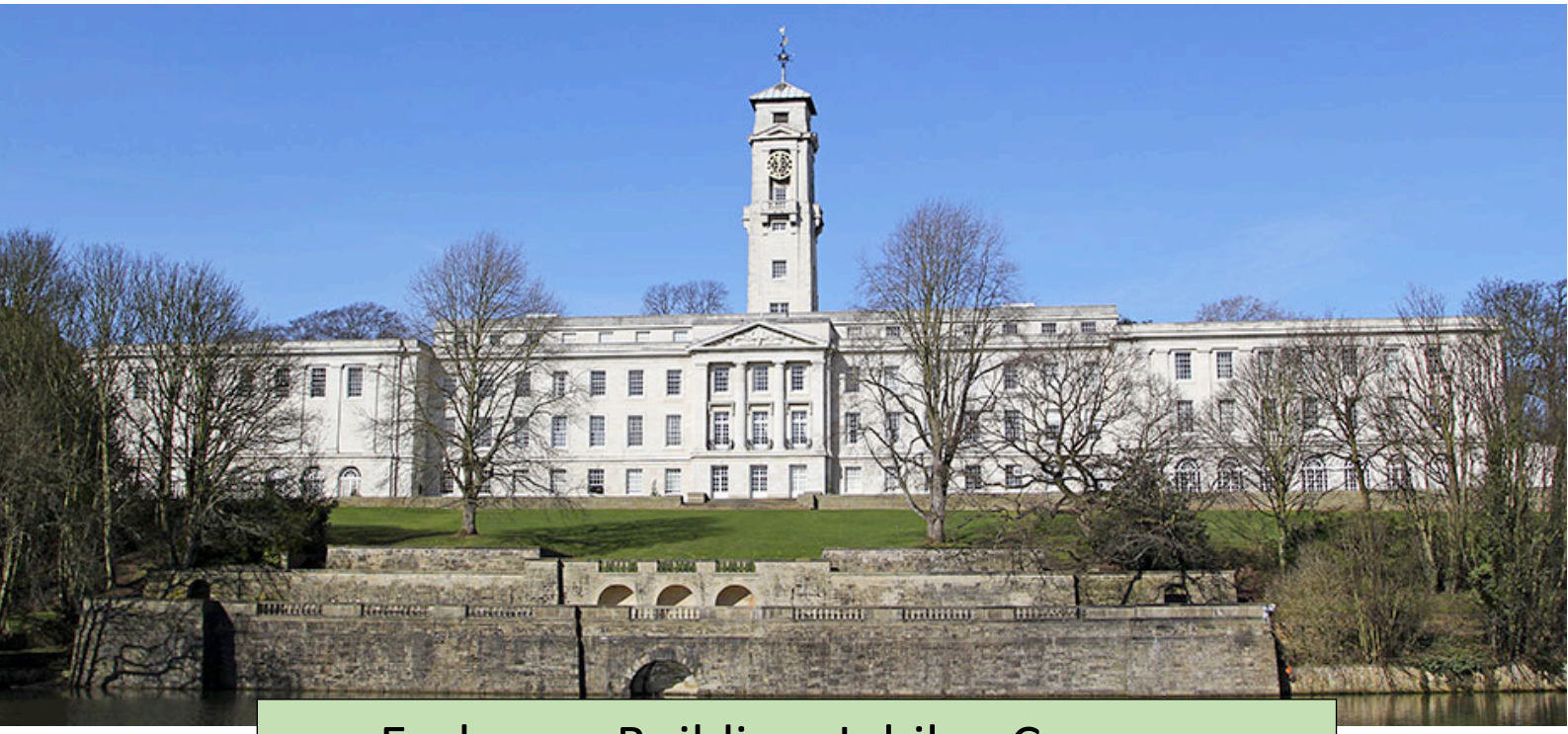
BMS 2018
British Microcirculation Society

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

British Microcirculation Society conference 2018 – 68th annual meeting

Monday 16th & Tuesday 17th April



Exchange Building, Jubilee Campus,
University of Nottingham, UK

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BMS 2018 - 68th annual meeting

University of Nottingham

Dear Attendees

It gives us great pleasure to welcome you to Nottingham for the 68th British Microcirculation Society meeting. I'm hoping that collaborations will be extended, and a broad range of knowledge disseminated through the following days. We wish to thank generous and ongoing support from the trade exhibitors (please visit them!) and the British Heart Foundation (please donate to them!) that has allowed us to have a selection of great UK and overseas speakers and what we hope is a very reasonable delegate fee.

This year's invited speakers' theme is Diabetic Vasculopathy which we hope will give a broad range of physiological information about different tissues eluding to great experimental protocols applicable across the microcirculation's disease portfolio.

On a personal note I'm thankful and excited that the BMS have extended the program to include a relatively informal workshop day on the endothelial glycocalyx, my subject of study. This free workshop day has brought excellent UK speakers, to join some of the main symposia speakers, from different fields (Mechano-transduction, permeability, proteoglycans etc) and is attended by >30 BMS members alone.

On behalf of myself and the organisation team:

Enjoy!

Dr Kenton Arkill

Local Organiser

Dr Kenton Arkill

Senior Research Fellow
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Organising Committee

David Bates
Lopa Leach
Nick Beazley-Long
Claire Allen
Marlene Da Vitoria Lobo
Naseeb Malhi
Hiten Mistry
Lesia Kurak
Andrew Benest
Jeanette Woolard
Sohni (Ria) Bhalla
Samantha Cooper

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General Information for BMS 2018

Registration open from 9:30am each day in the Exchange Building, Jubilee Campus, NG8 1BB

Commercial Exhibition: C33/C3 Exchange building
Trade exhibitors will be interspersed between the poster presentations. Please take some time to engage with exhibitors as without their support this meeting could not take place.

Oral Presentations
Oral presentations will take place in the in the Exchange Building. Bring Power Point presentation files on a USB memory stick formatted for PC / Mac and load on the meeting projection computer at the start of the day of the presentation in question. We kindly request personal laptops are not used for presentations unless absolutely necessary (e.g. if movies are shown) as this can cause delays in the schedule. Short Oral communications should no longer than **10 minutes** in length allowing **5 minutes** for questions.

Poster Presentations
Posters will be in C33/C3 Exchange building
Posters should be A0 Portrait (841x1189mm).
Posters should be mounted by Monday morning and be displayed for the whole meeting duration.
All poster presenters should be at their posters during lunch poster sessions on both days. Over lunch a panel of judges will select posters for several poster prizes so please be at your posters at this time.

Refreshments and Lunch
Coffees/teas and lunch will be served in C33 Exchange building

Accommodation
Halls Accommodation is in **Newark Hall,** Jubilee Campus, NG8 1BB

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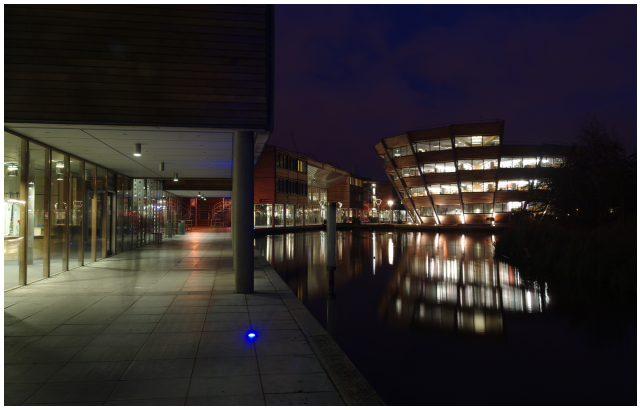
Venue: Jubilee Campus



The University of Nottingham
Jubilee Campus
Wollaton Road
Nottingham
NG8 1BB
UK

Link for Maps and directions to the Jubilee Campus

Or see map overleaf

More Information about Jubilee Campus

The Jubilee Campus is a modern purpose-built campus which now extends to 65 acres and is located only one mile from University Park Campus. The initial phase was opened by Her Majesty the Queen in 1999. The state-of-the-art facilities now include:

- the Schools of Education, Contemporary Chinese Studies and Computer Science
- The Nottingham University Business School
- The National College for Leadership of Schools and Children's Services
- a Sports Centre
- University of Nottingham Innovation Park
- 4000 third party purpose-built student residences within half a mile radius of the campus

Sustainability

Built on a site that previously had industrial use, Jubilee Campus is an exemplar of brownfield regeneration and has impeccable green credentials.

An important feature of the campus is the series of lakes which, as well as being home to a variety of wildlife, provide storm water attenuation and cooling for the buildings.

Less visible - but equally important to the sustainable and environmental credentials of the campus – are the:

- roofs covered by low-growing alpine plants which help insulate and maintain steady temperatures within the buildings throughout the year
- heat recovery mechanical ventilation systems
- lighting sensors to reduce energy consumption
- photovoltaic cells integrated into the atrium roofs
- lake source heating and cooling systems
- biomass boiler installation
- maximised use of passive ventilation engineering

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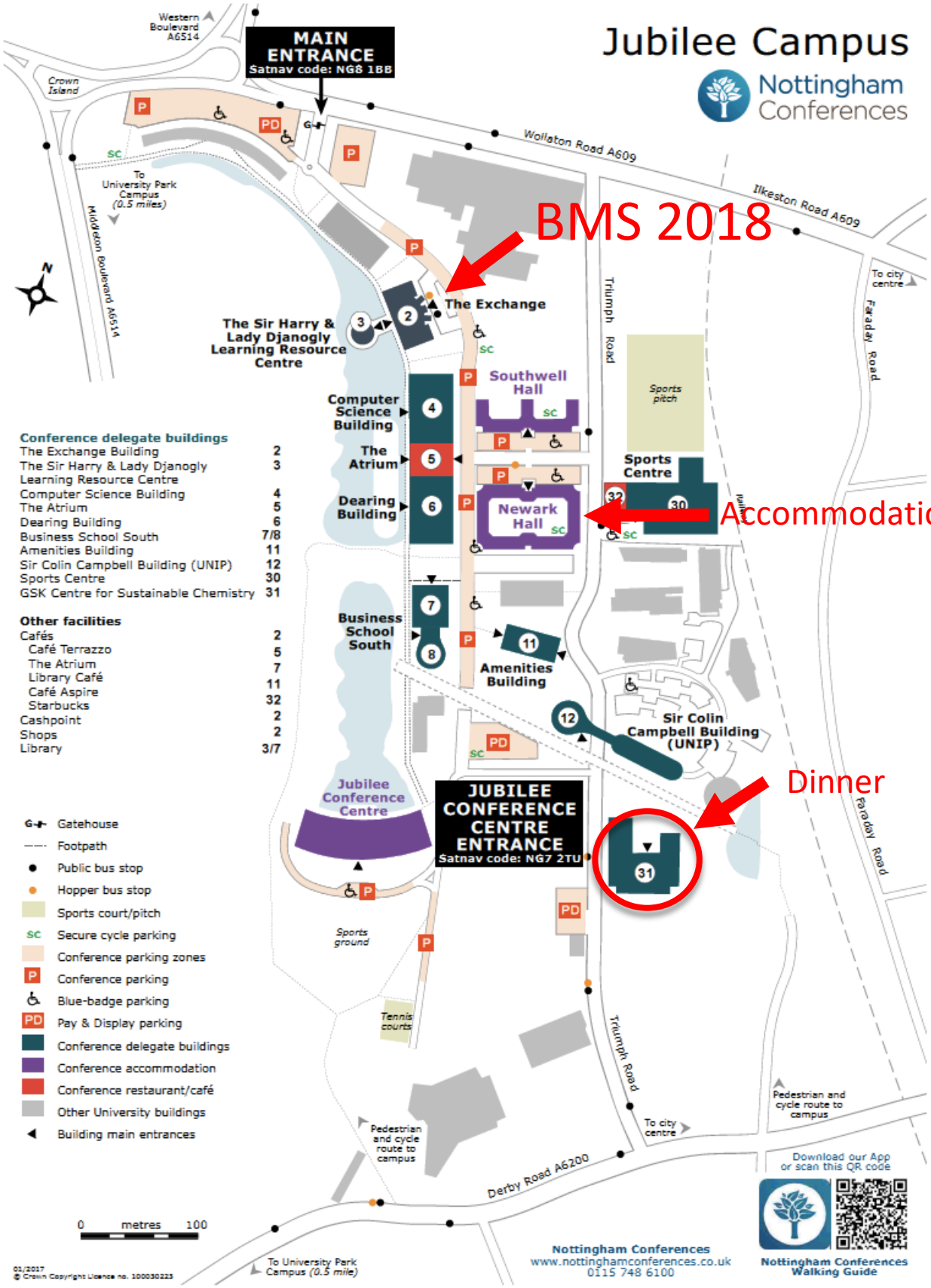
Monday 16th – Tuesday 17th April

BMS 2018, The Exchange Building, Jubilee Campus, NG8 1BB



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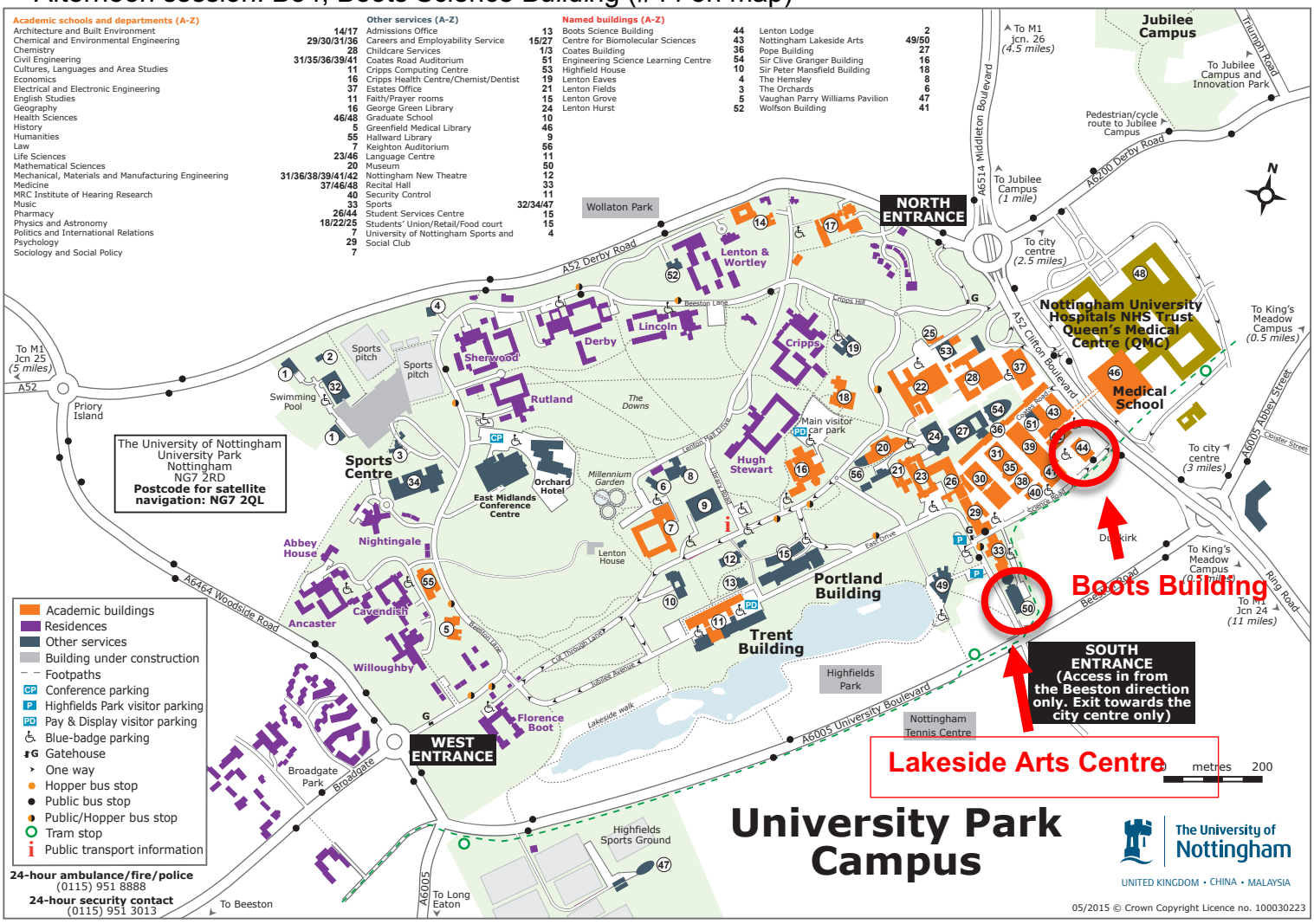
Glycocalyx Workshop

Wednesday 18th April, University Park Campus

Morning session: Lecture Theatre, Lakeside Arts Centre (#50 on map)

Afternoon session: B34, Boots Science Building (#44 on map)



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Trade Contributors:

Our Main Contributor this year is Moor Instruments

Other contributors are:

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Programme Venue: Exchange Building Jubilee Campus

Monday 16th April

09:30 **Registration, Poster set up**

10:25 **Welcome Address** Kenton Arkill

Morning Session - Chairs: Lopa Leach & Lesia Kurlak

10:45 **Keynote Speaker 1 - Carlos Escudero, Universidad del Bio-Bio, Chile**
Increased Pro-Angiogenic Status in Placentas Derived from Gestational Diabetes

11:30 **OC1 - Francesco Casanova** *Microvascular Functional Decline is Reduced in Type 2 Diabetes Patients Treated to Glycaemic Target: a Longitudinal Observational Study*

11:45 **OC2 - Colin Down** *Imaging the Placental Glycocalyx with Transmission Electron Microscopy*

12:00 **OC3 - Stephany D. Villota** *Occludin Expression in Human Placental Microvessels in Pregnancies Complicated with Gestational Diabetes*

12:15 **Lunch, Posters & Trade Exhibitors**

Afternoon Session 1 - Chairs: David Bates & Neena Kalia

14:00 **Keynote Speaker 2 -Mark Kearney, University of Leeds, UK**
Targeting a Novel Proatherosclerotic Signalling Loop in Insulin Resistance Related Endothelial Cell Dysfunction

14:45 **OC4 - Yan Qiu** *Diabetic Cardiomyopathy is Associated with Loss of Endothelial Glycocalyx in Coronary Microvessels and Angiopoietin 1 Restores Endothelial Glycocalyx and Corrects Cardiac Function*

15:00 **OC5 -Adam Lokman:** *Imaging the Vasculoprotective Effects of Haematopoietic Stem Cells (Hscs) Following Myocardial Ischaemia-Reperfusion (Ir) Injury in the Murine Beating Heart*

15:15 **OC6 - Laurienne Edgar** *Diabetic Hyperglycaemic Memory: How Diabetes Drives Long Term Inflammation and Atherosclerosis After Glucose Normalisation*

15:30 **Refreshment Break**

Afternoon Session 2 - Chairs: Nick Beazley-Long & Richard Hulse

16:00 **Keynote Speaker 3 -Lucy Donaldson, University of Nottingham, UK**
Nervous System Microvasculopathy and Contributions to Nociception

16:45 **OC7 - Marlene Da Vitoria Lobo** *VEGF-A-mediated Spinal Cord Vasculopathy and Hypoxia; Contributing Factors in the Development of Diabetic Neuropathic Pain.*

17:00 **OC8 - Peter Tickle** *Impaired Skeletal Muscle Performance After Cardiac Hypertrophy is Associated with Microvascular Rarefaction not Perfusion*

17:15 **OC9 – *Russell Hughes*** *Breast Cancer Metastasis to Bone: the Role of the Perivascular Niche in Regulating Tumour Cell Dormancy*

17:30 **BMS Annual General Meeting/ Career Discussion**

19:30 **Conference Dinner** – GSK Building Jubilee Campus

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Programme cont. Venue: Exchange Building Jubilee Campus

Tuesday 17th April

Morning Session 1 - Chairs: Claire Allen & Kim Gooding

09:00 **Keynote Speaker 4 - Heping Xu, Queen's University Belfast, UK**
The role of inflammation in diabetes-induced retinal microvascular degeneration

09.45 **OC10 - Mitra Tavakoli** *Measurement of AGEs products in the Crystalline Lens of the eye distinguishes subjects with prediabetes and Type 2 Diabetes and correlates with level of neuropathy: A Preliminary Study*

10:00 **OC11 - Naseeb Malhi** *The SRPK1 inhibitor SPHINX31 stabilises retinal permeability in vitro and in vivo models of diabetes*

10:15 **OC12 – Ulrich Rother** *Pilotassessment Of The Angiosome Concept By Intraoperative Fluorescence Angiography After Tibial Bypass Surgery*

10:30 **Refreshment Break**

Morning Session 2 - Chair: Angela Shore & Andrea Murray

11:00 **Keynote Speaker 5 -Hans Vink, Maastricht University, Netherlands**
Glycocalyx damage in diabetes: implications for microvascular function

11:45 **OC13 - Raina Ramnath** *Matrix Metalloproteinase)-Mediated Syndecan 4 Loss from The Endothelial Glycocalyx as a Therapeutic Target in Diabetic Nephropathy*

12:00 **OC14 - Anna Ogier** *Mineralocorticoid Receptor Inhibition in Diabetic Nephropathy Protects The Glomerular Endothelial Glycocalyx and Reduces Glomerular Permeability to Albumin*

12:15 **OC15 - Kim Gooding** *Examining the Relationship Between Skin Microvascular Reactive Hyperaemia and Urinary Albumin Excretion, Extending into the Low Level Range*

12:30 **Lunch, Posters & Trade Exhibitors –**

Afternoon Session - Chairs: Kenton Arkill & Becky Foster

14:15 **Keynote Speaker 6 -Bingmei Fu, The City College of New York, US**
Blood-brain barrier permeability determined by multiphoton microscopy and its modulation by physical and chemical stimuli.

15:00 **OC16 - Dilan Dabare** *Can Infrared Thermography be Used to Help Assess the Quality of KidneysfFor Transplantation?*

15:15 **OC17 - Karen Onions** *Angiopoietin-1 Induced Protection of Glomerular Function and the Role of Hyaluronan*

15:30 **OC18 - Andrea Murray** *Feasibility Study of Photoacoustic Imaging for Measurement of Digital Vascular Structure in Healthy Controls and Patients with Systemic Sclerosis*

15:45 **Prizes & Closing**

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Wednesday 18th April

Endothelial Glycocalyx Workshop
Where is it? What is it? When is it?

Morning Session:

Lecture Theatre A30 (Building 50) Lakeside Arts Centre, University Park Campus

09:30 **Registration and refreshments**

09:45 **The Point of Today** – **Dr Kenton Arkill** (University of Nottingham)

10:00 **Prof Charles Michel** (Imperial College)
The glycocalyx and microvascular permeability

10:45 **Prof Bingmei Fu** *(*The City College of New York)
Endothelial Surface Glycocalyx (ESG) Viewed by Confocal and Stochastic Optical Reconstruction Microscopy (STORM)

11:15 **Prof Anthony Day** (University of Manchester)
TSG-6-glycosaminoglycan interactions: implications for glycocalyx structure and function

11:45 **Refreshment Break**

12:15 **Prof Hans Vink** (Maastricht University, Netherlands)
Clinical assessment of glycocalyx damage and capillary red cell hemodynamics

12:45 **Lunch**

Afternoon Session- B34 Boots Building (Building 44), University Park Campus

13:30 **Ralf Richter** (University of Leeds)
Probing physical mechanisms of cell capture under vasculature-mimicking flow with mechanically and biochemically well defined environments

14:00 **Becky Foster (University of Bristol)**
Enhancement of endothelial glycocalyx in the diabetic microvasculature

14:30 **Xi Zhuo Jiang** *Large-Scale Molecular Dynamics Simulations of Flow and Glycocalyx: Towards Understanding Atomic Events on Endothelial Cell Surface*

15:00 **Refreshment Break**

15:15 **Discussion:** *Dissemination of the field*

15:55 **Round up and conclusions**

16:00 **Pub** – *The Johnson Arms, Abbey Street.*

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Symposium communication abstracts

Keynote speaker 1

Increased pro-migratory activation of VEGFR2 but reduced expression of VEGFR1 is associated with placental hypervascularization in gestational diabetes mellitus

Carlos Escudero[1,2]

[1]*Vascular Physiology Laboratory, Group of Investigation in Tumor Angiogenesis (GIANT), Department of Basic Sciences, Universidad del Bío-Bío, Chillán, Chile.* [2]*Group of Research and Innovation in Vascular Health (GRIVAS Health), Chillan, Chile.*

cescudero@ubiobio.cl

Placental hypervascularization observed in gestational diabetes mellitus (GDM) may be related with functional availability of vascular endothelial growth factor (VEGF) and its receptors. We aimed to test whether changes in phosphorylation of tyrosine 951 or tyrosine 1175 (pY951 or pY1175) of the vascular endothelial growth factor receptor 2 (VEGFR2) are associated with the proangiogenic state observed in placentas from GDM. We obtained placental samples from women with normal pregnancies (n=24) or GDM (n=18). We measured the relative expression of markers for endothelial cell number (CD31, CD34), VEGF, vascular endothelial growth factor receptor 1 (VEGFR1), VEGFR2, pY951 and pY1175 of VEGFR2 in placental homogenate. Immunohistochemistry of placental blood vessels were performed using CD34. Proliferation and migration of human umbilical vein endothelial cells (HUVEC) obtained from normal pregnancy and GDM were determined in absence or presence of conditioned medium (CM) harvested from GDM or normoglycemic HUVEC cultures. Placentas from GDM exhibited more CD31 and CD34 protein compared to normal pregnancy. High number, but reduced area of placental blood vessels was also found in GDM. Reduced VEGFR1 levels (mRNA and protein) are associated with reduced mRNA, but higher protein levels of VEGFR2 in placentas from GDM. No significant changes in Y951-or Y1175-phosphorylation of VEGFR2 in placentas from GDM were found. GDM did not alter proliferation of HUVECs, but enhanced migration. Conditioned medium harvested from GDM HUVEC cultures enhanced VEGFR2 protein amount, tube formation capacity and cell migration in HUVEC isolated from normoglycemic pregnancies. In conclusion, GDM was associated with reduced expression of VEGFR1 but high pro-migratory activation of VEGFR2 reflecting a proangiogenic state in GDM.

Symposium communication abstracts

Keynote speaker 2

Targeting a Novel Proatherosclerotic Signalling Loop in Insulin Resistance Related Endothelial Cell Dysfunction

Mark Kearney
Division of Cardiovascular and Diabetes Research, Faculty of Medicine and Health, University of Leeds, UK
m.t.kearney@leeds.ac.uk

Insulin resistance a well-established but poorly understood hallmark of type 2 diabetes leads to a disadvantageous alteration in the balance between endothelial cell generation of the antiatherosclerotic autacoid nitric oxide (NO), and cytotoxic concentrations of superoxide. This results in reduced NO bioavailability and a situation described as oxidative stress. We recently demonstrated that increased expression of insulin receptors in the endothelium leads to activation of a proatherosclerotic signalling loop in endothelial cells, composed of: endothelial NO synthase, Nox2 NADPH oxidase and proline rich tyrosine kinase 2, resulting in inhibitory tyrosine phosphorylation of eNOS, oxidative stress and accelerated atherosclerosis. In contrast increased insulin like growth factor 1 receptor (IGF-1R) expression in the endothelium despite insulin resistance protects against the development of atherosclerosis. Here we compare and contrast the effect of IGF-1 and insulin signalling in the endothelium on NO bioavailability and atherosclerosis.

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Symposium communication abstracts

Keynote Speaker 3

Nervous system vascular dysfunction in pain and diabetes

[1]Lucy F. Donaldson, Nikita Ved, [1]Nicholas Beazley-Long, [2]Marlene E. Da Vitoria Lobo, Samuel M. Bestall, [3]Kurt Ballmer-Hofer, [2]David O. Bates and Richard P. Hulse

[1]School of Life Sciences & Arthritis Research UK Pain Centre, [2]School of Medicine, University of Nottingham, QMC, Nottingham, NG7 2UH. [3]Paul Scherrer Institute, Villigen, Switzerland. [4]Science and Technology Building, Nottingham Trent University, UK

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Multiple diabetic complications affecting different organ systems can result from macro and microvasculopathies, hypothesised to be caused by poor glycaemic control. These vasculopathies can also affect the nervous system, most often recognised as peripheral microvasculopathy contributing to diabetic neuropathy, but more recently these vasculopathies are acknowledged to also affect the central nervous system, both brain and spinal cord. People with Type 2 diabetes have a 2-6 fold increased risk of cerebrovascular disease, and interestingly, people with proliferative diabetic retinopathy (DR) also have increased risk for multiple neuropsychiatric disorders including stroke, epilepsy and psychosis, compared to people with non-proliferative DR. These findings imply that the CNS vasculature, and therefore CNS function may also be affected in diabetes. Our recent findings suggest that there are changes in blood-DRG, blood-spinal cord, and blood-retinal barriers in animal models of diabetes and peripheral inflammation, that are vascular endothelial growth factor (VEGF) receptor dependent, and can be ameliorated by treatment with recombinant VEGF-A_{165}b or by VEGF receptor inhibition *in vivo*. CNS endothelial VEGF receptor dependent blood-spinal cord barrier function through VEGF receptors on central endothelial cells may be a contributory factor to pain in diabetic neuropathy and in other peripherally generated pain models.

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Symposium communication abstracts

Keynote speaker 4

The role of inflammation in diabetes-induced retinal microvascular degeneration

Heping Xu

The Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom
heping.xu@qub.ac.uk

Diabetic retinopathy (DR) is the progressive degeneration of retinal blood vessels and neurons resulting from long-term diabetes. Inflammation is known to play an important role in the pathogenesis of DR although the underlying mechanism remains poorly defined. During diabetes, metabolic disorder leads to the release of damage-associated molecular patterns (DAMPs) both in the retina and elsewhere in the body. The innate immune system provides the first line of defence against the DAMPs.

At the early stages of DR when the blood-retinal barrier (BRB) is intact, retinal microglia and the complement system are activated at low levels. This low-level of inflammation (para-inflammation) is believed to be essential to maintain retinal homeostasis and restore functionality. In the meantime, the phenotype and function of circulating immune cells, particularly the myeloid-derived innate immune cells such as monocytes and neutrophils are altered due to sustained hyperglycemic stimulation leading to abnormal leukocyte-endothelial interaction (leukostasis). As diabetes progresses, sustained stimulation by DAMPs leads to maladaptation of the innate immune system, which may turn the protective para-inflammatory response into detrimental chronic inflammation and contribute to DR development.

At the advanced stages of DR, vascular endothelial cells degenerate leading to the breakdown of BRB, which comprises retinal immune privilege. Circulating immune cells and serum proteins may infiltrate the retina. Such chronic inflammation further damages retinal vasculature and neurons and ultimately leads to the development of neovascular membrane (proliferative DR).

This presentation discusses how the systemic and local (retina) innate immune systems are activated in diabetes and the molecular pathways involved in DR-related inflammation.

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Symposium communication abstracts

Keynote speaker 5

The endothelial glycocalyx and control of microvascular flow and perfused capillary density

Hans Vink
Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, The Netherlands
hvink@microvascular.com

The endothelial glycocalyx extends up to more than 1 micron into the lumen of microvessels and is expected to affect microvascular blood volume and red cell hemodynamics in capillary blood vessels. In the current study, we determined the level of penetration of red cells in the vascular wall boundary region as a measure of glycocalyx damage in healthy controls and in individuals with type 2 diabetes. In addition, we measured red cell velocities in feed vessels and capillaries to determine the relation between microvascular blood flow and red cell perfused capillary density. Our findings demonstrate increased penetration of red cells into the glycocalyx boundary layer in type 2 diabetes and analysis of intra-individual variability of red cell hemodynamics revealed that glycocalyx damage is associated with impaired flow dependent control of capillary density. It is concluded that uncoupling of microvascular blood flow and capillary exchange capacity may contribute to microscopic areas of tissue injury and loss of organ function at early stages of glycocalyx damage.

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Symposium communication abstracts

Keynote speaker 6

Blood-brain barrier permeability determined by multiphoton microscopy and its modulation by physical and chemical stimuli

Bingmei M. Fu
Department of Biomedical Engineering, The City College of the City University of New York, 160 Convent Ave, New York, NY 10031
fu@ccny.cuny.edu

The blood-brain barrier (BBB) is a dynamic barrier essential for maintaining the micro-environment of the brain. It is compromised in brain diseases such as brain tumor, Alzheimer's disease and multiple sclerosis. It can also be regulated by physical and chemical stimuli. Many in vivo methods have been developed to quantify the BBB permeability since early 80's, but they either determined only the permeability of pial microvessels at the brain surface or just estimated the BBB leakage. With the recent development of laser scanning multiphoton microscopy, we are able to quantify the BBB permeability of brain parenchymal microvessels. Fluorescently labeled solutes with sizes from sodium fluorescein (376Da) to IgG (~160kDa) in 1% BSA mammalian Ringer was injected into the rat (SD, 250-300g) cerebral circulation via the ipsilateral carotid artery by a syringe pump at a constant rate of ~3 ml/min. Under the excitation wavelength of 800-850 nm, we collected the images of cerebral microvessels 100-200μm below the pia mater. The BBB solute permeability and the solute brain effective diffusion coefficient were determined from the rate of tissue solute accumulation and the radial concentration gradients around individual microvessels in the brain tissue. Employing this new method, we also quantified the modulation of the BBB solute permeability by ultrasound, transcranial direct current stimulation, as well as vascular endothelial growth factor and 3,5-cyclic monophosphate (cAMP). Quantification of BBB permeability and brain tissue transport is important in understanding disease and treatment mechanisms and in developing better drug delivery strategies. Supported by NIH SC1CA153325-01, R21EB017510-01 and RO1 NS101362-01.

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Selected oral communication abstracts

OC1

Microvascular Functional Decline is Reduced in Type 2 Diabetes Patients Treated to Glycaemic Target: a Longitudinal Observational Study

Casanova F., Aizawa K., Gooding K.M., Adingupu D.D., Mawson D., Ball C., Gates P.E., Lear R., Chui A., Shore A.C., Strain W.D.
Diabetes & Vascular Medicine Research Centre, NIHR CRF, Barrack Road, Exeter EX2 5AX
f.casanova@exeter.ac.uk

Background
Good glycaemic control from diagnosis delays onset of the microvascular complications of type 2 diabetes (T2DM). However, clinical trials of glycaemic control later in T2DM demonstrated conflicting results. We aimed to explore the natural progression of microvascular function in individuals with T2DM whose glycaemia was well and less-well controlled compared to healthy controls.
Methods
Microvascular function was assessed in 161 T2DM and 103 without T2DM, at baseline and after 3.1years. Laser Doppler imaging assessed maximum cutaneous hyperaemic response to heating (MHR), post 4-minutes occlusive reactive hyperaemia (PORH) and iontophoresis of Sodium Nitroprusside (SNP) and Acetyl-choline (ACH). Patients with diabetes were stratified by optimal (mean HbA1c over 3 years ≤53mmol/mol; 7.0%) and suboptimal (mean HbA1c ≥58mmol/mol; 7.5%) glycaemic.
Results
Median diabetes duration at baseline was 8yrs. As expected, those with T2DM had impaired baseline microvascular function. People with T2DM and optimal glycaemic control had less decline in SNP mediated endothelial independent function (p for interaction = 0.025) and PORH (p for interaction=0.021) compared to suboptimal control. There was a similar trend for MHR (p=0.066). This interaction was not dependent on changes in blood pressure, lipids, duration of diabetes or any specific medication choices over this period.
Conclusions
In a population with a significant duration of diabetes, good glycaemic control was associated with less age-related decline in microvascular function compared those with sub-optimal control. This was independent of other aspects of the metabolic syndrome. This is in favour of the hypothesis, that good glycaemic control will benefit people with diabetes at any stage in their disease process.

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Selected oral communication abstracts

OC2

Imaging the Placental Glycocalyx with Transmission Electron Microscopy

Colin J. Down, Christopher R. Neal, Anna Fabre-Gray, Rebecca R. Foster, Simon C. Satchell, Victoria L. Bills

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Background: There is increasing evidence of a significant glycocalyx present at the maternal-fetal interface in human placenta. Few studies have demonstrated this directly with transmission electron microscopy (TEM), as conventional processing does not allow for visualisation of glycocalyx.

Aim: To investigate a reliable technique to observe glycocalyx of the syncytiotrophoblast and fetal capillary endothelium in term human placenta with TEM.

Methods: Term human placentae were collected from uncomplicated pregnancies at caesarean section. Samples were processed in one of three ways. 1) Immersion Chemical Fixation. Placental biopsies fixed in glutaraldehyde with different cationic dyes (lanthanum nitrate and dysprosium chloride (LaDy), ruthenium red and alcian blue). 2) High Pressure Freezing. Biopsies frozen in liquid nitrogen under high pressure, followed by cryosubstitution with LaDy. 3) Perfusion Chemical Fixation. Maternal and fetal circulations of an isolated placental cotyledon perfused with a glutaraldehyde / alcian blue solution.

Results: Glycocalyx of 50 – 150 nm was demonstrated on the syncytiotrophoblast in placentae processed by immersion chemical fixation. This was consistent with all cationic dyes tested. Glycocalyx between 50 – 150 nm was observed on the fetal capillary endothelium in samples prepared by perfusion chemical fixation but not immersion fixation. Glycocalyx was inconsistent in the fetal capillaries and absent on syncytiotrophoblast in samples processed by high pressure freezing.

Conclusions: The placenta maintains a significant glycocalyx at the syncytiotrophoblast and fetal capillary endothelium in term uncomplicated pregnancy. Chemical fixation by immersion and perfusion is a reliable technique for imaging placental glycocalyx with TEM.

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Selected oral communication abstracts

OC3

Occludin Expression in Human Placental Microvessels in Pregnancies Complicated with Gestational Diabetes

Stephany D. Villota, Maria Toledo-Rodriguez, Lopa Leach
School of Life Sciences, University of Nottingham, E Floor Medical School, Queens Medical Centre, Nottingham, NG7 2UH, UK
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Introduction
The tight junctional (TJ) protein occludin is an important adhesion molecule involved in vascular maturity, restrictiveness and quiescence in human placental microvessels. Loss of junctional occludin is a feature of Type 1 and gestational diabetic (GDM) placentae. Recent studies showed several occludin (OCLN) splice variants whose translation results in isoforms with altered capability to translocate to TJ. However, it is still not known whether a change in the ratio of these splice variants is behind altered junctional occupancy in GDM placentae.

Objectives
Determine OCLN gene and protein expression in term placentae from well-controlled GDM pregnancies.

Methods
Samples from term placental microvessels were obtained from normal (n= 9), and GDM pregnancies treated by diet (n= 7) or metformin (n= 6). Gene expressions were determined by qPCR, protein expression by immunoblotting, and junctional occupancy by counting the % of CD-31+ vessels that were immuno-positive to occludin.

Results
Three OCLN splice variants were found in human placental samples. Diet-controlled GDM samples showed differential expression of gene and protein: OCLN var2 was reduced, while OCLN var3 was increased ($p < 0.05$). OCLN isoform A levels (fully functional isoform) were decreased ($p < 0.05$), and lower % of vessels showed junctional occludin ($p < 0.01$).

Conclusion
Our data suggest that, despite good glucose control, altered regulation of occludin expression may contribute to vascular dysfunction in GDM placentae.

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Selected oral communication abstracts

OC4

Diabetic Cardiomyopathy is Associated with Loss of Endothelial Glycocalyx in Coronary Microvessels and Angiopoietin 1 Restores Endothelial Glycocalyx and Corrects Cardiac Function

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Endothelial glycocalyx (eGlx) contributes to the microvascular permeability barrier and its dysfunction correlates with albuminuria in diabetic nephropathy. Albuminuria is a potent risk factor for cardiovascular disease. We therefore hypothesised that coronary microvascular eGlx damage also occurs in diabetic cardiomyopathy (DCM).

Diabetes was induced in FVB mice with streptozotocin (STZ). DCM was assessed with echocardiography by E/A ratio. A group of diabetic FVB mice received Angiopoietin 1 (Ang1) after DCM development.

FVB mice developed DCM at 7 weeks post STZ injection. Labelling with MAL-I, a specific lectin that binds to eGlx, was reduced in diabetic heart capillaries (DCM vs. ctrl: 1.64±0.27 vs. 2.70±0.27). Electron microscopy of diabetic heart capillaries showed decreased eGlx depth (14.54±0.79 vs. 27.88±5.82nm), increased perivascular space (2.08±0.22 vs. 0.54±0.11fold) and thickened endothelial cells (0.30±0.04 vs. 0.22±0.01μm).

Partial depletion of eGlx in rat hearts with the combination of heparanase and chondroitinase led to decreased cardiac output (enzymes: 63.47±10.14%; ctrl: 91.68±9.82%).

Ang1 improved diastolic function of FVB mice with DCM (DCM vs. DCM+Ang1: 104.63±7.66 vs. 135.28±8.78). In Ang1-treated diabetic mice, eGlx thickness in heart capillaries (13.87±0.87 vs. 24.55±2.02nm) and eGlx coverage (48.08±3.07% vs. 82.44±5.43%) were improved, and the increased perivascular space due to oedema in DCM normalised (2.08±0.13 vs. 0.23±0.03fold).

Thus, we have shown DCM development is associated with eGlx damage and that injury to eGlx impairs cardiac function. Recovered heart function with Ang1 treatment parallels reversal of eGlx loss. As such, correction of eGlx damage may have therapeutic potential for DCM and other diabetic vascular complications.

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Selected oral communication abstracts

OC5

Imaging the Vasculoprotective Effects of Haematopoietic Stem Cells (hscs) Following Myocardial Ischaemia-Reperfusion (ir) Injury in the Murine Beating Heart

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Introduction: The kinetics of HSC 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 (45min) and reperfusion (2hr) was performed on anaesthetised mice (Ket/Med; ip.). A 3D-printed stabiliser was attached to the beating heart to enable imaging of trafficking HSCs (CFSE), neutrophils (anti-GR-1 ab), platelets (anti-CD41 ab) and capillary perfusion (FITC-BSA). Laser speckle microscopy, performed for the first time on beating mouse hearts, was used to monitor blood flow. **Results:** Significant neutrophil ($p<0.001$) 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 HSCs freely circulated through injured hearts, their local retention was poor. Despite this, neutrophil ($p<0.001$) and microthrombus ($p<0.01$) presence was reduced resulting in a significant improvement in microcirculatory perfusion. HSCs also reduced endothelial oxidative damage ($p<0.05$) and ICAM-1 ($p<0.05$) and VCAM-1 expression as determined using a novel flow cytometry protocol on digested hearts. Laser speckle imaging demonstrated a functional hyperaemia in response to injury which was not affected by HSCs. **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 disturbances and whole heart blood flow *in vivo*. Despite poor local retention, HSCs therapeutically modified the thromboinflammatory events which benefited blood perfusion at a microvascular level.
Funded in part by the British Heart Foundation

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Selected oral communication abstracts

OC6

Diabetic Hyperglycaemic Memory: How Diabetes Drives Long Term Inflammation And Atherosclerosis After Glucose Normalisation

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Background: The mechanism by which diabetic hyperglycaemia increases atherosclerosis and cardiovascular disease risk after glucose normalisation remains unknown. We hypothesise (i) hyperglycaemia alters cellular metabolism; (ii) these changes drive long-lasting pro-inflammatory responses (iii) these increase atherosclerosis *in vivo*.

Methods and Results: Hyperglycaemia alters monocyte and macrophage metabolism, specifically the Warburg effect (FDR=0.046, non-targeted metabolomics). In vitro, hyperglycaemia increased pro- inflammatory macrophage gene expression upon lipopolysaccharide and interferon-γ stimulation (interleukin-6, $p<0.001$), decreased interleukin-4 anti-inflammatory gene expression (YM1, $p<0.005$ and FIZZ1, $p<0.05$) and enhanced both monocyte adherence to activated endothelium and macrophage uptake of modified lipid ($p<0.001$); all responses were normalised by the glycolytic inhibitor dichloroacetate (DCA). Diabetic bone marrow derived macrophages (BMDM) grown in physiological glucose mirror hyperglycaemic heightened pro-inflammatory responses, indicating hyperglycaemic memory in the haematopoietic stem cells (HSCs) as well as differentiated cells. To assess the effect of diabetic memory on atherosclerotic burden *in vivo*, control or diabetic murine bone marrow was transplanted into an atherosclerotic prone mouse model (LDLR-/-, "western" diet for 12 weeks). Analysis of atherosclerotic plaque burden showed diabetic bone marrow increased pathology ($p=0.036$) and increased plaque lipid content ($p=0.0076$), indicating augmented disease progression.

In conclusion: Diabetic hyperglycaemia alters HSC and macrophage metabolism to induce long lasting cellular changes which increases their pro-inflammatory responses and drives atherosclerotic disease *in vivo*.

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Selected oral communication abstracts

OC7

VEGF-A-mediated Spinal Cord Vasculopathy and Hypoxia; Contributing Factors in the Development of Diabetic Neuropathic Pain

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Neuropathy is a neurological complications in diabetic patients. The onset of this neurological disorder is strongly associated with a dysfunction in the neurovascular interaction within the central nervous system. This is a consequence of depletion in VEGF-A signalling. Our studies investigate a link between the loss of vasculature and the development of neuropathy.

Procedures were carried out in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986. 24 transgenic mice (C57.bl6) were used (Tie2CreER^{T2} mice were crossed with *vegfr2*$^{fl/fl)}$. All mice used were *vegfr2*$^{fl/fl}$ and either Tie2CreER^{T2} positive (n=12) or Tie2CreER^{T2} negative (n=12) and dosed once daily by i.p. with 1mg tamoxifen for 5 consecutive days. Nociceptive behavioral testing was done using VonFreys and Hargreaves test. After 8 weeks, hypoxyprobe (60mg/kg) i.p was injected 30 minutes before euthanasia in all animals. Spinal cord was collected and perfused fixed with 4% PFA. Spinal cords (40µm thickness) were stained using endothelial markers (IB_4, CD31), neuronal markers (NeuN) and Anti-hypoxyprobe. Confocal imaging of the dorsal horn of the lumbar spinal cord of all groups was performed. Imaris 8.1 software was used for 3D rendering.

Vascular Endothelial Growth Factor Receptor 2 Knockout (VEGFR2KO) mice showed an enhanced response to non-noxious stimuli, when compared to their respective controls. A reduction in blood vessels in the dorsal horn of the lumbar region of the spinal cord was observed in the KOs. The intensity and cell number of hypoxyprobe staining was increased in the dorsal horn of KOs.

Thus we show a loss in vessels and development of hypoxia, in the VEGFR2KO spinal cord is co-related to the development of neuropathy.

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Selected oral communication abstracts

OC8

Impaired skeletal muscle performance after cardiac hypertrophy is associated with microvascular rarefaction not perfusion

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Chronic heart failure is associated with the formation of skeletal muscle pathologies that drive a reduction in exercise tolerance. Using a rat model of cardiac hypertrophy (CH), we have investigated the interaction of microvascular rarefaction, muscle endurance and adaptive remodelling. CH was induced in healthy Wistar rats by surgical constriction (isoflurane anaesthesia) of the abdominal aorta. After 4 weeks, unilateral overload (OV) of the extensor digitorum longus (EDL) was imposed by synergist extirpation. Control (C) rats underwent OV only. Following a 2-week recovery period, fatigue resistance of EDL was quantified by electrical stimulation at 10Hz for 3 mins under anaesthesia (Alfaxalone). Mean femoral artery flow (ml min^{-1}) was monitored using Transonic perivascular flow probes. Heart mass (% body mass) was higher in CH (CH 0.31±0.01; C 0.27±0.01, P = 0.01) while EDL hypertrophy following OV was unaffected (CH 1.13±0.02; C 1.17±0.03, P = 0.249). Fatigue index in CH rats was reduced for both OV (CH 50.0%±3.6; C 65.0%±2.9, P = 0.03) and contralateral EDL (CH 40.7±2.3; C 50.0±3.6, P = 0.05). No effect on resting (OV P = 0.559; contra P = 0.890) or end-stimulation (OV P = 0.540; contra P = 0.308) femoral flow was detected. Quantification of capillary density (mm^{-2}) indicated that microvascular rarefaction in OV and control EDL accompanies development of CH (OV 656±31 contra 566±42; cf Egginton et al (2011) *J Physiol* 589, 195-206: OV 1038±60; C 827±79). Vessel rarefaction may impair exercising muscle by formation of localised regions of ischaemia, despite adequate arterial perfusion. We predict that increased rarefaction with disease progression may cause increased vascular resistance and reduction in perfusion, exacerbating declines in muscle performance.

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Selected oral communication abstracts

OC9

Breast Cancer Metastasis to Bone: the Role of the Perivascular Niche in Regulating Tumour Cell Dormancy

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Tumour cell dissemination to bone is an early event in breast cancer with approximately 30% of patients have disseminated tumour cells in their bone marrow at the time they receive treatment for their primary tumour. However, only a proportion of these patients develop metastatic disease, often following an extended period of dormancy. The perivascular niche within bone plays an important role in regulating the dormancy of DTCs, but the cellular and molecular components of this perivascular niche remain to be clearly defined.

We have established two *in vivo* mouse models of breast cancer bone metastasis using MB-MDA-231 cells, where breast cancer cells arriving in bone either undergo outgrowth (6-weeks old animals with high bone turnover) or enter dormancy (12-weeks old animals with mature skeleton). We use confocal microscopy, immunohistochemistry and qPCR to quantify differences in both the cellular composition and gene expression signatures in these two models, with particular emphasis on the perivascular niche.

The bone microvasculature and perivascular niche are markedly different in these two models. We show that significantly increased numbers of Thrombospondin-1$^+$ cells, vascular remodeling and reduced numbers of CD31$^+$ blood vessels, Osterix$^+$ and αSMA$^+$ osteoprogenitors, and CD169$^+$ macrophages are associated with the dormancy of DTCs in bone. We also show the differential expression of such dormancy-regulating genes as *Thrombospondin-1*, *Collagen-IV*, *Periostin*, *Tenascin-C* and *Osteopontin* between the outgrowth and dormancy-promoting niches in bone.

Our data indicate that tumour cell dormancy in bone is supported by the mature microvasculature, a reduced bone turnover and alterations in the immune system.

Funding from CRUK/EPSRC is gratefully acknowledged

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Selected oral communication abstracts

OC10

Measurement of AGEs products in the Crystalline Lens of the eye distinguishes subjects with prediabetes and Type 2 Diabetes and correlates with level of neuropathy: A Preliminary Study

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The accumulation of advanced glycation end products (AGEs) in the body contributes to pathogenesis of many diseases including complications of diabetes such as retinopathy and neuropathy. Proteins in the lens of the eye indicate the average glucose levels over a very long period of time through a unique measurable fluorescence, however non-invasive measurement of AGEs was not available until recently with introducing a new scanning confocal biomicroscope.
The main aim of the present study was to investigate if measurement of lens Auto fluorescence (FL) can distinguish subjects with impaired Glucose Tolerance (IGT) and Type 2 Diabetes (T2DM) from healthy subjects. Also to investigate the relationship between levels of FL. ratio, and corneal nerves morphology.

60 subjects including 20 IGT, 20 T2DM and 20 Healthy aged matched control subjects underwent comprehensive medical and neurological assessments including corneal confocal microscopy with using HRT-III and measurement of Crystalline Lens Autofluorescence by using ClearPath DS-120. There was a significant difference at the level of fluorescence ratios in Control subjects, IGT patient ($P=0.013$) and T2DM patients ($P<0.0001$). There was similar reduction in CNFL in IGT ($P=0.001$) and T2DM ($P<0.001$) subjects. There was a significant correlation between FL. Ratios and level of HbA1C ($r=0.496$, $P=0.01$), and CNFL ($r=-0.721$, $P<0.0001$).

The results of this preliminary study showed the Lens fluorescence is significantly greater in patients with IGT and T2DM compared to healthy subjects. The level of AGEs products was correlated with HbA1C and alterations in corneal nerves morphology. However the relationship with HbA1c was rather poor since HbA1c cannot completely reflect long-term glycation process.

Lens auto fluorescence could be a robust marker of long-term diabetes control predicting future complication risks. However, confirmation of such hypothesis will need larger and long-term clinical studies.

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Selected oral communication abstracts

OC11

The SRPK1 inhibitor SPHINX31 stabilises retinal permeability *in vitro* and *in vivo* models of diabetes

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Purpose: Microvascular damage results from ischaemia driven production of pro-angiogenic vascular endothelial growth factor (VEGF) during Diabetic Retinopathy (DR). VEGF induces angiogenesis and increased permeability in the retina. Small molecular inhibitors of serine-rich protein kinase-1 (SRPK1) have been shown to inhibit choroidal neovascularization (CNV) in mice by decreasing pro-angiogenic and increasing anti-angiogenic VEGF isoforms. SRPK1 inhibitors such as SPHINX31 may therefore switch splicing in DR and prevent increased vascular permeability.

Methods: Retinal epithelial cells were exposed to hyperglycaemia (HG) and hypoxia (Hx) and treated with SPHINX31. SRPK1 activation and monolayer permeability were assessed by immunofluorescence and Electrical Cell Impedance Sensing. Fluorescein Fundus Angiography was performed in Norway Brown rats on day 0 and 7, using the Micron IV retinal microscope. Animals received twice daily topical eye drops with control buffer or SPHINX31. On day 1 animals received a single dose of streptozotocin to induce type I diabetes. FFA was quantified using the ratio of interstitial to vascular fluorescence. An FFA time course was used to determine an estimate of permeability.

Results: HG and Hx induced a significant increase in SRSF1 phosphorylation, impeded with SPHINX31. Inhibition of SRPK1 decreased monolayer permeability in normoglycaemia but not HG. Retinal permeability was shown to significantly increase on day 7 compared to day 0 in the eye formulation control group. Following a weekly regimen of twice daily topical eye drop treatment with SPHINX31 retinal permeability stabilised on day 7 compared to day 0 and the control group.

Conclusions: SPHINX31 protected the retinal endothelial permeability barrier from diabetes-associated loss of integrity and reduced the progression. SPHINX31 may therefore be a potential alternative and more specific topical therapeutic for DR.

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Selected oral communication abstracts

OC12

Pilotassessment Of The Angiosome Concept By Intraoperative Fluorescence Angiography After Tibial Bypass Surgery

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Objectives
The angiosome concept as a model for decision-making in revascularization of patients with critical limb ischemia has fallen victim to a lively discussion in recent years. Therefore, aim of this prospective pilot study was to provide further data on the angiosome concept on the level of microcirculation after tibial bypass surgery by using intraoperative fluorescence angiography.
Design, Materials and Methods
Prospective analysis of 40 patients presenting with CLI stage Rutherford IV to VI before and after tibial bypass surgery was performed. Macrocirculation was measured by the ankle-brachial index. Skin microcirculation was assessed by intraoperative fluorescence angiography. The alteration of microcirculation was compared in direct and indirect revascularized angiosomes. Clinical follow-up investigations were performed and the wound healing rate was compared between the different revascularization methods.
Results
Cumulated microcirculation parameters showed a significant improvement after surgery (Ingress, Ingressrate $p<0.001$). Likewise, a general microcirculatory improvement was observed in each foot angiosome after revascularization, regardless of the particularly revascularized tibial artery. Furthermore, a comparison of the direct (DR) and the indirect revascularized (IR) angiosome did not show a significant difference concerning the improvement of microcirculation (difference DR-IR, Ingress: 1.69 (95%-confidence interval: -71.73-75.11), Ingressrate: 0.08 (95%-confidence interval: -12.91-13.07). The wound-healing rate was similar in both groups, although the time to wound healing was faster by on average 2.5 months in the DR group ($p=0.083$).
Conclusion
Microcirculatory improvement was recognized over the whole foot after tibial bypass operation. Therefore, the fluorescence angiography is a promising tool to evaluate the angiosome concept in larger future studies.

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Selected oral communication abstracts

OC13

Matrix metalloproteinase (mmp)-mediated syndecan (sdc) 4 loss from the endothelial glycocalyx as a therapeutic target in diabetic nephropathy(dn)

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The endothelial glycocalyx is a key regulator of vascular permeability. We have shown that MMP9-mediated SDC4 shedding contributes to glomerular endothelial cell (GEnC) glycocalyx dysfunction and increased albumin permeability in vitro.
Our overall hypothesis is that glycocalyx dysfunction in DN, caused by MMP-mediated SDC4 shedding, is amenable to therapeutic intervention.
DN was induced in DBA/2J mice by giving daily intraperitoneal (i.p) injection of streptozotocin (STZ) at 50mg/kg for 5 days. Six weeks post STZ injection, MMP2/9 inhibitor I was given daily by i.p injection at 5mg/kg for 21 days. The abdominal aorta was perfused with either Ringer solution or Alcian blue glutaraldehyde fixative. Plasma, urine, isolated glomeruli and FACS GEnC were used for either RNA or protein extraction for qPCR and ELISA respectively. Immunofluorescence and electron microscopy were also performed.
The mice became hyperglycemic at 2 weeks and significantly albuminuric at 8 weeks post STZ injection. In DN, SDC4 protein expression was significantly decreased in isolated glomeruli. This was accompanied by a corresponding increase in plasma and urine SDC4, suggesting systemic shedding of vascular glycocalyx SDC4. There was a compensatory upregulation of SDC4 mRNA synthesis in isolated glomeruli and GEnC. Sheddases MMP 2, 9 and 14 were significantly upregulated in DN. All the above coincided with an increase in albuminuria. Importantly, MMP blockade attenuated STZ-induced glomerular and systemic SDC4 shedding. It increased endothelial glycocalyx depth and coverage when compared to STZ-treated mice, resulting in significant reduction in albuminuria.
Potential therapies targeted at glycocalyx protection may be of benefit not only in DN but also in ameliorating systemic vascular disease in diabetes.

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Selected oral communication abstracts

OC14

Mineralocorticoid Receptor Inhibition In Diabetic Nephropathy Protects The Glomerular Endothelial Glycocalyx And Reduces Glomerular Permeability To Albumin.

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Mineralocorticoid receptor (MR) inhibitors reduce proteinuria in diabetic nephropathy (DN) and may slow disease progression. However, the mechanism of action is unknown and hyperkalaemia limits their utility. Proteinuria is classically caused by glomerular filtration barrier (GFB) defects. The endothelial glycocalyx, a luminal glycoprotein layer, is a key element of the GFB. We hypothesized that MR inhibition can prevent proteinuria by protecting this layer.

To induce diabetes mellitus, 16 male Wistar rats were injected with 50mg/kg streptozotocin, whilst 8 controls received citrate buffer. Blood glucose levels greater than 15mmol/L confirmed diabetes. At 4 weeks diabetic rats were randomised to receive 50mg/kg spironolactone (MR inhibitor), or vehicle, via daily subcutaneous injection. Rats were culled after 21 days treatment.

Spironolactone significantly reduced proteinuria (43 vs 21mg/mol, p=0.037) in the absence of significant changes in systemic blood pressure. An innovative glomerular permeability assay confirmed that spironolactone prevented any increase in albumin permeability in contrast to untreated rats where a significant increase was seen (1.7-fold, p=0.0008). Glomerular endothelial glycocalyx depth was measured using a novel peak to peak method (R18 (membrane) to MOA lectin (glycocalyx)). Glycocalyx depth in controls was 0.38+/-0.02µm with a significant reduction in diabetic rats 0.27+/-0.02µm p=0.0191. Diabetic rats treated with spironolactone had significantly thicker glycocalyx 0.44+/-0.03µm, a level equivalent to controls.

In summary spironolactone significantly reduces proteinuria and preserves the glycocalyx in the STZ model of diabetes. Blockade of MR activation-induced glycocalyx damage warrants further investigation as a therapeutic strategy in DN.

Funding from the Kidney Research UK Intercalated Degree Grant is gratefully received.

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Selected oral communication abstracts

OC15

Examining the Relationship Between Skin Microvascular Reactive Hyperaemia and Urinary Albumin Excretion, Extending into the Low Level Range

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Increasing urinary albumin excretion, commencing within the normal range, is a recognised risk factor for cardiovascular conditions in the general population. However, research into the relationship between early stages of vascular disease and low levels of albumin excretion is limited due to routine assays being unable to quantify low levels of urinary albumin.
Aim: to investigate the relationship between skin microvascular reactive hyperaemia and urinary albumin excretion, extending into the low level range, using a sensitive validated assay.
Methods: 186 participants were recruited (70% male; 65% with type 2 diabetes; 40% with cardiovascular disease, age range: 46-86yrs). Reactive hyperaemia (peak height and time to peak) was assessed on the dorsal surface of the foot following arterial occlusion (4mins) using laser Doppler fluximetry. Mean albumin excretion rate (AER) was reported from two overnight urine collections. AER data transformed by 1/square root, thus correlations are presented inversely.
Results: Peak height and time to peak were associated with AER (peak height: r=-0.14 p=0.051; time to peak: r=0.15 p=0.046). Further examination demonstrated that the association between peak height, but not time to peak, and AER remained after adjustment for age, gender, smoking status, HDL-cholesterol, systolic blood pressure, history of CVD, HbA1c and waist circumference (standardised beta= -0.21, p=0.008).
Conclusion: Increasing urinary albumin excretion, commencing in the previously undetectable range, is associated with an increase in the peak hyperaemic response to arterial occlusion; suggesting that increasing levels of albumin excretion is associated with a generalised impairment in microvascular autoregulation.

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Selected oral communication abstracts

OC16

Can infrared thermography be used to help assess the quality of kidneys for transplantation?

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Introduction: Kidney transplantation is the treatment of choice for end-stage kidney disease. However, with the growing demand, more marginal kidneys are being used, which carry a greater risk of ischaemia-reperfusion injury (IRI). Assessment of organ quality and predicting outcomes is still primarily based on basic donor factors and subjective visual assessment. Organ temperature during storage and reperfusion play a crucial role in IRI. Infrared (IR) thermal imaging provides a non-contact method of quantifying surface temperature. We aimed to assess whether either the average IR temperature of the kidney or the heterogeneity of temperature across the organ could be predictive of delayed graft function (DGF). **Methods:** In ten consecutive transplants, IR thermal images were taken by a non-operator using the FLIR One third-generation IR camera in the periods pre- and post-reperfusion. These images were analysed using the FLIR Tools software, with temperature readings taken at regular intervals across the visible range of the kidney. The average of these readings was then calculated, in addition to the standard deviation (SD), which was used as a measure of the heterogeneity of perfusion across the organ. These values were then compared between patients that did and did not develop DGF using t-tests. **Results:** Of the 10 patients, 5 developed DGF (50%). There were no significant differences in mean IR temperature across DGF vs non-DGF kidneys at any time point. The heterogeneity of temperature was significantly ($p<0.05$) raised at T10 in the DGF group ($p=0.02$), but this was not observed at any of the other time points. **Conclusions:** IR imaging has the potential to more objectively assess organ perfusion and may be a useful tool in testing organ viability. Further work, using larger numbers is required.

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Selected oral communication abstracts

OC17

Angiopoietin-1 Induced Protection of Glomerular Function and the Role of Hyaluronan

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Microalbuminuria, a hallmark of diabetic nephropathy, is caused by increased glomerular permeability. Angiopoietin (Ang)-1 (Desideri et al (2018) KI in press) and vascular endothelial growth factor (VEGF)C can restore barrier function to diabetic glomeruli with associated restoration of the endothelial glycocalyx (eGlx). *In vitro*, VEGFC increased hyaluronan (HA) turnover through up-regulation of HA synthesising enzyme (HAS)2 (Foster et al (2015) AJPath 183(2): 604-616). We hypothesised that Ang-1 also restored glomerular eGlx and barrier function through a HAS2 mediated increase in HA.
Glomerular endothelial cells (GEnC) were treated with ^{3}H-glucosamine (80 µCi, 48h) followed by Ang-1 (200ng/ml, 1h) and eGlx production analysed using ion exchange chromatography. HAS1-3 expression was analysed using QPCR. Isolated glomeruli from rats (60 µg/g sodium pentobarbital) and mice (3% isoflurane) were treated with a HAS-2/HA inhibitor 4-methylumbelliferone (4MU) (3mM, 20min) before addition of Ang-1 (60min) *ex vivo*. Glomerular apparent albumin permeability (Ps'alb) and eGlx depth (by lectin confocal imaging) were assessed.
Ang-1 increased the turnover of HA and the expression of HAS2 *in vitro* with no change in HAS3 or HAS1). *Ex vivo* glomerular Ps'alb was increased by 4MU. However, Ang-1 remained effective in the presence of 4MU, reducing Ps'alb to control levels.
We hypothesised that Ang-1, acting via HAS-2, would not be able to protect glomerular function in the presence of 4MU. However, glomerular permeability was restored. These results indicate that the ability of Ang-1 to protect glomerular function in diabetes may not be exclusively mediated by changes in HA. The other mechanisms through which Ang-1 restores glomerular barrier function warrant further investigation.

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Selected oral communication abstracts

OC18

Feasibility Study of Photoacoustic Imaging for Measurement of Digital Vascular Structure in Healthy Controls and Patients with Systemic Sclerosis

Murray AK, Cummings J, Zafar S, Moore TL, Manning JB, Morscher S, Dinsdale G, Berks M, Taylor C, Dickinson MR, Herrick AL.

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Systemic sclerosis (SSc) is a connective tissue disease affecting the structure and function of the microvasculature. One of the main features of SSc is digital ischaemia, which can lead to ulceration causing extreme pain, loss of hand function and a severe reduction in quality of life. Photoacoustic imaging, a combination of optical and ultrasound techniques, offers a non-invasive method for assessing vascular structure and oxygenation. This feasibility study aimed to assess photoacoustic imaging: (1) as a method to quickly and easily identify severity of digital ischaemia in clinical practice and (2) as a potential outcome measure in clinical trials.

Photoacoustic images were obtained in 32 healthy controls and 21 patients with SSc. Images were taken at the dorsal aspect of the middle phalanx on all 8 fingers. The system resolution (80 microns) allowed visualisation of digital arteries and larger microvessels. Images were post-processed and analysed for vascular volume and baseline oxygenation.

The intravascular volume of the digits was not found to be significantly different between healthy controls and SSc groups for any fingers (e.g. little finger: control, mean volume 151 [SD 20.5] mm^3; SSc, 92.0 [24.6]; p = 0.24). Baseline oxygenation (mean for all the fingers) did not differ between the two groups (control, 0.38 [0.02] arb units; SSc, 0.37 [0.02].

This study suggests that the patients with SSc in this study do not have significantly occluded digital arteries, lower digital microvascular volume or baseline oxygenation. Data on oxygenation under digital occlusion and vascular volume in those with severe digital ischaemia was also collected but is not yet analysed; this may provide further insight into suspected peripheral vascular abnormalities in SSc.

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Poster communication abstract

PC01

Endothelial Specific Silencing Of The Insulin Receptor Impairs Angiogenesis

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Introduction Insulin resistant disorders are associated with vascular complications, including inadequate angiogenesis. We set out to define the contribution of endothelial insulin resistance to this phenomenon.

Methods Developmental angiogenesis was defined in the retina of P5 mice with endothelial insulin receptor haploinsufficiency, driven by Tie2-Cre (referred to as ECIR$^{+/-}$, with Cre-negative littermates as control). *In vitro* cell-based assays assessed sprouting angiogenesis, scratch wound closure, cell adhesion and proliferation, in human umbilical vein endothelial cells transduced with insulin receptor shRNA (IR shRNA), or a control shRNA (Con shRNA). Data are expressed as mean [SEM]; $p<0.05$ is denoted by *.

Results ECIR$^{+/-}$ have reduced vascular density (46.5 [0.5] versus 44 [0.5] % vasculature/field*), branching complexity (47.7 [0.3] versus 41.7 [0.8] branches/mm^2*) and tip cell formation (21.5 [0.2] versus 18.7 [0.3] tip cells/mm*) at the emerging vascular front in the developing retina. *In vitro* sprouting from covered Cytodex microcarrier beads showed a reduction in sprout number with IR shRNA (6.5 [1.2] versus 3.4 [1] sprouts/bead*). Migratory wound closure was also diminished with IR shRNA (84.7 [4.7] versus 74 [3.7] % wound closure*), along with a reduction in cell adhesion (95.9 [8.7] versus 59.3 [8.4] cells/field*) and a trend towards reduced proliferation (population doubling times of 1.8 [0.1] versus 2.8 [0.4] days).

Conclusion Endothelial specific silencing of the insulin receptor is associated with abnormal angiogenesis *in vivo* and *in vitro*, along with deficits in cell migration, adhesion and proliferation. Additional studies are required to unravel the underlying mechanisms of these observations, and define whether therapeutic intervention is feasible.

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Poster communication abstract

PC02

Inflammatory Cytokines Implicated in Pulmonary Arterial Hypertension Development Upregulate Plasma Membrane Calcium ATPase 1 Gene Expression in Vascular Smooth Muscle Cells

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Pulmonary arterial hypertension (PAH) is a chronic, life-threatening and multifactorial disease characterized by a progressive narrowing and occlusion of small pulmonary arteries leading to increased pulmonary resistance and finally right ventricular failure. Experimental research has identified increased activity of pro-inflammatory cytokines in PAH development. Abnormal proliferation and migration of pulmonary arterial smooth muscle cells (PASMCs) play a pivotal role in the vascular remodelling characteristic of PAH. This is modulated by changes in cytoplasmic calcium levels. Thus, factors that regulate cytoplasmic calcium concentrations have emerged as crucial elements in PAH progression. The Plasma Membrane Calcium ATPase (PMCA) proteins extrude calcium from the cytosol to the extracellular environment and in so doing, actively participate in regulation of calcium homeostasis. Here, we investigated the effects of proinflammatory cytokines on the expression of *PMCA* genes in PASMCs. Treatment with TNF-□, and IL-1□ induced a significant increase in the RNA levels of *PMCA1*. Other inducers of PAH; Platelet Derived Growth Factor (PDGF) and monocrotaline also increased *PMCA1* gene expression *in-vitro* and *in-vivo.* Previous studies on *PMCA1* gene expression have identified functional binding sites for the transcription factors NFAT and NFKB in the *PMCA1* promoter region. We show here that cytokine dependent upregulation of *PMCA1* transcriptional expression does not involve binding of these factors to the *PMCA1* proximal promoter region. No significant changes were observed in the RNA levels of *PMCA4*, the other major PMCA isoform expressed in PASMCs. These results suggest an important role for PMCA1 in PASMC deregulation during PAH. Further functional studies will provide a full understanding of the role of PMCA1 on PAH development and progression.

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Poster communication abstract

PC03

Effects of Early Goal Directed Therapy on Endothelial Glycocalyx Integrity in Septic Compared with Non-septic Shock Patients in the Intensive Care Unit

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Background: Perturbation of the endothelial glycocalyx contributes to vascular dysfunction and the pathogenesis of sepsis. Early Goal-Directed Therapy (EGDT), involving intensive monitoring of specific EGDT circulatory parameters, may reduce mortality in sepsis patients compared with standard care. However, the effects of EGDT on glycocalyx integrity are unclear.

Method: Video images of sublingual microvessels were recorded using Sidestream Dark Field videomicroscopy at baseline and post EGDT in controls (n=6) and sepsis patients (total, n=19; survived, n=13; non-survived, n=6). Glycocalyx integrity was assessed by measurement of the standard perfused boundary region (PBR) and a new PBR parameter (corrected for flow differences in vessels with 5-25μm erythrocyte column width, 'corrected PBR') derived from GlycoCheck software. Perturbed glycocalyx has an increased PBR, a measure of glycocalyx permeability to erythrocytes.

Results: Non-significant trends with decreased PBR in microvessels were observed in control (2.61(±0.41)μm to 2.58(±0.30)μm) and all sepsis patients (2.65(±0.41)μm to 2.58(±0.41)μm) post EGDT vs. baseline. In contrast, corrected PBR increased in sepsis patients post EGDT vs. baseline (total, 2.00(±0.43)μm to 2.13(±0.51)μm; survived, 1.90(±0.42)μm to 1.94(±0.26)μm; non-survived, 2.21(±0.41)μm to 2.52(±0.72)μm) whilst a decrease in corrected PBR was observed in controls (2.18(±0.54)μm to 1.67(±0.40)μm).

Conclusion: The differences in glycocalyx response to EGDT in control and sepsis patients with adjusted flow differences in microvessels may reflect different levels of microvascular damage in sepsis compared with the non-septic condition, however, further investigations are required to confirm these observations.

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Poster communication abstract

PC04

The Potential Proangiogenic Role Of Galectin-1 In Human Omental Endothelial Cells

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Background: Ovarian cancer often metastasises to the omentum where the omental microvascular endothelial cells (HOMECs) undergo pro-angiogenic changes in response to factors secreted from the metastasised ovarian cancer cells. We previously showed that factors other than vascular endothelial growth factor (VEGF) may be responsible for these changes e.g. cathepsin L (CathL). Subsequent studies indicated that exogenous CathL increased HOMEC angiogenic activity *in vitro,* possibly by stimulating HOMEC secretion of galectin-1. Galectin-1 is a glycoprotein implicated in several cancers, although its role in ovarian cancer is unclear.
Aim: To examine whether galectin-1 secretion and resultant autocrine signalling induced pro-angiogenic changes in HOMECs.
Methods: HOMECs were isolated from omental tissue donated during elective surgery. Proliferation and migration were examined by WST1 (metabolic activity)/BrdU incorporation assays and scratch assays respectively. Galectin-1 localisation in HOMECs following CathL treatment was studied by immunocytochemistry.
Results: Intracellular galectin-1 in CathL treated cells was significantly decreased after 30 minutes from 13.2±4.4 to 9.5±2.1 ($p=0.0142$), and increased after 8 hours from 7.9±2.3 to 13.2±3.6 ($p=0.0028$). Extracellular galectin-1 in treated cells decreased after 10 minutes from 20.2±6.5 to 13.6±7.7 ($p=0.04$) (all $n=4$, AU 1 x 10^4). Galectin-1 (1-125nM) induced in a significant increase in proliferation after 24 hours, at all concentrations, using both WST1 and BrdU assays ($p=0.001$, $p<0.0001$ respectively). Galectin-1 did not significantly alter HOMEC migration.
Conclusions: These data suggest that CathL affects galectin-1 localisation in HOMECs, and that galectin-1 induces HOMEC proliferation, a key step in angiogenesis.

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Poster communication abstract

PC05

Glucagon-like Peptide-1 Analogues Increase Retinal Microvascular Endothelial Cell Proliferation

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Aim: Glucagon-like peptide-1 (GLP-1) is an incretin hormone that stimulates insulin release to reduce glucose levels following nutrient ingestion. GLP-1 analogues are being increasingly prescribed for the management of glycaemic control in patients with type 2 diabetes (T2DM). Recent studies have shown GLP-1 stimulated proliferation and differentiation of endothelial progenitor cells through upregulation of vascular endothelial growth factor (VEGF). However, there are currently no data for direct proliferative effect of GLP-1 analogues on microvascular endothelial cells in the retina. Our study attempts to determine this.
Methods: Relative cell cycle progression of human retinal microvascular endothelial cells (hRMECs) was assessed after being treated with therapeutic concentrations of exenatide and liraglutide (100pM and 10nM, respectively). Bromodeoxyuridine (BrdU), a thymine analogue, was added to the hRMECs to incorporate into newly synthesised DNA strands of actively proliferating cells. After 24 hours, the double stranded DNA was partially denatured and the BrdU was immunochemically detected allowing for the assessment of cells which were synthesising DNA through proliferation.
Results: A significant ($p<0.0001$) increase in cell proliferation was observed in hRMECs treated with exenatide and liraglutide (209±129% and 260±186%, respectively (n=17)) when compared to controls (100%).
Conclusion: These results indicate that GLP-1 analogues significantly increase hRMEC proliferation. Whether this may have harmful (e.g. exacerbate the progression of proliferative diabetic retinopathy), or beneficial effects (e.g. increase capillary density in diabetic ulcers) in patients with T2DM is unclear. Further research needs to be conducted to determine these effects.

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Poster communication abstract

PC06

Confluency of Human Bone Marrow Endothelial Cells Modulates Their Interactions with Breast Cancer Cells Measured by Atomic Force Microscopy (AFM)

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Purpose: Endothelial adhesion is essential for cancer cell extravasation in metastasis. However, how the integrity of the endothelial cell (EC) barrier influences cancer-EC interaction is still controversial. The hypothesis of this study was that formation of the EC monolayer influences cancer-EC adhesion. **Methods:** Human bone marrow ECs (HBMEC-60) were cultured as non-, sub- or fully-confluent and scratch-migrating to mimic different situations of the barrier integrity. A single human breast cancer cell, MDA-MB-231 (MB231) was attached on the tip of an AFM cantilever and brought into contact with an HBMEC-60 cells for cell-cell contact periods of 0.5, 10 and 60 sec. Force curves were recorded upon cantilever retraction and used to calculate adhesion forces. **Results:** The highest total adhesion forces with MB231 were seen in non-confluent HBMEC-60 cells (200.1±27.0, 649.7±76.7 and 1268.1±143.2 pN for 0.5, 10 and 60 sec contacts, respectively), while the lowest were seen in the full-confluent cells (74.2±10.0, 255.0±38.5 and 348.4±51.7 pN for 0.5, 10 and 60 sec contacts, respectively. Adhesions of the sub-confluent ECs were in between. Adhesions in migrating HBMEC-60 were the same as those in the non-confluent for 0.5 sec contact (199.0±29.1 pN), but between the non- and sub-confluent for longer contact periods (481.4±56.2 pN for 10 sec and 765.8±90.2 pN for 60 sec). **Summary:** 1. The data suggest the importance of an intact EC barrier in preventing metastasis; 2. In adhesion with cancer cells, a single migrating EC in wound healing does not have the same behavior as a single non-confluent cell. 3. Further studies to identify the role EC junctions regulate cancer-EC adhesions are required.

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Poster communication abstract

PC07

OxLDL Regulated Angiogenesis Is Potentially Mediated via S1P

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Sphingosine-1-phospahte (S1P) is a bioactive lipid implicated in a wide variety of diseases from cancer to atherosclerosis. New blood vessel formation via angiogenesis and the regulation of endothelial function are important factors in inflammation, cardiovascular disease and cancer spread. Lipids are potential regulators of the endothelium and we have shown that oxidised low-density lipoprotein (oxLDL) controls angiogenesis via a potential bioactive lipid signalling mechanism. We hypothesise that oxLDL activates endogenous S1P signalling and by modifying endogenous and exogenous lipids, we aim to find novel lipid targets that regulate angiogenesis. We are therefore investigating the role S1P plays in angiogenesis, endothelial migration and intracellular signalling.

Using exogenous S1P in in vitro angiogenesis assays, we observed that at high concentrations (10μM), S1P prevented endothelial migration, sprouting and angiogenesis, mimicking the effects of high oxLDL concentrations. S1P also caused the phosphorylation of Extracellular signal-regulated kinase (ERK 1/2), confirming previous work targeting upstream kinases in endothelial migration. Fingolimod, an analogue of the S1P precursor sphingosine used to treat multiple sclerosis, caused a deranged migration response suggesting perturbation of the S1P signalling cascade can regulate endothelial functions. Interestingly, receptor antagonists of the three S1P receptors had varying effects on regulating ERK phosphorylation, suggesting different receptors link to distinct downstream pathways or that S1P preferentially binds to one receptor over the others to control angiogenesis.

This study will provide insight into how bioactive lipids regulate endothelial function, providing a basis for future drug discovery programmes.

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Poster communication abstract

PC08

Free Flap Autonomy After Combined Defect Reconstruction Using AV Loop And Free Tissue Transfer

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Objective:
Transplantation of free flaps in combination with arterial reconstruction by means of arteriovenous (AV) loops or bypass grafts has meanwhile been established as a feasible therapeutic option in defect reconstruction for body areas without suitable recipient vessels, Against this background, our aim was to analyze the long-term performance, flap autonomy as well as the flap perfusion pattern after combined reconstruction.
Patients and Methods:
Patients receiving a combination of arterial reconstruction and free flap transplantation at a single center institution between the years 2004 and 2015 were included. During follow-up examination, patency of arterial reconstruction was investigated by duplex ultrasound. Flap microcirculation was assessed by a combination of laser Doppler flowmetry and white light tissue spectrometry (O2C) (parameters: sO2 and Flow) as well as by indocyanine green (ICG) fluorescence angiography (parameters: Ingress and Ingressrate).
Results:
23 patients could be clinically followed-up. Duplex ultrasound showed in four cases arterial pedicle occlusion in spite of vital flap. Comparison of the O2C perfusion parameters between those flaps with occluded pedicles and those with intact inflow showed no significant difference (parameters sO2: p=0.823; Flow: p= 0.314). Similar results were obtained by ICG fluorescence angiography; even here no significant difference could be detected between both groups (parameters Ingress p=0.130; Ingressrate p=0.539).
Conclusion:
Combined vascular reconstruction with free tissue transfer is a feasible option for body areas without suitable recipient vessels; it is associated with a good long term outcome. Even after flap transplantation to areas with critical tissue perfusion, such as the CLI, the flap can develop autonomy and thus, survive after pedicle occlusion.

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Poster communication abstract

PC09

The Role of the Systemic Microcirculation in Peritoneal Dialysis

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Background
Peritoneal dialysis (PD) is a treatment for patients with end stage renal disease. It utilises the semi-permeable peritoneal membrane as a dialyser through which uraemic solutes and excess fluid pass from the blood into the dialysate. The rate of this 'transport' is measured clinically using the Peritoneal Equilibration Test (PET), the ratio of creatinine in the dialysate compared with plasma after 4 hours. The rate of solute movement influences dialysis prescription and has prognostic implications. Transport rate exhibits significant inter-subject variability and cannot be predicted non-invasively priot to treatment. Experimentally the capillary wall of the peritoneal microcirculation appears to be the main barrier to solute movement. Patients with end stage renal failure have impairments in both the structure and function of their systemic microcirculation compared with healthy controls.

Research Question
Is peritoneal transport rate at baseline associated with structural or functional alterations in other microvascular beds?

Methods
In this study, 46 patients newly started on PD (<6 months) are being recruited. The results of their PET, conducted routinely in all new patients, will be compared to non-invasive measures of microcirculatory structure and function in dermal, retinal and sublingual vascular beds.

Impact
Insights gained from this study will add to our knowledge of the state of the systemic microcirculation in this high risk cohort and how it relates to movement of small solutes across the peritoneal vascular wall. Improved understanding of this relationship will aid development of therapies aimed at protecting the peritoneal microcirculation and improving the quality of dialysis received by patients.

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Poster communication abstract

PC10

A Novel Heparanase Inhibitor Protects Glomerular Endothelial Glycocalyx During Diabetes Mellitus

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An estimated 647 million people worldwide will have diabetes mellitus (DM) by 2040, which causes life altering microvascular complications, like diabetic nephropathy (DN) and retinopathy (DR). The endothelial glycocalyx (eGlx) contains proteoglycans (core proteins 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, an HS degrading enzyme, is upregulated during diabetes (Shafat et al, (2011), *PLoS1*, 6(2):e17312). We hypothesise that heparanase inhibition will prevent eGlx damage and associated microvascular permeability. Db/db mice (type 2 DM) were administered a novel heparanase inhibitor (HI) from 9-11wk of age. Anaesthetised mice (2.5 % isoflurane, 1.5 liter/minute) were cardiac perfused with Ringer's solution, to flush kidneys before using an *ex vivo* glomerular permeability assay, and then glutaraldehyde and Alcian blue for electron microscopy (EM). Db/db mice were diabetic and proteinuric and had significant changes to glomerular filtration barrier ultrastructure (quantitative EM). HI significantly reduced apparent albumin permeability (Ps'alb) in glomeruli isolated from db/db mice and EM quantification showed significant increase in eGLX thickness and coverage. Increased eGlx thickness correlated with decreased Ps'alb. Future work will investigate retinal eGlx depth from these mice. In summary, inhibition of heparanase during DM restores eGLX structure and function during DN, suggesting potential for this novel HI as a therapeutic treatment during DM microvascular complications.

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Poster communication abstract

PC11

Adiponectin Protects the Endothelial Glycocalyx from TNF-α Induced Disruption in Human Glomerular Endothelial Cells

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The glomerular endothelial glycocalyx is a hydrated mesh of proteoglycans, glycosaminoglycan (GAG) chains, and sialoglycoproteins that determinesvascular permeability through the selective sieving action of the capillary wall. Damage to the glycocalyx leads to an increase in albuminuria in disease states for example in diabetes.
Adiponectin is a hormone secreted primarily by adipocytes and known to have anti-inflammatory and protective effects on vascular endothelial cells. I have previously found that it activates these pathways in cultured glomerular endothelial cells (GEnC). Adiponectin also protects against the development of albuminuria including in diabetes. Therefore, we hypothesised that adiponectin would protect the GEnC glycocalyx from damage induced by the inflammatory mediators implicated in diabetes.
We used an in vitro model in which tumor necrosis factor alpha (TNFα) causes disruption to the glycocalyx in human conditionally immortalised GEnC. By quantitative PCR (qPCR) and Western blotting, we showed that there was an upregulation of syndecan-4 (SDC4) mRNA (2.8 fold) and protein levels (1.4 fold) in response to 2-hour treatment with 10ng/ml TNF-α. Adiponectin prevented this increase. There was also a significant increase in MMP2 and MMP9 mRNA expression in response to TNF-α but not in cells co-treated with adiponectin. Sulfated GAG in the culture medium were significantly increased by TNFα, suggesting increased glycocalyx component shedding, and again this was prevented by adiponectin treatment.
These findings show that adiponectin protects the glycocalyx in vitro through direct actions on GEnC and suggests that the decrease in albuminuria in diabetic models may be due to glycocalyx protection.

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Poster communication abstract

PC12

Investigating a Novel Role for the Il-36 / Il-36r Pathway in Mediating Inflammation in the Young and Aged Murine Heart After Ischaemia-Reperfusion Injury

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Introduction: Although reperfusion of the ischaemic heart is essential, extensive myocardial damage occurs by up-regulating inflammatory processes, a process termed ischaemia-reperfusion (IR) injury. Ageing renders the heart more susceptible to IR injury, which contributes to poorer prognosis in the elderly. Mechanisms contributing to these inflammatory events, and their varying response in the aged, are not yet fully understood. A new member of the interleukin-1 (IL-1) cytokine family, IL-36, has recently emerged as a strong promoter of inflammation in many diseases. However, its role in heart disease is currently not known. **Methods:** Expression of the IL-36 receptor (IL-36R) was investigated immunohistochemically on adult and aged murine heart tissues, vena cava endothelial cells (VCECs) and neutrophils. The effects of IL-36γ, one of three IL-36 isoforms (α, β and γ) on VCEC adhesion molecule expression and inflammatory cytokine production (IL-6, IL-1β) were investigated using ELISA. Neutrophil adhesion to VCECS was investigated *in vitro*. The impact of topical IL-36α and IL-36γ on neutrophils was also imaged intravitally within the beating mouse heart. **Results:** We are the first to identify cardiac IL-36R expression, its co-localisation with vasculature through co-staining with CD31 and, importantly, a significant up-regulation with ageing ($p<0.01$) and IR injury ($p<0.001$). Basal expression of IL-36R was noted in VCECs which increased after TNFα stimulation. Increased VCAM-1 was observed on IL-36γ stimulated VCECs as well as dose dependent increases in IL-6 release over 48hrs. Neutrophil adhesion to VCECs ($p<0.001$) increased with IL-36γ priming. Topical application of either IL-36γ or IL-36α increased neutrophil recruitment within beating hearts with striking effects demonstrated with the latter. **Conclusion:** These novel results indicate a pro-inflammatory influence of IL-36 isoforms on cardiovascular microvasculature both *in vitro* and *in vivo*. Further studies will determine whether the increased expression of IL-3R explains the increased susceptibility of aged hearts to IR injury.

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Poster communication abstract

PC13

Investigating the Impact of 2D vs 3D Culture and Tissue of Origin on the Adhesive Capacity of Mesenchymal Stem Cells *In Vitro* and to the Beating Mouse Heart *In Vivo*

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Background: Systemic injection of MSCs is a promising approach to treat myocardial infarction. However, clinical success is hampered by poor retention and survival of MSCs in ischaemic hearts. This may be due to culture conditions affecting adhesive potential or an inability to identify sub-populations with greater therapeutic efficacy. Although 2D culture is more traditional, 3D culture better resembles the natural *in vivo* environment. We evaluated whether culture technique or the tissue of MSC origin influenced their adhesion. Human Wharton's jelly MSCs were compared to bone marrow-derived sub-populations. Specifically, these were MSCs identified as likely perivascular (pv) niche cells and highly OAC differentitating or non-differentiating but highly immunomodulatory. **Methods:** MSCs were cultured using 2D methods or a 3D hanging drop technique. Adhesion to vena cava endothelial cells (VCECs) , ICAM-1/VCAM-1 and heart sections was tested *in vitro*. Homing to the beating mouse heart was imaged intravitally. Adhesion molecule distribution was investigated confocally. **Results:** All 3D cultured cells were significantly ($p<0.05$) smaller and showed significantly ($p<0.05$) improved adhesion to VCAM-1, healthy and injured cardiac tissue. Only pv-MSC adhesion to VCECs was significantly ($p<0.05$) enhanced. Although 2D culture clustered adhesion molecules (CD54/CD44) on one side of the cell, 3D cells expressed them uniformly over the whole cell surface. 3D culture also improved first-pass homing and subsequent adhesion of pv-MSCs within the healthy beating heart myocardial microcirculation *in vivo*. **Conclusion:** 3D cultured MSCs may be more suited for systemic delivery to the injured heart. Importantly, MSCs showed both inter- and intra-organ adhesive heterogeneity. The vasculoprotective consequences of this are yet to be determined.

Funded by a Thai Government Studentship to Miss Kobkaew Bumroongthai

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Poster communication abstract

PC14

Interplay between Fluid Shear Stress and the Glycocalyx in Regulation of Redox Homeostasis in Human Endothelial Cells

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The redox state of vascular endothelial cells (EC) is regulated by the hemodynamic fluid shear stress (FSS) acting on the vessel wall. Pro-oxidant EC phenotypes are native to complex vascular geometries exposed to low and oscillatory (OS) FSS, whereas high unidirectional FSS (US) is athero-protective. The redox-sensitive transcription factor nuclear factor E2-related factor 2 (Nrf2) regulates antioxidant gene expression, and its activity is enhanced in response to US. The glycocalyx (GCX), a rich meshwork of glycosaminoglycans, decorates the luminal surface of EC in athero-protected vascular regions, however is scarce in athero-susceptible areas. An abundant EC GCX is necessary for physiological nitric oxide and reactive oxygen species (ROS) production during FSS mechanotransduction. This study investigates the effects of FSS on Nrf2 signalling and the GCX, focusing on the role of the GCX as a mediator of redox signalling by US. Human umbilical vein EC (HUVEC) were subjected to either US (15 dynes/cm^2) or OS (±5 dynes/cm^2, 1Hz) for 48h using microfluidic slides (Ibidi GmbH) or grown in static conditions. The sialic acid (SA) component of the GCX (Wheat Germ Agglutinin fluorescence) and expression of the Nrf2-regulated antioxidant enzyme heme oxygenase-1 (HO-1) were differentially regulated by US and OS compared to static cultures. Removal of SA with Neuraminidase (Neur, 2U/ml, 30min) prior to US exposure abrogated HO-1 induction and enhanced mitochondrial ROS production (MitoSOX Red fluorescence) in response to FSS. Taken together, these findings demonstrate that FSS-sensitive Nrf2 signalling is dependent on mechanotransduction through the GCX. Enhancement of the GCX may therefore restore physiological redox homeostasis in atheroprone regions.
This work is funded by Biotechnology and Biological Sciences Research Council (BBSRC) and Unilever, UK.

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PC15

Evaluation of Protocols for Assessment of Flicker Induced Vasodilation

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Flicker induced vasodilation (FIV) assesses retinal vascular function; a flickering light stimulates an increase in metabolic demand, resulting in retinal vessels dilating. Though FIV is a well-known assessment there is no universal protocol.

This study examined 1: vessel selection (superior(SC) vs inferior central(IC) and 2: differing flicker intervals to inform protocol development.

Methods 1: Diameter of SC and IC vessels were continuously recorded during manufacturer's standard flicker protocol (3X 20sec flicker, 80sec recovery period between flickers) in 6 participants. FIV reported as mean of 3 FIV responses (%increase from min diameter during 30sec prior to flicker and peak diameter during flicker/10secs after) for artery (aFIV) and vein (vFIV). 2: Diameter of SC vessels were recorded during a standard (described above) and extended flicker protocol (1 cycle of 40sec) in 3 participants on 3 occasions. Extended protocol FIV was calculated as %increase from min diameter during 30sec baseline and peak diameter recorded within flicker and 10sec after.

Results 1: FIV were similar between SC and IC vessels (aFIV mean(SD): 6.3(1.70) vs 6.5(3.42); vFIV: 6.5(3.3) vs 7.2(2.59) respectively). 2: FIV were similar between protocols for each participant (standard and extended protocols respectively). P1, aFIV 6.4 & 12.2%, vFIV 9.6 & 5.6%; P2, aFIV 12.9 & 10.9% and vFIV 7.4 & 5.8%; P3, aFIV 9.4 & 19.4% and vFIV 7.5 & 7.6%. Intra-participant coefficients of variations ranged between 5-65% and 11-77% for aFIV and 4-40% and 27-37% for vFIV.

Preliminary data suggest that FIV in SC and IC are similar, though participants favoured superior selection. FIV and its variability were similar for the two flicker protocols.

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PC16

Association Between Beta-Amyloid, Diabetes and Endothelial Function in Humans

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Aims: β-amyloid production, via BACE1 activity, results in the formation of amyloid plaques, a hallmark pathology in Alzheimer's disease (AD). β-amyloid may also play a role in type 2 diabetes and cardiovascular disease progression. We have examined the association between plasma $A\beta_{42}$ levels and endothelial function in patients with type 2 diabetes.

Methods: Endothelial function was measured using an EndoPat©. Reactive hyperemia index (RHI) was calculated as a ratio of the post-occlusion to pre-occlusion signal. Human plasma $A\beta_{42}$ was determined in duplicate using the Simoa HD-1 analyzer and human $A\beta_{42}$ assay kit.

Results: The type 2 diabetes (n=220) and control (n=127) groups were matched for age, mean arterial blood pressure and gender. Body mass index was greater in patients with type 2 diabetes (32.5±5.67 and 28.4±4.3, respectively, P=0.001). Duration of diabetes was 11.2±7.4 years. $A\beta_{42}$ levels were greater in patients with type 2 diabetes versus controls (12.9±4.3 and 10.8±3.9 pg/ml, P<0.001). There were significant correlations between $A\beta_{42}$ levels and duration of diabetes (r=0.23, P=0.001), HbA1c (r=0.19, P=0.001) and endothelial function RHI (r=-0.14, P=0.008).

Conclusions: We previously reported that serum β-amyloid levels negatively correlate with endothelial function and NO signaling in mice. We now show that the same correlation is present in humans. With elevated plasma β-amyloid levels found in patients with type 2 diabetes, this could be a novel biomarker for early diagnosis of associated vascular dysfunction. Furthermore BACE1 inhibition could be a novel therapy for vascular dysfunction in type 2 diabetes.

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PC17

Examining the Contribution of Inflammatory Markers Towards the Relationship Between Obesity and Microvascular Dysfunction: a Cross-Sectional Study

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As the prevalence of obesity continues to increase, concerns are raised about the future burden of comorbidities, e.g. cardiovascular disease (CVD). Vascular dysfunction is an early marker for CVD, and is described in obesity. The pathophysiological mechanisms underlying obesity, vascular dysfunction and CVD are not fully understood, but inflammation has been implicated. This study investigated potential inflammatory mediators through which obesity may contribute to microvascular dysfunction.

236 participants (65% male, age range: 45-86 years, BMI range: 18-47, 71% type 2 diabetes, 46% CVD) were recruited. Body composition (BMI, waist circumference and % body fat), microvascular function (maximum hyperaemia and endothelial (in)dependent vasodilation) and inflammatory markers (interleukin-6 (IL-6), IL-1 receptor antagonist (IL-1ra), leptin and growth/differentiation factor 15 (GDF15)) were assessed. Mediation analysis examined the direct effect of obesity on vascular function, the indirect/mediated effect via proposed mediators and the total effect (indirect and direct), after adjusting for potential confounders: age, gender, blood pressure and diabetes. Inflammatory factors were considered significant contributors if they accounted for >20% of the total effect.

Leptin mediated the effect of obesity on all microvascular function tests after adjusting for confounders (e.g. for the relationship between BMI with maximum hyperaemia the total and indirect regression coefficients (β) were -2.91 and -0.92, respectively). After adjustment, IL-1ra mediated the effect of BMI on endothelial dependent vasodilation (total and indirect β: -5.42 and -1.48).

This study suggests inflammatory factors leptin and IL-1ra contribute to the relationship between obesity and microvascular function.

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PC18

Pericyte mediated reduction in spinal cord blood flow in diabetic neuropathic pain

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The role that the neurovascular network within the spinal cord plays in regulating nociception has not been investigated; especially in neuropathic pain. We have recently identified that blood vessels in the spinal cord of diabetic animals are narrower than in non-diabetic animals, and that this was accompanied by development of pain. We hypothesise that this reduction in vessel diameter could be a result of vasoconstriction, related to changes in the cells surrounding these vessels (pericytes) due to alterations in the hormone angiotensin II, and activation of its receptors. A rodent model of type 1 diabetes was induced in Female Sprague dawley rats (~200g) (n=5/group). Streptozotocin (intraperitoneal 50mg/kg) was administered and animals were insulin supplemented. All studies were carried out with age matched controls. Animals body weight was monitored and levels of blood glucose determined (hyperglycaemia>15mmol/l). All Experiments were designed in accordance with UK Home Office legislation and ARRIVE guidelines. 8 weeks following streptozotocin administration animals were terminally anaesthetised (intraperitoneal 60mg/kg Sodium Pentobarbital) and cardiac perfused with 4% paraformaldehyde. Lumbar spinal cords were extracted and processed for confocal microscopy. Spinal cord sections were incubated in primary antibodies including CD31, NG2, PDGFR and AT1R. In diabetic animals there was a significant reduction in vessel diameter in the spinal cord versus age matched controls. Furthermore, this vasconstriction in diabetic animals was significantly prevalent when in close proximity to pericytes (AT1R positive). This demonstrates that pericyte function has a role in modulating the neurovascular network and pain.

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PC19

Imaging Blood Brain Barrier Permeability in Alzheimer's Disease Mouse Models

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The cerebral microvasculature is surrounded by endothelial cells forming the blood brain barrier (BBB), a functional and structural barrier separating the circulation from the CNS parenchyma. In Alzheimer's disease (AD) the stability and function of the BBB deteriorates and is associated impaired Aß efflux and leakage of blood-derived molecules, which accelerates the disease progression.
Our aim was to quantify the BBB breakdown in mouse models of tauopathy (rTg4510) and amyloidopathy (J20). We compared quantitative analysis of BBB breakdown *in vivo* by imaging the extravasation of a fluorescent dye with *post mortem* analysis using Evans Blue.
A cranial window above the right somatosensory cortex was secured in male rTg4510, female J20 mice and wild-type (WT) littermate controls. Longitudinal *in vivo* two-photon imaging was carried out in anaesthetised mice at different ages. 15 min prior to imaging, fluorescein-labelled dextran and sulforhodamine B were injected i.v. in the tail vein for blood vessel visualisation. After the last imaging session 20 mg/kg of 2% Evans Blue was injected i.p. and transcardiac perfusion was performed 24h later. Formalin-fixed brains were sliced, photographs taken and two-photon imaging carried out.
There were no differences in the BBB permeability between J20s, rTg4510s and WT littermate controls with increasing age. This was shown by *in vivo* two-photon imaging of dye extravasation and confirmed by *post mortem* analysis of the brain slices using Evans Blue. Preliminary results indicate no difference in the total blood vessel volume in these animals.
Our results suggest that the BBB in not impaired in the J20 and rTg4510 mouse model and that the blood vessel volume is not altered in these animals.

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PC20

Characterisation of Fast Muscle Phenotype of Outbred and Inbred Rats

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Skeletal muscles contain different fibre types with specific contractile and metabolic properties. Composition is usually heterogeneous, typically utilising Type IIa and IIb fibres for phasic activity and Type I to maintain posture. Genetic background may contribute to biological variability in fibre type composition of individual, thought to be higher in outbred than in inbred strains. This study compared fibre type composition and capillarity between wild (Brown) and inbred (Wistar) rat strains in extensor digitorum longus (EDL) muscle. Wistar (n=3) and Brown (n=3) rats with body mass 207±3g and 209±2g, respectively, were sacrificed and cryosections (10µm at -20°C) stained to identify capillaries (*Griffonia simplicifolia* lectin I, Vector) and fibre type (monoclonal anti-MHC antibodies; BA-D5 for Type I fibre and SC-71 for Type IIa fibre, DSHB, University of Iowa) and visualised (20x magnification) under fluorescent illumination (Nikon E600), with systematic-random sampling. Capillary to fibre ratio (C:F), capillary density (CD, mm^{-2}), and mean fibre area (MFA, μm^2) were determined using in-house morphometric analysis programmes. Globally, MFA was greater in Brown than Wistar rats (1388±16 *vs.* 1108±93 µm², $P<0.05$), while capillary density (CD) and capillary to fibre ratio (C:F) were not significantly different. Type I numerical density (N_N) of Brown rat EDL was higher (0.058±0.003 *vs.* 0.038±0.003, $P<0.05$) and Type IIb lower (0.67±0.01 *vs.* 0.75±0.02, $P<0.05$) than Wistar rat. Areal density (A_A) of TI fibres was higher in Brown than Wistar rat (0.038±0.001 *vs.* 0.023±0.003, $P<0.01$). These data suggest that Brown rat locomotor muscles are more aerobic than those of Wistar rats, potentially increasing exercise tolerance.

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PC21

Synthesis of Heparin and Heparin mimics-Engineered Nanoparticles for Nanomedicine Applications

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Heparin, heparin derivatives and heparin conjugated nanoparticles (NPs) maintained within the circulation could be useful tools for example: By inhibiting cancer metastasis *via* blocking growth factors such as vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF); or detection of biomarkers. Heparin reduces heparanase enzyme activity facilitating tumour cell migration. Herein, heparin and two structurally related polymers, chondroitin and alginate serving as controls were grafted to the surface of silica NPs by coupling of amine functionalised NPs to the activated polymers using EDC as a coupling agent. Polymer grafted NPs had average particle size ~ 200 nm and negative zeta potential values (~ -56) compared to the positive value (~ +31) of amine functionalised NPs. Heparin, chondroitin and alginate conjugated NPs were fluorescently labelled with different fluorophores; TAMRA, FAM and Alexa 633, respectively, in order to investigate their cellular effects in a single experiment. Preliminary cell uptake studies of these functionalised nanoparticles were conducted using human umbilical vein endothelial cells (HUVEC). Both confocal microscopy and flow cytometry data showed that all NPs types were up-taken by the cells. Chondroitin and heparin conjugated nanoparticles were found to be co-localised within the same cellular vesicles, while alginate nanoparticles were deposited in alternate cellular vesicles as indicated by confocal microscopy. These findings could suggest different cellular uptake pathways of the NPs with different surface chemistries, thus eluding to specific pathways for glycosaminoglycans (GAGs) cellular internalisation.

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Glycocalyx Workshop Presentations

The Point of Today

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I've called today a workshop because my aim is for people to gather knowledge from many disciplines to have a broader understanding of the area. I've had a gap of 4-5years taking glycocalyx data and there has been a good deal of knowledge gained in that time, though I feel it has been disparate and fractious. My own work is in vascular imaging and permeability, but we also have proteoglycan, shear stress, model systems and disease specialists giving talks and additional expertise in the audience.
We still cannot satisfactory visualise the endothelial glycocalyx to determine its location: ***Where is it?*** We seem to argue over its components, certainly when we include the additional plasma molecules: ***What is it?*** We know it is disrupted in diseases, but not really how, or how many diseases, or the timeline in the diseases or if this disruption is important in these diseases: ***When is it?***

10:00

The Glycocalyx and Microvascular Permeability

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Recent thinking on the glycocalyx and permeability dates from 1965 with Luft's electron micrographs (EMs) of a ruthenium red staining layer on endothelial cell surfaces. Largely ignored initially, exclusion of ferritin molecules from the endothelial surface (ECS) and luminal caveolae and the rapid and reversible increases in permeability after completely removing plasma proteins from vessel interiors, led to the fibre-matrix theory (Curry & Michel 1980. MVR 20:96-99) which showed that the glycocalyx could act as the molecular sieve of endothelium. Later convincing evidence for the glycocalyx as a barrier, was the *in vivo* demonstration of exclusion of macromolecules from the ECS using confocal light microscopy (Vink & Duling. 1996. Circ.Res. 79: 581-589). The exclusion zone extended ~1.0 μm from the ECS and while later found consistent with resistance and red cell passage through small vessels, posed problems for permeability theory. Autocorrelation analysis of glycocalyx EMs revealed a quasi-periodic structure extending only ~ 0.1 μm from the EC surface but consistent with fibre matrix theory (Squire et al 2001. J,Struct.Biol 136:239-255; Arkill et al. 2001. Bioph.J. 101: 1046-1056). Some of these problems are considered in reviews (Weinbaum et al. 2007. Ann.Rev. Biomed.Eng. 9: 121-167) and will be discussed particularly in relation to a layered structure of the glycocalyx (Curry & Adamson. 2012. Ann, Biomed.Eng.40: 828-839).

Glycogalyx Workshop Presentations

10:45

Endothelial Surface Glycocalyx (ESG) Viewed by Confocal and Stochastic Optical Reconstruction Microscopy (STORM)

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In order to play important roles in vascular functions, the ESG should have an organized structure at the molecular level. Employing confocal microscopy, we estimated the thickness of ESG at the rat mesenteric and mouse cremaster muscle microvessels and that at aorta. Employing a newly acquired super high resolution fluorescence optical microscope (STORM), we revealed the ESG on bEnd3 (mouse brain microvascular endothelial cell) monolayer. The revealed ultra-structure of ESG by STORM suggests that heparan sulfate of ESG plays a major role in mechanosensing and hyaluronic acid of ESG plays a major role in forming the molecular sieve. Supported by NIH SC1CA153325-01 and R01HL094889-01.

11:15

TSG-6-glycosaminoglycan interactions: implications for glycocalyx structure and function

Anthony Day
University of Manchester

Glycosaminoglycans (GAGs) are polysaccharides that are critical components of the glycocalyx. TSG-6 is a multi-functional GAG-binding protein (often made during inflammation) that interacts with sulphated and non-sulphated GAGs, and has been implicated in their structural perturbation. For example, TSG-6 crosslinks hyaluronan (HA), leading to the rigidification and condensation of the HA polymer; this enhances the interaction of HA with cell-surface receptors and provides a mechanism for how TSG-6 communicates anti-inflammatory signals to a variety of cells. TSG-6 has also been implicated in the crosslinking of chondroitin sulphate and heparan sulphate chains, where this may affect the structure and permeability of the pericellular matrix and contribute to the regulation of chemokine-glycocalyx interactions.

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Glycogalyx Workshop Presentations

12:15

Clinical assessment of glycocalyx damage and capillary red cell hemodynamics

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The endothelial glycocalyx extends up to more than 1 micron into the lumen of microvessels and is expected to affect microvascular blood volume and red cell hemodynamics in capillary blood vessels. In this workshop, we will discuss the tools that we developed to measure penetration of red cells into the vessel wall boundary layer as a measure of glycocalyx damage and recent developments to measure red cell hemodynamics to assess the relation between microvascular blood flow and red cell perfused capillary density.

13:30

Probing physical mechanisms of cell capture under vasculature-mimicking flow with mechanically and biochemically well defined environments

Ralf P. Richter

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The endothelial glycocalyx acts as a 'gate keeper' that coordinates the selective capture of cells from the blood circulation towards their traffic into tissues. How the endothelial glycocalyx orchestrates is biomechanical properties (softness and thickness) and its biochemical signals (cytokine and receptor presentation) to accomplish this vital function is not well understood, and challenging to dissect *in vivo*. We study this question with *in vitro* model systems of the endothelial glycocalyx-circulating cell interface that are well defined and tunable and recapitulate selected aspects of the *in vivo* system. These enables quantitative analysis and integration of biology with soft matter physics. I shall present how we do this and show first results indicating that the endothelial glycocalyx acts as a biomechanically and biochemically integrated system.

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Glycogalyx Workshop Presentations

14:00

Enhancement of endothelial glycocalyx in the diabetic microvasculature

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Increased vascular permeability leads to an assortment of microvascular complications such as diabetic nephropathy, diabetic retinopathy and diabetic cardiomyopathy. All blood vessels are lined with a protective endothelial glycocalyx (eGlx). One of its properties is to restrict the passage of larger proteins from the blood into surrounding tissues. In diabetes, eGLX is shed from blood vessels, which is associated with increased vascular permeability. We hypothesise that this is key to microvascular complications in diabetes and we aim to protect or restore eGlx experimentally.

14:30

Large-Scale Molecular Dynamics Simulations of Flow and Glycocalyx: Towards Understanding Atomic Events on Endothelial Cell Surface

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The glycocalyx has a prominent role in orchestrating multiple biological processes occurring at the plasma membrane. In our research, an all-atom flow/glycocalyx system is constructed with the bulk flow velocity in the physiologically relevant ranges for the first time. The system is simulated by molecular dynamics (MD) using 5.8 million atoms. Flow dynamics, including velocity and shear stress distributions, and corresponding statistics in the presence of the glycocalyx are presented and discussed. Complex dynamic behaviours of the glycocalyx, particularly the sugar chains, are observed in response to blood flow. Furthermore, potential force transmission pathways are discussed based on the dynamics of the glycocalyx constituents, which provides new insight into the mechanism of mechanotransduction of the glycocalyx. The constructed system can also be applied to predict the behaviour of red blood cells (albeit not included in the system) on the endothelial glycocalyx layer from a new perspective. These findings will contribute to our understandings in the pathologies of glycocalyx-related diseases, for example in renal or cardiovascular conditions.

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