BMS 2018
British Microcirculation Society

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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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Glycocalyx Workshop Presentations

The Point of Today

Kenton Arkill

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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

C. Charles Michel

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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)

Bingmei M. Fu
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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

Hans Vink

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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

Rebecca Foster
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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.