Enhanced interstitial flow as a contributing factor in neointima formation: 
(shear) stressing vascular wall cell types other than the endothelium

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As blood flows through the vascular network, it creates fluid mechanical forces that can be predicted based on established physical principles of fluid dynamics. The major components of flow-generated force in the vasculature include pressure and shear stress, both of which are imparted directly onto the vessel wall. The realization that different patterns (laminar vs. oscillatory), magnitudes (high vs. low), and spatial gradients of shear stress can influence the overall vessel homeostasis has led to an intense investigation into how shear stress regulates the structural and functional phenotype of the blood vessel. Through the work of many dedicated laboratories, we are now certain that shear stress exerts a profound effect on endothelial cells which line all vessels. The endothelium, in turn, can communicate the condition of the hemodynamic environment to which it is exposed to underlying vascular components such as smooth muscle and adventitial fibroblasts, resulting in a propagation of shear stress effects to the entire vessel wall. To illustrate this point, changes in shear stress are perceived by the endothelium and converted in biochemical signals, which are then relayed to enzyme systems that generate vasoactive substances such as nitric oxide (NO). Through a paracrine effect, NO activates the molecular machinery that governs smooth muscle contractility, thereby translating changes in shear stress into an alteration of vessel diameter.

In the normal, intact vasculature, the endothelial layer forms an interface between the blood and the remaining components of the vascular wall. Thus vascular smooth muscle and adventitial fibroblasts are remote from the actual hemodynamic shear forces. As a result, the direct influence of shear stress on these mural cell types has received little attention. Rather, the stretch generated via the rhythmic expansion and contraction of the vessel wall with each successive pulse of pressure and blood flow appears to be the dominant hemodynamic stimuli for smooth muscle and, potentially, adventitial cells. Indeed, cyclic stretch has been shown to activate mechanotransduction pathways that lead to functional responses in these cell, and readers are directed to an excellent review in this area (4). So, if smooth muscle and adventitial cells are shielded, in a relative sense, from shear stress and are most responsive to hemodynamic-imposed stretch, the influences of shear stress would seem to be of little concern in these cell types. However, if one considers the forces produced by the flow of interstitial fluid through the vessel wall, then shear stress forces may in fact be an important factor in regulating smooth muscle cells and vessel wall integrity. This concept may have clinical ramifications given that factors which enhance interstitial flow (i.e., chemical or mechanical injury to the endothelium and inflammation and hypertension induced enhancement of vascular permeability) are associated with vessel remodeling and neointima formation.

The basic notion that vascular smooth muscle and fibroblasts are responsive to shear stress has been tested by several investigative teams in the past. Laminar shear stress ranging from 10 to 25 dyn/cm² decreases smooth muscle proliferation (10, 12) and migration (3, 7) and induces a phenotypic shift from a synthetic to a more differentiated and contractile morphology (6). Initial mechanotransduction responses to shear stress appear to involve calcium influx (8), production of prostaglandins (1) and NO (3), and regulation of matrix metalloproteinases (MMPs) (7). The few studies that have been performed with vascular fibroblasts show that these cells are also sensitive to shear stress and that cell confluency, hence phenotype, modulates the degree to which fibroblasts respond to shear stress (2). While these findings confirm that vascular cells other than the endothelium have the ability to sense and respond to shear stress, a major limitation develops in extrapolating these results to more physiologically relevant settings since the magnitudes of shear stress used in most of these studies were relatively high and unlikely to be encountered by these cell types in vivo, even under conditions where interstitial flow is substantially raised.

The study reported in the American Journal of Physiology-Heart and Circulatory Physiology by Tarbell’s laboratory (9) continues a line of inquiry aimed at understanding the effects of interstitial flow on smooth muscle and adventitial cell function. Their earlier study aptly modeled transmural flow through the arterial wall and calculated values of shear stress around smooth muscle cells residing in various locations (i.e., near or away from the fenestral pore of the internal elastic lamina) in the medial layer of the vessel (11). To address more basic questions regarding shear stress effects on smooth muscle and adventitial cells, this group created a system where vascular cell types are suspended in a three-dimensional (3-D) collagen-I ECM to more faithfully represent the in vivo environment (13). This methodology, which is used in the current study, represents a significant advance over past approaches performed with parallel plate, cone and plate, and similar devices where cells are grown two dimensionally and then subjected to laminar shear stress. While the 3-D model demonstrated higher Darcy permeability (Kₑ) and interstitial flow velocities compared with the normal aorta, the estimated shear stress on the embedded cells was in good agreement with expected values from an intact vessel. Interestingly, the magnitudes of shear stress are in the range of 0.05 to 0.36 dyn/cm², which is 100× lower than those used in the two-dimensional (2-D) culture studies described above, illustrating again that this system can recapitulate key aspects of the in vivo environment.

Initial studies to demonstrate that smooth muscle cells are responsive to changes in interstitial flow in the 3-D collagen gel model were reported by Tarbell’s group (13) in 2000. They
showed that smooth muscle cells produced prostaglandins when subjected to interstitial flows that generated 1 dyn/cm² or less of shear stress. Interestingly, the production rate of prostaglandins was ~10× lower than that observed in cells exposed to the same shear stress magnitudes in a 2-D model, indicating that smooth muscle cells may be more quiescent in 3-D cultures. To extend these studies, these investigators measured smooth muscle and adventitial cell motility, migration, and apoptosis, all of which contribute to vascular remodeling and neointima formation. The major finding reported in their current study is that low levels of interstitial flow enhanced the motility of all cell types embedded in the 3-D matrix, whereas higher levels of flow (and shear) at longer times of exposure suppress the migration rate of all cell types. Observations from the 2-D flow system by contrast showed an enhanced fibroblast migration at 10 dyn/cm² (2). The authors speculate that the discrepancy in the shear stimulation of fibroblasts migration between the systems may involve flow acting on elements other than the cell surface such as matrix structure and cell matrix adhesions, which could influence mechanosignaling.

To gain mechanistic insight as to how changes in interstitial flow may modulate cell motility, the authors focused on well-known cellular regulators of vascular remodeling and cell migration, namely, MMPs. Through a series of well-designed experiments, it was revealed that MMPs indeed played a central role in the shear-induced migration of both smooth muscle cells and adventitial fibroblasts. Surprisingly, MMP-1 was found to be the major metalloproteinase that regulated cell motility rather than the more highly expressed MMP-2. While the authors conclude that MMP-1 is the primary MMP operating under these experimental conditions, they do not completely rule out a role for MMP-2 in their system since MMP-2 has been reported to govern the migration events for smooth muscle and fibroblasts but at later stages in the process (5).

In addition to uncovering a key component in the induction of the flow stimulation of cell motility and migration, the studies also provide a mechanistic explanation for the suppression of the migration rate for each cell type at the highest flow rate and the longest time of exposure. Under these flow conditions, tissue inhibitor of MMP-1 (TIMP-1) expression is enhanced and acts to limit MMP-1 activity. In addition, a greater degree of apoptosis was detected within the high-flow 3-D cultures. Taken together, the combination of enhanced TIMP-1 expression and induction of apoptosis serves to counterbalance MMP activity and downregulate cell migration.

Given the paucity of information regarding the influence of interstitial flow-generated shear stress on cell types located deep within the vessel wall, the current findings provide a new perspective in considering basic processes that contribute to vascular responses associated with injury. With the use of a more physiologically precise 3-D culture system, the experimental results indicate that a direct link may exist between vessel injury and smooth muscle and adventitial cell migration. Based on their findings, the authors present a scenario where elevated interstitial flow results from injury increases shear stress on all cells in the vessel wall. Cells farthest from the vessel lumen would experience the lowest shear stress given the loose arrangement of connective tissue in this region, thereby promoting adventitial cell migration. As the injury resolves, the flow through the intima decreases. The low shear stress then activates the smooth muscle cell migration into the intima. The shear-mediated events coupled to inflammatory responses present at sites of vascular injury would collectively contribute to lesion formation.

As a whole, the study from the Tarbell laboratory (9), as well as others who have focused their attention on this particular research topic, has begun to shed light on the role of interstitial flow on vascular cell function. The continued development of the 3-D culture model where smooth muscle cells are positioned in concentric arrays so as to mimic their natural morphology in the medial layer may give a better approximation of how these cells respond to changes in interstitial flow in vivo. Additionally, other matrix components such as fibronectin, which is enriched in lesion-prone areas of the vasculature, may be incorporated into this model to evaluate the effect of various ECMs on flow-induced cell function. Finally, the ability to regulate interstitial flow through an intact or injured vessel seems feasible. Experiments in whole vessel preparations would serve to validate and guide future work with the 3-D culture model.

GRANTS

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