S4:The Glycocalyx – Its Structure and Function

S4-1 Multilayer structures of the endothelial glycocalyx: barrier functions versus red cell hemodynamics

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Loss of glycocalyx components is an early indicator of vascular dysfunction. Loss results in increased penetration of red cells into a wall boundary region and increased leakage of water and plasma proteins. A common assumption is that the same glycocalyx structures determine both outcomes. An accumulating body of evidence does not support this assumption. Fluid and plasma protein exchange across the endothelial barrier is accounted for in terms of a quasi-periodic inner endothelial glycocalyx layer (about 300nm thick) with hydraulic resistivity of the order of 10^{11}dynes sec/cm^4 (Darcy coefficient 10^{-13}cm^2). Glycocalyx layers that extend beyond 300nm must be more porous to be consistent with measured vascular permeability properties. Red cell flows over layers more than 1 micron thick are accounted by a hydraulic resistivity 10-100fold less than that of the inner layer. These observations, and independent evidence from tracer penetration and enzyme degradation, conform to a multi-layer model of glycocalyx structure. A prediction of the model is that changes in red cell hemodynamics within the boundary region 1 micron or more from the endothelial cell membrane may provide only limited data about the inner glycocalyx layers. Clinical strategies to evaluate loss of glycocalyx components that focus only on imaging red cell flows must be validated by better understanding of the interaction of red cells with a multilayer glycocalyx and the effect of changes in outer glycocalyx layers on transvascular exchange.

S4-2 Endothelial Surface Glycocalyx (ESG) Components and Ultra-Structures Revealed by Stochastic Optical Reconstruction Microscopy (STORM)

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The major glycosaminoglycans (GAGs) in the ESG are heparan sulfate (HS), hyaluronic acid (HA), and chondroitin sulfate (CS). In order to play important roles in vascular functions, e.g, as a mechanosensor for the endothelial cells (ECs) to sense the blood flow, a molecular sieve to maintain normal microvessel permeability and a barrier between the circulating cells and ECs forming the vessel wall, the ESG should have an organized structure at the molecular level. Due to the limitations of optical and electrical microscopy, the ultra-structure of ESG has not been revealed until recent development of a super high resolution fluorescence optical microscope, STORM. We used newly acquired STORM to observe the ESG on bEnd3 (mouse brain microvascular endothelial cells) monolayers. The ESG was immunolabeled with anti-HS, followed by an ATTO488 conjugated goat anti-mouse IgG, and with biotinylated HA binding protein, followed by an AF647 conjugated anti-biotin. The ESG was then imaged by the STORM with a 100x/1.49 oil immersed lens. Multiple Reporters of ATTO488 and AF647 with alternating illumination were used to acquire the 3D images of HS and HA. The field of 256 x 256 (40 x 40 μm^2) of HS and HA at the surface of ECs was obtained based on

totally 40,000 of EM-CCD captured images for each reporter at a capturing speed of 19 ms/frame. We found that HA is a long molecule weaving into a network, which is horizontal to the EC cell surface. In contrast, HS is a shorter molecule, which is perpendicular to the cell surface. The height of the HS is ~600 nm. HA and HS seem to overlap with each other at the EC surface. The revealed ultra-structure of ESG by STORM suggests that HS plays a major role in mechanosensing and HA plays a major role in forming the molecular sieve.

S4-3 In Vivo Studies of the Enzymatic Degradation and Structure of the Endothelial Glycocalyx

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The endothelial cell (EC) glycocalyx serves as a barrier to trans-vascular exchange of solutes and adhesion of leukocytes during the inflammatory process. Observations in post-capillary venules (rat mesentery) revealed that prolonged exposure to high shear results in a matting down of the glycocalyx and reduced thickness of the layer. Rapid reductions in shear resulted in an unfurling of its molecular structure and a significant increase in thickness. The surface of the glycocalyx was found to undergo large deformations in the direction of flow. Observations of lectin coated beads adhered to the EC under pure oscillatory flow revealed excursions of about 400 nm during changes in flow direction. Structural alterations arise from two principal enzymes that cleave glycans from the EC surface: matrix-metalloproteases that cleave core proteoglycans, and heparanase that cleaves heparan sulfate chains from the core protein. Shedding of glycans from the EC in post-capillary venules was observed by EC stimulation with the chemoattractant fMLP. Inhibition of MMP activity by topical application of the inhibitor doxycycline, or scavenging of heparanase by infusion of low molecular weight heparin (LMWH), significantly inhibited glycan shedding due to fMLP. LMWH also resulted in a dose-dependent clustering of glycans on the EC surface. The magnitude of WBC-EC adhesion in response to fMLP varied inversely with clustering of glycans. Although LMWH initially reduced the rate of WBC adhesion in response to fMLP, prolonged stimulation with fMLP resulted in a continued rise in adhesion, thus suggesting that other factors may have played a role in the adhesion response. Nonetheless, the therapeutic value of stabilization of the EC glycocalyx with LMWH appears promising.

S4-4 The endothelial glycocalyx and control of microvascular flow and perfused capillary density

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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 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.