is mediated by the endogenous XIP region of the same regulatory loop (Reilly et al., 2015). XIP interacts with a region near the palmitoylation site between 709-728 (Gök et al., 2020). Palmitoylation modifies XIP binding to this particular region by inducing local structural changes within the intracellular loop. Yet, relatively little is known regarding the physiological relevance of NCX1 palmitoylation.

Methods We engineered cell lines stably expressing tetracycline-inducible NCX1: palmitoylatable (WT-NCX1), unpalmitoylatable (C739A), non-functional XIP domain (K229Q and Δ229-232), missing XIP binding site (Δ709-728), and missing XIP domain (ΔXIP). NCX1 current was recorded using whole-cell patch clamp in response to voltage steps from -120mV to +100mV with 20mV steps from a holding potential of -80mV. We prepared FRET sensors for all mutated NCX1 lines listed above to study intermolecular structural changes between NCX1 dimers. We combined our patch clamp and FRET experiments with pharmacological approaches where we either manipulate NCX1 activity using a custom-made XIP peptide or deplete PIP₂ (to release the endogenous XIP domain) using a cocktail of Wortmannin/m-3M3FBS. We measured intracellular Ca²⁺ and Na⁺ dependent Ca²⁺ intake using Fluo-4 dye.

Results and Conclusion Disrupting NCX1 inactivation by either mutating the XIP domain (K229Q) or abolishing palmitoylation (C739A) increased NCX1 current amplitude compared to WT-NCX1. Basal intracellular Ca²⁺ was higher and Na⁺-dependent cellular Ca²⁺ uptake was larger in cells expressing K229Q than cells expressing WT-NCX1. However, treating K229Q cells with a custom-made XIP peptide fully compensated the Ca²⁺ overload caused by impaired inactivation. Strikingly, XIP peptide did not affect NCX1 current in patch clamp recordings made from cells expressing unpalmitoylatable NCX1. This suggests that XIP inactivates K229Q but not C739A because palmitoylation tunes the interaction between XIP and its binding site. Targeting palmitoylation therefore offers great potential to sensitize NCX1 to XIP, and hence devise novel pharmacological strategies to manage cardiac pathologies.

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Conflict of Interest None

BS28

OPTIMISING CULTURE OF IPSC-DERIVED CARDIOMYOCYTES ON A PRO-SURVIVAL SUBSTRATE

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Introduction Cell therapy is a potential novel treatment for cardiac regeneration. Numerous studies have attempted to transplant cells to regenerate myocardium lost during myocardial infarction. To date, only minimal improvements to cardiac function have been reported in clinical trials. This is likely to arise from low cell retention following delivery and high cell death after transplantation.

The current study aims to improve intramyocardial delivery of viable cells by using an injectable biodegradable substrate that supports cell attachment and growth of cardiomyocytes derived from induced pluripotent stem cells (iPSC).

Methods Highly porous microcarriers <250 μm were fabricated from 2% (w/v) 75:25 poly(DL-lactide-co-glycolide) using Thermally Induced Phase Separation (TIPS). Ultrastructural features of the microspheres were characterised using scanning electron microscopy and image analysis. A range of parameters were investigated for optimising conditions for iPSC attachment to TIPS microspheres. This included pre-conditioning with a protein solution containing recombinant human vitronectin (VTN-N) ranging from 0 to 20 μg/ml and 'wetting' with solutions of HBSS or F10 medium for up to 7 days. 5×10⁵ episomal iPSC were incubated with the pre-conditioned microspheres and cell attachment quantified after 24 hours. The distribution of iPSC attached to the surface of the microspheres was evaluated using fluorescence microscopy. Pluripotency of the attached cells was assessed by confocal microscopy and flow cytometry, as well as the ability to subsequently differentiate into cardiomyocytes.

Results TIPS microspheres exhibited a highly porous topography, with pore features ranging in shape and size (figure 1A). Pre-incubating the microcarriers with VTN-N; 0.5 μg/ml, resulted in an increase in iPSC attachment (93.4% vs 2.9% control; attached cells as % of seeded cells, n=5, P ≤

Abstract B528 Figure 1 (A) Scanning electron microscopy image of the microcarrier. (B) iPSC attachment to the surface of TIPS microspheres, 24 hours post-seeding

0.0001) (figure 1B). The attached cells proliferated (doubling time ≤ 24 hrs, $n=3$). iPSC attached to the microcarriers retained their pluripotent phenotype, demonstrated by positive expression of markers, SOX2, OCT4, TRA-1-60 and SSEA4. Pluripotency was further demonstrated by differentiation of TIPS-iPSC into a mixed cardiomyocyte-like population exhibiting a beating phenotype for up to 40 days in culture and positive expression of cardiac markers Troponin T and sarcomeric α -Actinin.

Conclusion This work demonstrates that TIPS microcarriers offer a supporting matrix for culturing and differentiating iPSC and may provide an injectable biomaterial for cardiac regeneration.

Conflict of Interest N/A

B529

SINGLE CELL TRANSCRIPTOMICS REVEALS REGENERATIVE EMBRYONIC PATHWAYS LOST IN THE ADULT EPICARDIUM

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Background Damage to the heart muscle often leads to chronic arrhythmia and heart failure. This is because the muscle of the adult human heart does not functionally regenerate after injury and is replaced by fibrous non-contractile and non-conductive scar tissue. However, the heart muscle of some vertebrates and developing mammals is capable of regeneration which restores the muscular function of the damaged tissue. Evidence suggests that the epicardium plays a crucial role in orchestrating this successful regenerative response in animals like the zebrafish and that epicardium cells are active in developmental stages of life. However, in adult humans the epicardium is putatively quiescent.

Objectives We hypothesise that the difference in the regenerative capacity of human adult and foetal myocardium may be explained in part by differences in the epicardium and reactivation of these molecular processes lost in adult epicardium may lead to effective myocardial regeneration. Using single-cell RNA sequencing, our aim was to capture the regenerative epicardial signalling present in the foetal epicardium that may be absent in adult hearts.

Methods Heart sections from 7 human embryos between 8 and 12 weeks of gestation were dissociated and sequenced with scRNA-seq. ScRNA-seq data from 5 human adult hearts between 55 and 75 years old were acquired from the Heart Cell Atlas. Data were integrated and epicardial cells in all samples were identified using supervised classification and canonical markers.

Results We compared the markers expressed in foetal and adult mesothelial epicardial cells and identified foetal, adult and stage-independent epicardial signatures. Among these were a number of pro-angiogenic factors specific to the foetal epicardium, including a group of WNT-signalling secreted factors, WNT2B, SFRP2 and SFRP5. Gene Ontology suggested a shift towards immune response of the epicardial cells during maturation. Additionally, an epicardial cluster with fibroblast-like markers was found only in the foetal heart (96 % foetal).

We examined organ-wide influences of epicardial signalling by mapping the potential ligand receptor interactions from the epicardium-derived secreted factors to receptors upregulated in other heart cell clusters. This revealed the prospective influence of reactivating foetal epicardial pathways for heart regeneration. Lastly, we demonstrated that epicardium derived from human embryonic stem cells exhibits some of these lost regenerative pathways, promoting their value as a model and as therapeutic agents.

Conclusions We have shown that the adult epicardium has major differences to the foetal epicardium. Firstly, a set of epicardium-specific angiogenic pathways are absent from the adult epicardium and secondly, foetal hearts contain another epicardial cell type that is not present in the adult heart. Recapitulating both the missing epicardial signals and the synergy between the foetal epicardial cell types may be essential for developing effective regenerative stem cell therapy.

Conflict of Interest None

B530

LIGHT SHEET IMAGING TO ANALYSE THE SPATIAL DISTRIBUTION OF PROTEINS IN ATHEROSCLEROTIC PLAQUES

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The mechanisms linking shear stress, endothelial physiology and plaque biology are currently poorly understood, but their elucidation could identify new strategies to reduce plaque growth and rupture. To address this, we use eNOS as high shear stress marker coupled to immunofluorescent staining, optical clearing and light-sheet microscopy, to develop a system for analysing the spatial distribution of proteins in murine plaques and correlate them with local WSS.

Results Confocal microscopy of concavity mounted slides revealed strong eNOS staining at the outer curvature of WT mice but significantly reduced staining at the inner curvature ($N=5$; $P<0.001$). This is consistent with high WSS induction of eNOS because the outer curvature corresponds to a HSS site (Suo et al) however precise correlations between eNOS and shear stress could not be made because the tissue geometry was lost during processing.

Light-sheet microscopy of cleared samples with preserved 3D structure confirmed elevated expression of eNOS at HSS regions of the outer curvature (figure 1A; $N=5$ WT; $P<0.01$). In aortic arches of ApoE^{-/-} mice, eNOS was observed at the outer curvature but was also present at portions of atherosclerotic plaques (figure 1B). Further analysis revealed that eNOS expression was higher at the proximal (upstream) part