REVIEW

Cellular and molecular effects of mechanical stretch on vascular cells and cardiac myocytes

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ABSTRACT

Cells in the cardiovascular system are permanently subjected to mechanical forces due to the pulsatile nature of blood flow and shear stress, created by the beating heart. These haemodynamic forces play an important role in the regulation of vascular development, remodelling, wound healing and atherosclerotic lesion formation. Mechanical stretch can modulate several different cellular functions in VSMCs (vascular smooth muscle cells). These functions include, but are not limited to, cell alignment and differentiation, migration, survival or apoptosis, vascular remodelling, and autocrine and paracrine functions. Laminar shear stress exerts anti-apoptotic, anti-atherosclerotic and antithrombotic effects on ECs (endothelial cells). Mechanical stretch of cardiac myocytes can modulate growth, apoptosis, electric remodelling, alterations in gene expression, and autocrine and paracrine effects. The aim of the present review is primarily to summarize the cellular and molecular effects of mechanical stretch on vascular cells and cardiac myocytes, emphasizing the molecular mechanisms underlying the regulation. Knowledge of the impact of mechanical stretch on the cardiovascular system is vital to the understanding of the pathogenesis of cardiovascular diseases, and is also crucial to provide new insights into the prevention and therapy of cardiovascular diseases.

INTRODUCTION

Cells in the cardiovascular system are permanently subjected to mechanical forces due to the pulsatile nature of blood flow and shear stress, created by the beating heart. These haemodynamic forces play an important role in the regulation of vascular development, remodelling, wound healing and atherosclerotic lesion formation. For the cardiovascular systems, ECs (endothelial cells), SMCs (smooth muscle cells) and cardiac myocytes are the major cells that face mechanical forces. Blood pressure is the major determinant of vessel stretch, and volume and pressure are the major determinants of cardiac stretch. VSMCs (vascular SMCs) are the main cellular component of the blood vessel wall. They are subjected to a dynamic mechanical environment modulated by pulsatile pressure

Key words: cardiac myocyte, endothelial cell, mechanical stretch, shear stress, smooth muscle cell.

Abbreviations: ANF, atrial natriuretic factor; AngII, angiotensin II; AP-1, activator protein-1; AT1, receptor, AngII type 1 receptor; ARB, AT1 receptor blocker; BAD, Bcl-2-associated death factor; BMP, bone morphogenetic protein; BNP, brain natriuretic peptide; Cdc42, cell division cycle 42; Cx, connexin; DDR-2, discoidin domain receptor-2; EC, endothelial cell; EGFR, epidermal growth factor receptor; ERK, extracellular-signal-regulated kinase; ET, endothelin; FAK, focal adhesion kinase; GADD153, growth-arrest and DNA-damage-inducible protein 153; GSK, glycogen synthase kinase; HIF-1α, hypoxia-inducible factor-1α; IGF-1, insulin-like growth factor-1; IGF-1R, IGF-1 receptor; IL, interleukin; JNK, c-Jun N-terminal kinase; KLF-2, Kruppel-like factor-2; LXR, liver X receptor; MAPK, mitogen-activated protein kinase; BMK-1, big MAPK-1 (also known as ERK-5); MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; NOS, NO synthase; eNOS, endothelial NOS; iNOS, inducible NOS; oxLDL, oxidized low-density lipoprotein; PI3K, phosphatidylinositol 3-kinase; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PPAR-γ, peroxisome proliferator-activated receptor-γ; ROS, reactive oxygen species; SCD-1, stearoyl-CoA desaturase-1; siRNA, small interfering RNA; SMC, smooth muscle cell; SRF, serum-responsive factor; TGF, transforming growth factor; TNF-α, tumour necrosis factor-α; VEGF, vascular endothelial growth factor; VSMC, vascular SMC.

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and oscillatory shear forces. VSMCs are primarily subjected to cyclic stretch resulting from pulsatile changes in blood pressure. ECs are mainly subjected to shear stress by the flowing blood. In addition, cardiac myocytes are primarily subjected to cyclic stretch due to pressure and volume overload. In vitro models have been developed to apply mechanical stretch mimicking in vivo haemodynamic overload [1]. Therefore knowledge of the impact of mechanical stretch on the cardiovascular system is vital for the understanding of the pathogenesis of cardiovascular diseases and can provide new insights into the development of therapeutic strategies. Over the past few years, several review articles have been published discussing the molecular mechanisms of mechanical stretch on ECs and VSMCs [2–9]. To date, only a few review articles have discussed the cellular and molecular effects of mechanical stretch on cardiac myocytes [10,11]. In the present review, recent findings on the cellular and molecular effects of mechanical stretch on vascular cells, including ECs, VSMCs and cardiac myocytes, will be summarized. Because several types of devices have been applied to induce mechanical stretch in vitro [12], the cellular and molecular responses in each type of vascular cell may be different. Most of the effects of shear stress on the ECs are beneficial (including anti-apoptotic, anti-atherosclerotic and anti-thrombotic effects); however, the effect of mechanical stretch on SMCs and cardiac myocytes may be beneficial or detrimental. Although various models of mechanical stimuli (static or dynamic) have been used in the past, the majority of research has used the Flexercell stress unit. This model, a two-dimensional cell culture, is controlled by a computer program and provides a physiological representation of the in situ environment of repetitive mechanical stimuli; however, the model is a poor representation of the natural tissue environment of vascular cells, which is three-dimensional, mechanically dynamic and involves the interaction of multiple cell types [13]. An organ culture model for studying the effects of mechanical stretch of vascular cells is the best in vitro representation of the vessel in its in vivo environment, where multiple cell types and the extracellular matrix participate in response to mechanical stimuli [14]. Regardless of the two- or three-dimensional model used, in vitro studies do not allow easy distinction between stretch effects due to transmembrane force transfer and stretch effects due to a global change in cell morphology, which cause generalized deformation of the plasma membrane and the cytoskeleton [15].

**EFFECT OF MECHANICAL STRETCH ON VSMC FUNCTION**

Mechanical stretch can modulate several different cellular functions in VSMCs. These functions include, but are not limited to, cell alignment and differentiation, migration, survival or apoptosis, vascular remodelling, and autocrine and paracrine functions. However, different kinds of VSMCs (venous or arterial) and several species of animals (mouse, rat, rabbit, swine and others) were used in different studies, resulting in sometimes controversial findings. Most of the studies used in vitro models. The cellular functions induced by in vitro mechanical stretch may not really represent cellular function in vivo. Further and detailed studies are needed to elucidate the real effect and mechanisms of mechanical stress on VSMC functions.

**Effect of mechanical stretch on VSMC alignment and differentiation**

Arterial SMCs are aligned primarily in the circumferential direction in the media of the artery. Mechanical stretch from pulsatile blood flow is one of the key factors in regulating vascular remodelling [16]. The mechanical environment in vivo modulates the distinct patterns of VSMC orientation in the arterial wall. There are at least three elements included in cyclic stretch: magnitude, frequency and duration. Cultured VSMCs in vitro can be induced to reorient to a uniform alignment almost perpendicular to stretch vector alignment by mechanical stretch [14,17]. The response of cell reorientation depends on the stretching magnitude and frequency [18–20]. The signalling pathways involved in stretch-induced VSMC alignment include p38 MAPK (mitogen-activated protein kinase) [20], NO and ROS (reactive oxygen species) [17,21]. The mechanosensor and outside-in signal of integrin-β1 is also involved in stretch-induced VSMC alignment [21]. In addition, an intact cytoskeleton is important for the stretch-induced VSMC alignment. Destroying the actin filament system by cytochalasin D inhibits the effect of stretch-induced alignment [20]; not only VSMC alignment is affected by stretch frequency, but also the phenotype of VSMCs. Cyclic strain increases smooth muscle and decreases non-muscle myosin expression in VSMCs [22]. Mechanical stretch increases both smooth muscle α-actin protein expression and promoter activity [23]. The induction of smooth muscle α-actin is mediated by the activation of JNK (c-Jun N-terminal kinase) and p38 MAPK pathways. Mechanical stretch could promote a frequency-dependent re-differentiation of synthetic VSMCs in vitro, mediated at least in part by the activation of p38 MAPK [24]. Mechanical stretch modulates cell shape, cytoplasmic organization and intracellular processes, leading to migration, proliferation or contraction. Rho and intact actin filaments play an important role in mechanical-stretch-induced ERK (extracellular-signal-regulated kinase) activation and cell growth [25]. RhoA signalling plays a major role in SRF (serum-responsive factor)-dependent regulation of SMC differentiation marker gene expression [26]. The primary genes encoding SMC contractile proteins are
regulated by the stretch-induced RhoA pathway and associated transcription factors, most importantly SRF [27]. In vitro, RhoA enhances actin polymerization and stimulates the binding of SRF homodimers to their CArG boxes [26]. SRF binds to the serum-response element region containing the 10-bp CArG box sequence, facilitating the activation of this motif alone or as a macromolecule bound to myocardin, its specific co-activator [28]. Myocardin increases the promoter activity of the CArG-dependent VSMC contractile markers. Stretch of the vascular wall can stimulate increased actin polymerization, activating the synthesis of smooth muscle-specific proteins via Rho-associated kinase and cofillin downstream of Rho [29]. Rho/Rho kinase, p44/p42 MAPK and PI3K (phosphoinositide 3-kinase) pathways are all involved in the stretch-induced HSVSMC (human saphenous vein SMC) proliferation and inhibition of either of them prevents stretch-induced SMC proliferation [30]. The effect of mechanical stretch on SMC phenotype has been reviewed by Halka et al. [28].

**Effect of mechanical stretch on VSMC migration**

VSMC migration is important in the development of vascular diseases, including atherosclerosis and post-angioplasty restenosis. SMC migration is found more frequently in curved and bifurcating blood vessels, which are exposed to non-laminar blood flow, than in straight arterial segments exposed to laminar blood flow [31]. In a vein graft model of SMCs, vortex blood flow induces SMC migration and neointimal hyperplasia in control vein grafts, whereas reduction in vortex blood flow in the vein graft strongly suppressed migration and hyperplasia [32]. In this model, SMC migration was regulated through the mediation of ERK1/2 and MLCK (myosin light-chain kinase). In vitro, mechanical stretch of arterial SMC translocates PKC (protein kinase C)-δ from the membrane to the cytoskeleton and increases the migration of SMCs [33]. In our laboratory, we have also demonstrated that mechanical stretch increased the migration of SMCs [34]. The increased migration of SMCs by mechanical stretch involves p38 MAPK and TGF (transforming growth factor)-β1 [33]. Inhibition of p38 MAPK and TGF-β1 activity decreased migration activity.

**Effect of mechanical stretch on proliferation, survival and apoptosis of VSMCs**

The effect of mechanical stretch on survival and apoptosis of VSMCs has been reviewed extensively by Kakisis et al. [2] and Haga et al. [9]. In the present review, I would like to extend those reviews by including results published recently. In a mouse SMC cultured model, Cheng et al. [35] reported that mechanical stretch prevents apoptosis of VSMCs in response to oxLDL (oxidized low-density lipoprotein). The mechanism of increased survival of VSMCs induced by mechanical stretch includes αVβ3 integrin expression, stabilization of PINCH-1, a survival protein that is linked with integrin and the cytoskeleton [36], and remodelling of the cytoskeleton. siRNA (small interfering RNA) against integrin β3, as well as VSMCs isolated from integrin β3-knockout mice, abolishes the anti-apoptotic effect of mechanical stretch. Down-regulation of PINCH-1 by siRNA enhances the ability of oxLDL to cause apoptosis of VSMCs, whereas up-regulation of integrin β3 stabilizes PINCH-1 and protects VSMCs from apoptosis. Disruption of the cytoskeleton also abolishes the anti-apoptotic effect of stretch. In cultured rabbit VSMCs, mechanical stretch also stimulates SMC growth and hypertrophy [37]. The increased SMC survival by mechanical stretch is induced by nuclear protein import and nuclear pore protein expression, which is mediated via MAPK. In cultured bovine pulmonary artery VSMCs, mechanical stretch stimulates the proliferation of VSMCs, and RhoA is essential for stretch-induced VSMC proliferation [38]. Blocking Rho completely inhibits the proliferation of VSMCs induced by stretch. In cultured vein VSMCs, mechanical stretch stimulates the proliferation of venous SMCs [39]. The proliferation of venous SMCs induced by mechanical stretch is mediated by the activation of IGF-1 (insulin-like growth factor-1) and IGF-1R (IGF-1 receptor). When IGF-1R is knocked-out, the mechanical-stretch-induced increase in VSMC proliferation is blocked. The IGF-1R level is increased in the neointima of vein grafts, and IGF-1R deletion reduces neointima formation in vein grafts.

In a porcine VSMC cultured model, Su et al. [40] have demonstrated that mechanical-stretch-induced VSMC apoptosis is phenotype-dependent. Mechanical stretch induces apoptosis in differentiated VSMCs, but not in proliferating VSMCs. The stretch-induced apoptosis in VSMCs is associated with BAD (Bcl-2-associated death factor) expression. VEGF (vascular endothelial growth factor) and overexpression of the anti-apoptotic protein Bcl-2 decrease BAD expression and apoptosis induced in response to stretch. Recently, we have also demonstrated that mechanical stretch induces apoptosis in VSMCs from rat thoracic aorta [41]. Mechanical-stretch-induced VSMC apoptosis is load-dependent. In contrast with 10 % stretch, stretch of 20 % induces apoptosis. The mechanism of apoptosis induced by mechanical stretch in our study is mediated by GADD153 (growth-arrest and DNA-damage-inducible protein 153), a component of endoplasmic-reticulum-stress-mediated apoptosis factor [42]. Caspase 3 is involved in the GADD153-induced apoptosis of VSMCs after mechanical stretch. An in vitro model of aorticaval shunt also increases aortic GADD153 protein expression [41]. These results indicate that GADD153 plays an important role in mechanical stretch-induced apoptosis.
role in stretch-induced VSMC apoptosis. Using cells from different species and modifications in intensity and duration of stretch may cause the differences observed in previous studies. Therefore the controversial effect of mechanical stretch on survival and apoptosis of VSMCs under mechanical stretch needs further investigation.

**Effect of mechanical stretch on vascular remodelling**

Vascular inward remodelling results in decreased lumen size and increased vessel resistance. The signalling cascades that modulate vascular remodelling process in response to mechanical stretch include ROS, NO, NF-κB (nuclear factor κB), EGFR (epidermal growth factor receptor), MAPK and PKC [43]. Mechanical force can be transduced via ROS-dependent autocrine and paracrine EGFR activation, and may regulate VSMC proliferation and synthetic activity through the NF-κB pathway [44]. TGF-α is a potential specific target for vascular remodelling induced by mechanical stretch. In vivo, increased haemodynamic forces in a model of hypertension by AngII (angiotensin II) infusion, activation of NF-κB and associated cell proliferation and wall thickening are reduced in TGF-α-mutant mice compared with wild-type animals. Syndecan-1 and -4 belong to a family of transmembrane proteoglycans, acting as co-receptors for growth factor binding as well as cell–matrix and cell–cell interactions, and are induced in neointimal SMCs after balloon injury [45]. Both syndecan-1 and -4 expression and shedding are up-regulated by mechanical stretch [45,46], which may contribute to the vascular pathology induced by the mechanical microenvironment in vivo. Recently, Albinsson and Hellstrand [47] have reported that remodelling of SMCs to stretch requires a dynamic cytoskeleton. The stabilization of actin filaments is essential for the growth and synthesis of contractile proteins in response to physiological levels of mechanical stretch. Mechanical stretch enhances VEGF and HIF-1α (hypoxia-inducible factor-1α) gene expression through transcriptional regulation in VSMCs [48,49]. The transient increase in VEGF and HIF-1α gene expression induced by mechanical stretch may be relevant to pathological complications in the cardiovascular system, including atherosclerosis, plaque stability and hypertension. The induction of VEGF and the HIF-1α gene by mechanical stretch may play a role in vascular remodelling.

**Autocrine and paracrine effect of mechanical stretch on VSMCs**

Mechanical stretch may induce secretion or synthesis of bioactive molecules from VSMCs. These secreted bioactive molecules can act on neighbouring cells or the cells secreting them. The autocrine and paracrine effect of mechanical stretch has been demonstrated recently and these effects regulate individual intracellular signalling pathways, VSMC growth, and initiate the cellular and molecular effect of mechanical stretch on VSMCs (Figure 1). PDGF (platelet-derived growth factor) was initially found to have an autocrine function in VSMCs after mechanical stretch [50]. Mechanical stretch using portal vein SMCs induces ET (endothelin)-1 release and promotes the synthesis of smooth-muscle-specific proteins by a mechanism requiring an intact cytoskeleton [51]. Mechanical stretch also stimulates autocrine IGF-1 production from arterial and venous VSMCs [39,52]. TGF-α has been shown to modulate the NF-κB activation and vascular remodelling under stress [44], and TNF-α (tumour necrosis factor-α) has been reported to modulate GADD153 expression and apoptosis in VSMCs under mechanical stretch [41]. The intracellular signalling pathways of the autocrine and paracrine effects of TGF-α under mechanical stress involve ROS and NF-κB. The intracellular signalling pathways of stretch-induced GADD153 expression involve JNK and AP-1 (activator protein-1) pathways. AngII and TGF-β1 have autocrine and paracrine actions on DDR-2 (discoidin domain receptor-2) expression in VSMCs under mechanical stretch [34]. DDR-2 can regulate cell proliferation and extracellular matrix remodelling mediated by MMP (matrix metalloproteinase) activities. The intracellular signalling pathways of stretch-induced DDR-2 involve the p38 MAPK and Myc pathways.

In an EC–SMC co-culture system, SMCs secrete IL-(interleukin)-1β and IL-6 after application of shear stress, resulting in the inhibition of E-selectin expression [53]. In this model, SMCs induce endothelial E-selectin expression, whereas shear stress inhibits SMC-induced E-selectin expression via the inhibition in SMC activation of IRAK (IL-1-receptor-associated kinase)/Gp-130 (glycoprotein-130), JNK/p38 MAPK and NF-κB [7,9].

**EFFECT OF SHEAR STRESS ON ECs**

The vascular endothelium is a dynamic cellular interface between the vessel wall and the bloodstream. It plays an important role by sensing the alterations in biological, chemical and physical properties of blood flow to maintain homeostasis. Disturbance of normal haemodynamic load can contribute to cardiovascular diseases, including hypertension, intimal hyperplasia, vascular restenosis and atherosclerosis [54]. Laminar shear stress, the frictional force created by the flowing blood, exerts a variety of cellular and molecular effects on endothelial structure and functions. The molecular mechanism of shear stress on EC has been reviewed extensively [2,6,7,54,55]. In addition to anti-apoptotic and anti-atherosclerotic effects of laminar shear stress on ECs, laminar shear stress has a profound impact on endothelial metabolism and can alter gene expression, leading to
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Figure 1  Response of VSMCs to mechanical stretch
Summary of the mechanical-stretch-induced autocrine or paracrine cytokine secretion and intracellular signalling leading to the modulation of gene expression and cellular function, as discussed in the text. Some of the signalling pathways are observed under in vitro conditions only. For further details, see [26,27,29,33,41,44].

IκB, inhibitor of NF-κB; IKK, IκB kinase; MEKK, MAPK/ERK kinase kinase; MKK, MAPK kinase; NIK, NF-κB-inducing kinase; ROCK, Rho-associated kinase; SRE, sterol-regulatory element.

changes in endothelial phenotype and vessel wall homoeostasis. The genes regulated by shear stress can modulate several endothelial functions, including vessel diameter, cell proliferation, migration and angiogenesis, cell–cell communication, coagulation and fibrinolysis, anti-inflammation, and immune modulation [2,7,54,55]. In the present review article, additional novel findings about the impact of shear stress on ECs are discussed.

Effect of shear stress on EC protein alteration
DNA microarrays have been used to analyse a large number of genes in ECs exposed to shear stress [56,57]. Proteomic analysis shows that a broad spectrum of proteins is altered by shear stress [58]. Wang et al. [58] found 142213 and 186 candidate proteins up- or down-regulated at least 2-fold after 10 min, 3 h and 6 h of shear stress respectively. These proteins included transcriptional regulators, enzymes, protein kinases, GPCRs (G-protein-coupled receptors), cytokines, protein-degradation-related proteins, and cytoskeletal and matrix proteins. These findings suggest that shear stress has profound effects on the molecular response and physiological function of the vascular endothelium.

Vasculoprotective effect of shear stress on ECs
The vasculoprotective effects of shear stress on ECs were reported more than a decade ago [59]. Novel findings extending this aspect have been reported more recently. BMP (bone morphogenetic protein) is a TGF-β family member cytokine that exerts pro-inflammatory effects on the endothelium and plays a role in atherogenesis. BMP is up-regulated at ‘atherosclerotic-prone’ regions in blood vessels and may contribute to vascular calcification and the development of atherosclerotic plaques [60]. Csizsar et al. [61] reported that laminar shear stress activates cAMP and PKA (cAMP-dependent protein kinase) and down-regulates BMP-4 expression in coronary artery ECs. This finding supports the anti-atherogenic and vasculoprotective effects of shear stress because BMP-4 elicits endothelial activation, dysfunction, hypertension and vascular calcification.

The transcription factor KLF-2 (Kruppel-like factor-2) is an important mediator of the anti-inflammatory
and antithrombotic properties of the endothelium [62,63]. Prolonged shear stress stabilizes KLF-2 mRNA and induces KLF-2 protein expression, especially in the presence of pro-inflammatory cytokine TNF-α stimulation [64]. The atheroprotective effect of prolonged shear stress is superior to statins, lipid-lowering agents, in the presence of TNF-α in an EC culture model [64]. This finding also supports the atheroprotective effect of prolonged shear stress on ECs. Vascular injury and atherogenesis can be induced by complement activation. The complement-inhibitory protein CD59 can be up-regulated by shear stress through PPAR-α activation [65] in venous and aortic ECs, indicating vascular protection by shear stress in complement-mediated injury.

SCD-1 (stearoyl-CoA desaturase-1) is a rate-limiting enzyme in the biosynthesis of mono-unsaturated fatty acids. SCD-1 converts palmitate and stearate into palmitoleate and oleate by catalysing the Δ⁹-cis desaturation of saturated fatty acids [66]. Palmitoleate and oleate are the predominant unsaturated fatty acids in membrane phospholipids. Qin et al. [67] have recently reported that shear stress increased SCD-1 expression in human vascular ECs through a PPAR-γ (peroxisome-proliferator-activated receptor-γ) mechanism. The metabolic effect of shear stress provides more evidence for its atheroprotective effect. LXRα and LXRβ (ligand-activated nuclear receptors) participate in cholesterol transport and lipid metabolism, resulting in atheroprotective effects. Laminar shear stress increases LXRα function via a PPAR-γ/CYP27 (sterol 27-hydroxylase)-dependent mechanism [68], supporting further the atheroprotective role of laminar shear stress in ECs.

Li et al. [69] have provided additional evidence for the atheroprotective effect of shear stress in ECs by demonstrating that shear stress decreases TNF-α-mediated VCAM-1 expression in human vascular ECs by inhibiting JNK through MEK-5 (MAPK/ERK kinase-5)/BMK-1 (big MAPK1; also known as ERK-5) signalling pathways.

Effect of shear stress on EC polarity
Mechanical forces regulate EC polarity and directional migration, and this is important for vascular function, remodelling and wound repair [70]. Shear stress can modulate the polarity of microtubule-organizing centres and microtubule stability in vitro and in vivo by activation of the GSK (glycogen synthase kinase)-3β signalling pathway [71]. The application of shear stress causes EC elongation in the direction of flow. Shear stress induces reorientation of the microtubule-organizing centre to the leading edge of migrating cells in a Cdc42 (cell division cycle 42)-dependent manner [72]. Recently, Simmers et al. [72] have demonstrated that shear-stress-induced directed migratory polarity is modulated by exogenous growth factors and is dependent on Par6 activity, a major downstream effector of Cdc42-induced polarity, and shear stress direction. Shear stress regulates EC bulk migratory characteristics as well as morphology.

Anti-inflammatory and antioxidant effects of shear stress on ECs
Endothelial inflammation is a major initiator of atherosclerosis. ECs exposed to disturbed flow experience oxidative stress, increased expression of markers of inflammation and monocyte recruitment as early signs of atherosclerosis [73]. Anti-inflammatory and antioxidant defences are critical for the protection of cellular macromolecules and the progression of atherosclerosis. Laminar shear stress up-regulates PRXs (peroxiredoxins) as important antioxidants in ECs [74]. Laminar shear stress increases ERK-5 and PPAR-γ transcriptional activity and decreases adhesion molecule expression in ECs. Laminar shear stress increases eNOS [endothelial NOS (NO synthase)] expression through ERK-5 to inhibit the formation of ROS [75]. Shear stress also activates PI3K to enhance eNOS expression. Laminar shear stress up-regulates antioxidant genes and activates the transcriptional factor Nrf-2 (nuclear factor-erythroid 2 p45 subunit-related factor-2), which is a major transcriptional factor for EC redox homeostasis [77]. These findings strongly support the crucial role of laminar shear stress as an anti-inflammatory and antioxidative force.

Effect of disturbed flow on ECs
Although laminar shear stress plays an atheroprotective role on ECs, cyclic stretch or oscillatory shear stress induces different cellular responses (Figure 2). Pulsatile flow is steady and laminar in the straight part of vessels, whereas disturbed flow is not steady with large oscillation near bifurcations and curvatures. Cyclic stretch is the repetitive mechanical deformation of the vascular cells as it rhythmically distends and relaxes with the cardiac cycle. These biomechanical forces promote atherosclerosis by increasing the formation of ROS in ECs and by up-regulating pro-atherogenic cytokine expression. We have reported that cyclic stretch augments TNF-α production and MMP expression in HUVECs (human umbilical vein ECs) [78]. CD40 is a co-stimulatory molecule playing an important role in controlling inflammatory responses, including atherosclerosis. Recently, Korff et al. [79] demonstrated that cyclic stretch increases the abundance of CD40 in ECs co-cultured with SMCs through TGF-β1/ALK-1 (activin-receptor-like kinase-1) signalling, whereas EC CD40 abundance is down-regulated by exposure to cyclic stretch in ECs alone. Cyclic stretch also activates Akt and GSK-3 to enhance survival of ECs [80]. Akt is important in preventing apoptosis, but is not involved in EC proliferation. These findings indicate that haemodynamic forces present in atherosclerosis-resistant
and susceptible regions of the vasculature induce different responses in the vessel wall. Atherosclerotic lesions frequently develop in areas of the vasculature exposed to disturbed flow, whereas areas that experience pulsatile laminar flow are relatively protected from plaque formation.

**EFFECT OF MECHANICAL STRETCH ON CARDIAC MYOCYTES**

Sustained haemodynamic stimulation by hypertension and volume overload elicits a series of functional and structural changes in ventricular myocytes that culminate in cardiac hypertrophy. Cardiac hypertrophy is often accompanied by cardiac remodelling characterized by cardiac myocyte loss, interstitial fibrosis and collagen deposition, leading to decreased compliance and an increased risk of heart failure [81]. Cardiac hypertrophy is an independent and powerful predictor of cardiovascular morbidity and mortality [82]. In *vivo*, biomechanical load, neurohumoral pathways, including adrenergic signals [83], the RAAS (renin–angiotensin–aldosterone system) [1] and ET-1-dependent signals [84] may also contribute to the development of cardiac hypertrophy. To exclude the involvement of neurohumoral effects on cardiac myocytes, mechanical stress is applied to cardiac myocytes to study the cellular and molecular mechanisms of gene expression and signal transduction induced by biomechanical load. Three models of mechanical stress have been used for cardiac myocytes: mechanical stretch [85], aortocaval shunt [86] and balloon dilation of the left ventricle in the isolated heart [87]. In cardiac myocytes *in vitro*, mechanical stretch has been shown to modulate growth, apoptosis, electric remodelling, alterations in gene expression, and autocrine and paracrine effects.

**Effect of mechanical stretch on cardiac myocyte growth**

Cyclic mechanical stretch can directly cause elongation and alteration of the orientation of cultured cardiac myocytes. Mechanical stretch has been reported to induce cell alignment and determine the cell polarity [88]. Recently, Yamane et al. [89] reported that Rac1 activity is necessary for cardiac myocyte alignment in response to mechanical stretch. Rac1 may play a role in cell alignment in

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**Figure 2  Response of ECs to shear stress or mechanical stretch**

Summary of the shear-stress-induced or mechanical-stretch-induced mechanosensing and intracellular signalling leading to the modulation of gene expression and cellular function, as discussed in the text. Shear stress induces atheroprotective effects (blue arrows), whereas elevated mechanical stretch can increase oxidative stress and damage ECs (red arrows). Some of the signalling pathways are observed under in vitro conditions only. For further details, see [61,69,73,78–80]. AC, adenylate cyclase.
response to mechanical stretch as a common downstream signal transducer of integrins as mechanoreceptors [90]. Mechanical stretch causes hypertrophy of atrial [91] and ventricular [84] myocytes. For the hypertrophic effect of mechanical stretch, cell size, sarcomeric organization and expression of the hypertrophic markers, such as ANF (atrial natriuretic factor), BNP (brain natriuretic peptide) and β-MHC (myosin heavy chain), genes are increased [92]. Torsoni et al. [93] have reported that RhoA and FAK (focal adhesion kinase) are key elements in the regulation of the hypertrophic genetic programme in cardiac myocytes in response to mechanical stretch. ERK1/2 mediates the effect of RhoA to promote cardiac hypertrophy by regulating the activation of GATA4, a cardiac-expressed transcription factor. PKC-α and -δ are important regulators in mediating the activation of Rho GTPases and MAPKs in the stretch-induced hypertrophic process [94]. The hypertrophy of cardiac myocytes induced by mechanical stretch is comparable with that induced by the pharmacological agent phenylephrine, an α-adrenergic agonist [94]. The hypertrophy induced by mechanical stretch also includes activation of immediate early genes, such as c-jun, c-fos, c-myc and skeletal α-actin [95,96].

Effect of mechanical stretch on apoptosis in cardiac myocytes
Mechanical stretch at a physiological level does not induce cell damage of cardiac myocytes; however, pathological stretch or stretch elongation of more than 20% may induce myocyte apoptosis. Apoptosis of cardiac myocytes is an important pathological factor in the transition from hypertensive heart disease to heart failure. Pimentel et al. [97] have reported that a low amplitude stretch of 5% induces myocyte hypertrophy, whereas a high amplitude stretch of 25% induces myocyte apoptosis. Myocyte apoptosis and the cell death signal gene bax are significantly induced by high amplitude stretch through ROS production and MAPK activation. ROS production is also stretch-amplitude-dependent. This stretch-induced apoptosis is reproduced in an isolated heart model by balloon dilation of the left ventricle during the early phase of reperfusion [87]. Liao et al. [98] have demonstrated another mechanism of stretch-induced apoptosis of cardiac myocytes. In that study, mechanical stretch induced a rapid and significant intracellular NO elevation in cardiac myocytes through Ca2+-dependent eNOS and iNOS (inducible NOS) activation. NO is a bidirectional regulator of apoptosis. Chronic overexpression of NOS in the maladaptive state could be associated with the progression of heart disease.

Effect of mechanical stretch on electric remodelling of cardiac myocytes
Cyclic stretch under pathological conditions may cause remodelling of conduction pathways and lead to arrhythmogenesis. Gap junctions are altered during mechanical stretch of cardiac myocytes. Mechanical stretch of atrial myocytes shortens the action potential duration and increases the expression of genes encoding I_{K1} (inward rectifier K+ current) and I_{Kor} (ultrarapid delayed rectifier K+ current) [91]. This mechanical stretch effect can be attenuated by the ARB [AT1 receptor (AngII type 1 receptor) blocker] losartan, indicating the mechanism of action of ARBs for the prevention of atrial fibrillation in clinical use. Gap junction proteins such as Cx43 (connexin43) have been shown to increase in cardiac myocyte after mechanical stretch [99,100]. AngII, VEGF and TGF-β may mediate the stretch-induced Cx expression because ARBs, and anti-VEGF and anti-TGF-β antibodies attenuate the stretch-induced up-regulation of Cx43 [99,101]. Zhou et al. [102] have reported that mechanical stretch activates ROS to decrease Kv4.3 transient outward current channel expression, which is pro-arrhythmogenic. The mechanism of stretch-induced Kv4.3 expression is similar to that of AngII. These findings indicate that the RAS (renin-angiotensin system) can play a critical role in the stretch-regulated changes in gap junctions and electrical remodelling in cardiac myocytes. Using an in vivo intraventricular balloon dilation model, stretch-activated channels and K+ channels have been shown to mediate mechanoelectric feedback in the heart [103]. Mechanoelectric feedback caused by mechanical forces may change the electrical properties and induce arrhythmias.

Effect of mechanical stretch on alterations in gene expression in cardiac myocytes
Mechanical stretch induces hypertrophy of cardiac myocytes, which is accompanied by an increase in cell size, enhanced sarcomeric organization and induction of the ‘fetal’ gene programme [92]. DNA microarray methodology has identified specific genes induced by mechanical stretch for 24 h in neonatal cardiac myocytes [92]. ANF, BNP, skeletal α-actin, HSP70 (70 kDa heat-shock protein), the proto-oncogene c-myc, CKS-2 (cyclin-dependent kinase regulatory subunit-2), intoxicative and cardioprotective genes, such as metallothionein-1 and HO-1 (haem oxidase-1), cytokine growth and differentiation factors are up-regulated, whereas lipocalin, Cx40, cell-adhesion molecules and phospholipase are down-regulated. Frank et al. [92] have reported that only 185 genes from more than 28000 genes analysed were significantly regulated by mechanical stretch.

Stretch-induced gene expression can be significantly attenuated by the ARB irbesartan, supporting the well-known crucial role of AngII in the induction of gene expression by mechanical stretch [1]. Zou et al. [104] have demonstrated that short periods of mechanical stretch (5–8 min) activate the AT1 receptor without the involvement of AngII. The studies demonstrating the involvement of AngII in stretch-induced gene expression of cardiac myocytes use a longer period (more than 4 h) of mechanical stretch. Different durations of mechanical stress...
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activate different mechanisms in cardiac hypertrophy. In fact, different magnitudes of stretch can also affect the pattern and types of gene expression. In our laboratory, we have demonstrated that 20% mechanical stretch of cardiac myocytes increases myostatin gene expression through the action of IGF-1 and increases serotonin 2B receptor expression to modulate BNP function [105,106]. The induction of myostatin, a TGF-β family member that plays an essential role in regulating skeletal muscle growth [107], by mechanical stretch may serve to ameliorate the effect of excess hypertrophy induced by mechanical stretch. More recently, we also found that mechanical stretch enhances resistin gene expression in cardiac myocytes [108]. This finding suggests changes in glucose uptake in cardiac myocytes due to the regulation of resistin. Naka et al. [109] have reported that 20% mechanical stretch in cardiac myocytes induces IL-18 expression through the ET₄ receptor and the AT₁ receptor. The induction of IL-18 from cardiac myocytes may cause a deterioration in cardiac function by autocrine and paracrine actions.

**Autocrine and paracrine effects of mechanical stretch on cardiac myocytes**

Mechanical stretch may induce secretion or synthesis of bioactive molecules from cardiac myocytes. These secreted bioactive molecules act on neighbouring cells or the secreting cells themselves. The autocrine and paracrine effects of mechanical stretch on cardiac myocytes were identified 16 years ago [1]. These secreted proteins interact with their receptors, activate intracellular signalling pathways, and initiate cellular and molecular effects in cardiac myocytes (Figure 3). AngII, IGF-1, TNF-α and VEGF have been reported to mediate the autocrine and paracrine effects on cardiac myocytes in response to mechanical stretch [1,101,105,108]. The autocrine and paracrine production in cardiac myocytes in response to mechanical stretch may activate the expression of other growth factors or cytokines. For example, mechanical stretch enhances resistin expression mediated by TNF-α in cardiac myocytes. This finding indicates an additional metabolic link between mechanical stretch and hypertrophy in the heart [108].

**SUMMARY AND CONCLUSIONS**

Mechanical stretch activates multiple intracellular signalling networks and regulates gene expressions and functional responses in ECs, VSMCs and cardiac myocytes. Specific cell types may respond differently to mechanical forces. Different mechanisms of response may be observed by using different durations, loads and frequencies.
of mechanical forces. Although the in vitro mechanical stretch model is assumed to mimic in vivo haemodynamic overload, the findings obtained from the in vitro mechanical models must be considered with caution because in vivo haemodynamic overload is more complex than the in vitro mechanical stretch models. The cellular and molecular effects of mechanical stretch on vascular cells may provide new insights in the pathogenesis of vascular diseases and therapeutic potentials. Understanding the molecular mechanisms regulating electrical remodelling under mechanical stretch supports the clinical application of ACEIs (angiotensin-converting enzyme inhibitors) and ARBs in cardiac protection and in the prevention of atrial fibrillation [110]. Recently, we have used siRNA technology in the carotid artery balloon injury model to demonstrate the potential therapeutic use of DDR-2 siRNA for the prevention of neointimal formation induced by balloon injury [111]. This finding supports previous studies indicating that DDR-2 increases SMC proliferation and migration in vitro and in vivo [112]. This work was supported, in part, by the National Science Council, Taipei, Taiwan; and the Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan.

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