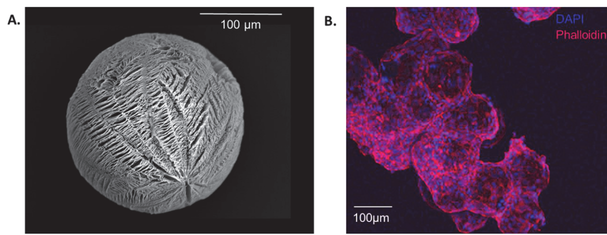

Abstract BS28 Figure 1 (A) Scanning electron microscopy image of the microcarrier. (B) iPSC attachment to the surface of TIPS microspheres, 24 hours post-seeding

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

BS29 SINGLE CELL TRANSCRIPTOMICS REVEALS REGENERATIVE EMBRYONIC PATHWAYS LOST IN THE ADULT EPICARDIUM

Vincent Knight-Schrijver, Laure Gambardella, Hongorzul Davaapil, Sanjay Sinha, Alexander Ross, Ludovic Vallier. *University of Cambridge, Cambridge, UK*

10.1136/heartjnl-2021-BCS.227

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 epicardial-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

BS30 LIGHT SHEET IMAGING TO ANALYSE THE SPATIAL DISTRIBUTION OF PROTEINS IN ATHEROSCLEROTIC PLAQUES

Ziqi Zhou, Nicholas Van-Hateren, Sheila Francis, Maria Fragiadaki, Paul Evans. *The University of Sheffield, Sheffield, UK*

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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

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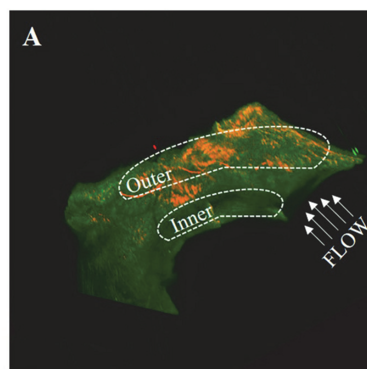

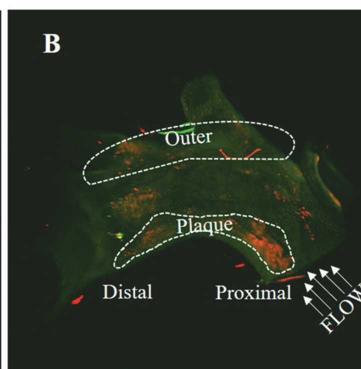

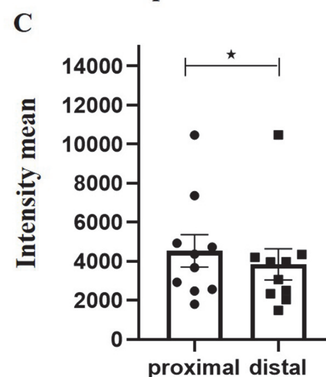

eNOS; VE-cadherin

Abstract BS30 Figure 1

of the plaque compared to distal (downstream) suggesting a potential correlation with WSS (**figure 1C**).
Conflict of Interest Atherosclerosis

BS31 VSMC CONTRIBUTION TO NEOINTIMAL LESIONS ARISES FROM THE CLONAL EXPANSION OF FEW PRIMED CELLS

Matt Worssam. *University of Cambridge, Cambridge, UK*

10.1136/heartjnl-2021-BCS.229

In healthy blood vessels, vascular smooth muscle cells (VSMCs) exist in a contractile, quiescent state but can switch phenotype to activate proliferation, migration and remodelling of the extracellular matrix. Phenotypically switched VSMCs contribute most cells within neointimal lesions, characteristic of atherosclerosis and in-stent restenosis, diseases that underlie heart attack and stroke. Using multicolour 'Confetti' VSMC-specific lineage tracing in animal models of vascular disease, we showed that the extensive VSMC contribution to these lesions results from the clonal expansion of few cells.

To understand how oligoclonal VSMC lesion contribution arises and to identify the signals activating VSMC proliferation in vivo, we used confocal microscopy to quantify VSMC clonal development over time in two models of vascular disease. We observed that the number and sizes of patches of clonally expanded VSMCs steadily increased, then plateaued post-injury. This suggests VSMC investment results from activation of a small number of VSMCs, rather than clonal competition following general VSMC activation. Selective VSMC activation in plaques was evidenced by the absence of plaques with high numbers of colours at any stage of plaque development.

In both models, VSMC activation was associated with vascular regions displaying elastic lamina alterations, medial acellularity and immune cell recruitment, implicating these as proliferation-triggering cues. However, not all VSMCs in these regions formed patches, suggesting that VSMCs must be primed to respond. In culture, few VSMCs gave rise to patches, suggesting cell-autonomous activation. This work supports the targeting of primed VSMCs in the healthy vessel as a therapeutic strategy against vascular lesion development.
Conflict of Interest None

BS32 THE ROLE OF BRANCHED-CHAIN KETO ACIDS IN MEDIATING INSULIN RESISTANCE IN THE FAILING HEART

Qutuba Karwi, Cory Wagg, Liyan Zhang, John Ussher, Gary Lopaschuk. *University of Alberta, Edmonton, Canada*

10.1136/heartjnl-2021-BCS.230

Brief introduction: Perturbed branched-chain amino acids (BCAA) oxidation positively correlates with the severity of cardiac insulin resistance in heart failure. We previously demonstrated that cardiac-specific deletion of the BCAA oxidative enzyme mitochondrial branched-chain aminotransferase (BCATm) increases cardiac BCAA levels and decreases branched-chain keto acids (BCKA) levels, enhances insulin-stimulated cardiac glucose oxidation rates. This increased cardiac insulin sensitivity is associated with an increase in the phosphorylation of protein kinase B (Akt) and activation of pyruvate dehydrogenase (PDH), the rate-limiting enzyme of glucose oxidation. However, whether it is the accumulation of BCAA or BCKA that is critical in mediating cardiac insulin resistance is unknown. How perturbed BCAA oxidation may mediate cardiac insulin resistance in heart failure is also unknown.
Explanation of basic Methods To address these questions, we first examined the effects of selectively enhancing cardiac BCKA levels on cardiac insulin-stimulated glucose oxidation. We perfused isolated working mice hearts (male and female C57BL/6N, n=8-10) with high levels of BCKA (α-keto-isocaproate 80 μM, α-keto-β-methylvalorate 100μM, α-keto-isovalerate 70 μM), levels that can be seen in diabetes and obesity.
Results High levels of BCKA completely blunted insulin-stimulated glucose oxidation rates and increased fatty acid oxidation rates. We also found that BCKA abolished insulin-stimulated mitochondrial Akt, an effect that was associated with PDH deactivation. We next determined the potential protective effect of reducing cardiac BCKA levels in the failing heart. We randomized WTCre+/+ and cardiac-specific BCATm-/- mice (male, 25-30g, n=6-8) to undergo either sham surgery or transverse aortic constriction surgery to induce heart failure. Five weeks post-surgery, there was a marked increase in insulin-stimulated glucose oxidation rates in the BCATm-/- failing hearts compared to the WTCre+/+ failing hearts, with no significant effect on glycolysis rates. Enhanced cardiac insulin

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