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Cellular Fluid Mechanics and Mechanotransduction

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Abstract—Mechanotransduction, the transformation of an applied mechanical force into a cellular biomolecular response, is briefly reviewed focusing on fluid shear stress and endothelial cells. Particular emphasis is placed on recent studies of the surface proteoglycan layer (glycocalyx) as a primary sensor of fluid shear stress that can transmit force to apical structures such as the plasma membrane or the actin cortical web where transduction can take place or to more remote regions of the cell such as intercellular junctions and basal adhesion plaques where transduction can also occur. All of these possibilities are reviewed from an integrated perspective.

Keywords—Shear stress, Endothelial cells, Mechanotransduction, Glycocalyx, Cytoskeleton.

INTRODUCTION

Fluid mechanics has played a central role in the biological response of cells to mechanical stimulus since early studies by Fry[15] and Caro and Nerem[4] using intact blood vessels suggested that the mechanical interaction of blood flow with the endothelial cells lining blood vessel walls might be a key event in atherogenesis and vascular remodeling. Subsequent studies using cultured endothelial cells in flow chambers, beginning with Dewey *et al*.,[11] were able to impose controlled fluid mechanical shear stresses on endothelial surfaces and demonstrate a wide variety of morphological and biomolecular responses as reviewed, for example, by Davies,[9] Tarbell[39] and others.[5,25]

These initial observations fueled years of research into arterial hemodynamics in search of a fundamental understanding of the processes by which cells sense and respond to fluid shear stress. The work has accelerated in recent years as a result of advances in our understanding of the biological processes involved in mechanotransduction, as well as studies that shed new light on the mechanisms of force transmission to and across the cell membrane.

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PERSPECTIVES OF MECHANOTRANSDUCTION

One new perspective on mechanotransduction that has emerged was emphasized in presentations by Vink, Tarbell and Weinbaum at this meeting. Although it has long been known that endothelial cells are coated by a thin, hydrated mesh of proteoglycans, glycosaminoglycans (GAGs) and associated plasma proteins (the glycocalyx) that is attached to the luminal membrane of vascular endothelial cells,[26] the role of this layer in mechanotransduction and other critical cell functions (white cell rolling and adhesion, red cell motion, and transendothelial transport) has only recently come into focus.

Vink reviewed recent estimates of glycocalyx thickness ranging between 0.2 and 3.0 μm depending on the visualization method (electron or fluorescence microscopy), vessel type (capillaries or large arteries), and tissue type (skeletal muscle or heart). The endothelial surface layer, or glycocalyx, prevents direct contact between flowing blood cells and the apical plasma membrane of capillaries. Degradation of the glycocalyx by the atherogenic risk factor, oxidized low-density lipoprotein (Ox-LDL), increases platelet–endothelial adhesion by degrading the glycocalyx.[7,43] In related studies it has been observed that cytokines also degrade the glycocalyx allowing enhanced leukocyte adhesion in an inflammatory response.[29] Degradation of myocardial capillary surface structures with hyaluronidase, an enzyme that degrades hyaluronic acid, a dominant GAG in these vessels, leads rapidly to myocardial tissue edema.[42] This further supports the concept that hyaluronic acid contributes to the permselectivity properties of the capillary glycocalyx.[20]

All of these physiological and pathophysiological functions of the glycocalyx derive from the fact that this surface layer provides the most apical aspect of the endothelial cell that constitutes the interface between blood and its cellular components and the endothelial cell's membrane and cytoskeleton. Because of this unique position, the glycocalyx also provides the physical structures that can sense fluid mechanical shear stress in flowing blood at its surface. Tarbell described an experimental study designed to test whether the glycocalyx serves as a mechanosensor for

fluid shear stress on endothelial cells. His group measured the production of nitric oxide by bovine aortic endothelial cells (BAECs) exposed to defined levels of shear stress *in vitro* after removing the dominant GAG component, heparan sulfate, with a selective enzyme (heparinase III). With a concentration of heparinase that removed only 46% of the fluorescence associated with an antibody specific to heparan sulfate, the substantial NO production induced by steady (20 dyn/cm^2) and oscillatory (10 $\pm$ 15 dyn/cm^2) shear stress over 3 h was completely inhibited, whereas the heparinase III treatment had no effect on agonist-induced (bradykinin, histamine) NO.[14] This was the first study to demonstrate that endothelial cells *in vitro* have a functioning glycocalyx and that it mediates the mechanotransduction of fluid shear stress.

Another recent study in isolated canine femoral arteries used the enzyme hyaluronidase to degrade hyaluronic acid GAG from the endothelial surface layer and demonstrated a significant inhibition of NO production.[28] An earlier study used the enzyme neuraminidase to remove sialic acid residues that are abundant in the glycocalyx from saline-perfused rabbit mesenteric arteries and observed that flow-dependent vasodilation was abolished.[32] Because flow-dependent vasodilation is mediated by NO release in many arteries, and neuraminidase degrades the surface glycocalyx, this study is consistent with the observations in Florian *et al*,[14] and Mochizuki *et al*.[28] These three studies[14,28,32] used different enzymes to degrade distinct components of the glycocalyx, but all blocked mechanotransduction by fluid shear stress. This draws attention to the fact that the glycocalyx is a multi-component matrix whose mechanical properties depend on the stability of the overall structure. It appears that degradation of individual components can degrade the mechanical function of the composite matrix. Other studies have shown that in addition to the integral components of the glycocalyx, the interaction of plasma proteins is required to stabilize the structure as well.[1]

In an extension of the studies described above, Tarbell reported (unpublished data) that the heparinase III treatment that was effective in completely suppressing shear-induced NO production in BAECs, did not inhibit the substantial production of prostacyclin (PGI_2) induced by steady shear stress (20 dyn/cm^2). These findings were consistent with an earlier study by Hecker *et al*.[19] that showed inhibition of shear-induced NO production, but not PGI_2 production, when intact segments of rabbit femoral arteries were pre-treated with neuraminidase. Again, different enzymes produced similar results, reinforcing the concept that the stability of the glycocalyx depends on the presence of several of its components.

Tarbell presented a hypothesis to account for the fact that degradation of the glycocalyx blocks shear-induced NO but not PGI_2 that is elaborated below. When the glycocalyx is intact, it senses fluid shear stress and transmits it to proteins in the apical plasmalemma of the cell that may activate signaling or transmit stress to the cytoskeleton and other cellular attachments including the intercellular adhesion junctions and the adhesion plaques that mediate cell adhesion to substrate. It has been demonstrated through calculations in a number of recent studies that the glycocalyx actually dissipates fluid shear stress and that the apical plasma membrane itself does not sense any significant fluid shear stress.[8,13,37] Rather, the stress is transmitted through the core proteins of the proteoglycans in the glycocalyx directly to the cytoskeleton (syndecans) or the plasma membrane (glypicans). When the glycocalyx is collapsed by enzymes or protein-free media, the fluid shear stress is directly sensed by the apical membrane and transmitted to the cellular structures. The hypothesis is that focal adhesion plaques on the basal surface of the cell feel the same stress whether or not the glycocalyx is intact because there is a mechanical equilibrium (balance of forces) that must be satisfied to hold the cell in place, but the cytoskeleton near the apical surface does not sense the same stress. This hypothesis is supported by related studies that show release of prostaglandins in response to fluid shear stress being mediated by focal adhesion,[30] and proteoglycans in the glycocalyx that have a transmembrane domain (syndecans) that can interact with eNOS in the apical cytoskeleton.[33]

Weinbaum presented a theoretical framework to describe the transmission of fluid shear stress to the actin cortical cytoskeleton underlying the apical plasma membrane. Using a structural model derived from the observations of Squire *et al*,[38] Weinbaum *et al*.[44] have shown that the core proteins in the bush-like structures comprising the glycocalyx are sufficiently stiff to act as transmitters of fluid shear stress without significant deflection. They propose that the fluid shear force that is dissipated in the outer region of the glycocalyx imposes a torque on the relatively stiff core proteins that is transmitted, via transmembrane domains (as in syndecans), to the actin cortical cytoskeleton. The calculations in Weinbaum *et al*.[44] suggest displacement of individual actin filaments in the actin cortical web on the order of 10 nm for typical fluid shear stresses, and this could drive intracellular signaling.

Weinbaum also described experiments to test the hypothesis that the glycocalyx plays a pivotal role in the transmission of fluid shear stress to the cortical cytoskeleton. Confluent monolayers of rat foot pat endothelial cells were exposed to 10 dyn/cm^2 shear stress in a parallel plate flow chamber for 5 h and fluorescent confocal images of the distribution of various cytoskeletal proteins were obtained including: actin, vinculin, paxillin, ZO-1, and connexin 43. Images were obtained for a control with no flow, a flow with Dulbecco's modified Eagle medium (DMEM) without any protein, a flow with DMEM plus 1% BSA, and a flow with DMEM plus 10% FBS. The latter two flow medias contained enough protein to support the glycocalyx, while the protein-free DMEM was expected to result in a collapsed

glycocalyx. The resuls showed a dramatic reorganization of the peripheral actin bands and the actin associated linker molecule vinculin in response to shear when the media contained BSA or FBS, but virtually no difference from controls in media without protein. This was strong evidence in support of the glycocalyx as a mechanosensor and transducer. In an extension of this work,[40] the flow experiments were repeated after the endothelial monolayers were pretreated with heparinase III as in Florian *et al*.[14] and the degradation of the heparan sulfate component of the glycocalyx by this enzyme led to results that were indistinguishable from those obtained in media without protein. This further supports the role of the glycocalyx as a mechanosensor. In this same paper,[40] Weinbaum presented a conceptual model, termed a "bumper car" model in which the actin cortical web and the dense peripheral actin bands are only loosely connected to basal attachment sites allowing for two distinct cellular signaling pathways in response to fluid shear stress, one transmitted by the glycocalyx core proteins, as described in Weinbaum *et al*.[44] and the other emanating from focal adhesions and stress fibers at the basal and apical membranes of the cell. This bumper car model provides a plausible mechanism to explain the hypothesis and data on NO and PGI_2 release described by Tarbell (above) and the data of Hecker *et al*.[19]

Other evidence points to focal adhesions as likely sites of mechanotransduction. Integrin receptors have been implicated for some time as mediators of many mechanotransduction events[6,34] and focal adhesions represent a site at which intracellular forces are concentrated.[21,27] Much recent work has focused on force transmission through transmembrane receptors to the cytoskeleton, and the potential for transduction into a biochemical signal as a consequence of force-induced conformational change in one of the load-bearing proteins.[16,2,45] This could apply either to forces transmitted to receptors via the glycocalyx, as described above, or to forces applied by other methods, such as by substrate strain or via externally tethered microbeads. In the experiments reported in Kaazempur-Mofrad *et al*,[24] and Mack *et al*.[27] magnetocytometry was used to stimulate sub-confluent endothelial cells grown on a rigid substrate and computational modeling was used to identify the stress distribution within cells. A precisely controlled magnetic trap delivered nano-Newton scale loads to individual cells via single 4.5-μm magnetic beads. Intracellular focal adhesion translocation, visualized with GFP-paxillin, served as a quantifiable biological marker to determine force magnitude thresholds relevant to mechanotransduction. A three-dimensional (3D) viscoelastic finite element model (FEM) was developed for the cell. Both the membrane/cortex and the cytoskeleton were modeled as Maxwell viscoelastic materials, but the structural effect of the membrane/cortex was found to be negligible on timescales corresponding to magnetocytometry. Computed stress and strain patterns were highly localized, suggesting that the direct effects of magnetocytometry were confined to a region extending $<10\ \mu$m from the bead. The endothelial cells exhibited a viscoelastic timescale of approximately 1 s and a shear modulus of 1000 Pa. Experimental results exhibited a distinct frequency dependence that differed for the near ($<7.5\ \mu$m diameter) and far regions of the cell, with different responses to inhibitors of tyrosine phosphorylation, suggesting different mechanisms of action, as well as a threshold for response of about 1 nN. These experiments highlight the complexity of the cellular response, that it is likely multifactorial, and that there is much we do not understand about the dynamics of the force transmission and transduction pathways.

Computational results using molecular dynamics were also presented for force-induced conformational changes in one of the proteins found in the load-bearing regions of the focal adhesion complex.[23] The hypothesis of this work is that forces acting on the cell and transmitted via transmembrane receptor proteins, or the cytoskeleton, create force-induced conformational changes in proteins, leading to altered binding affinity or kinase activity. Forces of 10–100 pN acting on individual proteins are considered sufficient to generate a signal by this mechanism. Any force transmission process, either via the glycocalyx or adhesion complex, could elicit such changes and thereby act as a means of transduction.

Such theoretical models provide a framework for understanding the distribution of mechanical forces within the cell, and the changes in protein conformation, that lead to mechanotransduction through focal adhesions.[24] By combining these approaches with the concepts of force transmission through the glycocalyx developed by Weinbaum[44] and the "bumper car" interaction of cells with an actin cortical web, dense peripheral actin band and focal adhesions in a confluent monolayer,[40] a more general quantitative description of mechanotransduction pathways should be possible.

The following guidelines for future studies of mechanotransduction have been derived from this review. For experimental studies of mechanotransduction *in vitro* and *ex vivo*, care should be taken to maintain the viability of all potential transmitting and transducing elements in the preparation. This means that (i) cells should be studied in appropriate media to maintain an extended glycocalyx, being sure that enough protein is present; (ii) methods to demonstrate the presence and integrity of the glycocalyx should be implemented routinely because this mechanosensor/transducer can be degraded through passage in culture; (iii) appropriate adhesion molecules should be applied to the supporting surface to insure realistic basal membrane adhesion linkages to the substrate; (iv) confluent monolayers should be employed to insure appropriate intercellular adhesion and interaction. Then it will be possible to effectively study the potential transducing elements for a wide variety of biomolecular end points.

This brief review has focused on the transmission of force to the cell membrane and across the membrane into the cytoskeleton, and has selectively mentioned only a few of the many mechanotransudcers that have been proposed, a more complete listing of which is given below:

(1) The actin cortical web on the apical surface that is linked to the glycocalyx through transmembrane core proteins (syndecans).[12,38]
(2) The apical plasma membrane that can be linked to the glycocalyx through GPI linkages (glypicans). Changes in membrane fluidity, reflected by altered diffusion and interaction of transmembrane proteins has been proposed as a mechanism of transduction in several studies.[3,17]
(3) Shear sensitive or stretch-activated ion channels (K^+, Cl^-) on the apical surface.[18] These have typically been studied *in vitro* in media without protein.
(4) Basal adhesion plaques in which integrins bound to the extracellular matrix transmit stress to the cytoskeleton.[35,10]
(5) Intercellular junctions where the cytoskeletons of neighboring cells are mechanically coupled through ICAMs.[36]
(6) Other elements of the cytoskeleton distributed throughout the cell.[31]
(7) Autocrine receptors, such as the EGF receptor, the activation of which can be modulated by changes in the volume of extracellular compartments.[41]
(8) Nuclear membrane proteins or DNA itself, that might change their conformation, thereby affecting gene expression due to forces transmitted directly to the nucleus.[22]

Fundamental research is needed to gain a better understanding of these mechanisms and their relative importance in mechanotransduction. By comparison, we know much more about the signaling cascades that are activated by shear stress than the mechanisms that elicit them. Based on current knowledge, we already recognize that the process of force transmission and transduction involves numerous pathways, and that further understanding will need to better elucidate the transition from continuum-level phenomena to the behavior of individual proteins.

We hope that the emphasis of this brief review on the decomposition of mechanotransduction into distinct sensing (transmitting) and transducing components will help clarify the many issues that remain to be resolved in this centrally important field that impacts vascular homeostasis, remodeling and pathology.

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