HIGH-RESOLUTION THREE-DIMENSION INTRAVITAL FLUORESCENCE MICROSCOPY REVEALS PARTIAL EXCLUSION OF POLYANIONIC PLASMA TRACERS NEAR THE CAPILLARY WALL AND PREDICTS GLYCOCALYX FIXED-CHARGE DENSITY

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INTRODUCTION

The interface between blood and the vascular endothelium represents one of the most complex, dynamic, and fundamentally important interfaces in mammalian physiology. Strategically located at this interface is a glycocalyx surface layer that is regulated by and expressed on capillary endothelial cells. In order to investigate the properties of this structure, we have combined analytical modeling with intravital brightfield and fluorescence microscopy. In our analyses [1-2], we have assumed that the glycocalyx consists of a mixture of electrostatically charged macromolecules hydrated in an electrolytic fluid. Disturbances arising from mechanical deformation are introduced as perturbations away from a nearly electroneutral equilibrium environment. In the case of mechanical compression of the layer, such as might occur on the passing of stiff leukocytes through capillaries, fluid flux into the compressed layer, driven by electrochemical potential gradients of the ions and glycocalyx macromolecules, results in rehydration of the layer and a restoration of its equilibrium dimensions. An analysis of the equilibrium configuration of the layer predicts that polyanionic fluorescent plasma tracers would be partially excluded by the glycocalyx by vitue of their charge. It further predicts, quantitatively, the degree to which this exclusion would occur as a function of tracer valence, glycocalyx fixed-charge density, and the ionic strength of blood plasma. This exclusion would result in a reduction in fluorescence intensity within the glycocalyx relative to luminal intensity levels, and could therefore be detected using epifluorescence illumination *in vivo*.

MODEL

The glycocalyx is here modeled as a continuously distributed isotropic anionic matrix made up of proteoglycans, glycoproteins, and glycosaminoglycans containing fixed-bound negative charges through which a solution of anionic molecular tracers in blood plasma can diffuse. The continuum model presented here has its origins in the Nernst-Planck equation. According to the model, in order to maintain a nearly electroneutral environment in equilibrium, gradients in the chemical potential of the mobile ions and anionic tracers in the blood balance gradients in the electrostatic potential generated by the fixed charges bound to the glycocalyx.

We assume that the molecular tracer solution has a monodisperse valence, i.e. it is characterized by a single valence, $-m$, per tracer molecule. In equilibrium, the flux of each species (mobile cations (+), anions (–), and polyanionic molecular tracers (L)) vanishes. Neglecting axial variations in the field variables, the one-dimensional axisymmetric equations governing the equilibrium distributions are given by

$$J_r^i = -D^i\left(\frac{\partial c^i}{\partial r} - \frac{z^i q}{k_B T} c^i E_r\right) = 0 \qquad \text{(Nernst-Planck Equation)}$$

$$\frac{1}{r}\frac{\partial}{\partial r}(rE_r) = \frac{q\rho}{\varepsilon} \quad E_r = \frac{q}{\varepsilon r}\int_0^r \rho(\zeta)\,\zeta\,d\zeta \qquad \text{(Gauss's Law)}$$

$$\rho(r) = z^+ c^+(r) + z^- c^-(r) - mc^L(r) - nc^F(r) \qquad \text{(Local Charge Imbalance).}$$

In the above equations, c^i (i = +, –, L, and F) is the concentration field, J_r^i is the radial flux relative to the solvent, D^i is the diffusion coefficient, E_r is the radial component of the electric field, k_B is Boltzmann's constant, T is absolute temperature, ε is the permittivity of water, q is the elementary charge, and z^+, z^- and $-n = z^F$ are the effective valences of the mobile cations, mobile anions and glycocalyx matrix, respectively.

The two most significant quantities that arise from the equations are

$$B = \frac{c_0^L}{c_0^-} \quad \text{and} \quad \Gamma(r) = \frac{nc^F(r)}{c_0^-}$$

where $c_0^- = c^-(0)$. Stace & Damiano (2001) derived a closed form expression for the ratio of the tracer concentration at some radial distance r to that at the center of the capillary under the assumption that the

quantity $mB << 1$ [1]. A simple generalization of that result that drops this requirement reveals that

$$x(\Delta(r)) = \frac{c^L(r)}{c^L(0)} = \left[\frac{1}{2}\left(mB + \Delta(r) + \sqrt{(mB+\Delta(r))^2 + 4(1-mB)}\right)\right]^m$$

where we define the quantity $x(\Delta(r))$ as the *exclusion factor*, since it gives the factor by which anionic molecular tracers are excluded from within the glycocalyx compared with the lumen. Defining $\Delta_0 = \Delta(r_0)$, where $r = r_0$ is where the glycocalyx is most concentrated, then $\Delta_0 = nc_0^F / c_0^+$ measures the ratio of the maximum glycocalyx fixed-charge density to the luminal concentration of cations in the blood, and $x(\Delta_0)$ is the maximum exclusion of tracer in the glycocalyx.

METHODS

To investigate the fixed-charge density and permeability of the glycocalyx, distributions of 20 and 40 kDa neutral and polyanionic plasma tracers were obtained over the cross section of capillaries in the mouse cremaster muscle using high-resolution, three-dimensional, intravital, digital optical microscopy. A computer-controlled piezo-electric focus device allowed rapid acquisition of 200 stacked sagittal sections centered around the mid-sagittal plane through the capillary. Image reconstruction by maximum likelihood iterative-constraint deconvolution algorithms provided the radial fluorescence intensity distributions of both tracers in the mid-sagittal plane where the fluorescence intensity of each pixel in the image contained very little light from outside of the plane or from neighboring pixels in the plane. Using different optical filters, fluorescence intensity distributions were obtained for both neutral and polyanionic tracers of the same molecular weight that were simultaneously present within the same capillary segment.

RESULTS AND DISCUSSION

Results are shown below in Figures 1–3 in terms of intravital fluorescence intensity distributions of dextran tracers within a 5.8 μm-diameter capillary of the mouse cremaster muscle. The simultaneous presence of both the neutral and polyanionic 40 kDa dextran tracers within the same capillary provides an opportunity to assess the influence of the fixed charges bound to the glycocalyx on the equilibrium distributions of the tracers. Results consistently reveal a charge-mediated exclusion zone of polyanionic plasma tracers that typically extends between 0.5 and 0.7 μm from the vessel wall. This exclusion was measured by the attenuation in fluorescence intensity of near-wall polyanionic tracers, which was typically found to vary between 30 and 50% relative to the centerline polyanionic tracer intensity and between 30 and 40% relative to the near-wall neutral tracer intensity. When combined with our electrochemical model of the glycocalyx [1], these measurements provide an estimate of the glycocalyx fixed-charge density of between 0.7 and 1.3 mEq/l. Based on this estimate, and a recovery time of the layer of approximately 1 s after the passage of a single leukocyte [3], our mechano-electrochemical model of the glycocalyx [2] estimates the glycocalyx hydraulic permeability to plasma to be between 10^{-11} and 10^{-10} cm^4/dyn-s. This result is consistent with an independent estimate [4] using a fiber matrix model based on the Brinkman equation.

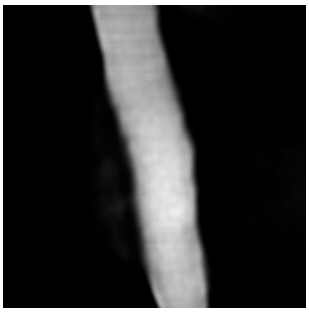

Figure 1. Sagittal intensity profile of neutral 40 kDa tracer in a 5.8 micron capillary.

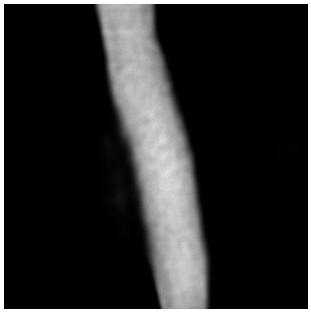

Figure 2. Sagittal intensity profile of polyanionic 40 kDa tracer in a 5.8 micron capillary.

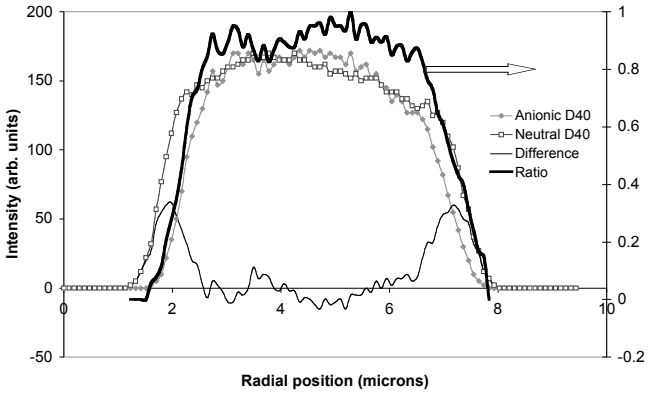

Figure 3. Plot of intensity profiles for the neutral (squares) and polyanionic (dots) plasma tracers, as well as the difference between the two (fine). The ratio of the anionic intensity to neutral intensity, scaled so its maximum is unity (thick) is referred to the right axis.

A layer with these properties has significant implications for microvascular physiology. In particular, if the hydraulic permeablility and restoring force we predict are accurate, our results would necessitate the revision of previous concepts of wall shear rate, wall shear stress, leukocyte adhesion, microvascular flow resistance, stress transmission to vascular endothelium, and endothelial mechanotrans-duction mechanisms.

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