

# **CRITICAL REVIEW: Mechanotransduction and flow across the endothelial glycocalyx**

Weinbaum S, Zhang X, Han Y, Vink H, Cowin S

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## Introduction

Effects of the glycocalyx, a proteoglycan, glycoprotein, and glycosaminoglycan containing matrix that lines the luminal surface of vascular endothelial cells, are often overlooked in studies investigating molecular transport, mechanotransduction, and red and white blood cell dynamics at the luminal endothelial surface. Recent studies show the glycocalyx functions as a molecular sieve, limiting the transport of molecules based on size and surface charge and generating oncotic pressure to offset hydrostatic. The glycocalyx also protects the endothelium from interacting with circulating red blood cells, but allows leukocyte attachment and rolling. Furthermore, the glycocalyx affects how fluid shear stress generated forces transmit to the endothelial cell membrane and underlying cytoskeleton.

Key experimental findings in recent years have defined the glycocalyx thickness as ~150 – 200 nm depending on the species, identified hyaluronan and chondroitin sulfate as important structural and sieving constituents, and shown biomolecule permeability of the negatively charged layer depends on particle charge and size (2). Due to the size scale of the glycocalyx, theoretical modeling has proved to be a powerful tool for exploring its mechanical and transport characteristics. Recent models have specifically addressed the effects of the glycocalyx on filtration coefficients, fluid permeability, and the pressure generated to oppose impeding red blood cells. These models depend heavily on the structural description of the glycocalyx mesh-like matrix and geometrical proportions of the core proteins and side fibers. Models also separate the glycocalyx and endothelial cell membrane with a ~400 nm thick cleft region. The cleft region is separated by a tight junction with transport orifices, is electrically neutral and is thought to reduce the molecular concentrations on the cellular side of the glycocalyx, thus establishing a negative concentration gradient across the glycocalyx.

Clearly, study of endothelium transport and force transmission in the context of the glycocalyx requires coordination of both experimental and theoretical results. Furthermore, a better description of the glycocalyx will add to the growing body of knowledge surrounding the dynamics of red and white blood cells circulating through vessels, as well as shear stress-induced mechanotransduction.

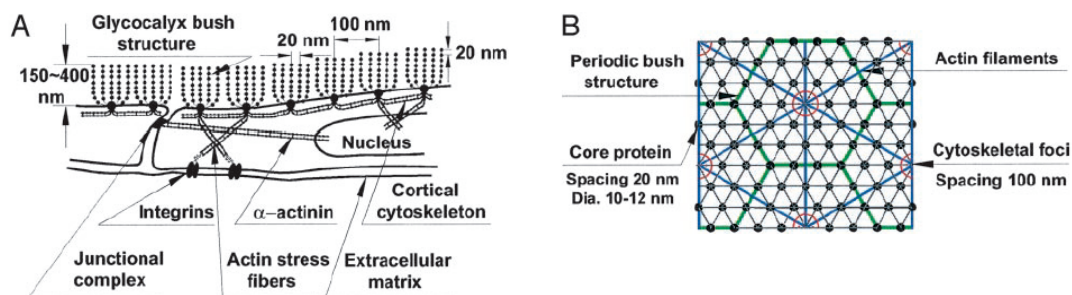
## Summary of model and results

An approach has been presented for modeling the glycocalyx structure and relating its structure to understanding transport, force transmission, and red and white blood cell interactions with the endothelium. The model uses a quasiperiodic ultrastructural approach proposed by Squire et al, 2001 to show that the glycocalyx contains stiff core proteins with a flexural rigidity capable of filtering plasma proteins and transmitting fluid shear forces to the endothelial cell (10). Furthermore, the model is extended to include force transmission from the glycocalyx directly to the endothelial cytoskeleton, which can ultimately be used to infer the mechanotransduction consequences of glycocalyx force transmission.

### *Quasiperiodic ultrastructural model*

The glycocalyx and underlying cytoskeleton are organized into repeating “bush-like” structures (see Figure 1). Core proteins in the glycocalyx, with perpendicularly extending fibers, penetrate the glycocalyx thickness and attach to foci on the cell membrane. The cytoskeleton foci and core proteins are evenly spaced a distance of 20 nm and 100 nm, respectively. The core proteins have a 10-12 nm diameter. The main modification of this model, compared to previous models, is the increase in core protein diameter. Previous models used a 1.2 nm diameter, typical of glycosaminoglycans, and 7-8 nm spacing between core proteins.

The model assumes a hexagonal arrangement for both the actin filaments and core proteins, with 27 core proteins associating with one cytoskeletal foci. It is also assumed that the foci connecting the core protein “bushes” to the cytoskeleton are composed of short, rigid linker proteins. This assumption is critical for modeling the core protein as a cantilevered beam.



**Figure 1:** (a) cross-section view of the endothelial surface layer (ESL) model and theoretical attachment to cytoskeleton, (b) hexagonal organization of core proteins and cytoskeletal foci.

### *Transport model*

The transport model describes passage of molecules to the endothelial cell surface. Based on previous models, a cleft region separates the glycocalyx and endothelial cell membrane (see Figure 2). The cleft is divided by a periodic tight junction



glycocalyx/lumen interface compared to the centerline velocity. The velocity within the glycocalyx decays to a nearly uniform velocity. Thus, there is no fluid shear stress felt directly by the plasma membrane because the velocity gradient at that surface is essentially zero. The fluid shear stress on the glycocalyx surface is then related to a drag force acting on the tips of the core proteins. Based on this analysis, the majority of the bending moment experienced by the core protein arises from the drag force on the core protein tip.

Assuming that the drag force acts on the core proteins and is localized to the tip, a flexural rigidity for the core protein can be calculated from the sudden deflection and time-dependent recovery of the glycocalyx. Such is a balance between the elastic recovery and viscous resistance. Experiments have shown the characteristic recovery time of a bent core protein, due to a passing white blood cell, to be  $\tau = 0.38$  s (11). Here, a viscoelastic model was employed to describe the recovery of the core protein that related the point load at the end of a cantilevered beam, where the point load is the effective drag force on the core protein tip, to the bending rigidity using differential beam bending theory. Accordingly, a relationship between recovery time, deflection, viscosity, permeability, and core protein geometric dimensions was developed. Using experimental and theoretical values, a flexural rigidity of  $700$  pN-nm<sup>2</sup> was calculated. This is 21-fold less stiff than actin. Using this value for flexural rigidity, a deflection of  $17.9$  nm was predicted from beam theory to result from hydrodynamic loading.

#### *Red blood cell interaction model*

The theoretical result for core protein flexural rigidity was further used to predict the mechanical interactions between the glycocalyx and passing red blood cell and consequences of red blood cell arrest. First, the lateral tip deflection was predicted for different red blood cell passing velocities. It was assumed that the core protein and red blood cell were separated by a non-zero distance and supported by elevated pressure, as in lubrication. It was found that tip deflection increased with red blood cell velocity. This can be explained by a drastic increase in pressure between the passing red blood cell and glycocalyx because the lubricating fluid does not have time to escape with the passing red blood cell. As the red blood cell velocity increases, so does that gap pressure and consequently the tip deflection.

Second, red blood cell arrest was examined. Assuming that the red blood cell remained spherical and did not take on a bi-convex configuration, it was found that the arrest force exceeded the critical buckling load (estimated as  $P_{cr} = 0.0108$  pN) in each of the core proteins. Using elastica (large deformation) theory and acknowledging that the core proteins become compacted as the pressure of the red blood cell increases, the fluid drainage time from the glycocalyx at the arrest location was significantly less for a constant permeability  $K_p$ , compared to a variable permeability. Accordingly, compaction of the glycocalyx allows for the retention of fluid during red blood cell arrest and is critical in maintaining red blood cell/endothelium separation.

#### *Summarized discussion of results*

A new structural model of the glycocalyx was presented, along with the effects of the model on transport, bending rigidity, red and white blood cell interactions, and mechanotransduction. These results better correlate with experimental results compared

to previous models and provide new insight into the potential structure of the glycocalyx and its effects on transport, the flexural rigidity of the core proteins of the glycocalyx, the glycocalyx protection of the endothelium against red blood cell contact, and the rolling capability of white blood cells. In summary, the model results lead to the following conclusions:

- 1) The model presented estimates for reflection and filtration coefficients more consistent with experimental results compared to previous models.
- 2) The flexural rigidity of the glycocalyx was found to be 20-fold less than actin filaments, which supports the initial assumption that core proteins are anchored firmly to cytoskeletal foci.
- 3) Contrary to previous assertions, the glycocalyx buckles under the pressure of an arrested red blood cell and it is the impaired expulsion of fluid that protects the endothelium from red blood cell invasion.
- 4) White blood cells, which roll along the glycocalyx surface, have microvilli tips that penetrate the glycocalyx. The present model predicts a shorter penetration depth compared to other models because of an increased core protein volume fraction and decreased hydraulic permeability.
- 5) The core protein acts as a lever arm to magnify the stress at the cytoskeleton foci due to the bending moment and thus deforming the cytoskeleton more than a direct  $10 \text{ dyne/cm}^2$  shear stress would. This magnification of stress transmitted to the actin cytoskeleton, along with evidence that the cytoskeleton acts as a mechanosensor (3), suggests that the glycocalyx has a role in mechanotransduction.

## Weaknesses and improved analysis

### *Assessment of pressure gradients*

Oncotic pressure forces, which arise from the filtering plasma proteins ( $> 7\text{nm}$  albumin), oppose fluid flow from the lumen across the ESL similar to how osmotic pressure drives fluid towards regions of elevated ion concentration. In order for fluid to travel from the lumen towards the tissue and transport essential molecules, hydrostatic forces must exceed oncotic. This is demonstrated with the present model, where a bi-directional pressure gradient is established to drive fluid towards the cleft orifice, and is a reasonable assessment for trans-capillary analysis. However, when the velocity profile is evaluated along the length of the capillary according to effective medium theory, the velocity distributions in the lumen and glycocalyx are based upon a pressure gradient only in the longitudinal direction of the capillary, neglecting the transverse pressure gradient. The pressure on the luminal side of the glycocalyx is taken to be  $15\text{ cm H}_2\text{O}$  and uniform across the glycocalyx except at an  $x$ -distance (refer to Figure 2) near the orifice. Consider normal diastolic blood pressure that is on the order of  $150\text{ cm H}_2\text{O}$  (1). If the luminal surface of the glycocalyx has a pressure one-tenth of the blood pressure, then there must be a local, transverse pressure gradient near the surface of the glycocalyx and typical Poiseuille flow would not hold outside the glycocalyx. The D'arcy permeability may also be affected within the glycocalyx in local regions containing transverse pressure gradients. Further evaluation of the model boundary conditions and scaling arguments is required for clarification.

### *Electrodiffusion and kinetic considerations*

The authors fail to acknowledge the effects of a negatively charged glycocalyx in regulating the transport of biomolecules. Experimental evidence shows that biomolecules similar in size have different permeability values based on their charge (2) and these results have been successfully incorporated into electrodiffusion models (5). Accordingly, it should be specified that the transport results in the present model pertain to neutral biomolecules. Further consideration could also be taken for the electrokinetic coupling through glycocalyx pores. With the negatively charged glycocalyx, a positively charged mobile double layer will develop along the glycocalyx surface and extend into the lumen a distance described by the Debye length. This generates a streaming potential and induces a positive electrical potential across the glycocalyx. The induced potential rise, and subsequent electrical field, would enhance transport of positively charged molecules and hinder the transport of negatively charged molecules.

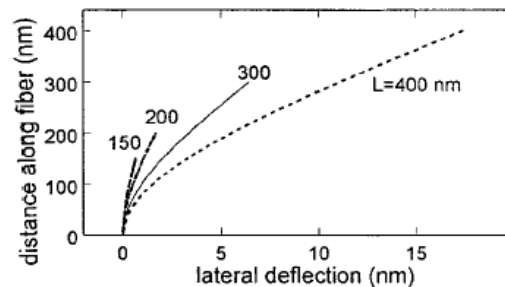
### *Viscoelastic approximation*

In deriving the flexural rigidity, the authors constructed an approximate viscoelastic model for the recovery of the glycocalyx after being deflected laterally by a passing white blood cell. This was a misused description of viscoelastic material behavior. Throughout the analysis, the core proteins were treated as an isotropic, homogeneous, linearly elastic material. It should be clarified that the recovery time for the core protein depended on viscous interactions with the surrounding fluid, but that the core protein remained isotropic, homogeneous, and linearly elastic. Approximating the core protein as viscoelastic implies that the elastic modulus, and thus flexural rigidity, has

time dependence for constant loads or displacements. A viscoelastic material would experience creep under a constant force or stress relaxation when subjected to a constant displacement. It may be possible to consider the entire glycocalyx as a continuum by neglecting the microstructure, but then it would not be possible to infer an elastic modulus, or flexural rigidity, specific to the core protein.

#### *Flexural rigidity and Brownian fluctuations*

The flexural rigidity calculation for the glycocalyx core proteins gives substantial insight into the behavior of those proteins, but does not address how the glycocalyx resists Brownian fluctuations. According to the worm-like chain model, which models polymers as thin, flexible and continuous rods, the polymer persistence length is related to the flexural rigidity over the thermal energy,  $k_bT$ . For a flexural rigidity of  $700 \text{ pN}\cdot\text{nm}^2$ , the polymer persistence length is approximately  $165 \text{ nm}$  at  $37^\circ\text{C}$ , which is consistent with experimental values for the core protein length. While the contour length of the polymer is equal to or less than the persistence length, the polymer behaves as a rigid rod and resists Brownian fluctuations. Also, as the length of the polymer increases, the polymer becomes more flexible, as was shown in the present model comparison of lateral deflection to fiber length (see Figure 3).



**Figure 3:** Core protein lateral deflection profile for protein lengths of 150, 200, 300, and 400 nm.

#### *Force transmission from the core protein tip to cytoskeleton*

The description of shear force transmission and magnification from the core protein tip to the cytoskeletal foci disobeys equilibrium. Shear force imposed on the core protein tip must be opposed by equal and opposite shear forces at the foci. The mechanical advantage can, however, increase the normal stress due to the bending moment. When a point load is applied to the end of a cantilevered beam, the maximum axial stress due to bending occurs at the base of the cantilever and increases with the length of the cantilever. In principle, then, the mechanical advantage argument is valid in that the underlying cytoskeleton experiences elevated stress in the presence of the glycocalyx, which could ultimately contribute to mechanotransduction by generating local regions of magnified stress within the cytoskeleton. It should also be recognized that the elevated stress is transmitted through the linker molecules, making the linker molecules candidate mechanosensors as well. Regardless, the transmission argument is flawed and requires further justification (it should also be noted that the reported bending moment units are not consistent with those typically used for bending moments).

#### *Stiffness of cytoskeletal foci and cantilever assumption*

The authors have sufficiently estimated the flexural rigidity of the glycocalyx and found the stiffness to be significantly less than the cytoskeleton. This evidence was used to support the assertion that the glycocalyx core proteins can be modeled as cantilevered beams because the connection with the cytoskeleton is sufficiently rigid and the linker molecules are sufficiently short. These assumptions, however, fail to acknowledge dynamic behavior inherent to transmembrane receptors that link to the cytoskeleton. Focal adhesion sites and selectins both link the cytoskeleton to extracellular ligands, but have different functions and assumed mechanical behavior. Focal adhesion sites assist in cell adhesion, migration, and proliferation (6), while selectins allow circulating white blood cells to adhere to the endothelium (4). Depending on the cytoskeletal foci, focal adhesion sites, L-selectins, or other transmembrane molecules, the core protein boundary condition at the foci may differ because a sufficiently stiff cytoskeleton does not guarantee that a moment will be supported. Receptor clustering and membrane fluidity could also significantly alter the arrangement of cytoskeletal foci (9). Steered molecular dynamics simulations have shown that forces applied directly to proteins unfold the protein domains (7). Such actions may allow for the core protein to pivot with the application of force, thus requiring electrostatic repulsion forces between the glycocalyx constituents for stability.

## **Conclusions and final recommendations**

An improved structural model for the glycocalyx has been presented that takes advantage of quasiperiodicity for organizing glycocalyx core proteins, protruding fibers, cytoskeletal foci, and the underlying cytoskeleton. A key feature of the model is its ability to estimate glycocalyx flexural rigidity and mechanical amplification of stress transmitted to the underlying cytoskeleton and its insightful basis for studying interactions between red or white blood cells and then endothelium.

Minor improvements are suggested for refining the viscoelastic and bending moment mechanical advantage discussions for consistency with material behavior theory, as well as the transverse and longitudinal pressure gradients and boundary conditions for proper evaluation of the fluid velocity profile. Major additions include incorporating cytoskeletal foci dynamics, accounting for the streaming potential and modified permeability of charged particles and a charged pore, and using molecular mechanics considerations and polymer chain theory to better characterize the mechanical behavior of glycocalyx core proteins.

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