Biomechanical properties of endothelial glycocalyx: An imperfect pendulum

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Abstract

Endothelial glycocalyx plays a crucial role in hemodynamics in health and disease, yet studying it is met by multiple technical hindrances. We attempted to outline our views on some biomechanical properties of endothelial glycocalyx, which are potentially amenable to mathematical modeling. We start with the null-hypothesis ascribing to glycocalyx the properties of a pendulum and reject this hypothesis on the grounds of multiple obstacles for pendulum behavior, such as rich decoration with flexible negatively charged side-chains, variable length and density, fluid fixation to the plasma membrane. We next analyze the current views on membrane attachments to the cortical actin web, its pulsatile contraction-relaxation cycles which rebound to the changes in tension of the plasma membrane. Based on this, we consider the outside-in signaling, the basis for mechanotransduction, and the dampening action of the inside-out signaling. The aperiodic oscillatory motions of glycocalyx and cortical actin web underlie our prediction of two functional pacemakers. We next advance an idea that the glycocalyx, plasma membrane, and cortical actin web represent a structure-functional unit and propose the concept of tensegrity model. Finally, we present our recent data suggesting that erythrocytes are gliding or hovering and rotating over the surface of intact glycocalyx, whereas the rotational and hovering components of their passage along the capillaries are lost when glycocalyx of either is degraded. These insights into the mechanics of endothelial glycocalyx motions may be of value in crosspollination between biomechanics, physiology, and pathophysiology for deeper appreciation of its rich untapped resources in health and pharmacotherapy in disease.

In this brief overview we shall consider several topics in biomechanics of glycocalyx which, in our opinion, deserve further exploration, and attempt to find up-to-date conceptual interpretations. Obviously, the biophysics of glycocalyx is a multifaceted subject. Yet, the mechanical engineering aspects of this subject are attracting growing attention. With the existing technical limitations for studying glycocalyx, several attempts have been published in recent years to model glycocalyx and apply diverse simulation techniques to offer glimpses into its behavior [1,2].

Nonetheless, the fragmentary character of the existing knowledge on the subject at hand is eye-catching and the discourse between physical, biophysical, and biological approaches, techniques, assumptions, and interpretations is conspicuously lacking in harmony. Therefore, we attempted to bridge some contentious points by summoning findings or analogies from different disciplines and extrapolating them to deformations of endothelial glycocalyx. We also offer in this opinion piece a few conjectures to provoke further scrutiny of this captivating field.

How perfect is the heparan sulfate proteoglycans (HSPG) pendulum?

A structure with one fixed end and another free is prone to oscillate in flow. One cannot assume, however, that the deformation of glycocalyx components is elastic, because long, flexible structures tend to exhibit viscoelastic deformational patterns [3]. In the case of in-series group of oscillators, their motility will be determined by the a) (non-)uniformity of their length; b) spatial layouts of the oscillators with/without interfering movements between each other; c) synchrony vs asynchrony of oscillations; d) interactions between flow and oscillators that may alleviate momentum transfer. Motility of EG in flow is constrained by the density and lengths of glycosaminoglycans (GAGs) in proteoglycans. In fact, it is probable that GAG chains may strengthen viscoelastic behavior of glycocalyx. Thus, the length of such side-chains as well as their crowding (i.e. a dense layout) should impose constraints on the amplitude of oscillatory motions (Fig. 1). Furthermore, although the abundance of proline residues tends to linearize syndecans, their N-terminal and juxta-membranous ectodomain portions are occupied by GAGs, whereas the central portion of ectodomains is free of them, is bendable, and that by itself creates the possibility of a double pendulum [4]. Moreover, the existence of different layers within the glycocalyx has been hypothesized [5,6]. Flow-induced bending of GAGs leads to the higher concentration of negative charges in the juxtamembranous space of 15–30 nm [7]. All these heterogeneities further support the notion of a pendulum with multiple bending joints. If this is the case, the system is prone to acquire chaotic behavior [8].

Standing on shaky grounds: how imperfect is HSPG pendulum?

Though it has been habitually accepted that glycocalyx is anchored to the plasma membrane or has the "fixed end" to qualify as being an oscillator, this assumption deserves more scrutiny in the light of recent discoveries – is it actually affixed and immobile? Considering prerequisites for the "fixed-end" pendulum-like behavior, such as a) steady insertion into the plasma membrane; b) stable attachment to cortical cytoskeleton; c) constant membrane tension; d) steady membrane-to-cortical actin attachment via adaptor proteins, it is clear that those preconditions are not met in living cells. The flow of membrane lipids, according to fluid mosaic model, is constrained by membrane-spanning domains of proteins and intercellular adhesions. Plasma membrane tension [described as $T = T_m + \gamma$; with $T_m = k(\Delta A/A)$ as non-elastic lipids. In the equation, T is the apparent membrane tension, T_m in-plane membrane tension γ surface tension, k the elastic modulus and A cell surface area] determines cell shape, the activity of exo- and endo-cytotic processes, as well as the transitions between plain and lipid-rich domains [10]. Among this multitude of functions, membrane tension as manipulated, for instance, by exposing endothelial cells to various ambient osmolarity, has been reported to regulate syndecan-1 expression via remodeling of the cortical actin cytoskeleton [11]. In turn, cortical actin is itself in the state of constant remodeling through polymerization-depolymerization. Measured as cortical stiffness by atomic force microscopy, actin cytoskeleton of endothelial cells strengthens with shear stress and hydrostatic pressure [12]. Cortical actin is organized as a dense cross-linked meshwork enriched with about
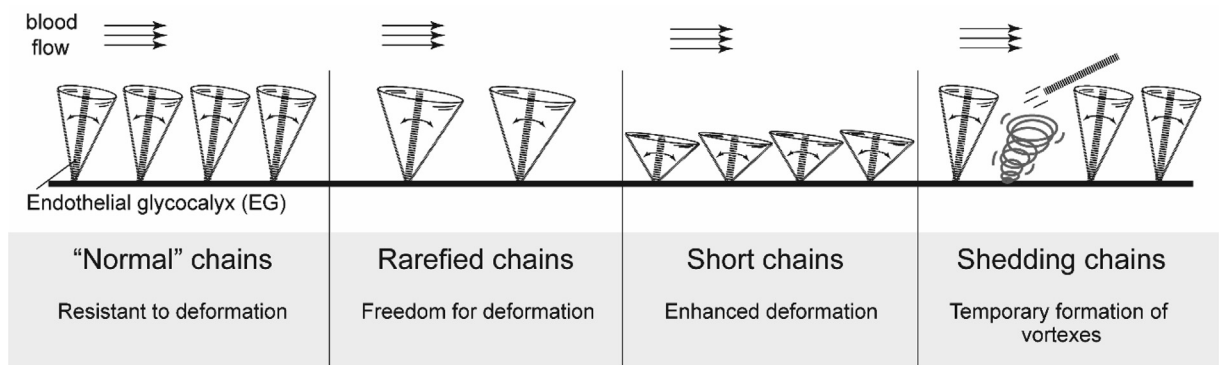

Fig. 1. Oscillation of GAG chains at varying GAG geometries (modified from Ref. [9] with permission.) In contrast to the classical unhindered pendulum oscillations, in the case of glycocalyx motility is modified by the length and density of heparan sulfate chains, as well as the same parameters of the core protein, e.g., syndecans. Vortices are expected to appear at the sites of shed proteoglycans, thus further disorganizing the oscillatory pattern.

hundred-fifty actin-binding proteins [13], among which the myosin II motors which are capable of contracting this network and initiating waves of contractions, thus reshaping plasma membrane and assisting in cell motility (Fig. 2). Other regulators of actin assembly and disassembly present in the cortical actin include capping proteins, actin-severing proteins (like cofilins) and promoters of actin polymerization, like profilin 1 [14]. In addition, actin filaments cross-linkers, like α-actinin, and proteins tethering cortical network to the plasma membrane, like ezrin-radixin-moezin proteins and myosin II motors, complete the molecular architecture of cortical cytoskeleton, which leaves a narrow gap of 20 nm between the lipid membrane and cortex. The thickness of cortical actin is quantified at 100–200 nm, mesh size of less than 100 nm [13].

The cytoplasmic domain of syndecans is bound, directly or indirectly, to the cortical actin network. This network is quite extensive occupying approximately 100 nm of submembrane space, engaging more than hundred actin-binding proteins (ABP), myosin II among them. Actomyosin contractility is in part responsible for the plasma membrane tension [13]. Generated gradients in cortical tension are transmitted to the plasma membrane and induce the flow of lipid bilayer. Considering the fact that syndecan-1 ectodomain uniquely binds to fibrillar collagens I, III, and V [15], the entire complex may account for signaling from the extracellular matrix to the cell interior.

As noted above, glycocalyx simulations consider the point of syndecan-to-plasma membrane interaction as an immutably restrained attachment, however, this misconception has been refuted by the recent work. Cruz-Chu *et al.* [7] in their large-scale molecular dynamics all-atom model of syndecan-4/heparan sulfate glycocalyx under the shear stress observed that moderate shear forces stretched heparan sulfate chains, thinned glycocalyx, and ectodomains were acting as levers capable of bending syndecan-4 cytoplasmic domains. This remarkable glycocalyx modeling insight into mechanotransduction initiated by shear stress and transmitted to cell interior appears to have not only an outside-in component, but may also have an inside-out periodic mechanical signaling, as detailed below.

Mechanotransduction: outside-in signaling

Mechanotransduction, the term describing the outside-in signaling may engage diverse components in its cascade. Tarbell's group advocates that glypican-1 is a main mechanosensor of shear stress to activate eNOS [16]. In addition, there is evidence supporting the involvement of PECAM-1 in mechanotransduction of shear stress to eNOS activation [17]. More recently, the role of such a transducer has been assigned to transient receptor potential (TRP)
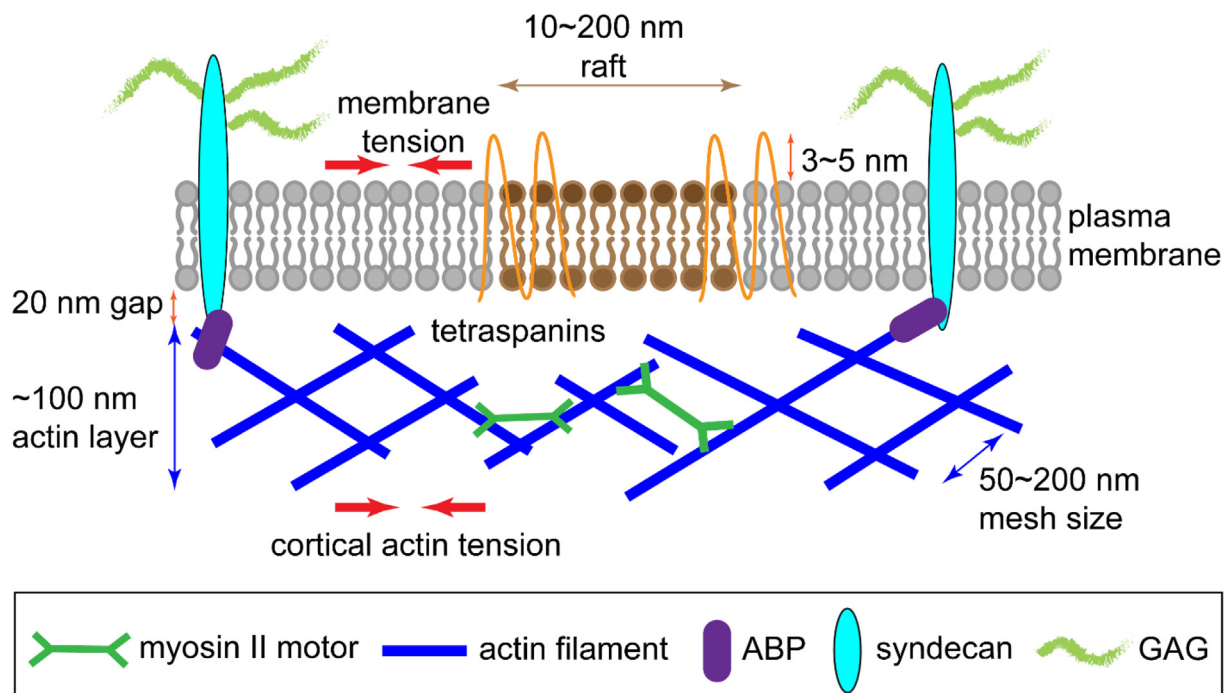

Fig. 2. Spatial relations between and dimensions of glycocalyx, lipid-rich domains of the plasma membrane, and cortical actin meshwork. (Note: dimensions are not to scale.)

non-selective cationic channels and cytosolic calcium transient associated with their opening [18]. Both heparan sulfate and hyaluronic acid are requisite for stretch-induced calcium transients and stimulation of NO production. One could envisage that tensile forces applied to these GAG chains activate opening of TRP channels, entry of calcium (Fig. 3, a), and activation of calmodulin-eNOS system with the release of NO. This sequence of events, initiated by the deformation of glycocalyx, translated by several putative transmitters (those are schematically represented by a series of universal joint shafts, Fig. 3, b) into intracellular events, and resulting in vasoaction represent the cascade of outside-in signaling. Furthermore, molecular dynamics simulation studies suggest that bending syndecan-4 ectodomain serves as a lever transmitting deformation directed opposite to the direction of flow to the cytoplasmic domain [7].

Inside-out signaling

The end-result of it is the generation of NO that partitions in the lipid membranes and, according to our earlier suggestion, should increase the fluidity of the plasma membrane [19]. This in turn leads to the reorganization/dispersion of lipid rafts, the loss of clustering platforms, dissociation of FGF and its distancing from the cognate receptor (among several other cognate ligand-receptor interactions), and termination of the original signal – the sequence of events conceptualized as "inside-out" signaling

and depicted in Fig. 3, **c**. In addition, NO-induced S-nitrosylation of actin isoforms reduces their sliding velocities and S-nitrosylation of muscle and non-muscle myosin reversibly inhibits them [20,21]. These data imply that mechanotransduction-induced NO release "relaxes" tension of cortical actin web and terminates its cytosolic $[Ca^{2+}]$-elicited contraction cycle.

The above inside-out model is resting on several experimental observations. The cortical cytoskeleton is a highly dynamic structure [22]. Cosgun, Fels and Kusche-Vihrog conceptualized relations between EG and cortical actin web as mutually interactive [23]. While cytochalasin B or D and jasplakinolide by halting or increasing actin polymerization, respectively, result in changes in the height of EG and shedding of EG leads to the loss of reorganization of the cortical actin web [24] and with it, in turn, the loss of shear stress-induced reorganization of cortical actin web. Hypoosmotic ambient stress temporarily reduces the expression of syndecan-1 in the endothelial glycocalyx, pari passu with depolymerization of F-actin cortical web [11], which partially recovers within 2 h in parallel with the reconstitution of syndecan-1. Permanent depolymerizing action of cytochalasin D, however, leads to the sustained downregulation of syndecan-1, whereas stabilization of F-actin by application of jasplakinolide prevents hypoosmotic stress-induced decline in syndecan-1. Hydrostatic pressure (as well as shear stress) elicits the initial rise in cytosolic $[Ca^{2+}]$ which leads to activation of the cortical myosin, actomyosin contraction and
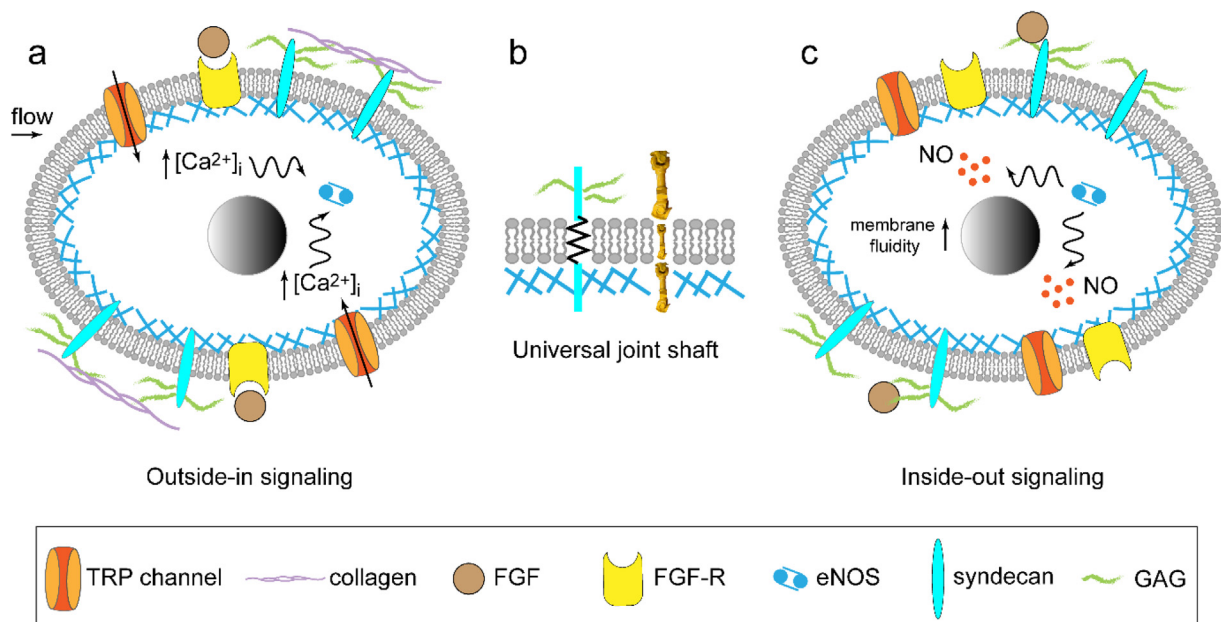

Fig. 3. Schematic presentation of the ideas of outside-in signaling central to mechanotransduction (panel a) and a reciprocal inside-out signaling essential for signal termination allowing to start the cycle anew (panel c). FGF in panels a and c represents FGF-2. See the text for details. Panel b illustrates the idea of interconnectedness of signaling components with a universal joint shaft.

cortical stiffening; chronic response to elevation in hydrostatic pressure to endothelial cells results in increased cortical actin density [12]. A prior study has elucidated that cortical actomyosin contraction (secretory vesicles used as a model) requires also cofilin-1 and alpha actinin, in addition to ROCK1 and myosin light chain kinase 1 [25].

As shear stress-induced mechanotransduction is essential for activation of eNOS, so is the NO production in the maintenance of EG [24,26]. Heparan sulfate proteoglycans prominently feature as mechanosensors mediating NO release [27], with the loss of EG being implicated in impairment of NO production and vice versa [28].

The above-proposed outside-in and inside-out signaling along the axis "shear stress-glycocalyx-cortical actin web" in endothelial cells portend the existence of pacemakers – external and internal. The external drive is provided by shear stress and periodic passage of erythrocytes to trigger oscillatory momentum to the ectodomain of syndecans, activation of TRP channels, cytosolic $[Ca^{2+}]$ transients with a pulse of contraction of cortical actin-myosin II and activation of eNOS. This cycle is terminated with generation of NO, increasing the fluidity of the lipid bilayer disengaging components of signaling cascades and relaxing cortical actin web. It remains to be clarified whether the latter events are capable of rebounding as "breaks" for the deformation of endothelial glycocalyx. The cycle reignites with the sequential near-field flow activation of EG. Thus, the described hypothetical external and internal pacemakers could sustain oscillatory activity of EG.

Glycocalyx, plasma membrane and cortical actin web behave as a functional unit – a tensegrity model

While the localization of different transmembrane proteins to lipid-rich domains remains controversial [29], there are observations suggesting that the boundary raft-non-raft regions serve as concentrators of transmembrane proteins, like tetraspanins [30]. Tetraspanins can cluster laterally with other tetraspanins and/or with partner proteins like integrins, metalloproteases and signaling molecules [31], thus forming large nanodomains limiting diffusion of lipids in the membrane and cortical cytoskeleton on the cytoplasmic side – known as a picket fence model. Despite these constrains, cortical actin web is a non-synchronously pulsating structure, constricting every 82 s and propagating to the neighboring cells, as observed in morphogenesis (*Drosophila* gastrulation) [32]. Cyclical pulses of assembly-disassembly of actomyosin web are a function of phosphorylation-dephosphorylation cycles of the non-muscle myosin II regulatory light chain [33]. More recent work showed that the cortical waves of contraction-relaxation are likely a general rule not limited to *Drosophila* [34]. The cortical

actomyosin web and lipid membrane tension are combined to produce mechanical tension counteracting intracellular osmotic pressure [34]. Plasma membrane tension appears to regulate itself via endo- and exocytosis: at higher tensions exocytosis is stimulated, thus relieving tension, whereas lower tensile pressures promote endocytosis [10,35,36]. When a motile cell spreads, membrane tension increases by 2-fold, leading to the activation of exocytosis, cortical contraction, followed by the relief of tension [37]. Tension gradients account for "Marangoni flow" shifting lipids and proteins toward areas with higher tension, thus forming a functional unit of cortical actin and transmembrane proteins, including core proteins in HSPG, like syndecans.

Notably, malfunction of cortical contractility has been causatively linked to defects in spinal neural tube closure [38] and other pathological conditions, as reviewed elsewhere[13]. Therefore, we are proposing that all 3 layers along the axis – EG, plasma membrane, and cortical actin web – behave as a structurally coupled and functionally interactive unit, which we envisage as a tensegrity model (Fig. 4). Disorganization of any of them leads to distortion of others culminating in architectural remodeling and compromised functional cooperativity.

Sliding vs gliding of erythrocytes over endothelial cells

Microvascular blood flow takes place in rather close quarters: RBC diameter of 6–8 um, capillary diameter varies between 5 and 10 μm. To accommodate a diameter of capillaries, RBC in flow attain a conical configuration. In the case RBCs slide on the surface of EC, the situation falls in the category of tribology with the consequent generation of friction, wear and heat. This is a less likely scenario. It is more likely that RBCs glide over the surface of EC without actually touching them. Gliding would reduce the resistance and propulsion force necessary for circulation in these terminal vascular beds. The same may be true for glycocalyx of these respective cells – though closely opposing each other, they are distancing from each other by the lifting force generated by oscillating glycocalyx and electrostatic repulsion as well. In our dissipative particle dynamics simulation of glycocalyxes of EC and RBC, we invariably predicted this lifting force potentially creating a cell-free zone. The similar conclusion was reached by Davies et al who have detected elastohydrodynamic lift on the endothelial surface [39]. Such a mutual preservation from friction under normal conditions may explain the phenomenon of "fingerprinting" observed under pathological conditions when glycocalyx of either EC or RBC was impaired [40]. In the context of the elastohydrodynamic lift, the observed fingerprinting would suggest that glycocalyx motion on the surface of both,
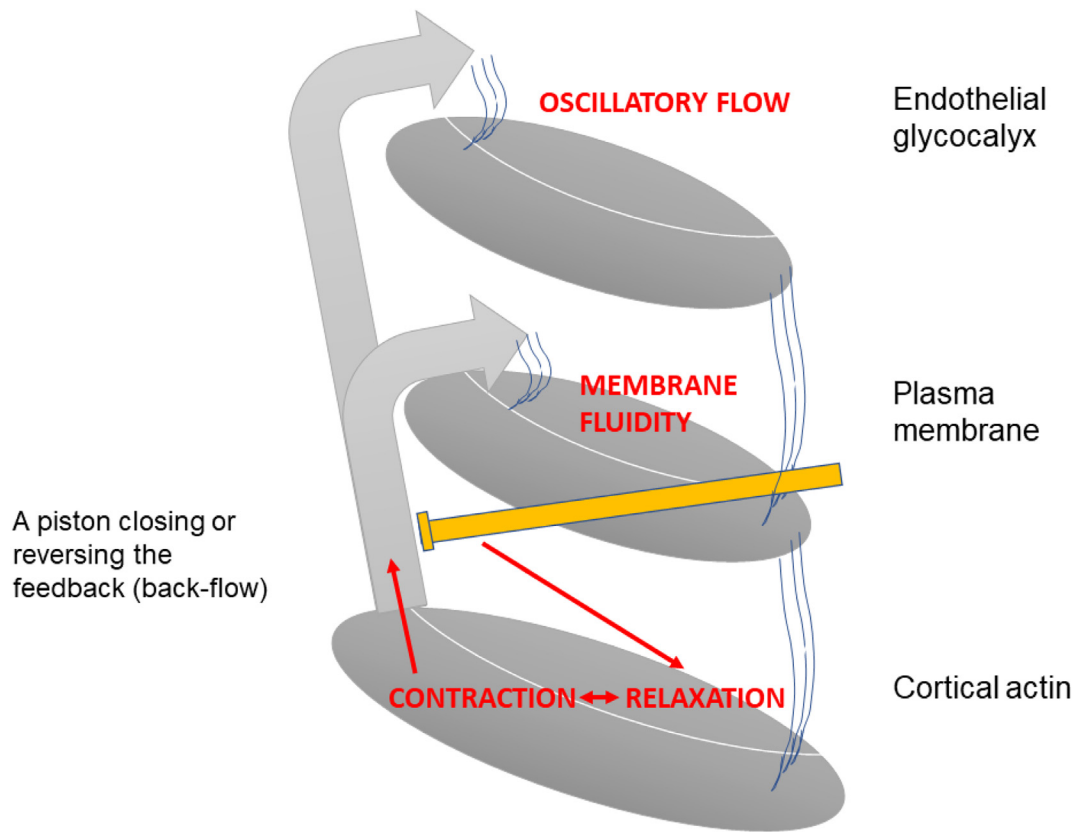
Fig. 4. Tensegrity model of the intrinsically interactive unit of glycocalyx – plasma membrane – cortical actin. Individual components of this unit are schematically displayed as containers with the fluid exchange between them, resulting in overflow and spillage to the lower container. Contraction-relaxation cycles of the container representing cortical actomyosin squeezes or retracts the back-flow to the upper containers, thus accomplishing feedback regulation.

the stationary endothelium and passaging RBC is required for creation of the cell-free zone and gliding. In this scenario, RBC are moving over the glycocalyx surface like hovercrafts as long as the integrity of glycocalyx in both is preserved, but when it is compromised, RBCs come in contact with the endothelium, encounter friction and require higher propulsive force to overcome the higher resistance (Fig. 5).

In this context it is conceivable that pathological conditions primarily affecting the rigidity of RBCs, such as sickle cell disease or invasion by *Plasmodium falciparum*, or use of blood after a long storage [41,42]; all could affect the hovercraft-like movement of RBCs in the capillary bed. The confirmation of such a prediction is provided by the fact that stiff RBCs are prone to margination [43]. Furthermore, the idea of a hovercraft-like movement of RBCs under normal condition and conversion from gliding to sliding under pathological conditions not only could explain the phenomenon of "fingerprinting", but is also buttressed by studies of sickled or deoxygenated RBC, both having increased rigidity, traverse glass capillaries 3–4 times slower than normal or oxygenated

RBCs [44]. In addition, the loss of endothelial glycocalyx predisposes to leukocyte transmigration and platelet aggregation, both prerequisites of inflammation. This latter subject is of immense medical importance, has been exhaustively reviewed [45–47] (and is beyond the scope of the present brief overview).

Concluding remarks

We summarized here a few opinions on some subtle biomechanical properties of endothelial glycocalyx. We acknowledge that endothelial glycocalyx functions as an imperfect pendulum due to multitude of obstacles including, but not limited to, the visco-elastic heparan sulfate side-chains, variable height and density, fluid nature of the plasma membrane points of fixation. We present the current views on the cortical actin web and its contraction-relaxation cycles capable of rebounding to invoke changes in tension of the plasma membrane. We consider the outside-in signaling, the basis for mechanotransduction, and propose the dampening or terminating action of
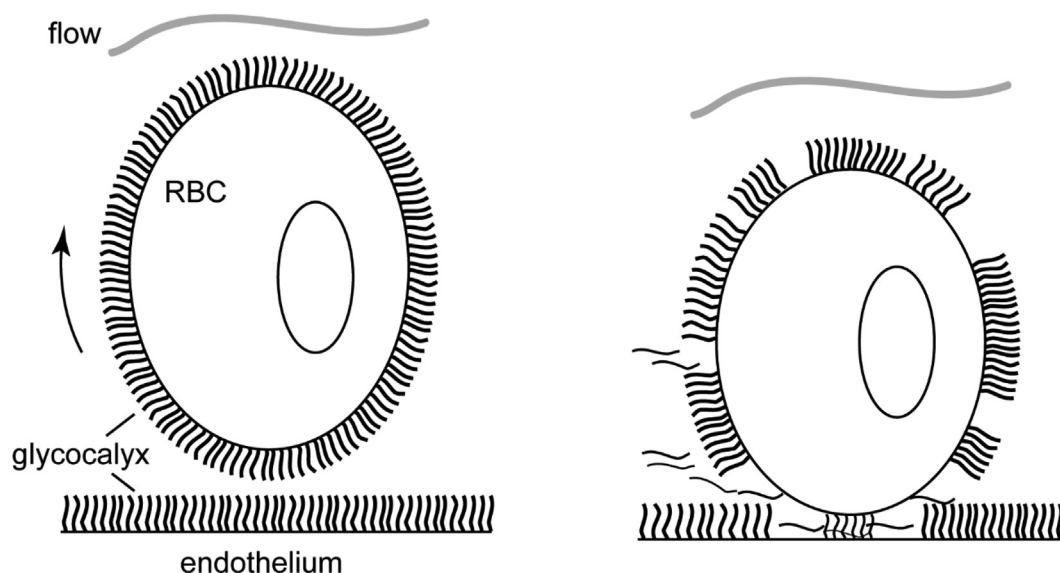
Fig. 5. Gliding (hovering) and rotation of RBC passaging capillary beds with intact glycocalyx and the loss of the lifting and rotational momentum in vessels with damaged glycocalyx.

the inside-out signaling. We offer our prediction of two functional pacemakers – glycocalyx and cortical actomyosin, coordination of which is by and large unknown. We next advance an idea that the glycocalyx, plasma membrane, and cortical actin web represent a structure-functional unit replete with multiple regulatory feedbacks which we propose to describe as a tensegrity model. Our recent mathematical modeling findings suggest that erythrocytes are gliding-hovering and rotating over the surface of intact glycocalyx, but lose their rotational and hovering motions passaging along the capillaries when glycocalyx of either endothelial or erythrocytes is degraded. These views into the mechanics of endothelial glycocalyx motions may offer potential benefits for crosspollinating physical and biological sciences and be of value in furthering our understanding of this captivating ultrastructure in health and its contributions in inducing or sustaining disease processes.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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