

REVIEW

Vascular dysfunction and pathology: focus on mechanical forces

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Abstract

The role of mechanical forces is emerging as a new player in the pathophysiologic programming of the cardiovascular system. The ability of the cells to ‘sense’ mechanical forces does not relate only to perception of movement or flow, as intended traditionally, but also to the biophysical properties of the extracellular matrix, the geometry of the tissues, and the force distribution inside them. This is also supported by the finding that cells can actively translate mechanical cues into discrete gene expression and epigenetic programming. In the present review, we will contextualize these new concepts in the vascular pathologic programming.

Key Words

- ▶ mechanotransduction
- ▶ vascular biology
- ▶ vascular pathology

Introduction

Blood vessels undergo continuous structural changes in response to physiologic alterations, or wall injury. In particular, blood flow exerts a crucial role in vessel remodeling, starting from vascular system development to adult life. Indeed, in the early post-implantation life period, blood flow, acting on endothelial cells, promotes intracellular signaling cascades leading to the formation of a branched, hierarchical structure of vessels with large and small caliber (1). The implication of hemodynamic forces in the vasculature development is suggested by evidences showing that in embryos with abnormal cardiogenesis, vessels of the yolk sac fail to remodel, resulting in embryo lethality (2, 3). Blood flow also contributes to vessel maturation by the specification of the arterial vs the venous compartments. For example, in zebrafish and mice, endothelial progenitor cells express markers of both venous and arterial phenotype (4, 5), and the exposure to blood flow promotes an increase in arterial markers expression and a parallel downregulation of the venous genes, contributing in this way to the differentiation into

arterial endothelial cells (ECs) (6). During the adult life, hemodynamic forces also play an important role in vessel homeostasis. Indeed, in physiological conditions, laminar shear stress, a tangential force acting on the endothelium, has vaso-protective effects, stimulating endothelial cells to release small molecules (e.g. nitric oxide) and cytokines with antithrombotic and vasodilator properties (7). An example of flow-dependent control of the vascular tone is the transcriptional regulation of genes encoding for mediators with vasodilator/vasoconstrictor effects, such as endothelial nitric oxide synthase, prostacyclin (*NOS1,PTGIS*) (8). Indeed, various mechanosensors exposed to shear stress, such as primary cilia, integrins, induce the activity of transcription factors, such as Krüppel-like factor (*KLF2*), thus promoting the expression of vascular tone-regulating genes (9). In the murine carotid artery collar model, it has been demonstrated that *KLF2* is highly expressed in high-shear stress regions and this regulates the transcription of flow-responsive genes, such as endothelin-1, adrenomedullin, and nitric oxide synthase

(eNOS), involved in controlling vascular tone (10). Although hemodynamic forces are considered determinants of vascular morphogenesis and physiology, it has been demonstrated that an altered blood flow, in conjunction with metabolic, genetic, or inflammatory-related diseases, may contribute to the pathological progression of the vascular tissue, such as atherosclerosis and vein graft disease (11, 12, 13). In the present contribution, we will describe the effects of perturbed hemodynamic forces on vessel wall remodeling with a specific focus on the relevance of cell-based-mechanosensing in the etiopathogenesis of several vascular diseases.

Altered shear stress is responsible for endothelium dysfunction and atherosclerotic plaque formation

Although laminar shear stress is considered crucial for physiological vascular functioning, disturbed or oscillatory flow emerges as an important feature of atherogenesis. Indeed, flow patterns and hemodynamic forces are different inside the vascular system: in the straight parts of the arterial tree, blood shear stress is high and laminar, while, in branches and curvatures, blood flow is disturbed with oscillatory/low shear stress (14). It has been demonstrated that atherosclerotic plaques usually tend to form in areas with low and oscillatory shear stress, probably due to the effects of disturbed flow on the endothelium (15). Indeed, oscillatory shear stress induces changes to endothelial gene expression, cytoskeletal arrangement, leukocyte adhesion, thus promoting an oxidative and inflammatory state in the vessel wall. The first evidence of the direct effect of altered hemodynamic conditions on endothelial structure and function is represented by the striking reorganization of endothelial cell morphology in response to shear stress. In particular, strain stimulation induces endothelial cell elongation and alignment in the direction of applied flow (16). This reorganization of actin fibers and cytoskeletal proteins requires a very short time and reaches maximal levels after 1 h of stimulation, favoring endothelial cell shape, over locomotion, in confluent monolayers (16). Therefore, in response to shear stress, EC stress fibers align with the direction of the flow (17), and this directionality is strictly determinant for the cellular response, such as proliferation (18). The early response of endothelial cells to altered shear stress is mediated, for example, by flow-sensitive ion channels, which are important components of EC mechanosensing system (19). In particular, oscillatory shear stress elicits minimal depolarizing current and

increases hyperpolarization in ECs, causing defects in cell membrane polarization and affecting ions influx (19). In this way, the production of molecules and bioactive agents with atheroprotective properties, such as oxide nitric (NO), is reduced (Fig. 1) (20). Recently, two transmembrane proteins, Piezo1 and Piezo2, have been identified as essential components of ion channels and new EC mechanosensors (21). Indeed, Piezo 1 channels, expressed by human placental arteries ECs, are activated by shear stress (22). Depletion of these two proteins affects the ability of ECs to align in the direction of shear stress, but not cell vitality, suggesting their possible implication in EC shear stress sensing. Another important player in EC mechanosensing is the glycocalyx, which lines the EC apical surface and is tightly related to atherosclerosis (23). Disturbed shear stress is able to physically displace the glycocalyx, inducing an intracellular response (24). Indeed, this mechanosensor is constituted by an extracellular domain for flow sensing and an intracellular sequence, which is directly connected to the cytoskeleton (24). Glycocalyx responds to external stimuli inducing a re-organization of the actin cytoskeleton and subsequent changes in EC morphology and functions, which lead to atherosclerotic plaque formation (Fig. 1) (25, 26). Once these mechanosensors are activated, they are able to transduce mechanical signals by increasing kinase activities and modulating the phosphorylation of many signaling proteins. For example, integrin activation results in the phosphorylation of focal adhesion kinases, paxillin and other mediators, leading to the activation of mitogen-activated protein kinases via Ras GTPase (27). This determines important morphological changes and affects cell proliferation and migration (28, 29). Rho family kinases activation

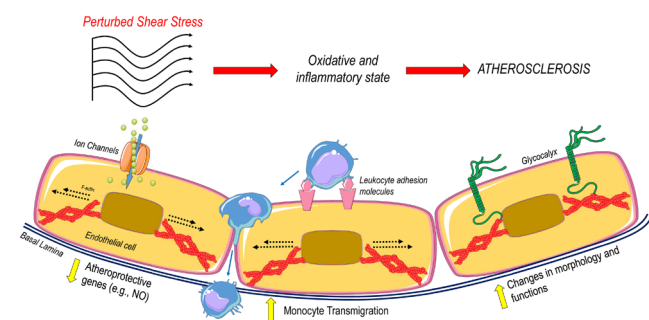


Figure 1

Effects of perturbed shear stress on vascular endothelial cells. Various mechanosensors (ion channels, glycocalyx), expressed at the luminal surface of endothelial cells, are activated by altered blood flow, with consequent changes in cell morphology and functions. This establishes an oxidative and inflammatory state in the endothelium, which promotes atherosclerotic plaque development.

mediates shear stress dependent-EC alignment, affecting stress fiber formation and cytoskeletal reorganization (30). Therefore, these kinases are also involved in the nuclear import of one of the most important mechanosensitive transcription factors in ECs, Yes associated-protein (YAP). In particular, nuclear YAP is transcriptionally active in human pulmonary artery ECs stimulated with high shear stress, and, in zebrafish, *in vivo* cessation of blood flow determines the exclusion of YAP from nuclei (31). In addition, it has been shown that disturbed blood flow leads to the nuclear translocation of YAP and consequent continuous activation of its transcriptional machinery. This also explains the increased expression of this factor in atherosclerosis-prone artery regions in mice, which are subject to perturbed shear stress (32). In support to these evidences, the depletion of YAP in mice endothelial cells prevents plaque formation, contributing to quiescent and anti-inflammatory EC phenotype (33). Altered shear stress contributes to establish a chronic inflammation in the endothelium also by inducing the expression of endothelial cell leukocyte adhesion molecules. This is associated with increased monocyte adherence and thus, enhanced vessel wall permeability (Fig. 1) (34). Altered hemodynamic flow is involved in making the pathological conditions worse. On one hand, forming plaque creates sections with perturbed flow inside the vessel, which may favor the rupture of the plaque (35). On the other hand, altered shear stress increases the expression of metalloproteinases (MMPs), promoting fibrous cap collagen degradation and affecting plaque vulnerability (35). The activity of MMPs, a family of proteolytic enzymes involved in ECM components degradation, is regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs) (36). The balance between MMPs and TIMPs plays an important role during late-stage progression and rupture of atherosclerotic plaques. For example, genetic deletion of MMP3 in ApoE mice stimulates the formation of plaques with reduced smooth muscle cell content and concomitant increase in fibrous layers (37). In addition, elevated levels of MMP9 in advanced plaques, associated with macrophage accumulation, do not affect plaque size or composition, but favor plaque disruption by promoting intra-plaque hemorrhages (38). Since endogenous levels of TIMPs are insufficient to contrast MMP activities, one possible therapeutic strategy to contrast flow-mediated plaque instability is to increase the expression of TIMPs (39). Indeed, overexpression of TIMP2 prevents plaque development and disruption, by modulating cellular content and functionalities in mice (40). In particular, TIMP2 interferes with MMPs-mediated atherogenesis

by inhibiting SMCs and macrophages-derived foam cells migration and apoptosis (40). The balance between MMPs and TIMPs should be taken into consideration to avoid plaque destabilization driven by perturbed mechanical forces.

Taken together, these evidences highlight that altered shear stress, in conjunction with other risk factors, is able to initiate atherosclerotic plaque formation through the activation of aberrant mechano-dependent machineries, which lead to endothelium dysfunction and pathology.

Cell mechanosensing participates to pathological vessel wall remodeling

Perturbed shear stress is not the only mechanical stimuli setting pathologic vessel remodeling. In fact, hydrostatic pressure and cyclic strain, due to pressure patterns, are also involved. In particular, altered compression forces, occurring during specific hemodynamic conditions, affect the deeper layers in the vessel wall, such as the media and the adventitia. For example, this is thought to occur in saphenous vein bypass grafts, possibly due to the vein shift from a low and constant flow to a high and pulsatile coronary pattern (41). Indeed, during exposure to arterial conditions, the saphenous vein undergoes a complete vessel wall remodeling, characterized by invasion and over-proliferation of vascular smooth muscle cells (VSMCs) in the intima layer (42). These pathological conditions, known as vein graft disease, lead to progressive bypass graft stenosis and consequent ischemic heart disease. Although this pathology has been considered for many years as an endothelial-inflammatory disease, nowadays, recent evidences highlight a strong implication of cell- and tissue-based mechanosensitivity in the initiation of this disease. Indeed, it has been demonstrated that the direct exposure of human saphenous vein to coronary pulsatile pressure causes a complete remodeling of the vessel wall, both at the cell and tissue level (43). In this study, cyclic strain induced a switching of VSMCs from contractile to synthetic phenotype, associated with a consistent release of thrombospondin-1, a pro-fibrotic matricellular protein, with chemoattractant effects on saphenous vein progenitor cells (SVPs) (Fig. 2) (43). In addition, this protein, in conjunction with TGFB1, increased the SVP proliferation rate and collagen production, resulting in myofibroblast-like differentiation of vein progenitor cells (Fig. 2) (43). The direct application of circumferential tension to SVPs revealed a potent mechano-dependent response of these cells, in terms of gene expression pathways involved in

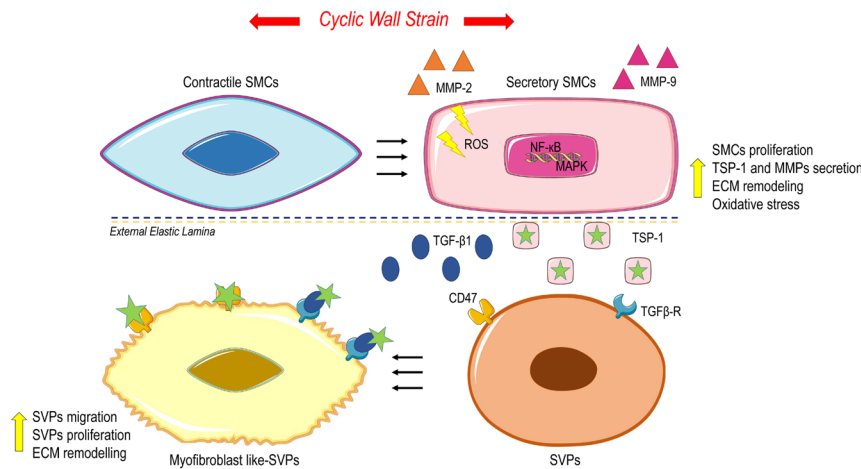


Figure 2

Effects of cyclic wall strain on vascular smooth muscle cells and adventitial progenitor cells. Vessel wall remodeling is associated with SMCs phenotypic switching and an increased release of TSP-1, a matricellular protein with chemoattractant effects on SVPs. TSP1, in conjunction with TGFβ, induces SVPs differentiation into proliferative myfibroblast like-cells deputed to ECM remodeling. In addition, cyclic strain increases smooth muscle cells proliferation, oxidative stress (ROS), and MMPs activities.

extracellular matrix remodeling and cell differentiation (Garoffolo *et al.* data not published). In particular, it has been demonstrated that a mechano-dependent enrichment of YAP/TEAD signature, with particular reference to genes implicated in profibrotic cell activation. Taken together, these evidences highlight the implication of cell and tissue mechanics in the human vein arterialization process. In particular, prolonged altered mechanics could cause remarkable changes in cell structure and gene expression, leading to pro-pathological cell activation and the onset of fibrosis. Beyond its effect on VSMCs phenotypic switching, pulsatile stretch is also able to stimulate SMCs proliferation by increasing oxidative stress and promoting DNA synthesis via NFKB (Fig. 2) (44). Indeed, direct exposure of SMCs to cyclic strain elicits a rapid increase in intracellular NAD phosphate (NAD(P)H), associated with elevated levels of reactive oxygen species (ROS, superoxide, and hydrogen peroxide) (Fig. 2) (45). One signaling target of oxidative stress is mediated by the mitogen-activated protein kinase (MAPK) family. In particular, extracellular signal-regulated protein kinase (ERK)1/2, p38 MAPK, and c-jun N-terminal kinase (JNK) are all activated by cyclic strain in SMCs and specifically inhibited by a specific NAD(P)H inhibitor, suggesting the implication of redox-sensitive signaling pathways activated by mechanical strain (45). Exposure of VSMCs to cyclic mechanical strain also increases collagen and fibronectin concentrations, metalloproteinases activity, and the release of TGFβ1, modulating in this way the fibrogenic activity of VSMCs (Fig. 2) (46, 47). However, SMCs derived from the internal mammary artery (IMA) or saphenous vein (SV) have different behavior under mechanical stimulation; pulsatile strain affects cell proliferation in SV, but not IMA, suggesting that

mechanical factors are potential mediators of aberrant cell proliferation during vein graft occlusion (48).

Mechanically strained VSMCs are also known to directly impact the vessel wall structure and organization by ECM production and deposition. In physiological conditions, VSMCs are surrounded by ECM consisting of collagens, elastin, and proteoglycans, all necessary to maintain vascular tissue integrity (49). Changes in hemodynamic forces increase the expression of enzymes involved in tissue remodeling and collagen production (50, 51), which are able to affect the structural composition of the vessel. Indeed, elevated cyclic strain is able to upregulate the production of matrix-degrading enzymes (MMPs 2 and 9) and the release of inflammatory and remodeling factors by VSMCs, such as TGFβ, angiotensin II, all associated with aneurismatic dilatation of the aorta (52, 53). Indeed, abnormal SMC mechanosensing has been implicated in thoracic aortic aneurysm progression (TAA) in a variety of genetically driven diseases (52); for example, Marfan syndrome, a heritable disorder of connective tissue, caused by mutations in fibrillin-1 gene (FBN1) (54). This protein is essential for the formation of elastic fibers, and mutations in this gene affect ECM integrity at the level of tunica media, in the aorta, leading to TAA and dissection (54). FBN1 mutation determines an altered mechanical compliance of the matrix, thus resulting in VSMCs morphological and functional changes (phenotypic switching and apoptosis), leading to aortic wall degradation (55). Stanford type A aortic dissection (STAAD) pathogenesis is characterized by changes in ECM mechanical stress and consequent VSMCs apoptosis, due to aortic media degeneration (56). How this disorganized mechanical stress contributes to

VSMCs apoptosis and the pathology remains unclear, but recent evidences suggest the implication of YAP-related mechanosensing pathway also in this disease. Indeed, the deletion of vascular smooth muscle-specific *Yap* in mice results in embryonic lethality with abnormal aorta development (57), and, in addition, altered mechanical stress is able to affect YAP expression (58). In normal aortas, YAP is mainly expressed by VSMCs at the level of the tunica media; instead, in the mouse model of STAAD, the expression of this transcription factor is completely reduced and it is negatively correlated with the ascending aorta diameter (59, 60). These results suggest that low mechanical compliance of the aortic matrix may cause YAP nuclear exclusion and degradation, making it transcriptionally inactive. This could also explain the increase of VSMCs apoptosis and the parallel downregulation of cell proliferation, one of the main YAP-mediated cell functionings. Also, elevated wall pressure can play an important role in the onset of vascular diseases. For example, cyclic strain induces arterial stiffening of large arteries, contributing to vascular calcification. Indeed, due to calcific plaque that affects vessel patency, the velocity of the blood flow increases with consequent effects on blood pressure (61). High pulsatile pressure affects VSMCs that actively differentiate to remodel arterial tissues after injury. Within the tunica media, undifferentiated VSMCs can shift phenotypically to osteoclast-like cells after environmental stimuli, such as increased matrix stiffness and elevated mechanical strain (62). Indeed, abnormal mechanical strain induces the overproduction of collagen and fragmentation of elastin fibers, causing complete structural disorganization of the vessel wall and a reduced vessel elasticity (63). This determines an increased matrix stiffness of the vessel and consequently an over-proliferation of hypercontractile SMC population with osteochondrogenic features (62). In addition to altered actomyosin activity, matrix stiffness is able to influence also VSMCs differentiation, creating in this way a feedback loop, which overstimulates remodeling processes and may be responsible for the growth of calcific plaque (64). WNT/ β -catenin signaling plays a crucial role in arterial medial calcification, through the induction of *Runx2*, which, in turn, regulates VSMCs osteoblast transdifferentiation in the high-phosphate environment (65). Inhibition of WNT/ β -catenin signaling using WNT antagonist, DKK1, abolishes calcium deposition stimulated by high-phosphate in VSMC (65). It is possible to speculate that pro-pathological VSMCs differentiation is affected by mechanical strain and matrix stiffness and

targeting WNT/ β -catenin signaling could be an effective strategy to prevent arterial medial calcification.

Conclusions

Hemodynamic forces play a crucial role both in vascular morphogenesis and disease. Vascular cells are able to respond to mechanical stimulation activating intracellular signaling pathways and regulating gene expression. Aberrant cell mechanosensing, in conjunction with metabolic, genetic and inflammatory risk factors, could be implicated in the initiation of several vascular diseases. Taken together, these evidences suggest the importance to better define the mechano-dependent mechanisms underlying these pathologies, in order to develop new therapeutic strategies for the treatment of vascular diseases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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