Physics Meets Molecules
Is Modulation of Shear Stress the Link to Vascular Prevention?
Ivo R. Buschmann, Kerstin Lehmann, Ferdinand Le Noble; on behalf of Art.Net.

Arteries and veins are permanently exposed to hemodynamic forces because of the pulsatile nature of blood pressure and flow. Hence, the endothelium is constantly detecting different biomechanical forces, cyclic stretch and shear stress in particular, and converts the latter stimuli into intra- and extracellular signals. Endothelial cells thereby modulate multiple of physiological and pathophysiological processes: production of growth-promoting and growth-inhibiting hormones, enzymes, cytokines, etc; mediation of inflammatory responses through the expression of chemotactic and adhesion molecules on the endothelial surface; modulation of hemostasis and thrombosis via secretion of procoagulant, anticoagulant, and fibrinolytic agents; and the regulation of vascular smooth muscle cell contraction through the release of vasodilators and vasoconstrictors.

This being the case, the equilibrium between physiological levels of blood flow (shear stress) and the endothelium is tightly counterbalanced. Thereby, the lumen radius of an artery is the most important denominator, which signifies that the smaller the lumen the higher the shear stress. However, once physiological shear forces are reduced, several pathological conditions may arise: proatherogenic and/or prothrombotic states and hence atherosclerosis and/or thrombosis. Inversely, high levels of shear forces play a key role in adaptive phases of arteriogenesis (collateral artery growth), the most clinically relevant mechanism of vascular remodeling.

In case of an arterial stenosis, these arterial/arteriolar anastomoses are the only anastomosis to the low-pressure artery is the most important denominator, which signifies that the smaller the lumen the higher the shear stress. However, once physiological shear forces are reduced, several pathological conditions may arise: proatherogenic and/or prothrombotic states and hence atherosclerosis and/or thrombosis. Inversely, high levels of shear forces play a key role in adaptive phases of arteriogenesis (collateral artery growth), the most clinically relevant mechanism of vascular remodeling.

Hence, this important role of shear stress and its modulation has stimulated researchers in the past to unravel the molecular mechanisms from physical forces to molecular medicine.

In this issue of Circulation Research, Woo et al elegantly demonstrate a role for the posttranslational protein modifier SUMO (small ubiquitin-related modifier) and its substrate, the mitogen-activated protein kinase extracellular signal-regulated kinase (ERK)5, in the progression of diabetes-induced endothelial dysfunction. The authors used streptozotocin-treated diabetic mice and detected increased endogenous ERK5-SUMOylation in the aortas of these animals. Using mediators of endothelial dysfunction (reactive oxygen species [H₂O₂] and AGE [advanced glycosylation end products]), in an in vitro approach in primary endothelial cells, ERK5-Sumoylation was induced. This led to the inhibition of flow-induced activation of transcription factors myocyte enhancer factor (MEF)2 and Kruppel-like factor (KLF)2, as well as their downstream target endothelial NO synthase. KLF2 is well known as a transcriptional inhibitor of endothelial mediated inflammation. Woo et al provide a proof of concept by using specific SUMO site mutants (K6, K22), preventing ERK5 kinase activity and thus activation of downstream effectors.

Thus, the outstanding question in the field of biomechanical shear forces and vascular biology is: Does a single or specific mechanoreceptor exist, or is the cell itself, through cytoskeleton rearrangements (the concept of tensegrity), able to sense physical stimuli? Many kinds of molecules, including receptors, ion channels, caveolins, Src, focal adhesion kinase (FAK), G proteins, transcriptional factors, and specific shear-responsive elements in the promoter region associate with sensing mechanical stimulation. It is postulated that mechanical forces can modify the conformation of proteins directly, leading to functional switches (opening of ion channels) or changes in phosphorylation status, resulting in activation of downstream signaling cascades.

In a classic study by Resnick et al, it was shown that shear stress activates gene expression via transcription factor interaction with a common promoter element. The authors noted that shear stress–induced platelet-derived growth factor-B transcription requires an essential 12-bp component containing a core binding sequence that allows binding of transcription factors. This common promoter element was...
called shear stress–responsive element (SSRE), which was subsequently identified in other shear stress–regulated genes including intercellular adhesion molecule-1, and transforming growth factor-β. Such SSREs were also identified in endothelial NO synthase, another factor essential for flow-mediated vasodilation.13

The transcription factor KLF2 is rapidly upregulated by shear stress. A substantial number of in vitro and in vivo studies have shown that KLF2 is important in hemodynamic regulation in response to arterial shear stress, both in the embryo and adult, as well as pathophysiological conditions including atherosclerosis. Using a genetic approach in both zebrafish and mice, it was shown that loss of KLF2 results in heart failure.14 A detailed hemodynamic analysis revealed that this heart failure is caused by a high cardiac output state, in the absence of structural vascular defects, indicating loss of peripheral resistance. Consistent with this hypothesis, in both KLF2a morpholino knockdown zebrafish and KLF2-deficient mice, the addition of phenylephrine (a potent α-adrenergic vasoconstrictor) could rescue these animals from lethal high-output heart failure.14 Based on these observations, it is hypothesized that fluid shear forces drive expression of endothelial KLF2 that regulates smooth muscle tone in the developing embryo.

At the structural level, it is suggested that adhesive receptors that physically link extracellular support scaffolds (the extracellular matrix) to the internal cytoskeleton may function as mechanoreceptors. The endothelial cytoskeleton is a candidate for shear stress transduction because of its fast and dramatic response to shear.15 At the protein level, integrins and adhesion receptors (including platelet endothelial cell adhesion molecule [PECAM]-1, E-selectin, cadherins) link the internal cytoskeleton with the extracellular matrix and provide mechanical coupling across the cell surface. Genetic deletion of fibronectin, α5β1 integrin, and components of the extracellular matrix, including thrombospondin-1 and -2 results in vascular remodeling and angiogenesis defects. A detailed analysis indicated that, in these models, lumenzation of vessels and establishment of a functionally perfused vasculature were disturbed.

Signals from the extracellular matrix must be transduced to the signaling machinery inside the cell. Integrins accomplish this by recruiting multimolecular complexes of cytoskeletal and signaling molecules at focal adhesion sites. The activation of non–receptor tyrosine kinase FAK depends on this integrin clustering. Cells exposed to cyclic stretch or shear stress show enhanced FAK tyrosine phosphorylation, indicating that FAK activity links to mechanical stimuli.16 It is suggested that FAK activity is essential for flow-driven arteriogenesis.17

The endothelial cell–cell adhesion site is also implicated as a potential site for mechanosensing. VE-cadherin is a major adhesive protein for the adherens junction and expressed specifically in vascular endothelial cells.18 It interacts with the cytoskeleton via anchoring molecules, including β-catenin. In mice, genetic deletion of VE-cadherin results in severe vascular remodeling defects and abolishes vascular endothelial growth factor receptor (VEGFR)-2 signaling. Short intervals of shear stress stimulate the formation of the VEGFR-2/VE-cadherin/β-catenin complex in vascular endothelial cells.19 Exposure of endothelial cells to laminar shear stress results in induction of VEGFR-2, VE-cadherin, and β-catenin. This intact complex appears essential for activating downstream SSRE-dependent gene transcription.

Adhesion molecules play a pivotal role in mediating mechanosensing upstream of the integrins.20 It could be demonstrated that the PECAM-1/VEGFR2/VE-cadherin complex acts as a mechanosensory complex in vitro. The phosphatidylinositol 3-kinase is essential for shear-driven activation of α5, β3 integrin involving c-Src. PECAM-1 is required for Src activation, whereas VE-cadherin is required for transmitting this signal to phosphatidylinositol 3-kinase. Neither VE-cadherin nor PECAM-1 cells showed alignment of actin filaments in response to flow. Interestingly, in this setting, the involvement of VE-cadherin in shear stress–dependent signaling appeared independent of cell–cell signaling.

Taken together, these studies suggest that the PECAM-1/VE-cadherin/β-catenin/VEGFR2 pathway is critical for mediating shear stress responses in endothelial cells. Here, Woo et al11 illustrate the importance of posttranslational modifications. Because SUMOylation appears to be involved in several cellular functions with a large number of cellular substrates and a highly diverse outcome, this study links shear stress–induced signal transduction with a clinical feature, diabetes.

In summary, beneficial effects of shear stress (antiatherosclerotic, proarteriogenic, improvement of endothelial dysfunction) receive more and more attention, both experimentally and clinically. The increased knowledge of the underlying molecular events of shear stress signaling is of key importance to understand and promote bench-to-bedside transfer of training and prevention programs.
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References


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