inflammatory genes and scavenger receptors, inducing an M2-like phenotype. Conversely, overexpression of miR-101 is associated with a pro-inflammatory phenotype (M1-like).

Conclusion miR-101-3p offers a potential target to simultaneously enhance the expression of ABCA1, MKP-1 and TRIB1, thus improving macrophage lipid metabolism and opposing inflammation, thereby attenuating atherosclerosis development.

Conflict of interest No

BS26 | **GENERATION OF A TISSUE ENGINEERED CONDUIT FROM HUMAN SAPHENOUS VEIN AND PORCINE BLOOD OUTGROWTH ENDOTHELIAL CELLS**

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Introduction Coronary artery bypass grafting (CABG) primarily uses autologous human saphenous vein (hSV), despite autologous arteries having better patency rates. However, a lack of arteries, and presence of disease in those which are available, has led to the need to develop alternative tissue engineered conduits. Here we aim to arterialise a vein, by re-seeding a decellularised hSV (DhSV) with arterial-like endothelial cells (EC), which will ultimately prevent early thrombosis and improve late graft patency.

Methods Blood outgrowth EC (BOEC) isolated from 350 ml pig blood were expanded for further use. BOEC were subjected to immunocytochemistry (ICC) for EC markers CD31, vWF, VE-Cadherin, and DBA-Lectin, and also vimentin (mesenchymal/fibroblast marker) and CD45 (leukocyte marker). BOEC seeded in plates were exposed to dynamic (D) culture conditions on an orbital shaker (94 hrs) and compared to static (S) culture. EC isolated from pig aorta (PAEC) were used as positive controls. qPCR was performed for CD31, vWF, VE-Cadherin, eNOS, DLL4 (arterial) and NR2F2 (venous).

hSV was decellularised using 0.01% SDS. 1×106 BOEC were seeded onto DhSV by tying the ends (T) to trap cells luminally, or placing BOEC in the lumen leaving ends untied (UT), before leaving under static (S) or dynamic (D: roller, 1rpm) culture conditions (93 hrs). Seeded DhSV were static cultured for 73 hours, fixed, paraffin wax-embedded and sections subjected to DBA-lectin staining to quantify maximum lumen coverage of BOEC, and anti-CD31 immunohistochemistry.

Results BOEC showed classic EC cobblestone morphology, and ICC staining revealed expression of CD31, DBA-Lectin, vWF, vimentin, VE-Cadherin levels were low and CD45 was not detected. CD31 mRNA levels were comparable in BOEC and PAEC (fold-change (FC) 1.2), while vWF (FC 228.3), VE-cadherin (FC 9.3) and NR2F2 (FC 5.5) mRNAs were increased and eNOS (FC 0.2) and DLL4 (FC 0.1) were decreased in BOEC compared to PAEC. Exposing BOEC to shear stress increased expression of all mRNAs investigated (FC CD31:2.6, vWF:1.8, VE-cadherin:1.4, eNOS:3.3, DLL4:1.4, and NR2F2:3.4) compared to static BOEC.

Seeding DhSV resulted in partial re-endothelialisation with greatest lumen coverage found by dynamic seeding with cells tied into lumen (91% coverage) and continued culture resulted in a near complete BOEC layer following (97%). CD31 was still expressed in seeded DhSV.

ConclusionS BOEC isolated from pig blood show expression of EC markers, and vimentin, and are negative for the leukocyte marker CD45. We have shown that it is possible to repopulate a DhSV with BOEC and, under the correct conditions, obtain a functional monolayer around the lumen. This preliminary data shows the translational potential for this approach in generating a tissue engineered saphenous vein, reendothelialised with BOEC from blood that could be used for CABG.

Conflict of interest None

BS27 | **DEVELOPMENT AND PRECLINICAL TESTING OF A LARGE HEART MUSCLE PATCH**

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Introduction The lack of efficacy of stem cell therapy for the treatment of heart failure may be related to the poor retention rates offered by existing delivery methods (intra-coronary/intramyocardial). Tissue engineering strategies improve cell retention in small animal models but data regarding engineered heart tissue (EHT) patches large enough for human studies are lacking.

Purpose To upscale EHT to a clinically relevant size and mature the patch in-vitro. Once matured to undergo preclinical testing in a rabbit model of myocardial infarction.

Methods We developed an upscaled EHT patch (3cm × 2cm × 1.5mm) able to contain up to 50 million human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) (figure 1A/B). Myocardial infarction model was performed by permanent ligation.

Results The patches began to beat spontaneously within 3 days of fabrication and after 28 days of dynamic culture (Late EHTs) showed the development of several mature characteristics when compared to early patches (<14 days from fabrication). For example, late EHTs contained hiPSC-CMs which were more aligned (hiPSC-CM accumulative angle change: early 2702 ± 778 degrees [n=4] vs late 922 ± 186 [n=5], p=0.042); showed better contraction kinetics (early peak contraction amplitude 87.9 ± 5.8a.u. versus late 952 ± 304a.u.; p<0.001) and faster calcium transients (time to peak: early 200.8 ± 8.8ms [n=5] vs late 147.7 ± 10.2ms [n=6], p=0.004; time to 75% decay: early 274 ± 9.7ms vs late 219.9 ± 2.7ms, p=0.0003).

We then tested the EHT patch in-vivo using a rabbit model (figure C). Patches were applied to normal (n=5) or infarcted hearts (n=8). Sham operations used non-cellular fibrin patches (n=5). The mean fraction of troponin positive cells in the graft was 27.8 ± 10.3% at 25.2 ± 1.7days relative to day 0 [n=5] and KU80 (human specific marker) staining confirmed that this was of human origin. CD31 (figure D) and KU80 staining revealed that the grafts were well vascularized and that the vasculature was not human in origin (therefore were originating from the host). Ex-vivo optical mapping revealed evidence of electrical coupling between the graft and host at 2 weeks and preliminary experiments indicated that the patch improved left ventricular function when grafted onto infarcted hearts. Telemetry recordings in vivo and arrhythmia provocation protocols (ex vivo) indicated that the patch was not associated with any significant changes in arrhythmogenicity.
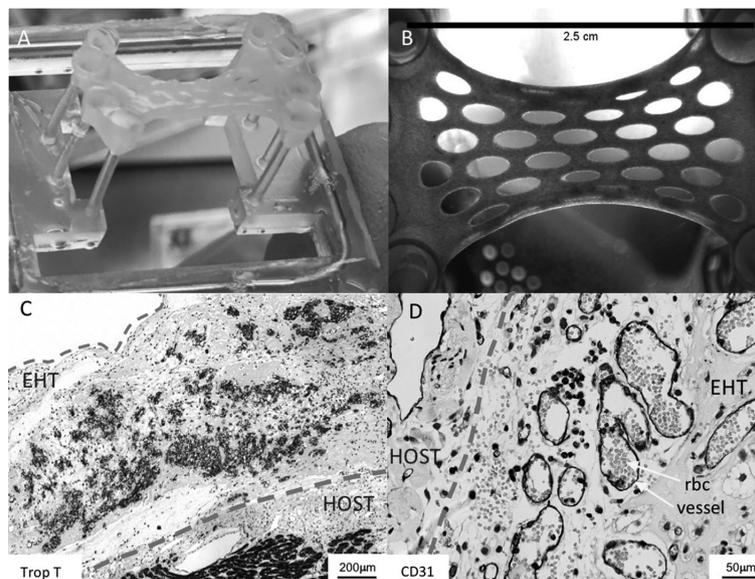
Abstract BS27 Figure 1   A/B) EHT Images; C) 20x troponin T(brown) of rabbit myocardium/EHT(2 weeks after grafting), blue counterstain=haematoxylin, red lines=EHT borders; D) 63x CD31 staining(brown) rabbit/EHT border zone(2 weeks after grafting), blue stain=haematoxylin, red lines=graft/host border zones

Conclusion We successfully upscaled hiPSC-CM derived EHT to a clinically relevant size and were able to demonstrate feasibility and integration using a rabbit model of myocardial infarction. Tissue engineering strategies may be the preferred modality of cell delivery for future cardiac regenerative medicine studies.

Conflict of interest none

ENDOTHELIAL STAT5A IS ENRICHED AT ATHEROPRONE REGIONS OF THE AORTA AND DRIVES INFLAMMATION IN RESPONSE TO LOW SHEAR STRESS

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Introduction Atherosclerosis is an inflammatory disease that develops at bends and branches of the arteries that are exposed to disturbed blood flow which generates low wall shear stress (WSS). These haemodynamic conditions lead to altered endothelial function and promote proliferation, apoptosis and inflammatory activation of endothelial cells (ECs), as well as other fundamental processes that drive disease. The Janus Kinase and Signal Transducer and Activators of Transcription (JAK/STAT) is an evolutionarily conserved pathway with key roles in the control of proliferation, apoptosis and inflammatory activation, yet its role in atherosclerosis is poorly understood despite the fact that these processes drive atherogenesis.

Methods Microarrays were performed on ECs from the porcine aorta to quantify stat5a expression in low WSS (atheroprone) and high WSS (atheroprotected) regions, and data were validated by qRT-PCR. En face immunostaining of the murine aortic arch was carried out to quantify Stat5a expression at low and high WSS. In vitro studies were performed using human coronary artery endothelial cells (HCAECs) and human umbilical vein endothelial cells (HUVECs) which were subjected to either retroviral-mediated shRNA (HCAECs) or siRNA (HUVECs) silencing of Stat5a. These cells were then exposed to low (5 dynes/cm^2) or high (10 dynes/cm^2) WSS for 72 hours on the orbital system and inflammatory molecule (ICAM-1, VCAM-1, E-Selectin and MCP-1) expression was studied by qRT-PCR.

Results qRT-PCR analysis of the porcine aorta identified that Stat5a was enriched at low WSS regions compared to high WSS regions ($P<0.05$). En face staining of the murine aorta also revealed that endothelial Stat5a expression was enhanced at regions of low WSS compared to high WSS ($P<0.05$). Similarly, Stat5a mRNA expression was enhanced in cultured HCAEC exposed to low compared to high WSS. Functional studies showed that silencing of Stat5a led to a reduction of ICAM-1 ($P<0.05$), VCAM-1 ($P<0.05$), E-Selectin ($P<0.05$) and MCP-1 ($P<0.05$), indicating that Stat5a is a positive regulator of EC inflammatory activation.

Conclusion Our data demonstrate that Stat5a is upregulated by low WSS both in vivo and in vitro, and that its silencing reduces inflammatory gene expression. These data therefore suggest that Stat5a may regulate the focal nature of atherogenesis by promoting inflammation.

Conflict of interest none

EXPRESSION OF STABLE SOCS3 IN HUMAN SAPHENOUS VEIN SMOOTH MUSCLE CELLS: POTENTIAL THERAPY FOR VASCULAR RESTENOSIS

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Introduction Suppressor of cytokine signalling 3 (SOCS3) limits JAK/STAT pathways involved in vascular inflammation and remodelling responsible for vein graft failure. However,