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EDITORIAL

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Syndecan-4, a model proteoglycan to study endothelial glycocalyx mechanosensing and signal transduction

In the present issue of *Acta Physiologica*, Jiang, et al[1] tested how the endothelial glycocalyx transmits the blood flow shear stress to the cytoskeleton. In a series of large-scale molecular dynamics computational experiments the dynamics of the proteoglycan syndecan-4 force transmission is examined under varying conditions, that is, blood flow velocity changes and proteoglycan sugar chain shedding. The authors identified that the main pathway of signal transmission within the syndecan-4 core protein manifests as a scissors-like motion with a transmitted force in the order of 10-100 pN. The authors further show that the main function of the sugar chains is to protect the core protein from severe conformational changes maintaining functionality of the endothelial glycocalyx.

The endothelial layer lining the inner part of blood vessels interacts with the forces arising from the flowing blood, such as a tangential shear stress and circumferential wall stretch.[2,3] Endothelial cells sense these forces and transmit the signals intracellularly given rise to a cascade of biochemical signals, resulting in cellular quiescence, which is of pivotal importance in maintenance of vascular health. As recently reviewed[4] a mechanosensing machinery complex is involved in this process, starting with the fact that cells are a tensegrity (tensional-integrity) structure where its membrane and cytosolic components acts as a single integrated structure, sensing physical forces and allowing these forces to be transmitted to other parts of the cell, to the extracellular matrix or adjacent cells. One of the fastest endothelial responses to shear is activation of flow-sensitive ion channels such as the inward rectifying K^+ channels and outward rectifying Cl^- channels, the transient receptor potential (TRP) family and mechanosensitive ion channels, the Piezo channels.[4] Cell-cell responsive complexes are formed by adhesion molecules such as the platelet endothelial cell adhesion molecule (PECAM-1) and vascular endothelial (VE)-cadherin in complex to other structural moieties.[4,5] In cell-to-extracellular matrix interactions transmembrane proteins such as integrins have been of specific interest as biomechanical sensors, because of their connectivity to both the extracellular matrix and the cytoskeleton. This was followed by observations that integrin-mediated cell adhesion and migration can be modified by cell surface proteoglycans such as syndecan-4 as well.[6,7] Like this, syndecan-4 localized at focal adhesions is able to provide signals required for focal adhesion assembly.[8-10] The first demonstration of syndecan-4 as a mechanically based initiation molecule to support cell attachment and spreading without the direct extracellular binding of integrins was given by Bellin et al,[11] arguing that syndecan-4 itself can be a mechanotransducer.

Shear signalling is initiated by the direct action of blood flow on structures on the apical membrane of the endothelium which are harboured within the endothelial glycocalyx. The endothelial glycocalyx, or endothelial surface layer, is a negatively charged gel-like surface structure of proteoglycans with their covalently bound sugar chains called glycosaminoglycans (GAGs), glycoproteins and glycolipids.[12,13] Its main GAGs are heparan sulphate (HS), chondroitin sulphate (CS) and hyaluronan (HA) of which the latter is not bound to a core protein. In general, GAGs within the glycocalyx function as a molecular scaffold facilitating protein binding and govern transcapillary fluid exchange. Another very important property of the endothelial glycocalyx is the ability to respond to external mechanical forces and to confer shear to the endothelium. Including the important luminal surface of endothelial cells in response to blood flow, recent modelling of the inter- and intracellular structures in response to various laminar and disturbed hemodynamic forces revealed that a mechanotransducer within the glycocalyx respond to forces of order 1 to several piconewton.[14]

Within the current article focusing on syndecan-4, the authors build further on the published all-atom glycocalyx model[15] to scrutinize the dynamics of this particular proteoglycan within the endothelial glycocalyx using direct

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simulation and molecular dynamics modelling. Following up on previous experiments showing a time constant for capillary endothelial glycocalyx dimensional recovery after compression by circulating cells,[16] the model predicts that because of the weak correlation of motion between the sugar chains and the core protein momentum changes cannot be compensated immediately and therefore leaves room for a time constant in recovery. According to the same published model,[16] assuming the endothelial glycocalyx as a rigid body in which the torque of the whole layer was studied, cytoskeletal deformation would be inhibited only when all sugar chains of the glycocalyx were removed, although other experiments[17] suggest a threshold in which about 30%-45% reduction already would inhibit cytoskeletal reorganization. Reconciling these differences, the soft matter model proposed in this study[1] shows that flexibility of the linkage between the ecto- and transmembrane subdomains of the core protein allows that forces can only partly be transmitted into the cytoskeleton. Thus by only removing 16.7% of the sugar chains, in terms of scissors angular variation, result already in a reduction of 55% in mechanotransduction. The authors question that as the endothelial glycocalyx is also essential in regulating endothelial permeability, would there be any connections between the role of the endothelial glycocalyx as a mechanotransducer and a microvascular barrier? For this, the study focusses on the importance of how the endothelial glycocalyx transmits forces from the ectodomain to the cytoplasm. In contrast to increased permeability upon hyaluronan loss in fenestrated glomerular endothelial cell glycocalyx,[18,19] the authors address the contrasting finding that increased permeability in continuous endothelial cells within the microcirculation only occurs when both the glycocalyx as well as cell-cell contacts are disturbed.[20,21] When testing this phenomenon in silico by removing 16.7% of the sugar chains, they hypothesized that the observed reduction of 55% in mechanotransduction only leads to a scissors angle change by 3%. This, in turn, would not affect the cytoskeleton in a way causing large enough cell cleft dimensional changes, necessary for changing the microvascular permeability.

In conclusion, in accordance with previous studies are the calculations by Jiang et al[1] of the uncovered transmitted force in the order of 10-100pN and the presence of a threshold in GAG reduction and changes in mechanotransduction. Together with earlier calculations[14] this could mean that syndecan-4 transduces force only at a certain threshold of shear stress to which endothelial cells will respond. The presented model also allows to revisit classic topics in understanding the functionality of the endothelial glycocalyx in terms of mechanotransduction, force transmission threshold and its possible relation to the microvascular barrier permeability. Finally, the observed time constant in recovery warrant further studies how endothelial cells sense flow direction and allow differential response to laminar verses disturbed shear stress exposure.

CONFLICT OF INTEREST

None.

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