Biomechanical regulation of vascular smooth muscle cell functions: from in vitro to in vivo understanding

Juhui Qiu1,†, Yiming Zheng1,†, Jianjun Hu1, Donghua Liao2,3, Hans Gregersen1, Xiaoyan Deng4, Yubo Fan4 and Guixue Wang1

1Key Laboratory of Biomechanical Science and Technology (Chongqing University), Ministry of Education, Chongqing Engineering Laboratory in Vascular Implants, College of Bioengineering, Chongqing University, Chongqing 400044, People’s Republic of China
2Clinical Institute, Aarhus University Hospital, Aarhus, Denmark
3Mech-Sense, Department of Gastroenterology and Surgery, Aalborg University Hospital, Aalborg, Denmark
4School of Biological Science and Medical Engineering, Beihang University, Beijing, People’s Republic of China

Vascular smooth muscle cells (VSMCs) have critical functions in vascular diseases. Haemodynamic factors are important regulators of VSMC functions in vascular pathophysiology. VSMCs are physiologically active in the three-dimensional matrix and interact with the shear stress sensor of endothelial cells (ECs). The purpose of this review is to illustrate how haemodynamic factors regulate VSMC functions under two-dimensional conditions in vitro or three-dimensional co-culture conditions in vivo. Recent advances show that high shear stress induces VSMC apoptosis through endothelial-released nitric oxide and low shear stress upregulates VSMC proliferation and migration through platelet-derived growth factor released by ECs. This differential regulation emphasizes the need to construct more actual environments for future research on vascular diseases (such as atherosclerosis and hypertension) and cardiovascular tissue engineering.

1. Introduction

The abnormal proliferation and migration of vascular smooth muscle cell (VSMC) are key components of various vascular diseases, including hypertension, atherosclerosis and vascular stenosis after vessel transplant [1–4]. In addition, VSMC apoptosis is one of the dominant factors that induce plaque vulnerability in atherosclerosis [5,6]. Haemodynamic factors within the vasculature, such as blood flow-induced blood vessel wall shear stress, cyclic strain and hydrostatic pressure, control the phenotype and function of VSMCs [7]. A normal haemodynamic environment balances the VSMC apoptosis and proliferation. However, the abnormal mechanical factors in the blood vessel wall destroy the balance between VSMC proliferation and apoptosis, resulting in vascular pathophysiology [8,9].

Endothelial cells (ECs) are the lining on the inside surface of vasculature and are capable of perceiving shear stress as a mechanical signal, transmitting this into the cell interior, triggering serial cellular signalling responsible for gene expression and then regulating the function of VSMCs [10,11]. This in vitro haemodynamic process, in which mechanical stimuli regulate the functions of VSMCs mainly through direct contact with ECs, is the physiological basis for in vitro models of EC–VSMC co-cultures. But in the case of endothelial injury or VSMC migration into the intima, blood flow may directly act on the superficial layer of VSMCs.

In this review, we provide an overview of VSMC functions and atherosclerotic plaque progression in physiological culture models with biomechanical stimulation. Elucidating how VSMC functions under mechanical stimulation will improve current understanding of vascular physiology/pathology and...
2. Haemodynamic factors and vascular smooth muscle cell function

2.1. Effects of shear stress and flow pattern on the two-dimensional cultured vascular smooth muscle cells

Most VSMCs in vivo and in primary cell cultures exhibit contractile phenotype. VSMCs at the contractile phenotype mainly express smooth muscle α-actin (α-SMA), smooth muscle myosin heavy chain (SM-MHC) and calponin1 [12]. Nevertheless, the synthetic-phenotype VSMCs mainly express vimentin; VSMCs undergoing passage in vitro or affected with diseases turn into the synthetic phenotype, and VSMCs span a continuum from contractile to synthetic phenotype [13]. The synthetic phenotype of VSMCs is critical for atherosclerosis [14–16]. Shear stress regulates VSMC function by changing their phenotypic behaviours, such as cell proliferation and differentiation [17].

VSMC aligns in a shear-stress-dependent manner and is relatively parallel to the shear stress direction in high lamina shear stress regions but is perpendicular to the shear stress direction in low shear stress regions in vitro [18]. This shear-stress-regulated VSMC alignment is controlled by the glyocalyx in vitro, particularly by heparin sulfate proteoglycans (HSPGs) [19]. In vitro investigations showed the VSMC proliferation is inhibited by high laminar shear stress [20,21] and it is dependent on the cell-cycle arrest [22]. On the contrary, the low shear stress induces VSMC proliferation [20,23]. Similar results are also observed in tissue culture studies [24–27], hence the low shear stress may act as a critical factor for intimal hyperplasia. Subnormal shear-stress-induced intimal thickening requires VSMC proliferation and migration [28], and it is well known that the mechanism of VSMC proliferation and migration regulated by shear stress involves platelet-derived growth factor (PDGF) [29] and matrix metalloproteinase-2 (MMP-2) through nitric oxide (NO) signalling pathway [30,31].

Oscillatory shear stress increases VSMC phenotypic transformation to the synthetic phenotype and then induces proliferation, mainly through activation of the phosphatidylinositol 3 kinase (PI3K)–protein kinase B (Akt) signal transduction pathway [32] and extracellular signal-regulated kinase 1/2 (ERK1/2) pathway [17] (figure 1 and table 1). Hence, the flow pattern is another critical parameter for cell proliferation and migration. It has been demonstrated in the vein grafts, the vortex blood flow induces VSMC migration and neointimal hyperplasia, whereas the reduced vortex blood flow in the modified vein graft strongly suppresses these effects. The suppression is achieved via the phosphorylation of ERK1/2 and myosin light chain kinase [28]. VSMCs can regulate platelet aggregation under haemodynamic forces, and oscillatory shear stress inhibits platelet reactivity and preserves blood fluidity at vascular injury sites [33]. Consistently, disturbed blood flow promotes thrombus formation in rabbit femoral arteries by inducing erosive injury to VSMCs-rich neointima in vivo [34] and induces atherosclerosis through enhanced VSMC proliferation, migration and thrombus formation.

Some previous studies showed that VSMC proliferation mainly localizes in high shear stress regions with sustained flow where atherosclerosis occurs [14,35,36]. After ECs become apoptotic, VSMCs are likely to have more direct contact with blood flow. As the internal elastic lamina remains localized on the VSMC surface when the endothelium is damaged, the VSMCs could be exposed to shear stress, which is derived from the transmural pressure gradient [37].

2.2. Effects of fluid shear stress on vascular smooth muscle cell at the three-dimensional level

Although the afore-mentioned results provide some explanations for atherosclerosis development by analysing VSMC...
functions at the two-dimensional level, they are insufficient to describe the actual behaviours and functions of VSMCs during atherosclerotic development. For example, the interstitial flow rate induces a shear stress of 1 dyn cm\(^{-2}\) on VSMCs through the internal elastic lamina [38–40]. When exposed to 8 dyn cm\(^{-2}\) laminar flow shear stress (two-dimensional) for 15 h, smooth muscle cell expressions of \(\alpha\)-SMA, SM22, SM-MHC, smoothelin and calponin are significantly reduced. However, when cells are suspended in collagen gels and exposed to interstitial flow for 6 h, expressions of SM-MHC, smoothelin and calponin are significantly reduced, whereas expressions of \(\alpha\)-SMA and SM22 are markedly enhanced [41]. These findings show that VSMCs on two-dimensional matrix mainly exhibit synthesized phenotype; by contrast, VSMCs sustain a contractile phenotype either on the three-dimensional matrix or with stimulation of interstitial flow [41]. These findings show that VSMCs on two-dimensional cultures with mechanical stimulation, VSMCs–gel model stimulation under three-dimensional cultures. For three-dimensional and quantitatively visualized in three-dimensional collagen lagen I is applied through exposure to interstitial flow induced MMP-13 [53,54]. The shear stress in the three-dimensional collagen matrix should be taken into account. As reported in a single cell three-dimensional culture under mechanical stimulation, single cell alignment and migration were dynamically and quantitatively visualized in three-dimensional collagen hydrogels under mechanical force [46].

VSMCs align perpendicularly to the interstitial flow direction under three-dimensional cultures. For three-dimensional cultures with mechanical stimulation, VSMCs–gel model withstands 90 cm H\(_2\)O differential pressure to form 1 dyn cm\(^{-2}\) [41]. Non-uniform shear induces formation of cell density gradients and alignment of VSMCs in the neointima of vascular polymer implants through the PDGF-\(\beta\) receptor and Src kinase [47]. Under a three-dimensional matrix, the expressions of prostaglandin I\(_2\) (PGI\(_2\)) and prostaglandin E\(_2\) (PGE\(_2\)) are approximately 10 times lower than those observed when the same cells are plated on collagen-treated glass slides (two-dimensional model) and exposed to 1 dyn cm\(^{-2}\) shear stress [37]. These results indicate that interstitial flow can affect VSMC biology in the three-dimensional matrix and that VSMCs are more quiescent in three-dimensional cultures than in two-dimensional cultures [37]. Thus, studying VSMC physiological behaviour in the three-dimensional matrix is necessary. Interstitial fluid flow also induces transdifferentiation of VSMCs into myofibroblasts and promotes collagen alignment in vitro [48]. Myofibroblasts have a strong migratory and contractile ability, and have been implicated in connective tissue remodelling [49–51]. As myofibroblast-mediated adventitial remodelling plays a critical role in arterial pathology [52], shear-stress-induced transdifferentiation of VSMCs into myofibroblasts might be involved in atherosclerosis development.

Interstitial flow induces vascular VSMC motility in three-dimensional collagen I via upregulation of MMP-1 and MMP-13 [53,54]. The shear stress in the three-dimensional collagen I is applied through exposure to interstitial flow induced by 1 cm H\(_2\)O pressure (shear stress = 0.05 dyn cm\(^{-2}\); flow velocity = 0.5 \(\mu\)m s\(^{-1}\); Darcy permeability = 10\(^{-11}\) cm\(^2\)). In fact, the glycocalyx layer is important in interstitial flow shear stress to embedded cells in the ECM [55]. Further research has indicated that this phenomenon is controlled by focal adhesion kinase (FAK)-ERK1/2-dependent and

### Table 1. Shear stress regulates the cell function of VSMCs by affecting gene expression.

<table>
<thead>
<tr>
<th>type of shear stress</th>
<th>cell type</th>
<th>genes regulated and time</th>
<th>functions</th>
<th>study</th>
</tr>
</thead>
<tbody>
<tr>
<td>orbital SS 9.8 dyn 210 r.p.m.</td>
<td>bovine ASMC</td>
<td>↑ERK1/2 phosphorylation at 15 min</td>
<td>increased proliferation by changing SMC phenotype</td>
<td>[17]</td>
</tr>
<tr>
<td>laminar SS 6 dyn</td>
<td>bovine ASMC</td>
<td></td>
<td>aligned along the direction of SS</td>
<td>[18]</td>
</tr>
<tr>
<td>physiological SS 12 dyn</td>
<td>rat ASMC</td>
<td>↑NO at 12–24 h</td>
<td>SS-suppressed proliferation and migration</td>
<td>[19]</td>
</tr>
<tr>
<td>laminar SS 3, 6 and 9 dyn</td>
<td>bovine ASMC</td>
<td></td>
<td>high SS inhibits SMC proliferation</td>
<td>[20]</td>
</tr>
<tr>
<td>laminar SS 14 dyn</td>
<td>rat ASMC</td>
<td>↑TFPI-2 at 24 h</td>
<td>inhibits SMC proliferation</td>
<td>[21]</td>
</tr>
<tr>
<td>laminar SS 5–25 dyn</td>
<td>human ASMC</td>
<td></td>
<td>high SS inhibits SMC proliferation</td>
<td>[22]</td>
</tr>
<tr>
<td>laminar SS 14 and 28 dyn</td>
<td>human ASMC</td>
<td>↓TGF beta 1 at 24 h</td>
<td>SS inhibited VSMC proliferation</td>
<td>[27]</td>
</tr>
<tr>
<td>laminar SS 12 dyn</td>
<td>bovine ASMC</td>
<td>↓PDGF receptor-beta and ↓MMP-2, ↓MMP-14 at 3 or 15 h</td>
<td>SS inhibits VSMC invasion and migration</td>
<td>[29]</td>
</tr>
<tr>
<td>laminar SS 10 or 20 dyn</td>
<td>rat ASMC</td>
<td>↓MMP-2 at 4 h</td>
<td>SS inhibits VSMC migration</td>
<td>[30]</td>
</tr>
<tr>
<td>laminar SS 11 dyn</td>
<td>bovine ASMC</td>
<td>↓Akt phosphorylation at 24 h</td>
<td>SS stimulates SMC apoptosis</td>
<td>[31]</td>
</tr>
<tr>
<td>oscillatory SS 14 dyn</td>
<td>bovine ASMC</td>
<td>↑Akt phosphorylation at 1, 3 or 5 d</td>
<td>oscillatory SS increases SMC proliferation</td>
<td>[32]</td>
</tr>
<tr>
<td>laminar SS 5, 10 and 20 dyn</td>
<td>rat ASMC</td>
<td>↑HO-1 at 1, 2, 4 and 24 h</td>
<td>high SS induces HO-1 expression through CD</td>
<td>[33]</td>
</tr>
</tbody>
</table>
c-Jun-mediated signalling [54,56]. The mechanotransduction can be transmitted by HSPGs and ERK1/2 [41].

2.3. Effects of vascular wall mechanical forces on vascular smooth muscle cell functions in two-dimensional environments

Cyclic stretch, arising from the periodic change of vessel circumference by pulsatile blood flow, is the predominant mechanical force that influences VSMC structural organization and signalling. Stretch induces profound changes in the cell phenotype, function and gene expression [57,58]. The physical cyclic stretch maintains a differentiated and fully functional phenotype of VSMCs. VSMCs use multiple sensing mechanisms to perceive the mechanical stimulus generated from pulsatile stretch and transduce it into intracellular signals, resulting in the modulations of gene expression and cellular functions, such as proliferation, apoptosis, migration and remodelling [59].

VSMC aligns perpendicularly to the direction of stretch via NO signalling [60] and redox-dependent activation of Notch3 [61]. Physiological cyclic stretch (10%, 1 Hz) inhibits VSMC proliferation [62] by inhibiting G1/S phase transition, which is associated with a decrease in retinoblastoma protein phosphorylation and with a selective increase in the cyclin-dependent kinase inhibitor p21, but not in the p27 [62]. Mechanical stretch stimulates activation of FAK, followed by its association with c-Src, which requires ion influx mainly via stretch-activated non-selective ion channels, thereby leading to activation of the p21 (Ras)/ERK1/2 cascade in VSMCs [63]. Cyclic strain (10%, 1 Hz) inhibits VSMC proliferation via Notch receptor signalling in VSMCs in vitro [64]. Parathyroid hormone-related protein participates in the regulation of stretch-induced renal VSMC proliferation [65]. Stretch-induced proliferation is achieved mainly through modulation of insulin growth factor 1 (IGF-1), mitogen-activated protein kinase (MAPK), PI3K, tyrosine kinase and nuclear factor-kappaB (NF-kB) [60,66–68]. In addition, stretch-induced hypertrophy also depends on the activated calcium channels [68,69], and the activated calcium channels necessitate PI3K/Akt signalling [70].

Cyclic stretch can also stimulate VSMC apoptosis. Periodical stretching (20% elongation, 0.5 Hz) induces VSMC apoptosis via endothelin B receptor [71]. Integrin-β1–rac-p38-p53 signalling pathways also participate in mechanical stress-induced VSMC apoptosis [72]. However, the phenotype change in VSMCs is also implicated in the VSMC functions. Differentiated VSMCs express a greater level of Bcl-2-associated death factor (BAD) and undergo significant cell loss when exposed to mechanical stretch (10% elongation, 1 Hz) for 24 h; by contrast, no changes are observed in the undifferentiated cells [73]. In addition, mechanical stretch (15%, 1 Hz) inhibits oxidized low-density lipoprotein (LDL)-induced apoptosis in VSMCs by upregulating integrin α (v)β (3) and stabilization of PINCH-1 (an adaptor protein essential for the regulation of cell–ECM adhesion). These findings provide evidence that mechanical stretch acts as a survival factor in the arches of aortas [74] (figure 2 and table 2).

In addition, mechanical stress is an important regulator for proinflammatory cytokine and reactive oxygen species (ROS) formation in VSMCs. It can induce interleukin (IL)-6 and C-reactive protein expression, and the signal pathway was induced by mechanical stretch in VSMCs via Ras/Rac-p38 MAPK-NF-kB signalling pathways [76,77]. Mechanical strain induces monocyte adhesion to the vascular wall by increasing the expression of monocyte chemoattractant protein-1, inter-leukin-6, keratinocyte-derived chemokine and vascular cell adhesion molecule-1 (VCAM-1) [84]. These results are consistent with the development of atherosclerosis through NF-kB induction under hypertension in vitro [84]. Additionally, mechanical stretch also induces ROS formation via the NAD(P)H oxidase, which then enhances MMP-2 mRNA expression and pro-MMP-2 release [78]. These observations may explain stretch-modulated vascular remodelling (i.e. in arterial hypertension) by stimulating MMP expression and activity via NAD(P)H oxidase pathway.

With advancing age and atherosclerosis, arterial compliance reduces leading to the development of systolic hypertension and a substantial increase in pulse pressure. Physiological cyclic stretch upregulates l-arginine transport and directs its metabolism to l-proline, which plays an important role in stabilizing vascular lesions by promoting VSMC collagen synthesis [79]. These results were also confirmed in tissue culture. Under normal pulse shear stress (6 ± 3 dyn cm⁻²) combined with a pulse pressure of 80 ± 10 mm Hg, mechanical stress reduces to levels less than 1% in porcine carotid arteries, facilitating both cell proliferation and migration, and also upregulating MMPs expression [75]. The molecular mechanism involved is the Rho/Rho-kinase pathway [75]. VSMC stiffness and focal adhesion area are time-dependently changed by 10% cyclic equibiaxial stretch [85]. In addition, VSMC stiffening leads to aortic stiffness with ageing [80]. Aortic stiffening reduces arterial compliance, causing a substantial increase in pulse pressure. An augmented pulse pressure can be a predictor of hypertension development, which has been linked to several cardiovascular diseases, including atherosclerosis [86]. In turn, vascular stiffness increases cell stiffness and matrix expression, which promotes vascular stiffening, forming a vicious cycle between cell stiffness and levels of arterial compliance. Therefore, research on vascular mechanical stress should be based on the experimental study and constitutive modelling of the passive mechanical properties [87].

VSMCs from different sources have different biological effects in response to mechanical stretch in vitro. Increased cyclic stretch is believed to be one of the major pathological factors of vein graft disease when moved from low-pressure venous to higher pressure arterial [88,89]. Aortic VSMCs do not show a significant increase in growth when compared with unstretched controls after stretching at 0.05 and 1 Hz. By contrast, stretchings of venous VSMCs both at 0.05 and 1 Hz result in a significant increase in growth when compared with static controls [90,91]. Mechanical stretch increases the synthetic-phenotype behaviour of injured VSMCs in the arterial intima in response to in vitro balloon angioplasty injury [16,81]. Venous VSMC proliferation was induced by mechanical stretch through IGF-1 receptor activation and serum-, glucocorticoid-regulated kinase 1 [82,92]. Mechanical stretch also stimulated rat aortic and coronary VSMCs to have differential DNA synthesis ability in vitro [83]. These results suggest that VSMC proliferation, which occurs in vein interposition grafts in vivo, is partially owing to the stimulatory response of venous VSMC to increased mechanical stimulation.
3. Haemodynamic factors regulate vascular smooth muscle cell behaviours in co-culture conditions

3.1. Co-culture model under a haemodynamic environment

VSMCs and ECs have critical interaction under physiological conditions in vivo through direct contact or via communication of growth factors. A co-culture system was used to investigate the synergistic effects of advanced glycation end-products on intercellular–cellular interactions [93,94], creating a more realistic platform for atherosclerosis research. Chiu et al. [95–97] developed an important EC–VSMC co-culture system in which ECs are not only in close proximity to VSMCs, but are also constantly subjected to shear stress. In this model, ECs and VSMCs are separated by a porous membrane, with only the ECs being subjected to flow conditions. That is, ECs and VSMCs are grown on two sides of the chamber, and one has no direct contact with the other, which is different from VSMCs migration into the intima under pathological conditions.

There is a new model of direct-contact co-culture between smooth muscle and ECs [98]. Phenotype-controlled VSMCs are also considered to illustrate in vivo conditions more precisely [99]. The perfusion bioreactor system is an important tool for investigation of cell–cell and cell–ECM interactions in vascular cell biology and tissue engineering [100,101]. A three-dimensional in vitro perfusion system allows direct investigation of shear stress effects on the development of microvasculature in vitro [102]. A new type of ‘on chip and in situ’ micropatterning technique has been developed [103–105] and this technique is even shown to control single cell co-cultures. Single cell co-cultures are in close proximity to the formation of connexion structures and the study of contact modes of communication [106]. Strain preconditioning results in rearrangement of the vessel wall that yields circumferentially oriented cells and collagen fibrils. Thus, ECs and VSMCs in response to fluid shear stress are influenced by the underlying substrate, suggesting that strain preconditioning should be considered [107]. Similarly, by comparing the ex vivo artery model and in vitro co-culture model, differences have been observed in the migration distance of ECs between the ex vivo and in vitro models, with ECs migration being slower in the ex vivo artery model [108]. The difference could be mostly attributable to stronger EC–ECM binding in the natural arterial structure than

Figure 2. Vascular smooth muscle cells mechanotransduction mechanisms in stretch-dependent interconnected signalling. Mechanical stretch signals are sensed by a variety of membrane mechanoreceptors, including transmembrane molecules such as integrin [72,74], notch [61,64], luminal structures such as ion channel (K⁺ and Ca²⁺) [68] and tyrosine-protein kinase [66]. Transmembrane integrins bind to specific ECM protein and associate with a variety of FAK proteins to trigger downstream RhoA/ROCK signalling cascades [75], and regulate the migration of SMC. Activated integrins also can lead to integrin-dependent activation of MAPKs via FAK through integrin-dependent Src-Ras-MEK1/2 activation [63]. In addition, integrin-dependent MAPKs (ERK1/2) activation mechanism involves Rho GTPase (RhoA, Rac1) [72,76,77]. Ultimately, integrin-dependent MAPKs pathway mechanically leads to activation of transcription factor NF-κB and p53 [72,76,77]. Thereby, integrin MAPK signalling pathway is a critical signal pathway for SMC proliferation and apoptosis under mechanical stimulation. Furthermore, tyrosine-protein kinase receptor and ion channel regulate the SMC proliferation and apoptosis through PI3K/Akt and ERK signal pathway [66,69,70]. ECM, extracellular matrix; FA, focal adhesion; FAK, focal adhesion kinase; c-Src, tyrosine-protein kinase; MAPKs, mitogen-activated protein kinases; ERK, extracellular signal-regulated kinase; Shc, SH2-combining adaptor protein; Ras, small GTPase; Rho, small GTPase related to Ras; NF-κB, nuclear factor-kappaB; IκB, IkappaB; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B.
**Table 2.** Mechanical stretch regulates the functions of VSMCs. SS, shear stress; SMC, smooth muscle cell; ASMC, aortic smooth muscle cell; CAKbeta, cell adhesion kinase beta; TFPI-2, tissue factor pathway inhibitor-2; PDGF, platelet-derived growth factor; MMP, matrix metalloprotease; HO, haem oxygenase; CO, carbon monoxide; NO, nitric oxide; ERK1/2, extracellular signal-regulated kinase1/2; AKT, protein kinase B; min, minute (or minutes); h, hour (or hours); d, day (or days); KC, keratinocyte-derived chemokine; VCAM-1, vascular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; P3K, phosphatidylinositol 3-kinase; SGK-1, serum-, glucocorticoid-regulated kinase-1; OxLDL, oxidized low-density lipoprotein; IL-6, interleukin-6; IEX-1, immediate early response gene X-1; MAPK, mitogen-activated protein kinase; TR3, TR3 nuclear orphan receptor.

<table>
<thead>
<tr>
<th>type of stretch</th>
<th>cell type</th>
<th>genes regulated and time</th>
<th>functions</th>
<th>study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexercell</td>
<td>rat ASMC</td>
<td>↑ Notch3, ↑ p21</td>
<td>VSMC alignment of 80–85° to the stretch via NO signalling at 48 h</td>
<td>[60]</td>
</tr>
<tr>
<td>uniaxial stretch</td>
<td>human SMC</td>
<td>↑ p38 MAPK at 3 h</td>
<td>cyclic stretch stimulates SMC a perpendicular to the stretch direction</td>
<td>[61]</td>
</tr>
<tr>
<td>FX 3000</td>
<td>rat ASMC</td>
<td>↑ p21 (cyclin-dependent kinase inhibitor) at 1 d</td>
<td>physiological cyclic stretch inhibits cell proliferation 1–5 day</td>
<td>[62]</td>
</tr>
<tr>
<td>FX 2000 6.25%, 12.5%, 25%, 1 Hz</td>
<td>rat ASMC</td>
<td>↓ NF-κB at 24 h</td>
<td>activation of cell adhesion kinase beta by mechanical stretch in ASMC</td>
<td>[63]</td>
</tr>
<tr>
<td>equibiaxial cyclic strain 15%, 1 Hz</td>
<td>rat ASMC</td>
<td>↓ Notch receptor at 0 to 24 h</td>
<td>cyclic strain inhibits SMC proliferation while enhancing SMC apoptosis</td>
<td>[64]</td>
</tr>
<tr>
<td>equibiaxial stretch 10%, 1 Hz</td>
<td>renovascular SMC and ASMC</td>
<td>↑ PThrP (parathyroid hormone-related protein) renovascular SMC at 23.7 h and ASMC at 42 h</td>
<td>stretch-inhibited renal VSMC proliferation through PThrP</td>
<td>[65]</td>
</tr>
<tr>
<td>Flexcell</td>
<td>rat ASMC</td>
<td>↑ insulin-like growth factor-1 receptor at 2 h</td>
<td>mechanical stretch augments insulin-induced VSMC proliferation</td>
<td>[66]</td>
</tr>
<tr>
<td>biaxial strain 4% strain, 1 Hz,</td>
<td>human ASMC</td>
<td>↑ PAI-1, ↑ VEGF, ↑ cyclooxygenase, ↑ tenasin-C, ↓ thrombomodulin, ↓ MMP1 at 24 h</td>
<td>stretch-induced genes have potential extracellular matrix or vasomotion roles to defence against excess mechanical load</td>
<td>[67]</td>
</tr>
<tr>
<td>biaxial strain 1–9%, 1 Hz</td>
<td>human ASMC</td>
<td>↑ iex-1 24 h</td>
<td>stretch inhibits VSMC proliferation through iex-1 gene</td>
<td>[68]</td>
</tr>
<tr>
<td>FX 3000</td>
<td>rat ASMC</td>
<td>↑ endothelin B receptor at 6 h</td>
<td>stretch induces VSMC apoptosis</td>
<td>[71]</td>
</tr>
<tr>
<td>FX 4000</td>
<td>rat ASMC</td>
<td>↑ p38 MAPK phosphorylation, ↑ beta1-integrin, ↑ p53 at 6 h</td>
<td>mechanical stretch induces apoptosis of VSMC on collagen I</td>
<td>[72]</td>
</tr>
<tr>
<td>FX 4000T</td>
<td>swine SMC</td>
<td>↑ Bcl-2-associated death factor (BAD) at 24 h</td>
<td>stretch-induced VSMC apoptosis is phenotype-dependent</td>
<td>[73]</td>
</tr>
<tr>
<td>FX 4000</td>
<td>mouse ASMC</td>
<td>↑ Integrin β3 at 2h</td>
<td>mechanical stretch inhibits OxLDL-induced apoptosis in VSMCs</td>
<td>[74]</td>
</tr>
<tr>
<td>FX 4000</td>
<td>mouse ASMC</td>
<td>↓ JwBcr</td>
<td>stretch induces IL-6 expression in SMC via Ras/Rac1-p38 MAPK-NF-κB signalling pathways</td>
<td>[76]</td>
</tr>
<tr>
<td>physiological stretch 80 mmHg or 150 mmHg</td>
<td>mouse carotid arteries</td>
<td>↑ VGCAM-1, ↑ MCP-1, ↑ IL-6, ↑ NF-κB at 1, 6, 12, or 24 h</td>
<td>high stretch promotes monocyte adhesion through high stretch promotes monocyte adhesion through MMP activation and activity in a NAD(P)H oxidase-dependent manner</td>
<td>[84]</td>
</tr>
<tr>
<td>FX-3000</td>
<td>mouse ASMC</td>
<td>↑ NAD(P)H oxidase subunit nox1, ↑ p47phox, ↑ MMP-2 at 0–24 h</td>
<td>stretch enhances MMP expression and activity in a NAD(P)H oxidase-dependent manner</td>
<td>[78]</td>
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(Continued.)
the polymer substrate in the co-culture model. Importantly, both in the ex vivo artery model and in vitro EC–VSMC co-culture model, normal arterial shear stress induced a higher rate of EC migration than under low shear stress [108]. Noteworthy is the influence of shear-mediated ECs on VSMC behaviour which is quite different between coculture and non-co-culture systems. As reported in an in vitro non-co-culture study, the authors found that arterial shear stress stimulated ECs to release several VSMC chemoattractants to induce VSMC chemotaxis via ERK1/2 pathways [109].

3.2. Shear-stress-regulated vascular smooth muscle cell biological behaviours via endothelial cells

VSMC functions are closely associated with VSMC phenotype change. Under EC–VSMC co-culture conditions, shear stress (12 dyn cm⁻²) induces synthetic-to-contractile phenotypic modulation in VSMCs via peroxisome proliferator-activated receptor α/β activations [110]. In the presence of physiological shear stresses, ECs align with the flow direction and VSMCs align more perpendicularly to flow [111]. Shear stress regulates the release of active substances by ECs, which then regulate VSMC functions. Those biologically active agents include endothelin-1 (ET-1), NO and angiotensin II as well as growth (cell) factors such as TGF-β, PDGF-A [112] and PDGF-BB [113].

EC–VSMC interactions lead to an increase in VSMC differentiation [114]. Atherosclerotic flow decreases VSMC gene expression (α-SMA and myocardin) and induces a pro-inflammatory phenotype in ECs and VSMCs by upregulating expression of VCAM-1, IL-8 and monocyte chemoattractant protein-1 (MCP-1) [111]. Shear stress downregulates pathophysiologically relevant gene expression under EC–VSMC co-culture [95,97].

Under static conditions, ECs induce VSMC migration in a co-culture system, but shear stress inhibits VSMC migration through regulation of ECs at 15 dyn cm⁻² [115]. The mechanism involved is that low shear stress increases endothelial PDGF expression [116–118] and the upregulated expression of PDGF-DD in ECs mediates VSMC phenotypic modulation [119,120]. High shear stress protects ECs, even shear exceeding 1500 dyn cm⁻² did not cause gross injury or denudation [121]. Shear stress induces the endothelial-derived release of NO [122,123], which inhibits VSMC migration and induces VSMC apoptosis [30,124,125] (figure 3). Shear stress also delays the migration of either cell type in a dose-dependent manner in an EC–VSMC co-cultured microsystem [104]. In a three-dimensional EC–VSMC co-cultured system, ECs with a high expression of Krüppel-like factor 2 significantly reduce VSMC migration [103].

Sustained pulsatile flow regulates endothelial NO synthase and cyclooxygenase expression in co-cultured vascular ECs and VSMCs [126], and NO decreases VSMC proliferation and increases apoptosis [125,127]. VSMC apoptosis is a critical factor for rupture-prone plaque formation. Clinical studies report that VSMCs mainly localize in the distal region of the vascular stenosis with low shear stress [128–130]. Increasing blood flow in vessel grafts induces endothelialization and inhibits neointimal hyperplasia [125,131], the molecular mechanism of which is based on the high expression of bone morphogenetic proteins (BMP4) [132] and NO [123]. However, segments of the artery wall exposed to lower endothelial shear stress are significantly thicker than segments exposed to higher shear stress within the same artery [133].

Oscillatory shear stress induces VSMC migration by increasing the expression of MMP-2 and MMP-9 and decreasing plasminogen activator inhibitor-1 (PAI-1) expression [134,135]. In vascular grafts, disturbed flow provides favourable conditions for VSMC proliferation with low shear stress, which may explain, in part, why intimal hyperplasia and atherosclerotic lesions develop preferentially in slow-flow regions [136]. In co-culture models, VSMCs and ECs influence each other, and fluid shear stress appears to have a greater effect on the EC elastic modulus than in the presence of VSMCs. Furthermore, the changes in the elastic modulus in the co-culture model are regulated through EC–VSMC communication [137]. Shear stress

<table>
<thead>
<tr>
<th>type of stretch</th>
<th>cell type</th>
<th>genes regulated and time</th>
<th>functions</th>
<th>study</th>
</tr>
</thead>
<tbody>
<tr>
<td>FX 3000 10%, 1 Hz</td>
<td>rat ASMC</td>
<td>↑ type I collagen</td>
<td>physiological cyclic stretch directs</td>
<td>[79]</td>
</tr>
<tr>
<td>equiaxial stretch 5%, 10%, 0.25 Hz</td>
<td>rat SMC</td>
<td>↑ paxillin</td>
<td>stretch changes SMCs stiffness and focal adhesion area</td>
<td>[85]</td>
</tr>
<tr>
<td>FX 3000 10%, 0.5 Hz</td>
<td>human venous SMC and ASMC</td>
<td>↑ TR3</td>
<td>cyclic stretch-induced proliferation of venous SMCs</td>
<td>[91]</td>
</tr>
<tr>
<td>FX 3000 0–20%, 1 Hz</td>
<td>mice venous SMCs</td>
<td>↑ IFG-1/IFG-1R</td>
<td>mechanical stretch simulates proliferation of venous SMCs 2h</td>
<td>[92]</td>
</tr>
<tr>
<td>FX 3000 15%, 1 Hz</td>
<td>mice venous SMCs</td>
<td>↑ SGK-1 at 2 h</td>
<td>the mechanical stress-induced</td>
<td>[82]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ pERK1/2, ↑ p38, ↑ pJNK at 10 min</td>
<td>neointima formation in vein grafts</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ p27kip1-ser10, ↑ pAKT at 30 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. (Continued.)
and VSMCs modulate EC migration and differentiation [138,139]. With increasing LDL concentration, shear stress increases LDL uptake by human ECs when co-cultured with VSMCs [140]. Shear stress inhibits VSMC-induced inflammatory gene expression in ECs [141].

3.3. Mechanical stretch-regulated vascular smooth muscle cell proliferation via endothelial cells

Mechanical stretch has been shown to cause EC and VSMC injury in experimental vein grafts [142]. Consistently, cyclic stretch inhibits VSMC growth by ECs through release of thrombospondin (TSP)-1, a natural antiangiogenic factor [143]. However, experimental evidence is available suggesting that proliferation of both ECs and VSMCs is increased in response to stretch [144]. The increased proliferation of VSMC could also be found in bovine aortic EC–VSMC co-cultured ePTFE vascular grafts [145]. In the EC–VSMC co-culture model, stretch owing to high blood pressure increases VSMC proliferation. ECs and VSMCs use different molecular mechanisms to respond to mechanical stretch [144], though it remains unknown how the interaction of endothelial and VSMCs in co-culture environments is regulated by mechanical stretch. Cyclic tensile strain regulates a sequence of autocrine and paracrine signalling in ECs [146], and then ECs appear to collaborate with mechanical conditioning to induce the VSMC contractile phenotype [147].

4. Concluding remarks

These findings suggest that VSMCs are exposed to more complex haemodynamic and chemical environments than previously anticipated. Therefore, future studies should focus on the systemically cooperative mechanisms by integrating knowledge of biomechanics, blood rheology, cell biology and molecular biology to study VSMC functions during atherosclerotic progression: (i) disintegrate and integrate multiple mechanical effects appropriately on VSMC to reveal the complex interplay of stress in cell behaviours. For example, based on physiological shear stress and pulse pressure, it is necessary to explore the effects of reduced cyclic stretch on smooth muscle function and within the arterial wall [79,148]. In addition to shear stress, substrate architecture [149] and cyclic strain [107,150] are also critical for cell function. The flexible tubular silicone substrate within the inner wall of the endothelium may be used to provide realistic mechanical pulsatile forces in the vasculature [151,152]. (ii) Further research should be directed at strengthening the current understanding of interaction between VSMCs and ECs in co-culture models in response to shear stress. ECs regulate VSMC functions, meanwhile VSMCs regulate EC functions. For instance, VSMC rigidity and ECM organization influence EC spreading and adhesion, and ECs regulate VSMC function in co-culture conditions [153,154]. (iii) Epigenetics is the heritable change in gene expression that occurs without changes in the DNA sequence. It includes DNA methylation, histone modification/chromatin remodelling and RNA-based machinery. Previous research proved that the mechanical environment regulates cellular functions by modifying the chromatin state of ECs and VSMCs [155]. Recent study provides direct evidence that shear stress regulates VSMC turnover by EC-secreted microRNA-126 in the EC–VSMC co-culture model [156].

Further research should consider the mechanical behaviour of VSMC as a research core. And the mechanical stress-regulated VSMC biological behaviours should be based on animal experiments in vivo combined with in vitro mechanical stress models. Many approaches are available to detect VSMC functions in the body, an example of which is polarization-sensitive optical coherence tomography that is used to detect
the cell content of VSMC [157]. This technique could be applied to
direct the therapy of cardiovascular disease. In the vein grafts
process, a biomechanical engineering approach with adaptive
training before implantation into the body could possibly pro-
mote graft patency when tensile stress was reduced [142].

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