LUBRICATION THEORY IN HIGHLY COMPRESSIBLE POROUS MEDIA: SKIING, TIP-TOEING AND SENSING YOUR WAY ACROSS THE ENDOTHELIAL GLYCOCALYX

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ABSTRACT

In this paper we shall provide an overview of the endothelial surface layer (ESL) or glycocalyx in several roles, as a transport barrier, as a porous hydrodynamic interface in the motion of red and white cells in microvessels and as a mechanotransducer of fluid shearing stresses to the actin cortical cytoskeleton of the endothelial cell (EC). These functions will be examined from a new perspective, the quasi-periodic ultrastructural model proposed in (Squire et al., 2001) for the three-dimensional organization of the ESL and its linkage to the submembranous scaffold. We shall show that the core proteins in the bush-like structures comprising the matrix have a flexural rigidity, *EI*, that is sufficiently stiff to serve as a molecular filter for plasma proteins and as an exquisitely designed transducer of fluid shearing stresses. However, *EI* is inadequate to prevent the buckling of these protein structures during the intermittent motion of red cells or the penetration of white cell microvilli. In these cellular interactions the viscous draining resistance of the matrix is essential for preventing adhesive molecular interactions between proteins in the endothelial membrane and circulating cellular components.

OVERVIEW

While the endothelial surface glycocalyx was first identified by special electron microscopic (EM) staining techniques nearly forty years ago (Luft, 1966), it is only relatively recently that this surface layer has been observed *in vivo* (Vink and Duling, 1996), and the importance of its multifaceted physiological functions recognized. Key among these functions are its role as a molecular sieve in determining the oncotic forces that are established across microvessel endothelium (Hu et al., 2000; Hu and Weinbaum, 1999; Michel, 1997; Weinbaum, 1998), its role as a hydrodynamic exclusion layer preventing the interaction of proteins in the red cell and endothelial cell membranes (Damiano, 1998; Feng and Weinbaum, 2000; Secomb et al., 2001a), its function in modulating leukocyte attachment and rolling (Zhao et al., 2001), and as a transducer of mechanical forces to the intracellular cytoskeleton in the initiation of intracellular signaling(Weinbaum et al., 2003).

It is widely recognized that fluid shearing forces acting on ECs have a profound effect on EC morphology, structure and function (Davies, 1995; Drenckhahn and Ness, 1997). It is now also clear from theoretical considerations (Damiano, 1998; Feng and Weinbaum, 2000; Secomb et al., 1998, 2001b) that the shear stress at the edge of the endothelial surface layer (ESL) is greatly attenuated by the extracellular matrix of proteoglycans and glycoproteins in the glycocalyx with the result that fluid velocities, except near the edge of the layer, are vanishingly small. Thus, the shear stress due to the fluid flow acting on the apical membrane of the EC itself is negligible. This paradoxical prediction has raised a fundamental question as to how hydrodynamic and mechanical forces, more generally, are transmitted across the structural components of the glycocalyx. How do these components deform under the action of these forces and how are these forces and deformations communicated to the underlying cortical cytoskeleton (CC).

Little was known about the specific proteins or generalized structure of the glycocalyx until recently (Henry and Duling, 1999, 2000; Squire et al., 2001). The state of knowledge prior to 2000 is summarized in (Pries et al., 2000). *In vivo* experiments demonstrated that hyaluronan and chondroitin sulfate play an important role in the assembly of the layer and its sieving properties (Henry and Duling, 2000). Using computed autocorrelation functions and Fourier transforms of EM images obtained from both new (Squire et al., 2001) and previous studies (Clough et al., 1988) of frog mesenteric capillaries, Squire et al. (2001) were able to identify for the first time the quasi-periodic substructure of the glycocalyx and the

anchoring foci that appear to emanate from the underlying CC. The computer enhanced images showed that the glycocalyx is a three-dimensional fibrous meshwork with a characteristic spacing of 20 nm in all directions and that the effective diameter of the periodic scattering centers was 10-12 nm. Using a freeze fracture replica from a rare section where the fracture plane passed parallel and close to the endothelial surface, they also showed that anchoring foci formed an hexagonal array with an intercluster spacing of typically 100 nm in frog lung capillary. This latter observation was consistent with the spacing of bush like structures seen on the plasmalemma of the fenestrated renal capillaries of the rat using a new fluorocarbon oxygen fixation technique which preserved the portion of the glycocalyx close to the EC surface (Rostgaard and Qvortrup, 1997).

Based on the foregoing observations Squire et al. (Squire et al., 2001) proposed a model for the structural organization of the ESL and its relationship to the EC cortical cytoskeleton. The model provides a new view of the organization of the matrix that forms the molecular sieve for the filtering of plasma proteins. The possible existence of an ordered structure was first proposed in (Michel, 1983) to explain why there is a sharp break in the solute permeability curve for molecules the size of albumin. These ideas will be used in the present paper to formulate a mathematical model for analyzing the transduction of mechanical forces and bending moments across the ESL. We first address a basic question: what is the bending rigidity EI of the core proteins comprising the glycocalyx that enables them to resist the randomizing forces of Brownian motion and deformation by fluid shear stresses? To answer this question we shall examine the time dependent recovery of the surface layer after it has been crushed by the passage of a white blood cell (WBC) (Vink et al., 1999). Theoretical models are then developed to explore the deformability of the matrix in both red and white cell interactions and in response to fluid shearing forces. The forces and torques exerted on the structural elements of the ESL by these mechanical loads are then used to predict the stresses transmitted to the CC.

A unique feature of the present analysis is the attempt to couple the dynamic response of the surface layer to mechanical loading to the stresses and deformations induced in the underlying CC. This CC has previously been explored in other contexts involving the movement of plasma proteins in the plane of the membrane using single particle tracking and optical traps (Edidin et al., 1991; Sako and Kusumi, 1995). These studies, summarized in (Sako and Kusumi, 1995), have led to a "fence" model construct in which one observes microdomains as small as 0.01 μm^2 restricting the movement of proteins due to the interaction of their cytoplasmic tails with the underlying cytoskeletal scaffold.

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