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15 THE EFFECT OF HYPOXIC MEDIATED PRO-MITOGENIC SIGNALLING ASSOCIATED WITH VEIN GRAFT FAILURE AND VASCULAR REMODELLING

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Tissue hypoxia is known to contribute to the hyperproliferative pathogenesis underlying vein graft stenosis with loss of luminal patency. Hypoxia has been reported to modulate PLC-χ leading to the activation of PKC dependent-cell proliferation through mitogen activated protein kinases (MAPK) signalling pathways. Additionally, hypoxia is positively correlated with the pro-mitogenic HIF-1α derived signalling pathway. This project aims to investigate the potential interaction of HIF-1α and PLC-χ in hypoxic mediated pro-mitogenic signalling associated with vein graft remodelling.

Vascular smooth muscle cells (VSMC) were cultured in hypoxic conditions (3%–10%) and compared with normoxic culture. VSMC proliferation was assessed through ^{3} hour thymidine incorporation assays, mRNA expression levels were measured using RT-PCR and MAPK signalling was evaluated by immunoblotting. Conditioned media experiments from VMSC and endothelial cells were performed along with exosome isolations from both cell types under experimental conditions.

Results confirmed a positive correlation between hypoxia and cell proliferation; cells cultured under 3% and 10% oxygen increased cell proliferation by 2.1 and 1.4 fold respectively. The PLC-χ inhibitor U73122 (1 μM) significantly reduced VSMC proliferation under normoxia however had no inhibitory effect in hypoxic culture. Experiments also showed hypoxia to downregulate mRNA expression levels of PLC-χ, by 49.9%.

Conditioned media from 3% hypoxic cultured endothelial cells increased VSMC proliferation by 5.8 fold at 24 hours. Initial experiments have confirmed a hypoxic-dependent-increase in exosomal trafficking where the measured exosomal yield from VMSC cultured under 3% hypoxia increased by 30% when compared with normoxic cultures.

Moderate hypoxia significantly drives VSMC proliferation in comparison to mild hypoxia. These results raise questions over the association with PLC-χ and hypoxia-dependent promitogenesis of VSMCs. This observation is backed up by the absence of hypoxic-dependent phosphorylation of the MAPKs. Of note, initial experiments have identified hypoxic culture modulates a pro-mitogenic endothelial-derived transferable factor(s) and increase in exosomal trafficking.

16 INVESTIGATING CALCIFIC AORTIC VALVE DISEASE USING NOVEL IMMORTALISED SHEEP AND RAT VALVE INTERSTITIAL CELL LINES

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Calcific aortic valve disease (CAVD) involves progressive valve leaflet thickening and severe calcification, resulting in impaired leaflet motion. Although much research has advanced our knowledge of this disease, the mechanisms underlying the initiation and progression of CAVD are still unclear, necessitating further studies to elucidate the underpinning processes in the early stages of this disorder.

Our present study aimed to (i) generate and (ii) evaluate the calcification potential of immortalised cell lines derived from sheep and rat aortic valve interstitial cells (VICs). We show that both sheep (SAVIC) and rat (RAVIC) cell lines expressed markers of VICs (vimentin and α-SMA). We also established that sheep VIC (SAVIC) calcification can be induced in the presence of increased calcium only (2.7 mM; 1.9 fold; $p<0.001$), with a synergistic effect of calcium and phosphate on VIC calcification noted at 2.7 mM and 2.0 mM, respectively (22.2 fold; $p<0.001$). Significant increases in mRNA expression of key genes associated with valve calcification were observed (RUNX2 and PiT1) when SAVICs were cultured under increasing calcium conditions. Contrastingly, the mRNA level of a potential calcification inhibitor (MGP) decreased under these conditions. Interestingly, we found very little alkaline phosphatase (ALPL) expression in these cells. Comparable data were observed in the RAVIC studies. Furthermore, SAVIC calcification levels were significantly reduced in the presence of known inhibitors of calcification, pyrophosphate (PPi; 1.7 fold; $p<0.01$) and etidronate (3.2 fold; $p<0.01$).

In conclusion, the use of immortalised sheep and rat VICs can provide a reliable model system to investigate aortic valve calcification *in vitro*, which will assist in our understanding of this pathophysiological process

17 FEMORAL ARTERY LIGATION INDUCES RAPID ARTERIOGENESIS THAT CORRELATES WITH RECOVERY OF PERFUSION IN THE FOOT

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Background and hypothesis Reperfusion following acute hindlimb ischaemia is mediated by both arteriogenesis (collateral growth) and angiogenesis. According to Poiseuille's Law, the arterial ability to conduct blood is proportional to the 4th order of its diameter. This suggests collateral arteries with a relatively large diameter are better suited to conduct blood than smaller vessels. We hypothesise that arteriogenic remodelling of collateral vessels has a greater role than angiogenesis in restoring blood supply to the ischaemic hindlimb.

Methods Acute hindlimb ischaemia was induced in 12-week-old male C57BL/6J mice by unilateral femoral artery ligation. Reperfusion in the foot pad was monitored by laser Doppler immediately before and after femoral artery ligation and subsequently on days 1, 3 and 7 (n=3 for each time point). Vascular resin casts plus optical projection tomography (OPT) were used to illustrate the arterial network before and after ischaemia.

Results Blood perfusion in the foot pad dropped by ~90% immediately following ligation but exhibited a 40% recovery over the next 7 days. This restoration of flow correlated with

the emergence and growth of collateral arteries in the thigh (upstream of the ligation point).

Conclusion A pre-existing collateral circulation provides the residual blood supply after femoral ligation. A rapid increase in the diameter of a small number of collateral arteries appeared to be the major mechanism for acute restoration of blood supply to the ischaemic lower leg and foot pad. Future work will use histology and immunohistochemistry to investigate the role of angiogenesis in reperfusion following femoral ligation.

18 TOWARDS NON-INVASIVE CHARACTERISATION OF RE-ENDOTHELIALISATION AND RESTENOSIS FOLLOWING CORONARY STENTING: AN IN VITRO INVESTIGATION USING IMPEDANCE SPECTROSCOPY

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Following the permanent implantation of a coronary stent, optimal arterial wall healing is characterised by re-endothelialisation, the regrowth of a functional Endothelial Cell (EC) monolayer over the exposed stent surface, which reduces the risk of thrombosis. However restenosis, arising from the proliferation and migration of medial Smooth Muscle Cells (SMCs) can cause luminal narrowing to reoccur. Previous research has suggested that the stent itself could be used as an electrode and, when combined with non-invasive impedance spectroscopy techniques, monitor post stenting recovery. This could then inform clinicians on cell regrowth without the need for invasive imaging techniques. In this study we investigated the feasibility of this concept using two *in-vitro* models representing the cellular regrowth scenarios: re-endothelialisation and restenosis.

Primary porcine ECs and SMCs were seeded onto platinum electrodes and electrical impedance spectroscopy measurements were made for up to 10 days in the frequency range 1 KHz to 100 KHz. Endothelium function was assessed through the measurement of the impedance response of confluent EC monolayers to the addition of a gap junction enhancer, dipyridamole, or an inhibitor (heptanol or carbenoxolone).

Our results show that confluent, stent surface comparable populations of SMCs and ECs give rise to distinct impedance signatures, providing a novel method of non-invasively characterising these cell types. Gap junction inhibition of EC monolayers dose dependently reduced total impedance. Conversely dipyridamole's enhancing effect on gap junction formation caused an increase in total impedance. These novel findings show the importance of intercellular gap junction communication in maintaining EC barrier function. Our current work is focused on the translation of this technology towards *in-vivo* monitoring of in-stent restenosis and recovery of a functional endothelium.

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19 ADVERSE CARDIAC REMODELLING UNDER PRESSURE OVERLOAD. A MAGNETIC RESONANCE IMAGING STUDY IN MICE

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Pressure overload, a hallmark of valvular heart disease and hypertension, is the leading cause of heart failure. With the progressive nature of this condition a better understanding of the process underlying the transition to heart failure is vital. Recent studies suggest that interstitial myocardial fibrosis occurs early in this transition and has a profound effect on cardiac function. The recently developed T1-mapping Cardiovascular Magnetic Resonance Imaging (CMR) technique has the potential to quantify the extracellular volume fraction (ECV) and therefore evaluate the expansion of the extracellular matrix (primarily diffuse fibrosis) over time.

We aimed to assess the feasibility of CMR (including functional and ECV imaging) to monitor cardiac remodelling using an animal model of pressure overload heart disease.

Fifteen mice were subjected to a 6 week Angiotensin-II infusion (AngII). CMR (cine and T1 mapping) was performed before and following Angiotensin II infusion at 2, 4 and 6 weeks. ECV was calculated from the T1 relaxation times pre and post-contrast infusion).

Mean blood pressure increased from 65±12 (baseline) to 84±14 mmHg ($p<0.001$) and ECV increased from 24.28% ±3.35% (baseline) to 30.03%±5.34% after 2 weeks of AngII ($p=0.011$). ECV plateaued at 4 and 6 weeks and stayed significantly higher compared to baseline ($p=0.001$). Cine imaging revealed left ventricular (LV) hypertrophy during infusion which remained stable at 4 and 6 weeks. Interestingly, systolic function was maintained after 2 and 4 weeks of AngII but was impaired at six weeks (EF 56.3% compared to 64.4% at baseline and 59.8%; 60.7%, at 2 and 4 weeks ($p=0.014$). This drop in cardiac performance was accompanied by a trend towards LV dilatation at 6 weeks compared to baseline (LV end diastolic volume 68 µl vs 63 µl, $p=0.056$).

Prolonged pressure overload results in ECV expansion, LV hypertrophy and subsequent systolic dysfunction. T1 mapping CMR shows promise in monitoring this transition.

20 THE POLYPHENOL DELPHINIDIN INDUCES ANTIOXIDANT EFFECTS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS THROUGH ACTIVATION OF ENDOGENOUS GLUTATHIONE: IMPORTANCE OF USING RELEVANT CONCENTRATION IN IN VITRO SYSTEMS

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