



## BSCR Autumn Meeting Abstracts

### Abstract Titles

Selected for oral presentation.

**S1: Early Intervention Using TGF $\beta$  Bolus Therapy Has an Anti-Inflammatory Effect on Endothelial Cells and Improves Outcomes Following Myocardial Infarction**

**S2: Neutrophil Dynamics in the Beating Embryonic Zebrafish Ventricle Following Localised Laser Injury**

**S3: The NF-kappaB Transcription Factor Subunit c-Rel Promotes Endothelial Proliferation and Inflammation in Response to Low Shear Stress**

**S4: Investigation of Toll-Like Receptors in Regulation of Mesenchymal Stem-Cell-Like Cardiac Fibroblast Phenotype**

**S5: Modulation of Macrophage Differentiation and Activation: Paracrine Signals from Cardiac Progenitor Cells**

**S6: Nox4 Regulates Endothelial Cell Signalling and Cardiac Remodelling in Experimental Diabetes**

Selected oral abstracts.

**S1: Early Intervention Using TGF $\beta$  Bolus Therapy Has an Anti-Inflammatory Effect on Endothelial Cells and Improves Outcomes Following Myocardial Infarction**

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**Background:** TGF $\beta$ 1 has well established anti-inflammatory properties that protect the body from exuberant inflammation. It suppresses inflammatory T cell differentiation and stimulates immunomodulatory Treg differentiation. However, vascular endothelial cells are important gatekeepers regulating leukocyte extravasation during the inflammatory response to injury. The potential of TGF $\beta$ 1 to modulate cardiac ischaemia/reperfusion (I/R) injury via an anti-inflammatory effect on endothelial cells remains almost completely unexplored. We hypothesise that early delivery of exogenous TGF $\beta$ 1 will dampen the pro-inflammatory effect of cardiac injury in endothelial cells and lead to improved outcomes.

**Results:** Using a preclinical model of cardiac I/R injury, we find that early systemic delivery of TGF $\beta$ 1 at the time of reperfusion leads to improved outcomes. The leucocyte infiltrate into the heart is reduced in both density and area and is associated with decreased infarct size. Myocardial expression of inflammatory cytokines IL1b and CCL2 is dramatically lowered. There are also long-term benefits of this early TGF $\beta$  treatment as adverse remodelling is reduced and cardiac function is improved. In vitro analysis of the effect of TGF $\beta$ 1 treatment on the endothelial cell transcriptome points to a major role for endothelial cells in the immunosuppressive effect. Furthermore, analysis of mice with endothelial-specific loss of the TGF $\beta$  receptor II is consistent with the requirement of endothelial cells for the immuno-protective role of TGF $\beta$ 1 therapy in cardiac I/R injury.

**Conclusion:** TGF $\beta$  has powerful anti-inflammatory effects on the endothelium during tissue injury that lead to long-term beneficial outcomes.

**S2: Neutrophil Dynamics in the Beating Embryonic Zebrafish Ventricle Following Localised Laser Injury**

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**Rationale:** Zebrafish possess a remarkable capacity to regenerate their hearts after injury. Innate immune cells, particularly neutrophils, are the first responders following myocardial injury. However, the role of neutrophils following myocardial injury is incompletely understood.

**Methodology:** We used a model of laser injury to the ventricular apex of embryonic zebrafish hearts at 3 days post-fertilisation. Cardiomyocyte and neutrophil reporting zebrafish (Tg(myl17:GFP;mpx:mCherry)) were serially imaged using epifluorescence microscopy to quantify the extent of heart injury (% ventricular GFP lost), heart function (% ventricular ejection fraction, VEF) and neutrophil dynamics (neutrophil number at wound site, NN) in vivo up to 48 h post injury (hpi). Single plane illumination microscopy (SPIM) combined with optical gating technology was used to analyse cardiomyocyte-neutrophil interactions at higher spatial and temporal resolution.

**Results:** Ventricular injury peaks at 6 hpi ( $3.75 \pm 0.635$  vs  $0 \pm 0$ ,  $p < 0.0001$ ) and decreases by 48 hpi ( $1.17 \pm 0.192$  vs  $0 \pm 0$ ,  $p < 0.5$ ). VEF falls at 2 hpi ( $14.9 \pm 1.23$  vs  $22.18 \pm 0.548$ ,  $p < 0.001$ ) and normalises by 48 hpi ( $25.44 \pm 0.849$  vs  $28.64 \pm 0.941$ , ns). NN increases by 2 hpi, peaks at 6 hpi ( $3.15 \pm 0.365$  vs  $0.1 \pm 0.069$ ,  $p < 0.0001$ ) and normalises at 48 hpi ( $0.85 \pm 0.244$  vs  $0.25 \pm 0.123$ , ns). SPIM tracking of heart-associated neutrophils demonstrates a similar dynamic response, with clear neutrophil involvement at the wound margin. Detailed 4D imaging illustrates ventricular injury size increasing up to 6 hpi and subsequent migration and bridging of cardiomyocytes across the wound margin.

**Conclusions:** Both cardiomyocyte injury and neutrophil chemotaxis peak at 6 hpi and alongside VEF resolve by 48 hpi. The specific role and potential therapeutic manipulation of neutrophils during this reparative response are currently under investigation.

**S3: The NF-kappaB Transcription Factor Subunit c-Rel Promotes Endothelial Proliferation and Inflammation in Response to Low Shear Stress**

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**Introduction:** Atherosclerosis develops preferentially at bends and branches of the vasculature, which are exposed to disturbed flow and low shear stress (LSS). Shear stress modifies endothelial cell (EC) function by regulating proliferation, inflammation and other processes. Although some members of the NF- $\kappa$ B pathway are known to respond to shear, the influence of haemodynamics on the c-Rel subunit and its role in atherogenesis are unknown and are a focus of this study.

**Methodology:** En face staining was performed in C57BL/6 wild-type and c-Rel knockout mice to quantify the expression of c-Rel and the proportion of proliferating EC (using anti-Ki67 antibodies) at LSS and high shear stress (HSS) regions of the murine aortic arch. Human umbilical vein EC (HUVEC) or human coronary artery EC (HCAEC) were exposed to flow for 72 h and c-Rel protein levels were measured by Western blotting. The expression of c-Rel was silenced in HUVEC under LSS using siRNA and EC proliferation and inflammatory markers were then measured by staining of proliferating cell nuclear antigen (PCNA) and qRT-PCR, respectively.

**Results and conclusions:** En face staining of murine aortas and Western blotting studies of HUVEC and HCAEC revealed that c-Rel was enriched at LSS compared to HSS regions ( $P < 0.05$ ). c-Rel silencing in HUVEC and c-Rel genetic deletion in mice resulted in decreased proliferation in EC exposed to LSS ( $P < 0.05$ ). c-Rel silencing in HUVEC also resulted in decreased EC inflammation under LSS ( $P < 0.05$ ). These data suggest that c-Rel might contribute to atherogenesis by promoting EC proliferation and inflammation under LSS.

#### **S4: Investigation of Toll-Like Receptors in Regulation of Mesenchymal Stem-Cell-Like Cardiac Fibroblast Phenotype**

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Using molecular and bioinformatic approaches, we have identified a subpopulation of resident myocardial fibroblasts which retain significant stromal/mesenchymal stem cell (MSC)-like properties and can express different pro- or anti-inflammatory phenotypes depending on the precise environmental conditions. We refer to these as F1 (pro-inflammatory/pro-fibrotic) and F2 (anti-inflammatory/anti-fibrotic). The F2 phenotype has MSC-like immunomodulatory and pro-resolution properties and is strongly associated with the down-regulation of fibrosis gene expression (i.e. CTGF and collagens) and the upregulation of cyclooxygenase-2 (COX-2), the COX-2-dependent prostanoids PGE2 and PGI2, M2/Th2 immune-regulatory genes, provisional (pro-)matrix genes, interferon regulated genes and DEXD-box RNA helicases. Importantly and surprisingly, the F2 phenotype is induced by activation of the innate immune receptor Toll-like receptor 4 (TLR4) by extracellular matrix (ECM) protein components (such as fibronectin EDA or sulphated proteoglycans). F2 induction via TLR4 is also potentiated by ss polyU RNA (via TLR8) or transfected (intracellular) ds (plasmid) DNA. In contrast, the F1 phenotype is induced by TGF $\beta$  as expected. Since TLRs 3, 7/8 and 9 are pathogen ssDNA/RNA sensors and DEXD-box RNA helicases are sentinel cytoplasmic dsDNA sensors, this suggests that F2/F1 switching is regulated by an ECM-sensing mechanism and modulated by pathogen- or damage-associated molecular patterns (PAMPs/DAMPs) which involve ssRNA or dsDNA. Therefore, we believe we have identified novel immune mechanisms in the regulation of cardiac fibrosis and myocardial tissue remodelling. This has the potential to generate new avenues of research which are relevant to the treatment of heart disease, particularly in the context of adverse myocardial remodelling in response to injury.

#### **S5: Modulation of Macrophage Differentiation and Activation: Paracrine Signals from Cardiac Progenitor Cells**

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**Background:** Myocardial infarction in adult mice is affected by both inflammatory and anti-inflammatory/repairative macrophages, whose modulation is a target towards enhancing heart repair. Previously, we demonstrated that injection of cardiac progenitor cells (CPC) improves cardiac function despite lack of long-term engraftment, via paracrine benefits. Here, we investigated CPC-dependent effects on macrophage phenotypes by single-cell qRT-PCR.

**Methods:** We developed an in vitro model to study macrophage gene expression and gain information about transcript prevalence, heterogeneities and co-expression. Mouse bone marrow-derived macrophages were stimulated with GM-CSF+LPS/IFN $\gamma$  versus M-CSF+IL-4/IL-13 to induce M1-inflammatory or M2-reparative macrophages, respectively. Cells were analysed by multi-parametric flow cytometry and > 60 single F4/80+CD11b+ macrophages were analysed by single qRT-PCR for 48 genes related to macrophage function.

**Results:** As expected, macrophages treated with GM-CSF+LPS/IFN $\gamma$  co-expressed Nos2, Cxcl9, Cxcl10 and Il-6 (inflammatory). Conversely, M-CSF+IL-4/IL-13 induced Arg1, Angpt2 and Igf1 (reparative). Notably, CPC-conditioned media (CPC-CM) antagonised pro-inflammatory macrophage activation by LPS/IFN $\gamma$ , whilst permissive for the anti-inflammatory signature evoked by IL4/IL13, even in the absence of exogenous M-CSF. Using complementary approaches and multiplexed ELISAs to de-convolute the cocktail secreted by CPCs, we identified M-CSF as a candidate to explain the benefits observed. Neutralising antibody against CSF1R impaired the M2-like phenotype, as shown by the loss of CD206 and Arg1 induction.

**Conclusions:** (1) Single-cell qRT-PCR defines dichotomous sets of genes enriched in M1- versus M2-driven macrophages. (2) Cardio-protective CPCs disrupt the pro-inflammatory (M1) programme and induce an M2-like macrophages phenotype. (3) CSF1R blocking antibody hampers the M2-like differentiation and activation of macrophages driven by CPC-CM.

#### **S6: Nox4 Regulates Endothelial Cell Signalling and Cardiac Remodelling in Experimental Diabetes**

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Chronic hyperglycaemia in diabetes increases reactive oxygen species (ROS) which drives cardiac remodelling. NADPH oxidases are ROS-producing enzymes implicated in cardiovascular disease development/progression. Here, we investigated the specific role of endothelial cell (EC) Nox4 as a major driver of diabetic cardiac remodelling. EC-specific Nox4-overexpressing (Tg) and wild-type (WT) mice were subjected to streptozotocin (STZ)-induced diabetes and studied at 6 months ( $n = 9-11$ ). Echocardiography indicated diastolic dysfunction in WT diabetic mice (E/A  $1.60 \pm 0.13$  vs control  $1.94 \pm 0.10$ ;  $P < 0.05$ ), whilst Nox4Tg diabetic mice exhibited similar diastolic function ( $1.55 \pm 0.06$  vs control  $1.54 \pm 0.09$ ;  $P = \text{NS}$ ). Similarly, WT but not Nox4Tg diabetic mice demonstrated increased cardiac fibrosis and profibrotic gene expression (qRT-PCR). Interestingly, Nox4Tg diabetic hearts exhibited increased antioxidant mRNA expression (e.g. SOD,  $3.12 \pm 0.43$  vs control

1.75 ± 0.32;  $P < 0.05$ ) not evident in WT hearts. Complementary experiments in human aortic ECs (HAoECs,  $n = 3$ ) subjected to high glucose (HG, 25 mM) for 2 days indicated that NOX4 overexpression (OE) increased antioxidant mRNA (e.g. catalase, HG OE 2.85 ± 0.40 vs HG empty vector (EV) 1.20 ± 0.11;  $P < 0.05$ ), whilst conditioned media from OE HAoECs promoted TGFβ-induced 3T3 fibroblast differentiation (HG OE 1.39 ± 0.16 vs HG EV 0.88 ± 0.04;  $P < 0.05$ ). Conversely, NOX4 knockdown (KD) reduced antioxidant mRNA expression (e.g. SOD, HG KD 1.38 ± 0.08 vs HG scrambled 0.94 ± 0.07;  $P < 0.05$ ) which was associated with normalisation of HG-induced superoxide (DHE fluorescence) and TGFβ-induced fibroblast differentiation. Together, these data indicate that Nox4 is a key mediator of EC function in diabetes which may regulate cardiac fibrosis/remodelling via modulation of paracrine signalling. However, further investigation is required to elucidate precise underlying mechanisms.

Selected for poster presentation.

**P1: Pathological Insights to Heart Failure with Preserved Ejection Fraction—a Comparison of Patients With and Without Diabetes**

**P2: Role of Vascular Smooth Muscle Cell-Derived Exosomes in Age-Related Vascular Disease**

**P3: Changes in Cardiac Inflammatory Signalling Pathways After Myocardial Infarction**

**P4: Fzd4 and β-Catenin Promote Inflammatory Signalling in Endothelial Cells Exposed to Disturbed Flow**

**P5: The Role of p47phox and Its Signalling Pathways in Angiotensin II (AngII)-Induced Cardiac Hypertrophy and Cardiomyocyte Apoptosis in Mice**

**P6: Endothelial Cells Form Transient Cystic Structures During Zebrafish Cerebrovascular Development**

**P7: Imaging the Vasculoprotective Effects of Haematopoietic Stem/Progenitor Cells (HSPCs) Following Myocardial Ischaemia-Reperfusion (IR) Injury in the Murine Beating Heart**

**P8: Nitrite Upregulates the Reperfusion Injury Salvage Kinase Pathway via Aldehyde Dehydrogenase During Myocardial Ischaemia-Reperfusion Injury**

**P9: The Role of Aldehyde Dehydrogenase in Myocardial Protective Effects of Inorganic Nitrite**

**P10: Platelet Microparticles Induce Endoplasmic Reticulum Stress in The Endothelium Through Delivery of the miRNA Let-7a**

**P12: Mathematical Modelling of Smooth Muscle Cell Migration Reveals Mechanisms of Early Fibrous Cap Formation in Atherosclerosis**

**P13: The Effect of Latent Cytomegalovirus Infection and CX3CR1+ T-Lymphocytes on Ventricular Remodeling After Myocardial Infarction**

**P14: The Soluble Epoxide Hydrolase Inhibitor GSK2256294 Decreases LPS-Induced Cytokine mRNA Expression in Human Peripheral Blood Mononuclear Cells**

**P15: Platelet Activation Causes Vesicle Release and Loss of Mitochondria**

**P16: The Role of Vessel Stiffening in Vascular Calcification and Vesicle Release**

**P17: Characterisation of Macrophage Response to Laser Injury in the Larval Zebrafish Heart: Implications for Repair and Regeneration**

**P18: The Effects of TNF-Α and IL-1β on Intracellular Calcium Waves in Sheep Ventricular Myocytes**

**P19: High-Fat Diet-Induced Nox2 Activation and Endothelial Dysfunction in ApoE Knockout Mice**

**P20: Human Cardiac Fibroblast-Secreted Exosomes Improve Efficiency of Human Cardiomyocyte Calcium Cycling**

**P21: Endogenous Circulating BMP9 Maintains Endothelial Cell Barrier Function**

**P22: Prelamin A Accumulation in Vascular Smooth Muscle Cells Accelerates Vascular Ageing**

**P23: Analysing the Role of Tyrosine Phosphorylation in Inflammasome Complex Formation and Function**

**P24: TNFα-Induced Inflammation Is Mediated by Wnt/β-Catenin Signalling in Endothelial Cells: Implications for Atherosclerosis**

**P25: Pro- and Anti-Inflammatory Macrophages Display Divergent Polarisation Towards Vascular Smooth Muscle-Like and Endothelial-Like Phenotypes**

**P26: Remodelling After Myocardial Infarction Involves Changes in Inflammatory Signalling Pathways**

**P27: Myeloid TRIB1 Controls Metabolic Tissue Resident Macrophage Phenotype Associated with Immuno-Metabolic Diseases**

**P28: Reduced Pro-Angiogenic Function of Cord Blood Endothelial Colony-Forming Cells in Hyperglycaemia Is Mediated by NOX4**

**P29: The Interaction Between Transforming Growth Factor-B and the Canonical Wnt Pathway in Post-MI Cardiac Remodelling**

**P30: Mechanosensitive Hox Genes Control Vascular Dysfunction in Adult Arteries**

**P31: The Role of Fractalkine in Cardiac Ischaemia-Reperfusion**

**P32: The Role of FAM176A in Endothelial Cell Responses to Flow**

**P33: Mitochondrial Hyperactivity, Impaired Matrix Adhesion and Functional Activity in Endothelial Colony Forming Cells Isolated From Patients with Diabetic Foot Ulcers**

**P34: Fibroblast-Specific Deletion of Interleukin-1 Receptor 1 (IL-1R1) Is Cardioprotective in an Experimental Model of Myocardial Infarction**

**P35: Freestyle Fluidics—a Novel Platform to Study Macrophage Chemotaxis**

**P36: Vulnerability of the Aged Heart to Ischaemia/Reperfusion Injury Before and After High-Fat Diet in Mice**

### P37: Porcine Blood Outgrowth Cells (BOCs) for Tissue Engineering of Decellularised Human Saphenous Vein Grafts

### P38: Endothelial Stat5a Is Enriched at Atheroprone Regions and Drives Inflammation in Response to Low Shear Stress

#### Selected poster abstracts.

#### P1: Pathological Insights to Heart Failure with Preserved Ejection Fraction—a Comparison of Patients With and Without Diabetes

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**Background and Rationale:** Despite the high prevalence of diabetes in heart failure with preserved ejection fraction (HFpEF), there are numerous uncertainties surrounding how these conditions interact. We aimed to determine whether patients with diabetes and HFpEF have a distinct pattern of cardiac remodelling compared to non-diabetic patients and whether remodelling was related to circulating markers of inflammation and fibrosis.

**Methods:** We prospectively recruited 140 patients with HFpEF (75 diabetic and 65 non-diabetic). Subjects underwent comprehensive cardiovascular phenotyping, including echocardiography, cardiac MRI, 6-min walk test and plasma biomarker profiling.

**Results:** Diabetic patients were younger (age  $70 \pm 9$  vs.  $75 \pm 9$  years,  $p = 0.002$ ), with evidence of more left ventricular (LV) concentric remodelling (LV mass/volume ratio  $0.72 \pm 0.15$  vs.  $0.62 \pm 0.16$ ,  $p = 0.024$ ) and smaller indexed left atrial (LA) volumes (maximal LA vol. index  $48 \pm 20$  vs.  $59 \pm 29$  ml/m<sup>2</sup>,  $p = 0.004$ ) than non-diabetics. Markers of inflammation (IL-8, SerpinE1 PAI1 and GDF15) and extracellular matrix remodelling (MMP7 and TIMP1) were elevated in diabetics. Overall, there were 45 hospitalizations for HF and 22 deaths over a median follow-up period of 47 (IQR 38–54) months. There was no difference in the primary composite endpoint of hospitalisation for HF and mortality between groups. On multivariable Cox regression analysis, age, prior HF hospitalisation, history of pulmonary disease and LV mass/volume were independent predictors of the primary outcome.

**Conclusions:** Patients with HFpEF and diabetes have increased concentric LV remodelling, smaller LA volumes and evidence of increased systemic inflammation compared to non-diabetics. This suggests the underlying pathophysiology for the development of HFpEF is different in patients with and without diabetes.

#### P2: Role of Vascular Smooth Muscle Cell-Derived Exosomes in Age-Related Vascular Disease

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**Background:** Exosomes have recently been recognised as mediators of formation. Age-related accumulation of amyloid is commonly associated with degenerative diseases, such as Alzheimer's disease. Exosomes have also been implicated in vascular smooth muscle cell (VSMC) calcification, a manifestation of ageing.

Aortic medial amyloid (AMA) is the accumulation of a peptide, medin, which forms in ageing vessel walls. Medin is formed by the cleavage of MFGE8.

The aims are to study (1) changes in exosome secretion with age, (2) amyloid protein loading in exosomes and (3) if MFGE8 and/or AMA can promote calcification.

**Methods:** Western blotting, qPCR and immunostaining were used to study medin and MFGE8 in VSMCs. FACS was used for the quantification of exosome secretion. Exosomes were isolated by differential ultracentrifugation. Extracellular matrix (ECM) was synthesised in vitro for staining and Western blotting.

**Results:** MFGE8 and medin were present in aortas of old subjects. MFGE8 was expressed by VSMCs and secreted by exosomes. Medin is deposited in the ECM and blocking exosome release decreased its deposition. The expression and secretion of MFGE8 increased in calcifying conditions and recombinant MFGE8 increased calcification while siRNA knockdown of MFGE8 decreased calcification.

**Conclusion:** Medin and MFGE8 are abundant in aged subjects and are secreted by exosomes into the ECM. Exosome release increases with age, which could contribute to the deposition of medin in the ECM and the formation of amyloid. MFGE8 may play a role in accelerating calcification by inducing an osteogenic phenotype. MFGE8 and medin secretion by exosomes could contribute to the age-related development of vascular calcification.

#### P3: Changes in Cardiac Inflammatory Signalling Pathways After Myocardial Infarction

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Cardiac remodelling post-myocardial infarction involves complex cellular and molecular mechanisms. It is proposed that there is a link between excessive inflammatory response triggered by cardiac insult and adverse remodelling. In this study, we used proteomic analysis to study signalling proteins mediating key inflammatory pathways following experimentally induced cardiac infarction. All experimental procedures conform to the European Directives and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and approved by the University of Bristol ethics committee. Adult male Wistar rats (220–240 g) were used. Surgically induced cardiac injury by left coronary artery ligation is described elsewhere. In sham-operated animals ( $n = 5$ ), no coronary ligation occurred. After 4 weeks, rats were killed, hearts extracted and Langendorff perfusion followed. Cardiac tissue from the apex was collected, and protein extraction ensued. Protein identification and quantification were completed using Tandem Mass Tagging (TMT) at the University of Bristol Proteomics Facility. Level of significance was set to  $P$  value  $< 0.05$ . Fold change (LAD/SHAM) either  $< 0.8$  or  $> 1.3$  was used. **Results:** Four thousand seven hundred eighty-six accession numbers were identified, of which 275 were significant. Several proteins involved in inflammatory signalling were found to change post-infarction. These include protein kinase C (PKC) ( $P = 0.019$ , FC = 1.4), tyrosine-protein kinase (0.036, 1.238), serine/threonine-protein phosphatase 2B ( $\beta$  isoform) (0.019, 1.9), integrin subunit  $\alpha V$  (0.034, 1.456), fibronectin (0.037, 1.34) and mitogen-activated protein kinase kinase 5 (MAP3K5) (0.023, 1.128). **Conclusions:** Rat hearts undergoing post-ischemic cardiac remodelling exhibit significantly higher abundances of certain proteins involved in inflammatory signalling pathways.

#### **P4: Fzd4 and $\beta$ -Catenin Promote Inflammatory Signalling in Endothelial Cells Exposed to Disturbed Flow**

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**Background:** Atherosclerotic lesions develop preferentially in regions of arteries exposed to multidirectional ‘disturbed’ flow that increases pro-inflammatory signalling. Here, we investigate the role of  $\beta$ -catenin signalling in mediating inflammatory responses to flow.

**Methods:** Human aortic endothelial cells (HAEC) were exposed to uniaxial (undisturbed) and multidirectional (disturbed) flow for 72 h using the orbital shaker method. To assess the role of Fzd4 and  $\beta$ -catenin in mediating flow-dependent responses, HAEC were transfected with siRNA (100 nM) prior to flow exposure using a Neon Electroporation System.

**Results:** We have previously demonstrated that the expression of Frizzled-4 and  $\beta$ -catenin is significantly increased in HAEC following exposure to disturbed flow (DF) for 72 h. DF is typically associated with the elevation of E-selectin, MCP-1 and VCAM-1 transcripts although this was significantly attenuated following knockdown of either Frizzled-4 or  $\beta$ -catenin. Similar results were obtained when HAEC were incubated with iCRT5, which blocks  $\beta$ -catenin transcriptional activity. iCRT5 also reduced the adhesion of THP-1 monocytes to HAEC and reduced nuclear localisation of NF- $\kappa$ B. Interestingly, application of DKK-1, which inhibits canonical Wnt signalling by disrupting the interaction of Frizzled with Lrp co-receptors, had no effect on inflammatory responses to DF. We observed a significant increase in Wnt5a expression (typically associated with non-canonical Wnt signalling) in cells exposed to DF at both the gene and protein level, which may promote activation of a Fzd4- $\beta$ -catenin pathway.

**Conclusions:** These data suggest that Frizzled-4 and  $\beta$ -catenin signalling regulate pro-inflammatory signalling in HAEC exposed to DF and that this may occur via a Wnt5a-dependent, Lrp-independent mechanism.

#### **P5: The Role of p47phox and Its Signalling Pathways in Angiotensin II (AngII)-Induced Cardiac Hypertrophy and Cardiomyocyte Apoptosis in Mice**

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A Nox2-containing NADPH oxidase by generating reactive oxygen species (ROS) is important in AngII-induced cardiac hypertrophy. p47phox is a key regulator of Nox2 enzyme. However, the role of p47phox and its signalling pathways in AngII-induced cardiac oxidative stress and hypertrophy remained unclear. In this study, we used C57/BL6 wild-type (WT) and p47phox knockout (KO) mice (male, 10–12 months,  $n = 10$ ) to investigate the role of p47phox in AngII-induced (mini-pump infusion, 1 mg/kg/day, 14 days) cardiac oxidative stress and hypertrophy. In WT mice, AngII increased significantly the blood pressure (SBP) (from  $127 \pm 13$  to  $172 \pm 11$  mmHg) and caused cardiac hypertrophy as demonstrated by increases in heart/body weight ratio (by  $\sim 17.9 \pm 0.1\%$ ) and cardiomyocyte size ( $\sim 10.3 \pm 0.1\%$ ). However, in p47phox KO mice, AngII only induced a mild increase in SBP (from  $119 \pm 9$  to  $149 \pm 10$  mmHg) without a significant increase in heart/body weight ratio or cardiomyocyte size. In AngII-infused WT hearts, there were significant increases in ROS production (2.81  $\pm$  0.50-fold), in the levels of Nox2 and p47phox expression and phosphorylation of ERK1/2, p38 MAPK and MAPK kinase 3/6 (MKK3/6). There was significant cardiomyocyte apoptosis as indicated by increased levels of gamma-H2AX and apoptosis signal-regulating kinase 1 (ASK1) phosphorylation. However, all of these AngII-induced

redox-changes and cardiomyocyte apoptosis were significantly reduced or absent in p47phox KO hearts. In conclusion, p47phox plays a key role in AngII-induced cardiac oxidative stress and hypertrophy. p47phox signalling through MKK3/6, p38MAPK and ERK1/2 phosphorylation is essential in gamma-H2AX and ASK1 phosphorylation and cardiomyocyte apoptosis.

#### **P6: Endothelial Cells Form Transient Cystic Structures During Zebrafish Cerebrovascular Development**

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**Introduction:** Angiogenesis is highly complex and dynamic. To study angiogenesis, transgenic zebrafish provide an excellent in vivo model for observation of endothelial cell behaviour and provide novel insight into vascular development. Here, we describe an unreported endothelial cell behaviour in zebrafish: the formation of transient cystic structures we term kugeln.

**Methods:** We used a Zeiss Z.1 or custom mSPIM lightsheet microscope to image the formation of cerebral vessels in the following transgenic zebrafish: tg(kdrl:HRAS-mCherry916) endothelial cell membrane, tg(fli1a:eGFPy1) endothelial cytoplasm, tg(flk1:nls-eGFPz109) endothelial cell nuclei marker and tg(fli1a:Lifect-mCloversh467) filamentous actin.

**Results:** Kugeln were spherical structures far larger than previously described endothelial vesicles (mean diameter  $10 \pm 0.5 \mu\text{m}$ ) arising from the membrane of cerebral vessels, particularly the middle mesencephalic central artery but never naturally in non-cerebral vessels. Kugeln (mean number/embryo  $5 \pm 1$ ) were present from 3-day post-fertilisation (dpf). Timelapse observation revealed that kugeln extruded and persisted for minutes or hours before retracting from parent vessels. Kugeln were not observed to detach, burst or anastomose. Kugeln contained no cell nuclei but filamentous actin was enriched in kugeln, particularly at the kugel neck. Inhibition of VEGF signalling by AV951 (250 nM) significantly increased kugel number, while inhibition of canonical Notch signalling by DAPT (50  $\mu\text{M}$ ) significantly decreased kugel number. When embryos were incubated in the vital stain DAF-FM, we observed a proportion of kugeln contained high levels of nitric oxide.

**Conclusion:** Kugeln represent a novel form of Notch-dependent endothelial cell behaviour specific to cerebral vessels. Further work is needed to establish whether they exist in mammals and their function.

#### **P7: Imaging the Vasculoprotective Effects of Haematopoietic Stem/Progenitor Cells (HSPCs) Following Myocardial Ischaemia-Reperfusion (IR) Injury in the Murine Beating Heart**

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**Introduction:** The kinetics of haematopoietic stem/progenitor cell (HSPC) homing and their ability to confer vasculoprotection within injured myocardial microcirculation in vivo is not known. This has been limited by an inability to directly image these events in a beating heart environment in real-time. This study performed intravital microscopy on the beating mouse heart to image these events.

Methods: LAD ligation (45 min) and reperfusion (2 h) were performed on anaesthetised mice (Ket/Med; i.p.). A 3D-printed stabiliser was attached to the beating heart to enable imaging of trafficking HSPCs (CFSE), neutrophils (anti-GR-1 ab), platelets (anti-CD41 ab), monocytes (FITC-microspheres) and capillary perfusion (FITC-BSA). Laser speckle microscopy, performed for the first time on beating hearts, monitored blood flow.

Results: Significant neutrophil ( $p < 0.001$ ), monocyte ( $p < 0.05$ ) and microthrombus ( $p < 0.01$ ) presence occurred primarily within injured capillaries. Capillary, but not larger blood vessel, perfusion was impaired as indicated by significant areas devoid of FITC-BSA. Although numerous HSPCs freely circulated through injured hearts, their local retention was poor. Despite this, neutrophil, monocyte and microthrombus presence was reduced resulting in improved microcirculatory perfusion. HSPCs also reduced endothelial oxidative damage and ICAM-1/VCAM-1 as determined using flow cytometry. Laser speckle imaging demonstrated a functional hyperaemia in response to injury which was not affected by HSPCs.

Conclusion: This is the first study to image the kinetics of stem cell homing to injured beating mouse hearts and the impact they have on myocardial microcirculatory and blood flow disturbances in vivo. Despite poor local retention, HSPCs therapeutically modified the thromboinflammatory events which benefited blood perfusion at a microvascular level.

#### **P8: Nitrite Upregulates the Reperfusion Injury Salvage Kinase Pathway via Aldehyde Dehydrogenase During Myocardial Ischaemia–Reperfusion Injury**

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Ischaemia–reperfusion injury (IRI) is a phenomenon of exacerbated cellular injury caused by the oxidative stress and abrupt metabolic changes that accompany reperfusion of the myocardium. A key mediator of IRI is the opening of the mitochondrial permeability transition pore (mPTP) and release of reactive oxygen species (ROS). During hypoxia/ischaemia, nitrite is reduced to nitric oxide (NO) by various nitrite reductases. Aldehyde dehydrogenase (ALDH2) is one such reductase within the vasculature and has been shown to be central to cardioprotection during myocardial IRI. Furthermore, experimental studies have shown nitrite to be protective when administered as a pre-conditioning agent, but the mechanism(s) remain uncertain. Herein, we assessed whether nitrite-mediated cardioprotection is dependent on active ALDH2 and if the Reperfusion Injury Salvage Kinase (RISK) pathway plays a role in this mechanism. Using a Langendorff model of global IRI, hearts isolated from the ALDH2 wild-type (WT) and knock-out (KO) mice were perfused with sodium nitrite ( $\text{NaNO}_2$ ) or placebo prior to 30 min ischaemia, followed by 2 h reperfusion. Western blot analysis was then performed using phosphospecific antibodies to Akt, GSK-3 $\beta$ (ser9) and ERK. Our data showed  $\text{NaNO}_2$  upregulated Akt and GSK-3 $\beta$ (ser9) phosphorylation but not ERK in ALDH2 WT mice compared to placebo/KO groups, thus suggesting that nitrite acts as a pre-conditioning agent to protect the heart against myocardial IRI by phosphorylation of RISK pathway mediators to activate the ALDH2 pathway and prevent opening of the mPTP. These findings support nitrite as a potential pre-conditioning pharmacological agent via its activation of ALDH2 and the RISK pathway.

#### **P9: The Role of Aldehyde Dehydrogenase in Myocardial Protective Effects of Inorganic Nitrite**

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Introduction: Strategies are vital to prevent myocardial ischaemia–reperfusion injury (IRI), a detrimental consequence of sudden restoration of blood flow to the myocardium, following acute myocardial infarction (AMI). Nitrite is cardioprotective, potentially via reduction of nitrite to nitric oxide (NO); however, the mechanisms remain uncertain. We have previously reported a role for aldehyde dehydrogenase (ALDH2) in nitrite-mediated vasodilation. Herein, we assess whether nitrite-mediated cardioprotection is dependent on active ALDH2.

Methods: We explored the effects of sodium nitrite ( $\text{NaNO}_2$ ) and vehicle control in isolated hearts from C57/BL6, ALDH2 wild-type (WT) and ALDH2 knockout (KO) mice, using an established murine Langendorff model of IRI. Measurements of coronary flow rate, infarct size and lipid peroxidation (4-hydroxynonenal (4HNE), biomarker of oxidative stress) were taken.

Results:  $\text{NaNO}_2$  improved coronary flow and decreased infarct size in C57/BL6 and ALDH2 WT hearts when compared to vehicle-treated C57/BL6 and ALDH2 KO mice, respectively. 4HNE protein adducts were decreased in ALDH2 WT hearts perfused with  $\text{NaNO}_2$  compared to ALDH2 KO.

Discussion and Conclusion: Nitrite mediates protection via the ALDH2 pathway. Our findings suggest that activation of ALDH2 may represent a method of harnessing the cardioprotective effect of nitrite when administered as a preconditioning agent.

#### **P10: Platelet Microparticles Induce Endoplasmic Reticulum Stress in the Endothelium Through Delivery of the miRNA Let-7a**

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There are an estimated seven million people living with cardiovascular disease (CVD) in the UK, accounting for over 26% of all UK deaths annually. Risk factors for CVD, including hyperglycaemia, dyslipidaemia and reactive oxygen species, induce endoplasmic reticulum (ER) stress within the endothelium. A wealth of studies implicate ER stress in inflammation and atherosclerosis progression. Acute ER stress evokes the unfolded protein response (UPR) that aims to restore homeostasis, but chronic activation further drives endothelial dysfunction. Given recent reports that platelet microparticles (PMPs), which are greatly increased in CVD, induce phenotypic responses in endothelial cells through the delivery of microRNAs (miRNAs), we investigated links with the UPR. Our data show that treating human umbilical vein endothelial cell (HUVEC) with PMPs increased mRNA levels of ATF-4, a key transcription factor underpinning one of the three arms of the UPR. Inhibiting miRNA Let-7a, which we have recently shown is required for PMP-driven angiogenesis, reversed this effect. Critically, PMP-let-7a signalling enhanced ATF-4 enrichment at the monocyte chemoattractant protein-1 (MCP-1) promoter, resulting in a significant increase in MCP-1 mRNA and protein. Given the well-documented role of MCP-1 in atherosclerosis progression, we investigated the functional impact of its production by PMPs. Treating HUVEC with PMPs increased endothelial migration, which was reversed by inhibiting Let-7a, and increased vascular permeability. Furthermore, PMPs induced an MCP-1-dependent increase in

HUVEC ICAM-1 expression and leukocyte adhesion. Control of endothelial cell permeability, leukocyte adhesion and inflammation are pivotal for atherosclerosis progression. Identifying the molecular mechanisms that underpin these responses has important implications for CVD treatment.

### **P11: Lysophosphatidylcholine 16:0: Pro-Inflammatory Cytokine or Metabolically Beneficial Adipokine?**

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**Rationale:** Lysophosphatidylcholines (LPCs) are a class of bioactive phospholipid and have previously been associated with low-grade inflammation, a characteristic of metabolic disease. However, the plasma concentration of LPC 16:0 is increased in individuals with greater amounts of active brown adipose tissue, which is associated with protection against obesity and cardiometabolic disease. In a lipidomic-based exploration of potential lipid brown adipokines, we have identified LPC 16:0 is secreted from human white primary (HWP) adipocytes as they undergo the ‘browning’ phenomenon: a switch from a white adipocyte to a brown adipocyte-like phenotype. Browning increases mitochondrial biogenesis, fatty acid and glucose oxidation, uncoupled respiration and energy expenditure within white adipose tissue, with subsequent anti-obesogenic effects.

**Methodology and Results:** We utilised a range of metabolic techniques to investigate in vitro autocrine/paracrine effects of chronic LPC 16:0 treatment on HWP adipocytes. LPC 16:0 (10, 15 and 20  $\mu$ M) increased basal, state II (succinate-stimulated) and state III (ADP-stimulated) oxidative respiration rates as determined by high-resolution respirometry. Consistently, LPC 16:0 increased citrate synthase activity (marker of mitochondrial density) and both the expression of brown adipocyte-specific genes (UCP-1, CIDEA, PRDM16) and fatty acid  $\beta$ -oxidation genes (CPT1a and CPT1b) in HWP adipocytes. Interestingly, LPC 16:0 was also inversely correlated with BMI in human adipose tissue and plasma.

**Conclusions:** Given LPC 16:0’s role in browning, effects on gene expression, oxygen consumption and mitochondrial density, LPCs may contribute to the anti-diabetic and anti-obesity effects of activated beige and brown adipose tissue and may have potential as therapeutic targets for cardiometabolic disease.

### **P12: Mathematical Modelling of Smooth Muscle Cell Migration Reveals Mechanisms of Early Fibrous Cap Formation in Atherosclerosis**

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The fibrous cap is a ubiquitous feature of mature atherosclerotic lesions that is formed by directed migration of collagen-synthesising smooth muscle cells (SMCs) within the vessel wall. By stabilising the plaque and sequestering thrombogenic plaque content from the bloodstream, the fibrous cap provides crucial protection against clinical sequelae such as heart attack and stroke. The mechanisms of cap formation, however, remain poorly understood. In particular, it is unclear why certain plaques remain stable and robust, while others become fragile and vulnerable to rupture. We present a differential equation model that studies the spatio-temporal dynamics of the SMC population in the vessel wall during early fibrous cap formation. Platelet-derived growth factor is assumed to

diffuse into the plaque from the injured endothelium and the model SMCs respond by migrating from the media and proliferating in the intimal tissue. Model simulations indicate that formation of a stable cap requires a critical balance between the relative rates of cell supply from the media, chemotactic migration in the intima and cell loss by apoptosis (or phenotype change). Moreover, we identify a number of disease-associated parameters that may be linked to variations in cap stability. The model represents the first detailed in silico study of fibrous cap formation in atherosclerosis and establishes a framework that can be readily extended to investigate other aspects of plaque development.

### **P13: The Effect of Latent Cytomegalovirus Infection and CX3CR1+ T-Lymphocytes on Ventricular Remodeling After Myocardial Infarction**

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**Introduction:** Coronary artery disease is largely an inflammatory process and lymphocytes are involved in its entire natural history, from the fatty streak to plaque rupture and ventricular remodeling. Cytomegalovirus (CMV), a ubiquitous herpes virus which inflates the cytotoxic T-lymphocyte compartment, is associated with worsened cardiovascular mortality in the elderly.

**Aim:** Investigating how CMV serostatus affects left ventricular remodeling after ST-elevation myocardial infarction (STEMI).

**Methods:** One hundred one STEMI patients were recruited, with blood samples taken immediately before and at six timepoints after reperfusion. Flow cytometry determined absolute counts of T-lymphocyte subsets and expression of the chemokine receptor CX3CR1. ELISA quantified its ligand, CX3CL1 (fractalkine), and CXCL10. Cardiac MRI determined acute infarct size, and 48 patients had another MRI 12 weeks post-infarction to assess remodeling.

**Results:** Acute infarct size ( $15.8 \pm 1.1\%$  of LV) was identical in both groups. At 12 weeks post-infarction, CMV+ patients showed less reduction in end-systolic volume ( $-2.0$  vs  $-9.1$  ml;  $p=0.27$ ) and increased end-diastolic volume ( $+10.7$  vs  $-6.1$  ml;  $p=0.02$ ) compared to CMV- patients. CMV+ patients had more cytotoxic CD4+ and CD8+ T-lymphocytes, strongly expressing CX3CR1. The number of CD8+CX3CR1+ T-cells correlated with increased end-diastolic volume ( $p=0.02$ ), while CD4+CX3CR1+ T-cells correlated with increased end-systolic volume. CXCL10, an anti-inflammatory chemokine inducible by interferon- $\gamma$ , was initially higher in CMV+ patients ( $p=0.02$ ) and correlated with better remodeling.

**Conclusion:** CMV+ patients remodel poorly following STEMI, despite similar acute infarct sizes, possibly mediated by CX3CR1+ T-lymphocytes and an exaggerated early inflammatory response. Interestingly, cytotoxic CD4+ and CD8+ T-cells seem to affect different aspects of adverse remodeling.

### **P14: The Soluble Epoxide Hydrolase Inhibitor GSK2256294 Decreases LPS-Induced Cytokine mRNA Expression in Human Peripheral Blood Mononuclear Cells**

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A vast variety of lipid mediators are known to be present and mediate inflammatory onset and resolution. One such family, the CYP450-derived epoxygenase products (epoxy-oxylinipins), has been recently shown to be anti-inflammatory and pro-resolution in rodent models of inflammation. These epoxy-oxylinipins are rapidly metabolised by soluble epoxide hydrolase. In order to prevent the breakdown of epoxy-oxylinipins and potentiate their endogenous activities, a number of sEH-inhibitors (sEH-I) have been developed. One such sEH-I GSK2256294 (GSK) has recently been tested in humans (Lazaar et al., 2016). Here, we investigated whether GSK had anti-inflammatory effects in human peripheral blood mononuclear cells (hPBMCs). hPBMCs were isolated from volunteers as previously described (Bystrom et al., 2013). hPBMCs ( $n = 4$ ) were pre-incubated (2 h) with or without GSK (300 nM) and then stimulated with or without LPS (10 ng/ml) for a further 4 h. Total RNA was extracted and IL-6, IL-1 $\beta$ , IL-8, TNF- $\alpha$  and IL-10 measured by TaqMan qRT-PCR. The LPS-induced increases in mRNA expression (fold change from control) of IL-6 (GSK,  $1.617 \pm 0.7885$ ; LPS,  $16069 \pm 6772$ ; GSK/LPS  $78.17 \pm 25.36^*$ ), IL-8 (GSK,  $1.212 \pm 0.2724$ ; LPS,  $414.8 \pm 138.6$ ; GSK/LPS,  $92.86 \pm 23.83^*$ ) and TNF- $\alpha$  (GSK,  $1.537 \pm 0.9659$ ; LPS  $96.25 \pm 26.32$ ; GSK/LPS,  $19.71 \pm 3.6^*$ ) were significantly decreased by co-incubation with GSK ( $*p > 0.05$ ; one-way ANOVA followed by Tukey's post-test). These results show that in hPBMCs, the sEH-I GSK2256294 can potentially decrease pro-inflammatory cytokine expression. GSK2256294 and related sEH-I may therefore be novel candidates for inflammatory disorders in humans. Bystrom J, et al., *PLoS One*. 2013 Sep 13;8(9):e75107. Lazaar AL, et al., *Br J Clin Pharmacol*. 2016 May;81(5):971–9.

#### P15: Platelet Activation Causes Vesicle Release and Loss of Mitochondria

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Platelets are central to the physiological process of haemostasis. Platelet activation, aggregation and secretion are extremely energy-dependent processes; therefore, platelets are highly metabolically active and rely on mitochondria to be fully functional. Here, we investigated the consequences of platelet activation on mitochondria.

Immunostaining and confocal microscopy were used to visualise platelet mitochondria (MitoTracker Orange CMTMRos) and microtubules (anti-alpha Tubulin) in unstimulated and TRAP-6-stimulated platelets. Samples were imaged using a Zeiss LSM880 Airyscan microscope. Image Stream X MKII analysis was used to characterise extracellular vesicles (EVs) produced from unstimulated and TRAP-6-stimulated platelets, staining with MitoTracker Orange CMTMRos, CD61-APC and Annexin V FITC. Results were analysed using FlowJo v.8, IDEAS 6.2 and GraphPad Prism. Results are expressed as mean  $\pm$  SEM, with significance established at  $p < 0.05$ .

Microscopy revealed that platelet activation by TRAP-6 caused a significant decrease in the number of mitochondria per platelet from  $8.1 \pm 0.5$  in resting platelets to  $3.5 \pm 0.1$  in activated samples ( $n = 6$ ,  $p < 0.001$ ), accompanied by a decrease in platelet cross-sectional area from  $5.8 \pm 0.5$  to  $4.3 \pm 0.2 \mu\text{m}^2$ . Using Image Stream X analysis, we were able to visualise the presence of mitochondria within EVs produced by TRAP-6 stimulation, accounting for  $21 \pm 4\%$  of the total population of vesicles.

Platelet stimulation causes mitochondrial loss as well as reduction in platelet size. Supportive of this, we have shown that mitochondria are present within EVs produced by platelets following stimulation. Further work is required to establish why platelets release their mitochondria into vesicles and the functional significance of this process to other cells.

#### P16: The Role of Vessel Stiffening in Vascular Calcification and Vesicle Release

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Vascular calcification is a detrimental pathology induced by vascular smooth muscle cell (VSMC) deregulation. Environmental stresses induce VSMC apoptosis, osteogenic differentiation, vesicle release and loss of mineralization inhibitors that promote extracellular matrix (ECM) mineralization. In the vessel wall, VSMCs are exposed to pathological mechanical stress such as stretch/strain and increased vessel stiffening in ageing, both of which are associated with increased calcification. Studies show that ECM stiffness determines mesenchymal stem cell differentiation via mechanosignaling. We hypothesized that mechanosignaling regulates VSMC calcification in response to matrix stiffness. Human VSMCs were cultured on polyacrylamide hydrogels to mimic physiological (12 KPa) and pathological vessel wall stiffness (24 KPa, 71 KPa and tissue culture plastic of 2 GPa) and treated with mineralization media to induce calcification. VSMCs grown on soft hydrogels were resistant to mineralization and osteogenic differentiation and expression of the mineralization inhibitor Matrix Gla Protein (MGP) increased by a 1.6-fold. VSMCs showed increased myosin light chain phosphorylation in response to increasing substrate stiffness indicating increased actomyosin activity. Treatment of VSMCs grown on plastic with a ROCK inhibitor significantly increased MGP expression and simultaneously reduced alkaline phosphatase expression. Moreover, VSMCs showed reduced vesicle release in response to increasing substrate stiffness under calcifying conditions but conversely showed increased vesicle release in response to mineralization treatment on each substrate. Preliminary data indicates that VSMCs grown on 12 KPa and their vesicles contain higher levels of fetuin-A compared to plastic. These data suggest that matrix stiffness influences vascular calcification via mechanosignaling potentially by regulating vesicle release and content.

#### P17: Characterisation of Macrophage Response to Laser Injury in the Larval Zebrafish Heart: Implications for Repair and Regeneration

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Heart regeneration occurs in many species and is dependent on immune cells. Macrophages exert both beneficial and detrimental effects during human heart repair, but therapeutic modulation of their phenotype remains untapped mainly due to a lack of understanding of their precise role and which phenotype(s) is most desirable for optimal recovery. Zebrafish have the remarkable ability to regenerate their hearts after injury therefore offering a potential roadmap of optimal macrophage phenotype during heart repair and regeneration.

We used a model of heart laser injury in the transparent zebrafish embryo to characterise macrophage phenotype after cardiac injury. Real-time optically gated light-sheet microscopy facilitated imaging of macrophages infiltrating the beating heart 0–48 h post-injury (hpi). Our in vivo imaging approach allowed direct examination of interactions between inflammatory and regenerative process in injured cardiac tissue.

Our laser injury induced a necrotic region of myocardium to form, surrounded by apoptotic cells. These wounds expand within the first 4 h, eventually reaching  $6.67 \pm 1.5\%$  ( $n = 8$ ) of total ventricular volume

but are then completely regenerated by 48 hpi. Regeneration occurs by proliferation and budding and bridging of cardiomyocytes from opposite wound borders. Coincident with the regenerative response, macrophages infiltrate the wound from 2 hpi and undergo a change in phenotype, becoming less motile, rounded and upregulating TNF  $2.95 \pm 1.7$ -fold ( $n = 14$ ).

During embryonic heart regeneration, macrophages undergo a phenotypic switch coincident with upregulation of TNF, suggesting that wound-associated macrophages transition to an inflammatory activation state. This zebrafish injury model replicates the apoptosis of peri-infarct cardiomyocytes observed in human myocardial infarction.

### P18: The Effects of TNF- $\alpha$ and IL-1 $\beta$ on Intracellular Calcium Waves in Sheep Ventricular Myocytes

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Cytokines including tumour necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-1 beta (IL-1 $\beta$ ) are known to mediate systolic and diastolic myocardial dysfunction in inflammatory disease such as sepsis. In severe cases, this myocardial depression leads to death. Previous work found that 50 ng/ml TNF- $\alpha$  and IL-1 $\beta$  reduced sarcoplasmic reticulum (SR) Ca content by 27% and 41%, respectively, accounting for a 17% and 24% reduction each in the amplitude of systolic Ca. In certain cells, the decrease in systolic Ca was preceded by an immediate potentiation lasting ~1–3 beats, suggesting altered ryanodine receptor (RyR) open probability. To investigate this further, in the present study, we measured the effect of 50 ng/ml TNF- $\alpha$  and IL-1 $\beta$  on the frequency and amplitude of Ca waves in sheep ventricular myocytes. All procedures used were in accord with the Animals (Scientific Procedures) Act, UK, 1986 and Directive 2010/63/EU of the European Parliament. Ventricular myocytes were isolated from young sheep then loaded with the ratiometric Ca indicator Fura-2. Waves were evoked using 0.3 mM ouabain and 5 mM extracellular Ca. Intracellular Ca and contractility dynamics were measured by epi-fluorescent photometry and video sarcomere detection, respectively. By 5 min, TNF- $\alpha$  and IL-1 $\beta$  reduced the Ca wave amplitude by 46% and 40%, respectively. Ca wave frequency was increased by 41% and 14%, respectively. That both cytokines reduced Ca wave amplitude but increased Ca wave frequency suggests they increase RyR open probability and this may account for the previously reported decrease of SR Ca content despite no change to SERCA activity.

### P19: High-Fat Diet-Induced Nox2 Activation and Endothelial Dysfunction in ApoE Knockout Mice

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Dietary obesity is implicated in the systemic oxidative stress and endothelial dysfunction. However, the role of Nox2 activation in response to metabolic disorders in the pathogenesis of vascular dysfunction and atherosclerosis remains unclear. In this study, we used littermates of ApoE knockout (KO) and ApoE/Nox2 double knockout (dKO) mice (C57Bl6/J background, male, 6 weeks,  $n = 9$ ) to examine the role of Nox2 in high-fat diet (45% kcal fat, 20% kcal protein and 35% kcal carbohydrate, for 10 weeks) induced endothelial dysfunction and aorta atherosclerotic lesion formation. Body weights were measured weekly and mice were used at 16 weeks of age. Compared to age-matched normal chow diet (NCD) mice, high-fat diet (HFD) ApoE KO mice had a significant increase in body weight ( $31.9 \pm 2.6$  to  $48.4 \pm 2.2$  g) and blood pressure ( $113 \pm 5$  to  $130 \pm 7$  mmHg). However, HFD ApoE/Nox2 dKO mice had no significant increase in body weight ( $29.1 \pm 0.8$  to  $29.5 \pm 0.7$  g) and the blood pressure was well maintained. The metabolic disorders seen in HFD ApoE KO mice were accompanied by significant increases in aorta

ROS production as measured by lucigenin chemiluminescence and DHE fluorescence, decreases in endothelium-dependent vessel relaxation to acetylcholine, reduced NO bioavailability and significant increases in atherosclerotic lesion in the aortas (all  $P < 0.05$ ). However, all these HFD-induced vessel abnormalities were reduced or absent in Nox2/ApoE dKO mice under the same HFD. In conclusion, Nox2-derived oxidative stress plays an important role in the pathogenesis of dietary obesity-associated metabolic disorders, endothelial dysfunction and atherosclerosis. Targeting Nox2-derived ROS represents a valuable therapeutic strategy to these diseases.

### P20: Human Cardiac Fibroblast-Secreted Exosomes Improve Efficiency of Human Cardiomyocyte Calcium Cycling

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**Rationale:** A key feature of healthy, adult human cardiomyocytes is efficient calcium-induced calcium release (CICR), mediating excitation-contraction coupling. This is poorly utilised in developing cardiomyocytes. Cardiomyocyte excitation-contraction coupling in a multicellular environment is poorly understood. Cardiac fibroblasts form extensive interactions with cardiomyocytes and regulate turnover of extracellular matrix proteins that bind to cardiomyocyte integrins, allowing dynamic interaction between cardiomyocyte extracellular environmental cues and intracellular events. Relatively unexplored is the role of fibroblast-secreted exosomes in modulating cardiomyocyte excitation-contraction coupling.

**Methodology:** Extracellular vesicles in human cardiac fibroblast-conditioned media were isolated by size exclusion chromatography and quantified by micro BCA. Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) (30,000 cells/dish) were incubated for 24 h with two conditions, (1) 20  $\mu$ g fibroblast-secreted exosomes or (2) fibroblasts and 20  $\mu$ M of a chemical inhibitor of exosomes release, GW4869, and compared to control. Optical mapping with Fluo4-AM visualised cardiomyocyte cytoplasmic calcium transients.

**Results:** HiPSC-CM treatment with fibroblast-secreted CD63-expressing exosomes abbreviated calcium transient time to peak vs fibroblast-naïve exosome-depleted media ( $p < 0.05$ ,  $-10.52 \pm 4.02\%$ ) and exosome-free control ( $p < 0.0001$ ,  $-26.6 \pm 3.95\%$ ). The abbreviation of hiPSC-CM calcium transient time to peak in fibroblast-cardiomyocyte co-culture ( $p < 0.001$ ,  $-101.8 \pm 9.257$  ms vs control) was attenuated by exosome inhibition ( $p < 0.05$ ,  $+33.49 \pm 2.847$  ms vs fibroblast co-culture).

**Conclusions:** Cardiac fibroblast-secreted exosomes induce calcium cycling in hiPSC-CMs that closely recapitulates that of the healthy, adult human cardiomyocytes. Further experiments must investigate the mediators of the effects. Overall, we show that fibroblast exosomes, at least in part, may play a role in the development of the adult cardiomyocyte phenotype induced by human cardiomyocyte-fibroblast co-culture.

### P21: Endogenous Circulating BMP9 Maintains Endothelial Cell Barrier Function

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**Rationale:** Bone morphogenetic protein 9 (BMP9) is a circulating vascular quiescence factor, signalling on vascular endothelial cells (ECs). Since recombinant BMP9 affords protection from endothelial permeability induced by inflammatory mediators, we questioned whether endogenous BMP9 plays a constitutive role in maintaining endothelial barrier function.

**Methods:** Wild-type mice were treated with BMP9 neutralising antibody to assess (1) rapid vascular leak by live imaging in mouse cremaster muscle and (2) sustained vascular leak by Evans Blue extravasation, neutrophil accumulation and histology in lung. Changes in endogenous BMP9 during endotoxemia were investigated by mRNA expression and circulating protein levels. Anti-BMP9 effect on VE-cadherin was assessed by immunostaining. BMP9 signalling on EC receptors were profiled by microarray. Effects of BMP9 administration were investigated in intranasal LPS lung injury model.

**Results:** Selective inhibition of circulating BMP9 led to a rapid increase in vascular permeability after 5 min. Prolonged effects of BMP9 inhibition include lung interstitial oedema and neutrophil extravasation. Circulating BMP9 levels were decreased markedly in murine endotoxemia, due to a temporary reduction in hepatic BMP9 mRNA expression coupled with the cleavage of BMP9 by plasma proteases. BMP9 neutralisation led to a rapid loss of endothelial VE-cadherin junction in ECs and BMP9 signalling in ECs effectively suppressed AQP1 and KDR expression. Pharmacological administration of BMP9 prevented the heightened vascular leak and acute lung injury induced by inhaled LPS.

**Conclusions:** Augmentation of BMP9 may be a useful therapeutic approach in conditions associated with microvascular leak such as sepsis and acute lung injury.

### **P22: Prelamin A Accumulation in Vascular Smooth Muscle Cells Accelerates Vascular Ageing**

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Prelamin A, the precursor of the nuclear lamina protein lamin A, has been identified as a biomarker of ageing in human vascular smooth muscle cells (VSMCs) *in vitro* and has also been detected *in vivo* in atherosclerotic and calcified arteries. This study sought to investigate how prelamin A accumulation *in vivo* affects VSMC phenotype and function and how this impacts vessel structure and function.

A smooth muscle cell (SMC)-specific *Zmpste24* knockout (KO) mouse model was generated to restrict the accumulation of prelamin A to SMCs only. A significant reduction in *Zmpste24* mRNA levels and consequent prelamin A accumulation was observed in the vasculature of KO mice which displayed reduced lifespan and weight loss prior to death at 6 months of age. Echocardiographic analysis revealed KO mice exhibited aortic root dilatation, reduced cardiac function and systolic hypertension with 60% also exhibiting aortic regurgitation (AR). Histological analysis of aortic sections revealed marked VSMC loss, extracellular matrix (ECM) deposition and extensive DNA damage accumulation—all hallmarks of vascular ageing. Further analysis using qRT-PCR revealed increased expression of senescence markers and senescence-associated

inflammatory cytokines in the aorta. Analysis of DNA microarray and ECM proteomics identified signalling pathways involved in vascular ageing such as oxidative stress, inflammation, DNA damage repair, and ECM remodelling. Our findings suggest this mouse model is an invaluable tool to identify and test novel therapeutic pathways involved in vascular ageing.

### **P23: Analysing the Role of Tyrosine Phosphorylation in Inflammasome Complex Formation and Function**

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The inflammasome is a multi-protein intracellular complex formed following detection of immune ‘danger’ signals that leads to the release of pro-inflammatory cytokines or activation of pyroptosis. Protein phosphorylation, a reversible post-translational modification of proteins that results in the addition or removal of phosphoryl group to a specific amino acid residue(s), is important in regulating cellular processes. Tyrosine phosphorylation has been shown to be essential in the regulation of the inflammasome and occurs at the level of the receptors (e.g. NLRP3, AIM2, NLRC4) and adaptor protein, apoptosis-associated Speck-like protein (ASC). In order to identify candidate tyrosine phosphatases involved in inflammasome function, the protein tyrosine phosphatase inhibitor, phenylarsine oxide (PAO), was used. Furthermore, site-directed mutagenesis of ASC was carried out to aid in the identification of putative tyrosine residues on ASC required for ASC function and subsequently inflammasome function. With these two approaches, we have shown that the nigericin-induced formation of ASC specks, processing and release of caspase-1 and IL-1 $\beta$  is perturbed by PAO in both human and murine cells. We have also shown that mutation of tyrosine to a non-phosphorylatable tyrosine mimic, phenylalanine, at residues 60, 137 and 146 of ASC results in attenuated IL-1 $\beta$  release. Taken together, we have shown that PAO is a potent inhibitor of the NLRP3 inflammasome suggesting an important role for tyrosine dephosphorylation in the activation of the inflammasome. Phosphorylation of ASC at Y60, Y137 and Y146 is required for inflammasome function.

### **P24: TNF $\alpha$ -Induced Inflammation Is Mediated by WNT/ $\beta$ -Catenin Signalling in Endothelial Cells: Implications for Atherosclerosis**

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Atherosclerosis leads to coronary artery disease and affects over 2.3 million individuals in the UK and is the main cause of death worldwide. Atherosclerosis is a chronic and inflammatory condition involving deposition of low-density lipoprotein and formation of a plaque within the arterial wall which can rupture and lead to thrombosis and blockage of the artery. Atherosclerosis involves endothelial dysfunction and associated inflammation. Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) is believed to play a major role in the initiation of endothelial dysfunction and inflammation. In this study, we determined whether TNF- $\alpha$ -induced inflammation was regulated by Wnt/ $\beta$ -catenin signalling. To examine this, HUVECs were treated with 10 ng/ml TNF- $\alpha$  for 24 h. Using qPCR, inflammatory gene expression was quantified. TNF- $\alpha$  significantly increased MCP-1 mRNA by 41  $\pm$  9-fold ( $p < 0.05$ ,  $n = 9$ ). ELISA confirmed that MCP-1 protein expression was significantly increased to 24,319  $\pm$  1207 pg/ml from 550  $\pm$  550 pg/ml ( $p < 0.05$ ,  $n = 4$ ). Interestingly TNF- $\alpha$  significantly reduced Axin-2 mRNA expression (a suppressor of Wnt/ $\beta$ -catenin signalling) by over 40% ( $p < 0.05$ ,  $n = 9$ ), suggesting that Wnt/ $\beta$ -catenin signalling was enhanced as a result of TNF- $\alpha$ . To test the involvement of Wnt/ $\beta$ -catenin signalling in TNF- $\alpha$  response, it was inhibited with

iCRT14 ( $\beta$ -catenin inhibitor) and ETC-1922159 (Wnt inhibitor) and agonised with Wnt3a and Wnt5a. Inhibition of Wnt/ $\beta$ -catenin signalling significantly attenuated the induction of MCP-1 protein by TNF- $\alpha$ , while recombinant Wnt3a and Wnt5a increased MCP-1 protein expression to  $1734 \pm 883$  and  $936 \pm 540$  pg/ml ( $p < 0.05$ ,  $n = 4$ ). In conclusion, TNF- $\alpha$ -induced MCP-1 expression is mediated at least in part by Wnt/ $\beta$ -catenin signalling and this may contribute to the inflammation of endothelial cells in atherosclerosis.

### **P25: Pro- and Anti-Inflammatory Macrophages Display Divergent Polarisation Towards Vascular Smooth Muscle-Like and Endothelial-Like Phenotypes**

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Current research has demonstrated that monocyte/macrophages can co-exist as diverse phenotypes which display an array of differing properties. The aim of this study is to robustly assess the ability of pro- and anti-inflammatory macrophages to polarise towards smooth muscle cell (SMC)-like and endothelial cell (EC)-like phenotypes. Human peripheral blood mononuclear cells were differentiated into pro- or anti-inflammatory macrophages through 7 days of culture with granulocyte-macrophage colony-stimulating factors (GM-CSF) or M-CSF, respectively. Utilising well-characterised SMC-related markers, pro-inflammatory macrophages demonstrated a significant up-regulation of mRNA and protein expression of caldesmon (twofold,  $p < 0.05$ ), calmodulin (threefold,  $p < 0.05$ ), smoothelin (twofold,  $p < 0.05$ ) and vimentin (fourfold,  $p < 0.05$ ), compared to stimulated anti-inflammatory macrophages, all of which were further augmented upon 3 days of PDGF $\beta$  and TGF $\beta$  co-stimulation. Interestingly, employing well-characterised endothelial cell-related markers, 3-day stimulation of pro-inflammatory macrophages with FGF2 or VEGFA induced a significant up-regulation of mRNA and protein expression for CD31 (PECAM1) (fivefold,  $p < 0.05$ ) and VWF (20-fold,  $p < 0.05$ ), compared to stimulated anti-inflammatory macrophages. Despite no change in CDH5 (VE-cadherin) mRNA expression, protein expression was significantly increased upon FGF2 stimulation (fourfold,  $p < 0.05$ ), suggesting a novel post-transcriptional regulatory mechanism.

These findings imply that blood-derived macrophages in culture can exhibit smooth muscle cell- and endothelial cell-like features, which are pre-dominant in pro-inflammatory macrophages, and accentuated by SMC or EC-related growth factors. As such, divergent macrophages as sites of vascular injury may harbour the potential to trans-differentiate into VSMC-like and EC-like cells to facilitate healing and stabilisation of vascular pathologies such as atherosclerotic plaques and aortic aneurysms.

### **P26: Remodelling After Myocardial Infarction Involves Changes in Inflammatory Signalling Pathways**

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Cardiac remodelling post-myocardial infarction involves complex cellular and molecular mechanisms. It is proposed that there is a link between excessive inflammatory response triggered by cardiac insult and adverse remodelling [1]. In this study, we used proteomic analysis to study signalling proteins mediating key inflammatory pathways following experimentally induced cardiac infarction.

All experimental procedures conform to the European Directives and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and approved by the University of Bristol ethics committee. Adult male Wistar rats (220–240 g) were used to surgically induced cardiac injury by left coronary artery (LAD) ligation as

described elsewhere [2]. In sham-operated animals ( $n = 5$ ), there was no coronary ligation. After 4 weeks, rats were killed, hearts extracted and briefly perfused in the Langendorff. Cardiac tissue from the apex was collected for protein extraction. Protein identification and quantification were performed using Tandem Mass Tagging (TMT) and LC-MS/MS at the University of Bristol Proteomics Facility. Level of significance was set to  $P$  value  $< 0.05$ . Fold change (LAD/SHAM) either  $< 0.8$  or  $> 1.3$  was considered. Four thousand seven hundred eighty-six accession numbers were identified, of which 275 were significant. Several proteins involved in inflammatory signalling were found to change in hearts after 4 weeks post-infarction (all increased post-infarction). These include protein kinase C (PKC) ( $P = 0.019$ , FC = 1.4), tyrosine-protein kinase (0.036, 1.238), serine/threonine-protein phosphatase 2B ( $\beta$  isoform) (0.019, 1.9), integrin subunit  $\alpha$ V (0.034, 1.456), fibronectin (0.037, 1.34) and mitogen-activated protein kinase kinase5 (MAP3K5) (0.023, 1.128). These preliminary data indicate that rat hearts undergoing post-ischemic cardiac remodelling exhibit significantly higher abundances of certain proteins involved in inflammatory signalling pathways.

### **P27: Myeloid TRIB1 Controls Metabolic Tissue Resident Macrophage Phenotype Associated with Immuno-Metabolic Diseases**

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Tribbles-1 (TRIB1) has been identified in GWAS to be associated with all major plasma lipid traits and is associated with myocardial infarction risk. Studies *in vivo* have shown hepatic expression of Trib1 reduces circulating lipids. Additionally, Trib1 is a regulator of alternative-macrophage polarisation. In this study, we hypothesised that TRIB1 regulates metabolic tissue-resident macrophage polarisation.

This study investigated the effects of Trib1 on Kupffer cells (KCs) and adipose tissue macrophages (ATMs) and its consequences on lipid homeostasis and metabolic inflammation. We developed myeloid-specific Trib1 knockout (Trib1 fl/fl x Lyz2Cre; mTrib1KO) and over-expressor mice (Rosa26Trib1.Tg x Lyz2Cre; mTrib1Tg) maintained on a chow diet. Plasma lipids were measured and macrophage phenotype was characterised in ATMs and KCs by IF and in bone marrow-derived macrophages (BMDMs) by qPCR. Additionally, ATMs were isolated and evaluated for phenotypic markers with qPCR. Furthermore, potential TRIB1-regulated cytokines were investigated by microarray on human MDMs. mTrib1KO mice had significantly elevated lipid levels ( $P < 0.05$ ). Loss of Trib1 promoted IRF-5 and reduced YM1 expression in KCs ( $P < 0.01$ ), ATMs ( $P < 0.01$ ) and BMDMs ( $P < 0.05$ ), while opposing changes were found in mTrib1Tg mice. Analyses of human MDMs showed up-regulation of pro-inflammatory cytokines that are implicated in fatty liver disease and adipocyte lipolysis. In agreement, we found increased hepatic TG in mTrib1KO mice ( $P < 0.01$ ) and reduced expression in Trib1Tg BMDMs ( $P < 0.05$ ).

Myeloid Trib1 is a potent regulator of tissue-resident macrophage phenotype *in vivo* at steady state; the loss of which promotes metabolic inflammation in the liver and adipose tissues, which may initiate the development of immuno-metabolic syndromes.

### P28: Reduced Pro-Angiogenic Function of Cord Blood Endothelial Colony-Forming Cells in Hyperglycaemia Is Mediated by NOX4

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Endothelial colony-forming cells (ECFCs) are an endothelial progenitor cell (EPC) subtype which can be isolated from umbilical cord blood (CB-ECFCs) and possess great therapeutic potential for ischaemic cardiovascular disease. Emerging evidence indicates that CB-ECFCs are sensitive to hyperglycaemia, e.g. gestational diabetes, and that their angiogenic function is regulated by NADPH oxidase (NOX)-derived reactive oxygen species (ROS). The aim of this study was therefore to investigate the specific influence of CB-ECFC NOX-dependent ROS signalling in experimental diabetes. CB-ECFCs were cultured in high glucose (HG, 25 mmol/L) or normal glucose (NG, 5.5 mmol/L) for 72 h. HG treatment increased CB-ECFC ROS generation (DHE fluorescence: NG 35.3 ± 1.9, HG 63.1 ± 2.2;  $n \geq 3$ ,  $P < 0.001$ ) whilst reducing tube formation (Matrigel assay) under both basal conditions (tube length: NG 11990 ± 530, HG 9631 ± 244;  $n = 3$ ,  $P < 0.05$ ) and after PMA (500 nmol/L) stimulation (tube length: NG+PMA 10,968 ± 125, HG+PMA 6349 ± 336;  $n \geq 5$ ,  $P < 0.01$ ). Furthermore, upregulation of antioxidant (e.g. HMOX1) and NOX4 mRNA (qRT-PCR) was observed in PMA-treated CB-ECFCs in NG, but this response was lost in HG, indicating a key role for NOX-derived ROS. Indeed, NOX4 overexpression (OE) rescued the pro-angiogenic response of CB-ECFCs to PMA in HG, as indicated by increased tubulogenesis versus empty vector (EV) control (tube length (all HG): EV+vehicle 8104 ± 136, EV+PMA 7454 ± 141, OE+vehicle 9435 ± 420, OE+PMA 12212 ± 718;  $n = 3$ ,  $P < 0.001$ ). Taken together, these data indicate that CB-ECFC dysfunction in hyperglycaemia is mediated by reduced NOX4 signalling, thus highlighting this major NOX isoform as a viable target to enhance the reparative capacity of CB-ECFCs in diabetes, thereby increasing their potential for treatment of ischaemic cardiovascular disease.

### P29: The Interaction Between Transforming Growth Factor- $\beta$ and the Canonical Wnt Pathway in Post-MI Cardiac Remodelling

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Adverse cardiac remodelling following myocardial infarction (MI) can result in cardiac dysfunction and eventually lead to heart failure. Fibrosis, characterised by excessive collagen deposition, is a key feature of this process and is known to be regulated by transforming growth factor- $\beta$  (TGF- $\beta$ ) and the canonical Wnt pathway. This study examined the involvement of the canonical Wnt pathway and TGF- $\beta$  in collagen regulation in human cardiac fibroblasts and whether TGF- $\beta$ -induced collagen production was mediated by canonical Wnt signalling. Treatment with recombinant Wnt3a significantly increased collagen protein levels (1.56 ± 0.15-fold,  $p < 0.05$ ,  $n = 4$ ), and inhibition of the canonical Wnt pathway with 25 nM iCRT14 ( $\beta$ -catenin inhibitor) significantly attenuated this effect (0.14 ± 0.07-fold,  $p < 0.05$ ,  $n = 4$ ). Collagen protein levels also significantly increased in response to TGF- $\beta$  (2.17 ± 0.17-fold,  $p < 0.05$ ,  $n = 3$ ) and this was significantly attenuated with iCRT14 (0.43 ± 0.13-fold,  $p < 0.05$ ,  $n = 3$ ), indicating involvement of  $\beta$ -catenin in TGF- $\beta$ -mediated collagen production. To understand whether TGF- $\beta$  regulates  $\beta$ -catenin via activation of the canonical Wnt pathway, the effects of TGF- $\beta$  on Wnt secretion and binding were investigated. Inhibition of Wnt secretion by ETC159 (porcupine inhibitor) had no effect on the increase in collagen in response to TGF- $\beta$ . Furthermore, phosphorylation/activation of the Wnt co-receptor, LRP6, did not significantly increase in response to TGF- $\beta$  treatment. In conclusion, this study provides evidence of the involvement of  $\beta$ -catenin in

TGF- $\beta$ -mediated fibrosis, but future studies are required to define the mechanism for this interaction. Interestingly, iCRT14 potentially attenuated collagen levels induced either by Wnt3a or TGF- $\beta$  and may provide a potential therapy against adverse cardiac remodelling post-MI.

### P30: Mechanosensitive Hox Genes Control Vascular Dysfunction in Adult Arteries

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Atherosclerosis is a chronic inflammatory disease marked by the hardening and thickening of the arteries. The disease develops predominantly at arterial branches and bends. These atheroprone areas are subjected to disturbed blood flow, which generates low and oscillatory wall shear stress (WSS), a frictional force directly exerted on endothelial cells (EC). Low WSS increases EC pro-inflammatory activation and proliferation. Here, we investigated the role of Homeobox (Hox) transcription factors, which are major regulators of morphogenesis during embryonic development, in EC dysfunction. Our analysis revealed that several Hox genes (HoxA1, A9, A10, B4, B7, B9, D8 and D9) are enriched at atheroprone areas due to enhanced expression in response to low WSS. We tested whether these genes influence EC dysfunction at atherosusceptible sites by silencing them in EC exposed to low WSS in vitro. Knock-down of HoxD8, HoxD9 and HoxB9 reduced expression of inflammatory markers, while knock-down of HoxB4 and HoxB7 significantly reduced EC proliferation. Silencing of HoxB9 reduced both proliferation and inflammation. The molecular mechanisms involve reduced activation of Gata4 and TNF expression and NF- $\kappa$ B pathway. By using a knock-out mouse model, we demonstrated that HoxB9 is a positive regulator of Gata4 expression and inflammatory molecules at atheroprone area in vivo. Finally, we showed that BMP signalling, which is induced at atheroprone areas, controls HoxB9 activation. In conclusion, our data reveal that an early developmental Hox signature is activated in adult EC and leads to EC dysfunction in response to low WSS at atheroprone areas.

### P31: The Role of Fractalkine in Cardiac Ischaemia-Reperfusion

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Background: Fractalkine and its receptor (CX3CR1) have been proposed to be key mediators of cardiac injury after myocardial infarction (MI). We have observed a marked drop in T-lymphocytes and CX3CR1+ non-classical monocytes in the circulation of MI patients following reperfusion, consistent with rapid recruitment of these cells to the site of injury. This project aims to investigate whether CX3CR1-expressing leukocytes are recruited early to the injured heart and whether fractalkine/CX3CR1 play a critical role in myocardial ischaemia-reperfusion injury (IRI) and repair.

Methods: We used a mouse model of cardiac IRI to study CX3CR1+ leukocyte infiltration following MI. Immunofluorescence staining, cardiac magnetic resonance imaging (MRI) and flow cytometry were used to study leukocyte recruitment and cardiac function.

Results: At 2 h post-reperfusion, there is reduced cardiac infiltration of leukocytes in heterozygous CX3CR1-EGFP ( $p = 0.02$ ) and CX3CR1 knockout (KO) mice ( $p = 0.003$ ) compared with wild-type (WT) controls. By 24 h post-reperfusion, leukocyte infiltration in heterozygous and KO mice returns to, and surpasses, WT levels. Cardiac MRI showed no difference between heterozygous and KO mice in the weeks following IRI. However, there is a trend towards worse outcomes compared to WT mice.

**Conclusion:** These findings suggest that CX3CR1 KO leads to delayed recruitment of leukocytes following cardiac IRI, which may result in an altered immune cell repertoire recruited to the injured tissue, and worse cardiac function in the longer term. We are continuing these studies to evaluate the role of CX3CR1 in cardiac IRI and explore the potential of fractalkine signalling as a therapeutic target in MI patients.

### P32: The Role of FAM176A in Endothelial Cell Responses to Flow

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Haemodynamic wall shear stress (WSS) exerted on the endothelium by flowing blood determines the spatial distribution of atherosclerotic lesions. Low/oscillatory WSS promotes atherosclerosis by regulating endothelial cell (EC) viability and function, while high WSS is athero-protective. We previously defined EC transcriptome at low/oscillatory and high WSS regions of the porcine aorta and identified ~60 putative regulators of apoptosis differentially expressed at the two regions. We hypothesised that these genes include key regulators of flow-regulated EC survival and performed their functional screening. One of the positive hits from our screening was FAM176A, a lysosome- and ER-associated protein.

We found that FAM176A is induced by low/oscillatory WSS in the mouse aorta and human ECs. FAM176A silencing using siRNA resulted in decreased EC apoptosis under low/oscillatory WSS. Additionally, we assessed the effect of FAM176A knockdown on EC proliferation and inflammation, another key processes underlying EC dysfunction. FAM176A silencing had no effect on EC proliferation, while it resulted in decreased expression of a number of inflammatory markers under low/oscillatory WSS.

FAM176A was described to play a role in autophagy. We tested the effect of FAM176A depletion on EC autophagy by analysing the expression of autophagy markers, LC3 and p62. We found that FAM176A knockdown had no effect on EC autophagy, suggesting that the observed function of FAM176A in ECs is autophagy-independent.

In conclusion, we identified FAM176A as a novel flow-sensitive gene which mediates the effects of low/oscillatory flow on EC apoptosis and inflammation; the underlying mechanism is the focus of our ongoing studies.

### P33: Mitochondrial Hyperactivity, Impaired Matrix Adhesion and Functional Activity in Endothelial Colony Forming Cells Isolated from Patients with Diabetic Foot Ulcers

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**Introduction:** Patients with diabetes have impaired endothelial colony forming cell (ECFC) function leading to poor vascular endothelial repair, potentially contributing to diabetic foot ulceration, a major health and economic burden. The objective was to characterise the functional deficit in these cells in order to understand the pathogenic mechanism and identify novel therapeutic targets.

**Methods:** ECFCs were isolated from the peripheral blood of controls and diabetic patients with neuroischaemic foot ulcers. Functional and biochemical analyses of the migratory, angiogenic and metabolic activity were performed. In addition, a neotissue array was used to compare ECFC binding and growth to different matrix ligands, including soft and fibrous matrices to represent distinct environments.

**Results:** Neuroischaemic ECFCs demonstrated impairments in colony formation, nitric oxide production (46% of control) and migratory response to SDF-1 (77% of control). Scratch closure and tube formation were also compromised (87% and 38%, respectively). Seahorse extracellular flux analysis identified a twofold increase in mitochondrial function and maximal oxygen consumption in neuroischaemic ECFCs. Furthermore, the neuroischaemic ECFCs bound less, and to a reduced repertoire of matrix peptides, in the neotissue array compared to controls, suggesting a diminished binding capacity.

**Discussion:** This study is the first to describe the defect in matrix adhesion and metabolic changes in neuroischaemic diabetic ECFCs, which may contribute to impaired endothelial repair in vivo. Further work to characterise the mechanism of this binding deficiency will support development of improved disease models in vitro, leading to identification of new therapeutic targets and stem cell therapies for wound healing.

### P34: Fibroblast-Specific Deletion of Interleukin-1 Receptor 1 (IL-1R1) Is Cardioprotective in an Experimental Model of Myocardial Infarction

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**Rationale:** Interleukin-1 (IL-1) plays an important role in driving inflammation and cardiac remodelling after myocardial infarction (MI), but the specific role of cardiac fibroblasts in this process is not known. We developed a mouse model in which the IL-1 receptor (IL-1R1) was inducibly deleted in fibroblasts and investigated the impact on cardiac function and remodelling post-MI.

**Methodology:** A tamoxifen-inducible fibroblast-specific IL-1R1 hemizygous knockout mouse line (Col1a2-CreER(T)-Il1r1 fl/-) was created and characterised. Cre-negative littermates served as controls. Cardiac fibroblasts from fibroblast-specific knockout mice exhibited a 75% reduction in Il1r1 mRNA expression and a similar reduction in IL-1 responses. Mice were injected with corn oil (vehicle) or tamoxifen (100 mg/kg/day) i.p. for five consecutive days and underwent experimental MI (permanent left anterior descending coronary artery ligation) at 10–12 weeks of age. Cardiac function was determined 4 weeks later by conductance pressure-volume catheter analysis. Molecular markers of remodelling were evaluated by real-time RT-PCR and histological staining.

**Results:** Control mice had significantly reduced ejection fraction 4 weeks after MI compared with sham animals (48% versus 62%;  $P=0.02$ ;  $n=11/12$ ). Importantly, ejection fraction was only partially reduced to 56% after MI in fibroblast-specific IL-1R1 knockout mice and was not significantly different to sham controls ( $P=0.45$ ;  $n=11/12$ ). IL-1R1 knockout mice showed reduced mRNA levels of remodelling genes (Col1a1, Col3a1, Mmp3, Tnc) after MI; differences were confirmed at the histological level.

**Conclusions:** Cardiac fibroblast IL-1R1 plays a key role in regulating post-MI remodelling. Inhibition of IL-1R1 signalling may therefore be beneficial post-MI.

### P35: Freestyle Fluidics—a Novel Platform to Study Macrophage Chemotaxis

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Chemotaxis is defined as directed migration of cells along a chemoattractant gradient. Chemotaxis plays an important role in atherosclerosis because it controls monocyte influx into arterial lesions and macrophage migration within plaques. Many widely used platforms to study chemotaxis have limited physiological relevance, no flexibility in experimental design or, in the case of most microfluidics devices, cause cytotoxicity (due to polydimethylsiloxane use) and are hard to reproduce. The aim of this study was to develop a highly reproducible chemotaxis platform that addressed these technical challenges.

Freestyle fluidics is a novel robotic device that can print fluid circuits in any shape using cell media. Overlaying with fluorocarbon oil FC40 stabilised the circuits whilst allowing the addition of new media. Our current chemotaxis protocol involves printing two chambers connected by seven channels. Bone marrow-derived macrophages (BMDMs) are plated in one chamber and the chemoattractant complement C5a at a final concentration of 10 nM is added to the other. A diffusion gradient is established and cells migrate towards the source of C5a. A protocol has been established which allows live cell imaging using IncuCyte Zoom and quantification of migration of viable BMDMs for up to 72 h towards the C5a chamber.

Freestyle fluidics provides a novel alternative to current chemotaxis systems, which allows multiplicity of designs and real-time imaging of cell migration under more physiological conditions with no cytotoxicity. We plan to extend our protocols to develop competition assays between different chemoattractants or different cell types by adding additional compartments to the circuits.

### **P36: Vulnerability of the Aged Heart to Ischaemia/Reperfusion Injury Before and After High-Fat Diet in Mice**

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The ageing heart is associated with metabolic, structural, and functional changes. Feeding rodents high-fat diet (HFD) is also known to induce structural and functional changes in the myocardium. The aim of this work is to compare the vulnerability of the ageing heart (to the adult heart) to cardiac insults including ischaemia and reperfusion (I/R) and whether this vulnerability changes after high-fat feeding. C57BL/6 adult (8 weeks), middle-aged (50 weeks), and aged (80 weeks) male mice were used in this work. For the HFD, aged mice were fed for a period of 20–24 weeks (starting at 60 weeks old). The animals were humanely killed using the Schedule 1 procedure: stunning followed by cervical dislocation and the hearts were excised, rinsed in ice-cold buffer, and perfused (Langendorff perfusion). Vulnerability to I/R injury was not affected in neither middle-aged nor aged hearts when compared to adults. Hearts from all three age groups showed similar degrees of functional impairment, creatine kinase (CK) release, and infarction by the end of reperfusion. Feeding aged mice a HFD was associated with significant weight gain but no change in heart weight. Hearts from HFD mice had better functional recovery early after reperfusion compared to age-matched mice. However, there was no difference in final degree of necrosis at the end of reperfusion. In conclusion, regardless of ageing being associated with changes in the heart, these changes do not render the heart more vulnerable to I/R injury, neither before nor after high fat feeding.

### **P37: Porcine Blood Outgrowth Cells (BOCs) for Tissue Engineering of Decellularised Human Saphenous Vein Grafts**

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**Introduction:** Autologous saphenous vein (SV) graft failure following coronary artery bypass grafting occurs in 10–20% of patients within the first year

and ~50% remain patent after 10 years. Tissue engineering strategies have the potential to improve graft patency. We propose that seeding decellularised SV with blood outgrowth cells (BOCs) may yield an improved conduit.

**Methods:** Peripheral blood mononuclear cells were isolated from porcine blood ( $n = 2$ ) using density centrifugation and cultured in collagen-coated plates in endothelial cell growth media MV2. Non-adherent cells were removed after 24 h, and adherent cells were cultured following standard culture protocols until BOC colonies emerged.

Human SV (hSV) segments were decellularised in 0.01% SDS and the acellular scaffolds seeded with BOCs (P2 or P4,  $\sim 1 \times 10^6$  cells/segment, either inserted into the lumen or by bathing in cells) and placed under static or dynamic culture conditions in EGM-2 media for 72 h, followed by static conditions for 96 h.

Seeded hSV were stained with H&E, EVG, and picrosirius red (collagen). Endothelial (CD31 and DBA-Lectin) and fibroblast (vimentin) markers were detected by immunohistochemistry.

**Results:** Adherence of BOCs to the lumen of hSV was observed after initial seeding and prolonged culture, with no differences between seeding conditions. Adhered cells were DBA-positive, had minimal CD31 expression, strong vimentin expression, and deposited collagen.

**Discussion:** dhSV can be seeded with porcine blood-derived cells which may be beneficial in the clinical setting. Surprisingly, despite following human BOEC protocols, we derived fibroblast-like cells that deposit collagen, which may be used for increasing mechanical strength of vascular grafts.

### **P38: Endothelial STAT5A Is Enriched at Atheroprone Regions and Drives Inflammation in Response to Low Shear Stress**

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**Background:** 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, survival and inflammatory activation, yet its role in atherosclerosis is less well known. Atherosclerosis is an inflammatory disease that develops at bends and branches of arteries, exposed to disturbed blood flow which generates low wall shear stress (WSS). These haemodynamic conditions induce inflammatory activation of endothelial cells (ECs).

**Methods:** Microarrays, qRT-PCR, immunostaining and en face staining were used to quantify stat5a expression in EC at low WSS (atheroprone) and high WSS (atheroprotected) regions of the porcine and in murine aorta and in human coronary artery endothelial cells (HCAECs). Stat5a was silenced using retroviral-mediated shRNA and inflammatory activation was studied by qPCR analysis.

**Results:** Stat5a mRNA expression was enriched at low WSS regions of the porcine aorta, and likewise, murine stat5a was higher at low compared to high WSS regions of the mouse aorta. Immunostaining revealed that Stat5a protein is nuclear under low WSS conditions, while its localisation was predominately cytoplasmic under high WSS, thus indicating that low WSS activates STAT5. Silencing of Stat5a in HCAEC significantly reduced inflammatory gene expression, indicating a role for STAT5 in inflammatory activation of endothelial cells exposed to low WSS.

**Conclusions:** Stat5a is preferentially upregulated at low WSS atheroprone regions and its silencing reduces inflammation. These data therefore suggest that Stat5a may regulate the focal nature of atherogenesis by promoting inflammation.

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