**PROGRAMME FOR MONDAY 15th JUNE 2020 AT 10:00AM (UK)**

Join by Zoom from 9.45am onwards. To register for this free meeting: [https://docs.google.com/document/d/1Alvqb751cgX8Ey9GXmxCe9LWx5zywyByf7op9DJ_w/edit](https://docs.google.com/document/d/1Alvqb751cgX8Ey9GXmxCe9LWx5zywyByf7op9DJ_w/edit)

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:00 – 10.05</td>
<td>Welcome Address</td>
<td>Neena Kalia &amp; Dean Kavanagh – Organisers</td>
<td></td>
</tr>
<tr>
<td>10:00 – 11.05</td>
<td>Scientific Session 1: Chairs</td>
<td>David Bates &amp; Neena Kalia</td>
<td></td>
</tr>
<tr>
<td>10:05 – 10:20</td>
<td>Selected Oral Communications</td>
<td>Eva Clavane – University of Leeds</td>
<td>INVESTIGATING THE ROLE OF BACE1 IN ANGIoGENESIS</td>
</tr>
<tr>
<td>10:20 – 10:35</td>
<td></td>
<td>Lillian Wallis – University of Oxford</td>
<td>INVOLVEMENT OF TRP CHANNELS IN ENDOCARDIAL-MYOCARDIAL INTERACTION</td>
</tr>
<tr>
<td>10:35 – 10:50</td>
<td></td>
<td>Monica Gamez – University of Bristol</td>
<td>ENDOThelial GLYCOCALYX HEPARAN SULPHATE PLAYS A KEY ROLE IN GLOMERULAR FILTRATION BARRIER FUNCTION IN HEALTH AND IS AMENABLE TO THERAPEUTIC TARGETING IN DIABETES</td>
</tr>
<tr>
<td>10:50 – 11:05</td>
<td></td>
<td>Sohni Bhalla – University of Nottingham</td>
<td>IMPAIRED REVASCULARISATION AFTER ISCHAEMIA IN MONOCYTIC- WNT5A IS SRPK1 DEPENDENT</td>
</tr>
<tr>
<td>11:05 – 11:35</td>
<td>Scientific Session 2: Chair</td>
<td>Tim Millar</td>
<td></td>
</tr>
<tr>
<td>11:05 – 11:35</td>
<td>Keynote Speaker</td>
<td>Dr Andries van der Meer</td>
<td>ORGAN-ON-A-CHIP DEVICES IN VASCULAR RESEARCH</td>
</tr>
</tbody>
</table>

**British Microcirculation Society Mini-Meeting**

MONDAY 15th JUNE 2020 @ 10:00am

- 2.5 hour interactive online meeting.
- Opportunity to learn about microcirculation research and engage with peers.
Scientific Session 3: Chairs – Jacqueline Whatmore & Dean Kavanagh

Selected Oral Communications

<table>
<thead>
<tr>
<th>Session</th>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC5</td>
<td>11:35 – 11:50</td>
<td>Juma El-Awaisi – University of Birmingham</td>
<td>Investigating a novel role for the IL-36 / IL-36R pathway in mediating inflammation in the adult and aged heart after ischaemia-reperfusion injury</td>
</tr>
<tr>
<td>OC6</td>
<td>11:50 – 12:05</td>
<td>Valeria Mastrullo – University of Surrey</td>
<td>The vasculature circadian clock affects endothelial cell/pericyte interaction</td>
</tr>
<tr>
<td>OC7</td>
<td>12:05 – 12:20</td>
<td>Tamara McErlain – Queen’s Uni. Belfast</td>
<td>Neurovascular coupling in the retina: Do retinal astrocytes sense vascular stretch?</td>
</tr>
<tr>
<td>OC8</td>
<td>12:20 – 12:35</td>
<td>Regis Joulia – William Harvey, London</td>
<td>Microvascular leakage disrupts the localisation of chemotactic cues and neutrophil diapedesis in vivo</td>
</tr>
</tbody>
</table>

END

Presentation of 2 best oral communication prizes, one in each session, sponsored by the BMS and Moor Instruments

Announcement of BMS 2021
Meeting Closes

http://www.microcirculation.org.uk/
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#BMS2020
OC1: Investigating the Role of BACE1 in Angiogenesis

Eva Clavane¹, Jane Brown¹, Melanie Taylor¹, Colin Murdoch², Paul Meakin¹

¹Discovery & Translational Science Department, Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, LS2 9DA, UK; ²Systems Medicine, School of Medicine, University of Dundee, DD1 9SY, UK.

umemc@leeds.ac.uk ; P.J.Meakin@leeds.ac.uk

Background: β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) is a transmembrane aspartyl protease notorious for its contribution to the pathophysiology of Alzheimer’s disease (AD). Noticeably, BACE1 cleaves receptors, VEGFR1 (Cai et al., 2012) and insulin receptor (Meakin et al., 2018), both involved in angiogenesis. Also, BACE1 is elevated in diseases displaying aberrant angiogenesis, including type 2 diabetes mellitus (T2DM) (Meakin et al., 2018). These findings suggest BACE1 downregulates angiogenesis. Here, we hypothesise that BACE1 cleaves Flt-1 to a soluble, high affinity, decoy receptor (sFlt-1) which in turn reduces VEGF bioavailability, preventing binding to VEGFR2 to elicit an angiogenic response.

Methods: To investigate the anti-angiogenic role of BACE1, we performed an in vitro angiogenic bead assay with human umbilical vein endothelial cells (HUVECs) treated with and without potent BACE1 inhibitor, M-3 (Nakatsu, 2007).

Results: BACE1 inhibition increased sprouting by 17.1% ± 3.4 (P<0.05). To determine whether BACE1 inhibition enhances VEGF signalling, we stimulated HUVECs and primary lung endothelial cells (PECs) with 2ng/mL or 20ng/mL VEGF (30 mins) and quantified the levels of phosphorylated eNOS, downstream signalling protein of VEGFR2, using Western blots. BACE1 inhibition increased phosphorylation of eNOS at Serine 1177 in HUVECs and PECs (51% ± 0.14, P<0.001). In support of our hypothesis, M-3-treated HUVECs had 3-fold increased levels of Flt-1 compared to untreated cells. Likewise, mice deficient of BACE1 had decreased levels of sFlt-1 in their blood serum compared to wild type mice (-87.35% P<0.05).

Conclusion: These findings suggest that BACE1 inhibitors, previously trialled to treat AD, could be repurposed to improve microvascular complications in individuals with T2DM.

Eva Clavane is currently a Masters student who will continue the above project as a BHF funded PhD with Dr Paul Meakin in Leeds.
OC2: The Involvement of TRP Channels in Endocardial-Myocardial Interaction

Lillian Eleanor Wallis, Lludmyla Borosova, Christopher J. Garland, Kim A. Dora
Department of Pharmacology, University of Oxford, Oxford, OX1 3QT
Lillianw@pharm.ox.ac.uk; kim.dora@pharm.ox.ac.uk

Background: Like the vascular endothelium, the intracardiac endothelium has been shown to modulate both the frequency and force of heart contraction through its release of various substances including ET-1, NO and PGI\textsubscript{2}. The release of these modulators can be adjusted by circulating humoral agents and blood pressure changes detected by mechanosensitive ion channels. Through the activation these pressure-sensitive channels, most notably of the TRP superfamily, it is thought that the endocardial endothelium detects gross blood pressure changes within the heart, which then communicate with adjacent cardiomyocytes to modulate their contractility. A disruption in the paracrine communication between the endocardium and the myocardium will impact the contractility of the heart, potentially resulting in diseases such as cardiac hypertrophy and arrhythmias.

Methods: Adult male Wistar rats were humanely killed by anesthetic and the hearts removed. The right-atrial appendage was dissected in HEPES buffered solution, and cross-sectional strips of up to 1mm were cut. A novel technique was used to image endocardial cells, where cardiac strips were loaded with Ca\textsuperscript{2+} indicator, followed by washing out with HEPES solution containing 10µM blebbistatin. Immunolabelling was utilized in order to confirm the presence of various TRP channels in both endocardial and myocardial layers.

Results: Endocardial cells were activated by both GSK101 and GSK170; TRPV4 and TRPC6 agonists respectively, which was determined by an increase in intracellular Ca\textsuperscript{2+}. Immunolabelling confirmed the presence of TRPV4 and TRPC6 channels in the endocardium, but not TRPC3 channels.

Conclusions: It is clear that TRPV4 and TRPC6 channels are involved in endocardial cell activation, indicating that the inner-most layer of cardiac tissue is able to detect blood pressure changes. Potential further research includes identification of the chemical mediators released from endocardial cells following their activation, and the effects that these have on cardiomyocytes.

Lillian Wallis is a pharmacology Masters student based at the University of Bath but is currently doing a 12-month placement in Professor Kim Dora's lab in Oxford.
Background: Diabetes mellitus (DM) causes life altering microvascular complications, such as diabetic nephropathy (DN). The endothelial glycocalyx (eGlx) contains proteoglycans with glycosaminoglycan (GAG) sidechains that help maintain vascular permeability and are damaged during DM. Heparan sulfate (HS) is the most abundant GAG in the eGlx and heparanase (HPSE), an HS degrading enzyme, is upregulated during diabetes (Shafat et al, (2011), PLoS1,6(2):e17312). The aim of this study was to show that eGlx HS is important in glomerular barrier function and preventing its shedding with a novel class of HPSE inhibitors (HI) is protective in DM.

Methods: EGlx HS was removed in anesthetized mice by i.v. injection of heparinase III (H3), or by knock-down of endothelial Ext-1/- (an HS biosynthesis enzyme). Db/db mice (type II DM) were used whereby HI or vehicle was given daily from 9-11wk of age and urine albumin creatinine ratios (uACR) measured at endpoint. Anesthetized mice were Ringer perfused for glomeruli permeability studies (Desideri et al, Kidney International 2018:93:1086-1097) or Glutaraldehyde/Alcian blue perfused for electron microscopy (EM) studies.

Results: A significant reduction in glomerular eGlx depth and coverage was seen by H3 treatment, in Ext-1/- mice and in db/db mice by EM. These were associated with significant increases in glomerular albumin permeability (Ps’alb). In db/db mice treated with HI, uACR was no longer significantly increased, eGlx depth and Ps’alb was significantly restored.

Conclusion: We confirmed that endothelial glycocalyx HS plays a direct role in the glomerular filtration barrier. We also demonstrated that heparanase inhibition in DN, using a novel and clinically relevant inhibitor, directly enhances the glomerular eGlx, resulting in normalised glomerular albumin permeability.

Dr Monica Gamez recently completed her PhD in the group of Dr Becky Foster in Bristol and recently secured a postdoctoral position with the same group.
**OC4: Impaired Revascularisation after Ischaemia in Monocytic-Wnt5a is SRPK1 Dependent**

**Sohni R Bhalla, Claire L Allen, Amy P Lynch, Lydia Teboul, David O Bates.**
Tumour and Vascular Biology Group (TVBG), University of Nottingham, BioDiscovery Institute, Science Road, University Park Campus, Nottingham, NG7 2RD, UK.
Sohni.Bhalla@nottingham.ac.uk ; David.Bates@nottingham.ac.uk

**Background:** Peripheral artery disease (PAD) is associated with vascular insufficiency resulting in tissue ischaemia and reduced blood flow. In response, circulating monocytes produce vascular endothelial growth factor (also known as VEGF-A), a key regulator of angiogenesis. Murine models of ischaemia and PAD patients have increased wingless-type MMTV integration site family 5a (Wnt5a) activity. As a result, pro-inflammatory monocytes overexpress anti-angiogenic VEGF-A_{165b}. The splicing of VEGF-A isoforms has been shown to be regulated by the phosphorylation of splicing factor SRSF1 by the kinase, SRPK1. To determine if this was the case in monocytes, we investigated the effect of revascularisation after ischaemia in a novel transgenic mouse model with Wnt5A overexpression and SRPK1 knockout in the monocytes.

**Methods:** Wnt5a gain of function (LysMCre-Wnt5a\textsuperscript{GOF}, abbreviated to WC++), Cre-negative LysM-Wnt5a with homozygous SRPK1\textsuperscript{loxP} (essentially wild-type) and LysMCre-Wnt5a\textsuperscript{GOF} with SRPK1 knockout (WCSS) littermate mice underwent left femoral artery ligation. Blood flow to the paw was measured by Moor FLPI-2 Laser Speckle Imaging system before and after surgery and on post-operative days 3, 7, 14 and 21.

**Results:** The blood flow recovery in WC++ (day 3: 20.9± 2.6%; day 7: 42.6 ± 3.2%) was slower than wild-type mice (day 3: 35.8 ± 8.7%; day 7: 57.6 ± 8.4%). This impaired revascularisation was rescued in WCSS mice on day 3 (40.7 ± 6.9%) and day 7 (64.5± 7.7%) post-surgery (p<0.0001, two-way ANOVA).

**Conclusion:** Overexpression of monocytic-Wnt5a in a hindlimb ischaemia mouse model results in impaired blood flow recovery. This is reversed with SRPK1 knockout in the monocytes suggesting that the impaired revascularisation in PAD patients is a result of Wnt5a over activity and ischaemic mouse models are dependent on SRPK1 in the monocytes.


Sohni Bhalla is a MRC IMPACT funded 3rd year PhD student in the lab of Professor David Bates in Nottingham (talk will not be recorded)
Dr. Andries D. van der Meer is an Associate Professor at the Faculty of Science and Technology of the University of Twente, The Netherlands. He is the leader of the Organs-on-Chips research theme in the Applied Stem Cell Technologies group of the Bioengineering Technologies cluster, supervising six Ph.D. candidates and coordinating multiple national and European research projects on the development and application of organ-on-chip technology.

Before joining the University of Twente in 2015, Dr. Van der Meer worked as a Senior Research Fellow at Harvard Medical School and the Wyss Institute for Biologically Inspired Engineering of Harvard University, Cambridge, MA, USA. He actively developed organ-on-chip models of the blood-brain barrier and the alveolus for the Defense Advanced Research Projects Agency (DARPA) Microphysiological Systems program and coordinated a collaborative project between the Wyss Institute organ-on-chip start-up company Emulate, Inc. and Janssen Pharmaceuticals. Before joining Harvard University, he was a Post-Doctoral Fellow at Prof. Albert van den Berg’s BIOS/Lab-on-a-Chip group of the University of Twente, The Netherlands. During that time, he also served as an Assistant Coordinator for the project ‘Beyond Borders: Organs-on-Chips’ of the Dutch Royal Academy (KNAW). This project led to the founding of the Dutch Human Organ and Disease Model Technologies (hDMT; www.hdmt.technology) Organ-on-Chip consortium, for which Dr. Van der Meer is currently his university’s representative.

Dr. Van der Meer obtained his Ph.D. in Biomedical Engineering from the University of Twente, The Netherlands in 2010, and received his M.Sc. degree in Medical Biology from the University of Groningen, The Netherlands in 2005.

**Abstract:** Organs-on-chips are plastic microdevices the size of a USB-stick with microchannels and small chambers that are filled with liquid. The devices contain multiple human cell types which are cultured in a technologically controlled microenvironment that artificially mimics aspects of the human body like morphology, movement, flow, electrical stimuli and liquid gradients. The resulting device emulates human organ functions and can be used to study biomedical phenomena in the lab.
OC5: Investigating a Novel Role for IL-36 / IL-36R Pathway in Mediating Inflammation in Adult and Aged Heart after Ischaemia-Reperfusion Injury

Microcirculation Research Group, Institute of Cardiovascular Sciences and *Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT, UK
JXE839@bham.ac.uk; n.kalia@bham.ac.uk

Introduction: Whilst blood flow restoration is critical following myocardial infarction (MI), our intravital imaging of beating mouse heart showed age-dependent microcirculatory perturbations post-reperfusion. This may explain the poor outcome of some MI patients, particularly the aged. The newly discovered cytokine interleukin-36 (IL-36) may mediate these microcirculatory disturbances. However, its role in cardiovascular disease is not known. This study determined whether IL-36 isoforms (IL-36α/β/γ) and its receptor (IL-36R) were present in the heart, whether expression varied with injury/age and whether this cytokine could mediate inflammatory responses in beating coronary micro-vessels.

Methods: Myocardial ischaemia-reperfusion injury (IRI) was induced in young (3 mth) and aged (>18 mth) mice. IL-36α/β/R and VCAM-1 expression was investigated immunohistochemically or using western blotting. IL-36 isoforms were topically applied to the beating mouse heart and thrombo-inflammatory responses intravitally imaged.

Results: IL-36α/β/R and VCAM-1 were expressed on CD31+ vasculature. Expression increased with both injury and age (see table), specifically on micro- and not macro-vessels. Western blotting confirmed the IL-36R increases. All IL-36 isoforms induced remarkable increases in neutrophil infiltration in the beating coronary microcirculation, with minor platelet involvement. Similar responses were observed in aged hearts.

Conclusion: These novel results demonstrate injury and age-dependent myocardial presence of IL-36/IL-36R. Furthermore, this is the first study to directly demonstrate the potent inflammatory nature of IL-36 in the beating heart in vivo. These exciting results may explain why MI leads to larger infarcts and a worse prognosis in older patients.

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<th>Adult sham vs Adult IRI</th>
<th>Adult sham vs Aged sham</th>
<th>Adult IRI vs Aged IRI</th>
<th>Aged sham vs Aged IRI</th>
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<td>IL-36α</td>
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<td>VCAM-1</td>
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*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 when compared using an ANOVA test; N=4 mice for each group.

Juma El-Awaisi is BHF funded (FS/18/45/33862) 2nd year PhD student in the lab of Dr Neena Kalia in Birmingham
Background: Circadian rhythms regulate many physiological processes such as sleep/awake patterns, feeding/fasting, behaviour and mood, and are synchronised by the suprachiasmatic nuclei in the brain, which dynamically responds to environmental stimuli. Peripheral tissues, such as the vasculature, are also circadian, and dysregulation of their rhythmicity affects fundamental processes, specifically angiogenesis. In the capillaries, physical and paracrine communication between endothelial cells (EC) and pericytes (PC) drives angiogenesis. The presence and role of circadian rhythms in PC, and their effect on EC/PC interactions, remain unexplored.

Methods: The expression of circadian clock genes in primary human umbilical vein endothelial cells (HUVEC) and saphenous vein pericytes (SVP) was assessed by qPCR and luciferase reporter assays following synchronisation, and angiogenic potential was tested by Matrigel assay. Circadian clock disruption was performed by shRNA silencing.

Results: SVP presented rhythmic expression of the principal circadian genes with a circa 24h period. Silencing of the Aryl hydrocarbon receptor nuclear translocator-like (ARNTL), a key component of the molecular clock, reduced SVP viability. In our experimental setting, HUVEC did not show circadian rhythmicity, opening up the possibility that a secondary form of EC molecular clock regulation exists. Co-culture of HUVEC with synchronised SVP in Matrigel increased the pro-angiogenic potential of the HUVEC, indicating that the circadian clock may influence angiogenesis through the PC component.

Conclusion: This study defines for the first time the existence of an endogenous molecular circadian clock in PC, and suggests implications for circadian clock synchronisation on physiological and therapeutic angiogenesis.

Valeria Mastrullo is a 3rd year PhD student in the lab of Dr Paola Campagnola in Surrey
Background: The relationship of astrocytes with the retinal vasculature is a complex and intimate one, with astrocytic end-feet being observed to engulf the vessels of the retina. Traditionally retinal blood flow is regulated intrinsically via vascular dependent (myogenic) activity and via neurovascular coupling, with the astrocyte acting as an intermediate in communicating metabolic needs to the vasculature. This study aimed to determine if astrocytes have the mechanical architecture and chemical means to sense stretch induced by changing vascular perfusion pressure and thus contribute to myogenic tone development.

Methods: Retinal flatmounts (C57BL/6 mice) were utilised for immunohistochemical studies to determine the presence of astrocyte-specific proteins, and for simultaneous pressure- and Ca^{2+}- recording to determine if retinal astrocytes sense vascular stretch.

Results: Immunohistochemistry revealed the expression of the novel mechanosensitive Piezo2 channel on retinal astrocytes. Additionally, CaV3.2 and connexin 43 expression was observed in regions of contact between astrocytic end-feet and the retinal vasculature. Direct mechanical pressure applied to flatmount retinas resulted in an increase in Ca^{2+} activity in astrocytes (p=0.005) and was followed by a vasoconstriction (p=0.0003) in the accompanying retinal arterioles. Similarly, when an arteriole within the retinal flatmount was cannulated and pressurised, Ca^{2+} activity increased in the astrocytes surrounding the vessel (p=0.026) and was followed by vasoconstriction (p=0.021).

Conclusion: This study provides the first evidence that retinal astrocytes can respond to vascular stretch via a Ca^{2+} dependent mechanism (possibly through Piezo2, CaV3.2 and Cx43) which has the potential to contribute to the myogenic response in retinal arterioles.

Tamara McErlain is currently a Masters student but will continue the above project as a PhD with Professor Tim Curtis in Belfast (talk will not be recorded)
Background: Acute inflammation is characterised by enhanced vascular permeability and leukocyte infiltration. Whilst temporally associated, the impact of stimulated microvascular leakage on neutrophil trafficking remains contentious.

Methods: Through the application of confocal intravital microscopy to analysis of inflamed cremasteric microvessels, we have obtained compelling evidence for the ability of vascular leakage to promote aberrant neutrophil transendothelial cell migration (TEM). Specifically, in tissues stimulated locally with both IL-1β (to induce neutrophil recruitment) and histamine (to increase vascular permeability), we noted a high frequency of neutrophils that had initiated diapedesis to exhibit reverse TEM and re-enter the microcirculation.

Results: In addressing the mechanism, we found that whilst histamine had no effect on levels of CXCL1 in IL-1β-stimulated tissues, it elevated plasma levels of this chemokine (~40% increase). Conversely, blockade of histamine-induced vascular leakage using an anti-VE-PTP antibody reduced plasma levels of CXCL1. These results indicated that enhanced vascular leakage may promote the trafficking of CXCL1 from the extravascular tissue into the bloodstream, a concept supported by mathematical modelling. We confirmed this possibility by tracking the movement of locally injected hCXCL8 from the interstitium to the vascular lumen in IL-1β+histamine-stimulated tissues. Here, whilst both IL-1β and IL-1β+histamine-stimulated tissues retained similar levels of hCXCL8, plasma levels of hCXCL8 was significantly increased (~30%) in mice treated locally with histamine.

Conclusion: Collectively, our findings suggest that induced vascular leakage can promote rapid translocation of chemotactic cues from the interstitium into the circulation resulting in disrupted directionality of neutrophil TEM.

Funded by the BHF, Wellcome Trust, and the EU.
A. dSTORM super-resolution imaging of collagen and LAT in platelets
B. State-of-the-art super-resolution Lattice sheet microscopy
C. Confocal image of endothelial tube formation
D. State-of-the-art spinning Nipkow confocal-based intravital microscope
E. Intravital imaging of thrombus formation in vivo post-laser injury
F. Intravital imaging of neutrophils in beating murine heart in vivo
G. Lattice lightsheet imaging of actin and tubulin movement during proplatelet formation
H. Multiphoton intravital imaging of murine spleen in vivo
I. SIM and dSTORM imaging of protein phosphorylation at actin nodules
J. Laser speckle contrast imaging of beating heart and lungs in vivo
K. Beating murine heart echocardiography