

Conference Report

Abstracts of the 2021 Autumn Meeting of the British Society for Cardiovascular Research [†]

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[†] Presented at the 2021 Autumn Meeting of the British Society for Cardiovascular Research, Online, 6–7 September 2021.

Abstract: The Autumn Meeting of the British Society for Cardiovascular Research in 2021 was a virtual meeting organised by Andrew Bond, Anita Thomas, Elisa Avolio, Michele Carrabba, Raimondo Ascione, and Paolo Madeddu of the Bristol Medical School. The theme of the meeting was ‘Tissue engineering and regenerative medicine in cardiovascular disease’ and included an early career symposium. The Annual Bernard and Joan Marshall Distinguished Investigator Lecture was given by Professor Toshiharu Shinoka. This paper presents the abstracts selected for oral and poster presentations.

Keywords: tissue engineering; regenerative medicine; cardiovascular disease

1. Selected Oral Abstracts

1.1. Modulation of the Hippo Pathway via the Serotonin Receptor 2B (5HT2B) to Induce Cardiac Regeneration

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Background: Due to the limited regenerative capacity of the adult mammalian heart, survivors of myocardial infarction (MI) undergo adverse cardiac remodelling, leading to irreversible heart failure. The Hippo pathway has emerged as a key endogenous regulator of cell proliferation and survival in cardiomyocytes. Through a candidate gene screening, we have identified serotonin receptor 2B (5HT2B) as a potential modulator of the Hippo pathway.

Purpose: To investigate whether overexpression of the 5HT2B receptor promotes cardiac regeneration.

Methods and Results: Adenovirus-mediated overexpression of 5HT2B in neonatal rat cardiomyocytes significantly enhanced YAP activity (the major Hippo effector) by inhibiting the LATS1 kinase, which was associated with increased cardiomyocyte proliferation in basal conditions and decreased apoptosis following oxidative stress. Conversely, transgenic mice overexpressing 5HT2B in cardiomyocytes had a lower survival rate (46.2%) 4 weeks post-MI compared to wild-type littermates (90%). Echocardiography and histological analysis of each genotype thus far cannot account for this survival disadvantage, and we are conducting 2 day MI experiments to address this. Furthermore, to investigate whether short-term overexpression of 5HT2B is beneficial post-MI where long-term overexpression is not, we have synthesised and validated modified mRNA transcripts coding for the 5HT2B protein or control luciferase, which are transiently translated when injected into the mouse heart.



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Conclusions: 5HT2B is a novel and potent regulator of the Hippo pathway that improves proliferation and survival of cardiomyocytes in vitro, while chronic overexpression of 5HT2B is deleterious following MI. Work is ongoing to determine if transient overexpression of 5HT2B proves to be more suitable in promoting regeneration.

1.2. Regulation of Vascular Smooth Muscle Cell Phenotype by Retinoic Acid: Implications for Abdominal Aortic Aneurysms

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Background: Abdominal aortic aneurysms (AAA) are a dilatation of the aorta. They cause over 5000 deaths per year in the UK, but there is currently no treatment for developing aneurysms. In AAAs, vascular smooth muscle cells (VSMCs) undergo a phenotypic switch from a contractile phenotype, in which there are high levels of contractile proteins such as alpha smooth muscle actin (α -SMA) and calponin, into a synthetic state which is more proliferative and migratory. We hypothesise that a derivative of vitamin A, all-trans retinoic acid (RA), induces a contractile phenotype in human aortic smooth muscle cells (HAoSMCs), reducing the progression of AAA.

Methods: HAoSMCs ($n = 4/5$) were treated with platelet-derived growth factor-BB (PDGF-BB; 20 ng/mL) and RA (0.1 μ M) for 48 h. Western blotting and immunocytochemistry were used to detect contractile markers calponin and α -SMA to determine HAoSMCs phenotype.

Results: PDGF-BB significantly decreased the amounts of contractile proteins, α -SMA and calponin, in HAoSMCs to $69 \pm 7\%$ and $32 \pm 7\%$ of control, respectively, indicative of de-differentiation to a synthetic phenotype. Treatment with RA retarded the PDGF-induced loss of contractile proteins; α -SMA and calponin protein levels increased to $93 \pm 6\%$ and $67 \pm 11\%$ of the control, respectively. Matrix metalloproteinase 2 (MMP-2) is elevated in AAA and is associated with the synthetic phenotype. RA treatment significantly suppressed PDGF-induced increase in MMP-2 by $38 \pm 12\%$ compared to PDGF-BB treated cells.

Conclusion: RA suppressed PDGF-BB-induced synthetic phenotype, maintaining cells in a contractile phenotype and decreased MMP-2, which may be beneficial for the treatment of AAA.

2. Selected Poster Abstracts

2.1. Engineered Pulmonary Artery Tissues (EPATs)—A Novel Technique to Assess Vascular Contractility In Vitro

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Background/Introduction: Conventional monolayer culture of vascular smooth muscle cells suppresses their contractile phenotype, a property crucial to cardiovascular function and disease. Therefore, we developed a novel in vitro three-dimensional culture platform using hydrogels containing human pulmonary artery smooth muscle cells—'engineered pulmonary artery tissues' (EPATs).

Purpose: To allow for rapid and direct measurement of vasoreactivity in vitro.

Methods: EPATs were produced using custom-made racks fabricated from polydimethylsiloxane (PDMS) using a 3D-printed resin mould. Primary human pulmonary artery smooth muscle cells were suspended with fibrinogen and seeded between pairs of posts hanging from the PDMS racks. Three-dimensional constructs were formed after fibrin polymerisation by thrombin. Auxotonic stretch exerted by the PDMS posts is designed to re-establish contractility within smooth muscle cells. This method was adapted from a published protocol for 'engineered heart tissues'.

Results: EPATs demonstrated a gradual reduction in length (i.e., flexion of the PDMS posts) over 3–4 weeks, indicative of enhanced smooth muscle cell contraction. Crucially, EPATs also responded to vasoactive drugs when viewed using time-lapse video microscopy. We observed modest but consistent changes in length (3–10%), both with constriction following administration of potassium chloride (75 mM) and dilation in the presence of nifedipine (100 nM) and sodium nitroprusside (50 μ M). Changes occurred steadily with a peak response after approximately 10 min.

Conclusion(s): EPATs may be suitable for testing contractile responses to novel drug therapies for cardiovascular diseases, such as pulmonary arterial hypertension.

2.2. The Novel RNA Binding Protein ANKHD1 Is Required for Vascular Health via Stabilisation of eNOS and PTGIS

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Background: Atherosclerosis is a lipid-driven inflammatory disease in arterial bends and branches. This has been attributed to low-shear stress (LSS) exerted on the endothelium at those sites. In contrast, regions exposed to high-shear stress (HSS) are atheroprotected. HSS enhances the atheroprotective endothelial NO synthase (eNOS) and prostacyclin synthase (PTGIS). Ankyrin repeat single KH domain-1 (ANKHD1) is an RNA-binding protein. The potential role of endothelial ANKHD1 has not been studied.

Methods: Primary human ECs (human umbilical vein ECs [HUVECs] and human coronary artery ECs [HCAECs]) were stimulated with HSS (~ 11 dyne/cm²) or LSS (~ 4 dyne/cm²). ANKHD1 was quantified via RT-qPCR and immunoblotting. The effect of ANKHD1 on eNOS and PTGIS was studied using ANKHD1-siRNAs. ANKHD1 function was examined in Ankh1-KO versus wild-type mouse aortas by assessing aortic diameter and en-face staining of the endothelium with eNOS or PTGIS antibodies. The molecular mechanisms whereby ANKHD1 controls eNOS and PTGIS were studied in HUVECs using RNA immunoprecipitation (RIP) assay and Actinomycin-D.

Results: ANKHD1 was expressed at higher levels at HSS compared to LSS regions in Ankh1-wild-type mice ($p = 0.0446$, $N = 4$), in HUVECs ($p = 0.008086$, $N = 4$), and in HCAECs ($p = 0.0331$, $N = 3$). Ankh1 influenced aortic diameter (Ankh1-KO: 0.8434 μ m; Ankh1-WT: 0.9502 μ m; 20-weeks-old; $n = 7-8$, $p = 0.0371$). eNOS and PTGIS expression were lower in Ankh1-KO compared to Ankh1-WT (eNOS: $n = 6-8$, $p = 0.0397$; PTGIS: $n = 5-11$, $p = 0.005$) and in primary human ECs with different ANKHD1siRNAs. ANKHD1 controls eNOS and PTGIS stability possibly via direct binding to their mRNAs.

Conclusion: ANKHD1 is induced by HSS and linked to vascular function through enhancing eNOS and PTGIS stability.

2.3. Changes in Vascular Smooth Muscle Cell Phenotype Regulate Extracellular Matrix Stiffness and Transmission of Mechanosensory Signalling

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Background: Aortic stiffening occurs due to the changes in vascular smooth muscle cell (VSMC) phenotype and the integrity of their extracellular matrix (ECM). VSMCs modulate gene expression to adapt to the ECM by transmitting mechanosensory information from the cytoskeleton to the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. The LINC consists of Nesprin1/2, which bind the cytoskeleton through their CH- domains and transmit mechanosignals via their KASH domain to the SUN1/2 proteins, which bind the Lamin proteins. These then alter the gene expression by making direct contact with the chromatin.

Aim: Investigate the changes occurring in the ECM and LINC Complex with VSMC ageing and examine the mechanosensitivity of the LINC Complex.

Methods: The stiffness of synthesised ECM from young and senescent VSMCs was measured via atomic force microscopy. VSMCs were plated on hydrogels of varying stiffness, and changes in the LINC Complex components were analysed on a gene and protein level.

Results: VSMC senescence results in stiffening of the ECM. Senescent VSMCs upregulate expression of the Nesprin1/2-KASH domain and downregulate expression of the Nesprin 1/2 CH-domain. Both SUN1/2 proteins are also downregulated. Lamin A/C and Nesprin1-KASH consistently exhibit strong mechanosensitivity, their expression scaling with matrix stiffness.

Conclusions: The ageing of VSMCs results in significant alterations to the LINC Complex and an increased matrix stiffness, which independently drives the upregulation of Nesprin1-KASH, thus further disrupting the LINC function and downstream mechanosignalling.

2.4. *FAM176A Is a Novel Shear-Sensitive Driver of Endothelial Cell Dysfunction*

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Background/Introduction: 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 differentially expressed putative regulators of apoptosis.

Purpose: We hypothesised that these genes include key regulators of flow-regulated EC survival and performed their functional screening in zebrafish embryos and human ECs. FAM176A, a lysosome and ER-associated protein linked to autophagy, was found to promote EC apoptosis in response to flow. Our aim was to elucidate the role of FAM176A in flow-regulated EC dysfunction.

Methods: FAM176A siRNA-transfected and control ECs were exposed to athero-prone and athero-protective WSS using the orbital shaker model. The effect of FAM176A knockdown on EC dysfunction was assessed using immunofluorescence, qPCR, a transwell permeability assay, and Western Blotting. Transcriptional changes induced by FAM176A silencing were studied using RNA sequencing.

Results: FAM176A was induced by athero-prone WSS in the mouse aorta and human ECs. In vitro, FAM176A silencing resulted in decreased EC apoptosis, inflammation, and permeability under athero-prone WSS, while it had no effect on EC proliferation. Autophagy was not affected by FAM176A knockdown, as demonstrated by unchanged expression of LC3 and p62. RNA sequencing indicated the AP-1 pathway in acting downstream from FAM176A.

Conclusion: In conclusion, we identified FAM176A as a novel flow-sensitive gene which mediates the effects of athero-prone WSS on EC dysfunction.

2.5. *Development of a New microRNA-Functionalized Composite Scaffold for Valve Regeneration*

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Currently, employed valve prostheses have limited durability and bear the risk of thromboembolism and bleeding. Tissue engineering aims to generate a native-like valve using exogenous material to guide self-regeneration. A current challenge is the formation of a complete endothelial lining in the shortest possible time to avoid complications deriving

from the interaction between blood and exogenous surfaces, like thromboembolism and prosthesis calcification. MicroRNA is particularly appealing for regenerative medicine since it is able to deeply influence cell processes, restoring cells' normal metabolism once it is degraded. MicroRNA-132-3p helps valve endothelial cells (ECs) to resist calcification, enhances angiogenesis, and stimulates ECs proliferation and migration. This project aims to exploit the microRNA potential for tissue regeneration. The idea was to integrate the microRNA-132-3p (bounded to a suitable vector) in a biodegradable scaffold to guide in situ valve regeneration. It was demonstrated that microRNA-132-3p increments the ECs proliferation rate by 30%. A composite scaffold was designed with a coating that transfects cells with microRNA during its degradation—around 18 days. The valve function is supported by an inner polymeric layer that degrades in a couple of years, ensuring a time to let the newly generated tissue mature. At the current stage, experiments on the microRNA-functionalized scaffold aim to verify the capability of the microRNA integration to enhance the scaffold colonisation speed and quality using aortic ECs. A regenerative platform able to locally administer different microRNAs would allow for medical research and clinical practices to address the problematic aspects of the valve regeneration process.

2.6. Use of Perfusion PET/CT Imaging to Assess Cardiac Function and Repair in Murine Diabetic Cardiac Models and Subsequent Refinement and Validation of Predictive In Silico Cardiac Models

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** Project funder: Kuwait University

Background: Mice models are used widely to study human cardiovascular system diseases and various conditions effect such as ageing. The quantification of Myocardial Blood Flow (MBF) and glucose metabolism in aged myocardium are vital markers which can be used to assess certain treatment trials or therapeutic interventions. Indeed, measuring MBF in small animals is a challenge because it requires high spatial resolution modality as well as great technical considerations that should be taken into account, such as tail vein cannulation and the effect of anaesthesia. This study aimed to use a micro-PET/CT scanner to quantify MBF and subsequently assess the validity of longevity gene therapy (LAV) in the treated (LAV) group and compare it to three controls groups: No virus group (NV), Wild-type group (WT), and Green Fluorescent Protein marker group (GFP).

Methods: A 1 day scanning protocol was developed to measure MBF in rest and under stress conditions following pharmacological stress administration with the animal being under terminal anaesthesia. Following termination, the heart and other organs were collected for longitudinal studies and further histological analyses to assess the correlation between different imaging modalities.

Findings: MBF and cardiomyocyte function was improved significantly in the treated LAV group.

Conclusion: The quantification of MBF in mouse hearts was successfully assessed through micro-PET/CT scanning using dynamic ¹³N-ammonia radiotracer and a one-compartment tracer kinetic model applied in PMOD as medical analysis software. The data produced promising results in using the LAV gene to improve blood flow and cardiac function in ageing mice, which can be translated to later human trials.

Key words: Myocardial Blood Flow (MBF), PET/CT imaging, Longevity gene therapy (LAV), Pharmacokinetic modelling, PMOD medical analysis software.

2.7. Understanding the Role of Wnt Signalling in Abdominal Aortic Aneurysms

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Introduction: Abdominal Aortic Aneurysms (AAAs) are the tenth leading cause of death in the UK, with no medical treatment available except surgery. The Wnt pathway may play an important role in AAA pathogenesis, but its involvement is undefined.

Aim: To determine the expression of the Wnt pathway in human AAAs and identify its potential as a target for retarding AAA development.

Methods: Human AAAs were compared to healthy aortic tissues using immunohistochemistry and image analysis ($n = 12$). ETC-159 (an anti-cancer therapy in phase I trials) was investigated as a potential inhibitor of AAAs.

Results: When comparing AAA tissues to healthy aortas, the activity of the Wnt co-receptor (pLRP6) was increased ($1.10 \pm 0.004\%$ vs. $0.09 \pm 0.0004\%$, $p < 0.05$). AAAs also had a higher level of the downstream effector of pLRP (β -catenin: $7.24 \pm 0.02\%$ vs. $1.72 \pm 0.004\%$, $p < 0.05$). Furthermore, significantly higher levels of active β -catenin were detected in AAAs compared to healthy aortas ($1.43 \pm 0.006\%$ vs. $0.09 \pm 0.0004\%$, $p < 0.05$). NOTUM, a target gene of Wnt/ β -catenin signalling, was also detected at higher levels in AAAs than in healthy tissues ($32.32 \pm 0.07\%$ vs. $12.69 \pm 0.02\%$, $p < 0.05$). Porcupine, which is essential for the exocytosis of all Wnts, was present at significantly higher levels in AAAs than healthy aortas ($7.14 \pm 0.02\%$ vs. $0.82 \pm 0.002\%$, $p < 0.05$) and was expressed by macrophages and smooth muscle cells. Consequently, it is a potential target for Wnt/ β -catenin inhibition. We validated the porcupine inhibitor ETC-159 and found that NOTUM was significantly decreased both *in vitro* ($21.0 \pm 0.12\%$, $n = 5$, $p < 0.05$) and *in vivo* ($69.4 \pm 0.07\%$, $n = 3-4$, $p < 0.05$) models. Ongoing experiments are assessing whether ETC-159 can retard AAA formation.

Conclusion: Increased Wnt/ β -catenin pathway activity occurs in human AAAs. ETC-159 is an effective inhibitor of the Wnt pathway and may be beneficial for impeding AAA development in the future.

2.8. Microtubule Stabilisation Protects against Vascular Smooth Muscle Dysfunction in Response to Matrix Stiffness

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Background: Aortic stiffening decreases arterial compliance, the ability of blood vessels to modulate their shape in response to changing blood pressure. Decreased compliance is a major risk factor for the development of cardiovascular disease. Age-related arterial stiffening occurs when elastin, an elastic extracellular matrix component of the vascular wall, is degraded and replaced by collagen I, thereby enhancing the tensile strength and rigidity of the aortic wall. Mechanosensitive vascular smooth muscle cells (VSMCs) are the principal cell type of the aortic wall. VSMCs respond to increased matrix stiffness by generating enhanced actomyosin forces that contract the aorta and further decrease compliance. Increased actomyosin force generation strains VSMCs, leading them to accrue DNA damage, promoting VSMC dysfunction and dedifferentiation.

Purpose: Remodelin, a NAT10 acetyltransferase inhibitor, has been shown to increase the functional lifespan of VSMCs by preventing age-dependent DNA damage accumulation. We hypothesise that remodelin will protect against matrix-stiffness-induced DNA damage accumulation and maintain the contractile VSMC phenotype.

Methods: Human aortic VSMCs were seeded onto polyacrylamide hydrogels, mimicking physiological (12 kPa) and pathological (72 kPa) aortic stiffnesses. Cells were pre-treated with pharmacological compounds before being stimulated with the contractile agonist, Angiotensin-II.

Results: Remodelin pre-treatment blocked morphological changes and DNA damage accumulation in VSMCs grown on hydrogels of pathological stiffness. We show that the protective action of remodelin arises through increased stabilisation of the microtubule cytoskeleton and confirm this mechanism using the clinically approved microtubule stabiliser, Taxol.

Conclusions: We identify a novel means of pharmacologically restoring VSMC function that displays specificity to VSMCs adapted to pathological matrix rigidities.

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