Review

Effects of static and cyclic loading in regulating extracellular matrix synthesis by cardiovascular cells

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Abstract

Extracellular matrix (ECM) provides several structural and functional characteristics to tissues including cell support, mechanical integrity and biological signaling. In cardiovascular tissues, cells produce various ECM components such as collagen, elastin, proteoglycans, matrix metalloproteinases, growth factors and signaling molecules. The cardiovascular cells (cardiac fibroblasts, cardiomyocytes, endothelial cells, and vascular smooth muscle cells) sense the changes in mechanical strains applied to them, through cell-surface receptors such as integrins and ion channels, and adjust their expression and synthesis of ECM molecules in order to adapt their environment to these changes. ECM changes due to altered mechanics are evident in numerous pathological situations including hypertension, cardiac hypertrophy, myocardial infarction, myxomatous heart valve disease, and atherosclerosis. In hypertrophic conditions, for example, increased mechanical loading is involved with enhanced collagen synthesis, whereas in myxomatous and atherosclerotic conditions reduced mechanical strains are accompanied by an accumulation of proteoglycans. Therefore, investigating the effects of various strain patterns on cardiovascular cells can enhance our understanding of ECM regulation and pathologies. This review focuses on the in vitro modulation of the synthesis of various ECM molecules through static or cyclic stretching of cardiovascular cells.

Keywords: Connective tissue; Mechanotransduction; Extracellular matrix; Matrix metalloproteinases; Stretch

1. Introduction

Mechanical stimulation is an important modulator of cell function and plays a critical role during tissue development and repair. Mechanical stimuli are transmitted to cells via the extracellular matrix (ECM), which provides an adhesive surface for cells and structural organization to tissue. Cells sensing mechanical strains will then reciprocate by remodeling their surrounding ECM. The role of mechanical stimuli was described first in bone remodeling and is now being actively investigated for many tissue types. Cells within the cardiovascular tissues have been shown to respond to mechanical stimuli by modulating the synthesis of almost all major components of the ECM, including collagen, elastin, proteoglycans (PGs), glycosaminoglycans (GAGs), matrix metalloproteinases (MMPs), glycoproteins, and various soluble proteins such as growth factors.

The major cell types found in cardiovascular tissues include cardiac fibroblasts, cardiomyocytes, endothelial cells (ECs), and smooth muscle cells (SMCs); all of these cells interact dynamically with the ECM in response to mechanical strains during development and disease [1,2]. Fibroblasts are the major cell type in cardiac muscle, representing two thirds of cardiac cells in number, and are mainly responsible for cardiac matrix production [3]. The other cell type in the cardiac muscle is cardiomyocytes, which primarily have a contractile role [4]. Blood vessels, in contrast, predominantly contain SMCs and fibroblasts in concentric layers. All these cardiovascular tissues are lined with endothelial cells, which act as a semi-permeable barrier between the tissues and body fluids.

These various types of cardiovascular cells experience complex mechanical strains, which are either mainly static or...
cyclic in nature with myriad amplitudes and frequencies. Because distinctive strain patterns differentially affect ECM synthesis according to the structural and functional needs of the tissues, it is important to understand the role of various types of mechanical stimulation. This review will focus on the modulation of ECM synthesis by cardiovascular cells in response to mechanical stresses and strains, with a particular emphasis on the effects of static vs. cyclic mechanical strains.

2. Extracellular matrix and non-matrix proteins relevant to mechanical loading of cardiovascular cells

Each ECM component fulfills a different structural or functional need in connective tissues but has also been shown to influence cell growth and migration. Collagen is the most abundant protein in cardiovascular tissues; it is secreted by cells to provide tensile strength and serve as an organizational scaffold. The other major fibrillar ECM protein is elastin, which provides elastic recoil and is therefore an essential component of arteries. PGs consist of one or more GAG chains attached to a core protein; PGs and GAGs serve diverse biological functions, including as acting as “space fillers,” within cardiovascular tissues [5]. The GAG hyaluronan and the large PG versican, in particular, sequester large volumes of water and provide resistance to repeated compressive loading, while the small leucine rich PGs such as decorin and biglycan have been shown to contribute to collagen fibrillogenesis [6,7]. In many pathological conditions of the cardiovascular system (i.e., hypertension, atherosclerosis, and myxomatous mitral valve disease) significantly altered profiles of GAGs and PGs have been found to accompany alterations in mechanical strains within the tissue, which has spurred investigations into the effects of mechanical strains on GAG and PG synthesis [8,9]. PGs also influence cell proliferation, migration, and phenotype and their synthesis is in turn regulated by growth factors and mechanical strains [10]. Tissue homeostasis is maintained by the synthesis of new matrix by cells and the degradation of matrix by MMPs and other proteases. Mechanical stimuli normally vary physiologically, but increased strains resulting from stenting, hypertension, or atherosclerosis may lead to enhanced matrix degradation and remodeling such as by MMPs [11]. The gelatinases MMP-2 and MMP-9 are the easiest to study using zymography and hence are the most commonly characterized MMPs in these reports. Other proteases such as MMP-1, -8 and -14, which have collagenase activity, have also been investigated. In addition to synthesizing ECM proteins and proteases, cells also secrete a variety of signaling and adhesion molecules including growth factors. Of these non-matrix mediators, transforming growth factor (TGF-β), which can influence proliferation, differentiation, and many other cell functions, and fibronectin, a glycoprotein involved in cell-matrix attachment and thus cell growth and migration, have been the most widely studied with respect to mechanical loading [12–14]. The mechanical modulation of cardiovascular cells’ secretion of vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), endothelin-1 and angiotensin-II has also been investigated since these proteins mediate cell growth, migration and signaling [15,16]. The ECM itself directs the gene expression of many transcribed proteins [17] by changing the physical and chemical environment of the cells and the cytoskeleton association with mRNA through transmembrane receptors.

3. In vivo mechanical strains experienced by cardiovascular tissues and relation to ECM remodeling

Cells in the cardiovascular system are exposed to a complex variety of shear, tensile, and compressive strains. Vascular endothelial cells constantly experience shear strains from blood flow, whereas pulsatile pressures result in both tensile and compressive strains on the subendothelial cells within vascular and cardiac tissues. The nonhomogeneous and multiaxial strains experienced by the cardiac wall cells are about 10% in magnitude on average [18,19]. All cardiovascular cells experience these strains with every heartbeat, i.e., at pulsatile frequencies close to 1 Hz.

Alterations in these physiological mechanical loads may cause compensatory remodeling of the ECM, a common process in cardiovascular pathologies such as hypertension, cardiac hypertrophy, myocardial infarction, myxomatous heart valve disease, and atherosclerosis [2,20]. In hypertension, the arterial mechanical strains reportedly increase by 15% with a corresponding increase in collagen [21]. Cardiac hypertrophy is also associated with excess collagen deposition (fibrosis) due to mechanical overload. Atherosclerosis, which develops in part due to reduced and disturbed shear stresses, is characterized by accumulation of collagen, GAGs and cholesterol [8]. Myxomatous mitral heart valves, which are enlarged and flail and thus are speculated to be under reduced tissue tension, have an overabundance of GAGs [9]. To understand these pathologies in more depth, many investigators have studied the effects of mechanical strains on ECM synthesis in vitro to clarify the relationships between tissue microstructure, cell mechanotransduction, and loading conditions using either commercial devices such as the Flexcell system [22] or custom built devices to apply mechanical strains in two-dimensional (2D) [23–26] or three-dimensional (3D) culture [27–29].

Mechanical strains regulate ECM synthesis through cell-matrix interactions, cytoskeletal rearrangements, and by opening stretch-activated ion channels, thereby activating membrane-bound enzymes or releasing growth factors in an autocrine or paracrine manner [13,30]. The transmembrane receptor integrins, which connect the ECM to the cytoskeleton, have been shown to play key roles in transducing mechanical signals to the cell interior [31,32]. The subject of cell mechanotransduction pathways has been widely reviewed [15,16,33–35]. Mechanical stimuli also affect other aspects of cell phenotype such as their orientation, growth and differentiation [33]; the overall effects of
mechanical strains on these characteristics of vascular smooth muscle cells (SMCs) and cardiac fibroblasts have been reviewed elsewhere [33,36].

4. Effect of various types of mechanical strains on ECM synthesis by cardiovascular cells

4.1. Cardiac fibroblasts

Cardiac fibroblasts experience high mechanical loads and remodel the ventricular and atrial ECM during development, growth, and pathogenesis. These cells are always under cyclic strains whose magnitude and frequency vary with heart rate and pressure load. Cardiac chamber walls contain both cardiomyocytes and fibroblasts with the latter serving as scaffolding primarily through maintaining a network of collagen fibers. Correspondingly, cyclic strains applied to cardiac fibroblasts have been found to modulate collagen synthesis and to cause the secretion of various growth factors into the ECM. When these cells were subjected to cyclic loading, their collagen I gene expression increased up to 4-fold compared to no loading conditions [37–40]. This response was similar whether the cells were grown on untreated elastomeric membranes or membranes coated with collagen, fibronectin or laminin [40]; collagen type III mRNA expression by these same cells, however, did not change appreciably. Cyclic stretching has been shown to accelerate the degradation of procollagen, but not to the same extent that new procollagen is expressed, resulting in a net increase [37]. In this same study, the exogenous addition of TGF-β to these stretching conditions further increased procollagen mRNA expression by 4.3-fold. In a different study, the cyclic-strain-induced increase in collagen synthesis was accompanied by increased secretion of TGF-β [38]. TGF-β can enhance collagen expression in a direct or indirect manner, often via the renin-angiotensin system, which is highly relevant to collagenous scarring in the myocardium. There has been only one direct comparison of the effect of static and cyclic strains on collagen synthesis by cardiac fibroblasts [41]. In this study, 5% cyclic strain (0.33 Hz) induced a 70% increase in the ratio of collagen type III to type I, whereas 5% static strain increased the ratio by only 5% compared to nonstretched controls [41].

One additional report investigated the effect of static strains alone on ECM synthesis. Cardiac fibroblasts subjected to uniaxial static strains showed a significant increase in collagen I, collagen III, and fibronectin expression with 10% strain, while 20% strain decreased collagen III and fibronectin expression when compared to nonstretched controls [42]. Similarly, when tensile equibiaxial strains were applied, cells subjected to 3% strain expressed more collagen III and fibronectin but at 6% strain the mRNA levels for collagen III decreased as compared to nonstretched controls [42]. In contrast, compressive equibiaxial strains of either 3% or 6% caused decreased expression of collagen III and fibronectin, suggesting that tensile strains might be the primary signal for stretch-induced matrix synthesis by cardiac fibroblasts. In the same study, TGF-β activity was increased at both 10% or 20% uniaxial strain and 6% biaxial strain (either tensile or compressive); 3% biaxial strain had no effect on TGF-β activity.

In general, mechanical strains increase the synthesis of collagen and release of growth factors by cardiac fibroblasts, which can cause myocardial hypertrophy [43]. This regulation of collagen synthesis is mainly mediated by TGF-β and MAP kinase pathways [38,39]. However, there is a paucity of information regarding cardiac fibroblasts’ synthesis of other ECM components and proteases in response to mechanical strains.

4.2. Cardiomyocytes

The cardiomyocytes are mainly involved with muscle contraction [4] yet they also produce various ECM molecules and release growth factors. Physiologically, cardiomyocytes are exposed to cyclic loading but can also experience hemodynamic (pressure) overload manifested as an increase in baseline static stretch. This hemodynamic overload causes cardiac hypertrophy (increase in cell size), which can significantly affect cell structure and function [16]. Therefore, the effects of both cyclic and static strain are relevant to these cells. Interestingly, much less attention has been given to cardiomyocytes than to other cardiovascular cells; only three studies have investigated the effect of mechanical strains on their synthesis of extracellular proteins relevant to ECM and tissue remodeling, likely because chemical (altered blood chemistry) and electrical (heart contraction) stimulations are more relevant to cardiac muscle. Regardless, mechanical stimulation has been shown to be important in hypertrophy. In two in vitro models of load-induced hypertrophy, static uniaxial strains of 20% were applied to cardiomyocytes, resulting in significant increases in the secretion of endothelin-1 and angiotensin-II [44,45], both growth-promoting factors whose release would activate phosphorylation cascades, initiate cell growth, and subsequently cause hypertrophy [45]. A third study reported that 20% cyclic stretch caused an increase in MMP-2 and MMP-14 expression via the angiotensin II-JAK-STAT1 pathway; the JAK/STAT pathway has been demonstrated to be involved in cell-specific MMP expression [46]. Although mechanotransduction pathways may initiate signal transduction through many possible mechanisms, growth factor secretion results in the release of second messengers in stretch-induced cardiac hypertrophy [15,16,47]. At this time, no study has explored how other ECM constituents such as collagen, elastin, and PGs are synthesized by cardiomyocytes in mechanical stretch-induced hypertrophic conditions, presumably because cardiac fibroblasts bear the majority of this function.

4.3. Endothelial cells

All cardiovascular tissues are lined with a layer of endothelial cells, which experience both pulsatile pressures
(normal tensile stresses) and shear stresses imposed by the flowing blood in the vasculature [48]. Shear stresses are transmitted to the ECs through the glycocalyx (a thin layer of glycoproteins and PGs surrounding the plasma membrane), stretch-activated ion channels, or integrin binding among other possible pathways [49]. Because cyclic strains and shear stresses on ECs regulate vascular architecture, tone, and remodeling, their effects on the synthesis of various ECM molecules have been widely investigated. ECs mainly synthesize GAGs, PGs, MMPs, fibronectin and a variety of growth factors such as PDGF and FGF. These ECM components and growth factors are critical to anticoagulation, anti-atherogenicity, and growth of the underlying SMCs.

The range of shear stresses on ECs, which normally vary with the flow rate of blood during diastole and systole, can affect the synthesis of various proteins. Shear stresses equivalent to laminar flow (1 dyn/cm²) decreased PG secretion whereas high shear stresses (5 to 40 dyn/cm²) increased GAG and PG synthesis compared to static no-flow conditions. These results were shown both in non-pulsatile conditions for durations of 24 h or less [50–52] and in pulsatile conditions of 72 h duration [53]. These ranges of high shear stresses were similar to those of veins (~5 dyn/cm²) and arteries (~23 dyn/cm²) [53], which indicates that high shear stresses are necessary for normal synthesis of GAGs and PGs and to maintain homeostasis within vascular tissue. When pulsatile shear stresses were applied bidirectionally, as compared to static or unidirectionally, ECs increased MMP-9 expression, which has been shown to promote atherosclerosis [54].

Cyclic strains have also been shown to modulate ECM synthesis by ECs. High strains (4.9–12.5%) increased total protein synthesis and decreased fibronectin secretion into the medium [24]; the authors speculated that these high strains possibly caused cell injury. In other studies, cyclic strains of 10–24% decreased collagen and non-collagenous protein, but increased PDGF-B expression [55,56]. The expression of MMP-2 and membrane type I MMP, partially mediated by p38 and ERK-dependent pathways, was found to increase with cyclic strains in a magnitude and time-dependent manner [57,58]. When aortic ECs were co-cultured with SMCs in tubular constructs, the cells decreased collagen and GAG deposition after 15 days under pulsatile shear stress conditions [59]. This study demonstrated the significance of cell–cell interactions as the presence of ECs caused SMCs to express more of a contractile phenotype compared to a synthetic phenotype. Similar to growth factors, the cytoskeletal proteins are major intermediates in transmitting the strains through integrins and regulating gene expression; Davies has comprehensively reviewed hemodynamic mechanotransduction within ECs [48]. Hemodynamics are also important in the development of pathogeneses such as atherosclerotic lesions. The heparan sulfate GAGs that are synthesized by ECs, located in their glycocalyx and underlying basement membrane, have anticoagulation activity; hence, the ECs lining vessels that are subject to high shear stresses are each surrounded by a thick glycocalyx for improved anti-atherogenicity [60].

There has been only one study to directly compare the effects of shear and cyclic strains on ECs. This study found that shear stress increased PDGF-B and bFGF expression, whereas cyclic strains had no effect [61]. These results indicate the importance of fluid shear in regulating EC secretion of growth factors, which then regulate mitogenic activity and govern the vascular structural response during atherosclerosis.

Overall, investigators have shown that low shear stresses and cyclic strains decrease the synthesis of ECM molecules by ECs. Static strains, which are less relevant to ECs, have not been investigated in this context. Cyclic strains on ECs decrease matrix building and cell-matrix binding proteins (collagen, fibronectin) and increase matrix degrading proteins (MMPs), consequently reducing net ECM. In contrast, high shear stresses variably regulate ECM expression and synthesis and maintain arterial wall tone and architecture.

4.4. Vascular smooth muscle cells

Smooth muscle cells, located in the medial layer of the vasculature, are responsible for vessel contractility and remodeling during growth and pathogenesis. In vivo, SMCs mainly experience cyclic tensile strains due to pressure forces of the blood and compression due to thinning of the vessel wall during inflation. Shear stresses experienced by the ECs in the blood vessels can also be transferred to SMCs but the magnitudes of these transferred stresses are low compared to the tensile stresses [48]. As SMCs are the predominant cell type in blood vessels, there are abundant studies on the mechanical regulation of ECM synthesis by SMCs.

Cyclic strains tend to increase collagen and elastin synthesis by vascular SMCs but these responses are sensitive to the strain magnitude, frequency and duration [28,62–64]. In a study of very high strains (25%) at 0.05 Hz, a significant increase in total protein and collagen synthesis could be seen only after 5 days of stretching [65]. The authors attribute this delay to the extremely high strain, which might have damaged the cells; the frequency may have also been too low to provide proper mechanical stimulus. Another study showed the dependence of total protein and collagen synthesis on strain magnitude; synthesis was increased between 5% and 10% strain, but then was unchanged between 10% and 20% strain, compared to nonstretched controls [66]. In a different investigation using 4% cyclic strain, collagen I expression increased to a maximum at 12 h, then remained constant up to 48 h [67]. Although the majority of these reports noted that strain increased overall protein synthesis, there is one exception. Kulik and Alvarado reported a slight decrease in protein and collagen synthesis when pulmonary arterial SMCs were subjected to cyclic stretch using strains of 10 or 20% and frequencies of 0.33–0.5 Hz [26]. Possible explanations for these unexpected findings included the loss of cell surface receptors, a lack of cell adhesion
molecules, or insufficient stretching time (3 or 6 h only). The culture environment is also highly relevant. For example, the addition of serum did not cause any increase in total protein or collagen synthesis by cyclically stretched aortic SMCs, whereas in serum-free medium the total protein and collagen synthesis doubled over stationary controls [68]. When seeded in 3D tubular collagen scaffolds and cyclically stretched (2.5–10%, 0.5 Hz), SMCs demonstrated no change in collagen synthesis, but elastin synthesis increased enormously [27]. In another 3D study, when SMCs were seeded together with ECs on a polyglycolic acid (PGA) scaffold, collagen content increased under pulsatile conditions (5% radial distension, 2.75 Hz) compared to non-pulsatile conditions [69]. These variations in elastin and collagen contents alter tissue material properties, emphasizing the critical role of mechanical strain in tissue remodeling and tissue-engineering applications.

Cyclic mechanical strains generally increase the GAG and PG synthesis by cardiovascular cells but the increases are often specific to certain types of GAGs. For example, cyclic stretching of aortic SMCs in 2D culture tripled the synthesis of the GAGs hyaluronan and chondroitin 6-sulfate over stationary cultures, but did not affect the synthesis of chondroitin 4-sulfate and dermatan sulfate [62]. Similarly, cyclic stretching increased the expression of the PGs versican, biglycan, and perlecan, but decreased the expression of the PG decorin [67]. These two reports are consistent as versican contains abundant chondroitin 6-sulfate and aggregates with hyaluronan, whereas decorin is associated with dermatan sulfate. SMC expression of the heparan sulfate PG syndecan-4, a cell-adhesion molecule colocalized with integrins in focal adhesions, was increased after 1 h of biaxial cyclic stretching (10%, 1 Hz) but then decreased over 24 h [70]. The expression of syndecan-4 did not change when strain was lowered from 10% to 3%. Mechanical stretch also caused syndecan-4 shedding from the cell surface, which served to promote cell motility via decreased focal adhesions.

MMP synthesis by SMCs has also been modulated with the application of cyclic strains, but different types of MMPs are regulated in distinct ways. One study found a significant downregulation of MMP-1 (collagenase) with 4% cyclic stretching [71] whereas another study reported no changes in the expression of MMP-2 or MMP-9 (both gelatinases) [72]. Two other studies, however, did find an increase in MMP-2 with cyclic strains ranging from 10% to 16% [64,73]. In a tubular collagen construct, which provides more of an in vivo-like 3D environment, the application of 10% cyclic stretch to the embedded vascular SMCs increased MMP-2 levels more than 5-fold compared to nonstretched controls [29].

Cyclic stretching has almost uniformly been found to increase SMC synthesis of growth factors and signaling molecules. The expression and secretion of TGF-β, PDGF, and VEGF increase substantially with cyclic stretching [64,71,74–76]. Cyclic strain-induced secretion of TGF-β and FGF-2 into the culture medium was found to be time and strain dependent [25,77]. These growth factors regulate SMC growth, differentiation and gene expression in an autocrine or paracrine manner. For example, secreted TGF-β1 promoted I-proline transport and hence collagen synthesis and cell growth during arterial remodeling in hypertension [77].

Although the role of static stretching on ECM synthesis has received less attention than cyclic stretching, several reports have examined its effect on SMCs. Static stretch has been reported to increase SMC synthesis of tropoelastin, the precursor of elastin [78]. Three studies in particular have directly compared the effects of static and cyclic strains [11,79,80]. In the first study, cyclic strain (10%, 0.86 Hz), compared to 10% static strain, caused a 2-fold increase in total protein and collagen synthesis [79]. Moreover, artificially generating an increase in intracellular cAMP levels (by adding theophylline, inhibitor of cAMP degrading enzyme) inhibited the collagen synthesis in cyclically stretched cultures only, suggesting that cAMP was involved in the response to cyclic stimuli but not static stimuli. cAMP mediates protein synthesis by SMCs; its levels decrease in response to cyclic stimuli but not static stimuli. cAMP regulates protein synthesis by SMCs; its levels decrease during hypertension and are inversely related to increased collagen synthesis [81]. In the second study, a 50-fold increase in MMP-2 mRNA was found after 24 h of 5% static stretch but no change was found in the absence of stretch or in cyclic stretch (1 Hz) [11]. Additionally, secretion of MMP-2 and MMP-9 increased with static stretch, but decreased with cyclic stretch. The stretching protocol of the third study simulated an arterial balloon injury to explain the tight regulation of syndecan-4 expression and cell migration during the injury process [80]; the high (30%) static strains rapidly increased syndecan-4 expression compared to 10% cyclic strains but subsequent application of 5% cyclic strains downregulated the expression. All three of these studies clearly demonstrated that static strains promote ECM degradation and adhesion molecules whereas cyclic strains enhance SMC synthesis of fibrillar ECM proteins.

Alternative mechanical loading conditions such as centrifugal force and shear stresses have also been used to investigate ECM synthesis by SMCs. Although centrifugal forces are less physiologically relevant to SMCs than the methods discussed above, they serve as a simple tool to apply circumferential stresses, as found in hypertension. Centrifugal forces applied to vascular SMCs increased total GAG synthesis, predominantly affecting heparan sulfate (found in the glycosalyx and basement membrane) but minimally affecting hyaluronan (which can be present in either the pericellular matrix or in the ECM) [82,83]. These are the same trends found with the application of static strains, which show some mechanical equivalence to centrifugal forces. Shear stresses have also been investigated because they are transferred from ECs to SMCs in vivo. Shear stresses (10–20 dyn/cm²) applied to SMCs decreased MMP-2 activation and significantly inhibited cell migration; SMC migration from the media is a characteristic finding in intimal hyperplasia [84,85].

Overall, cyclic strains applied to SMCs increase ECM building proteins, degrading proteases, and signaling molecules
whereas static strains and shear stresses downregulate ECM degrading proteases. ECM synthesis also depends on strain magnitude and durations. The results were also found to be sensitive to cyclic frequency and the culture conditions such as 2D vs. 3D or biaxial vs. uniaxial strain.

5. Summary and future directions

Cardiovascular cells show selective responses to different types of strains (Table 1). In general, the literature reviewed here reports that strains enhance the synthesis of most ECM components in cell culture. Although cells tend to respond rapidly to strains in a magnitude dependent manner, there are no overwhelming trends regarding time dependence. Cyclic strains are the obvious choice to apply mechanical stimuli to cardiovascular cells, which experience cyclic stresses under pulsatile blood flow. However, in certain circumstances such as when failing hearts are supported by continuous-flow left ventricular assist devices (LVADs) [86], the cardiac cells will experience predominantly static tensile stresses. The growing use of these continuous-flow LVADs mandates understanding the effect of static strains on ECM synthesis. From the limited investigations regarding static strains published to date, the comparative effects of static vs. cyclic strains appear to vary for different ECM macromolecules. Cyclic strains tend to affect collagen and elastin synthesis more profoundly than do static strains whereas the reverse is true for MMP-2 synthesis. Given that MMP expression increases in heart failure and that many patients are put on LVAD support [87], the potential for alterations in MMP expression warrants continued in vivo and in vitro research to understand the effect of various loading conditions on ECM synthesis and degradation. Furthermore, although SMCs and collagen were the focus of most of these reports, changes in other ECM components such as GAGs, PGs and MMPs are prominent in many cardiovascular pathologies, so the effects of strains on their synthesis should be given more emphasis in the future.

Many culture conditions have been used to apply mechanical strains to cardiovascular cells. 2D cell culture is appealing due to easy handling, maintenance, and manipulation of the mechanical strains. In addition, tissue-engineering techniques have recently been used to characterize vascular cell phenotype and modulation of ECM synthesis [88,89]. The cell shape and pericellular environment in 3D cultures is more like those found in native tissues and cells may utilize cell-matrix interactions (i.e., focal adhesions) and mechanotransduction signaling differently than when they are in 2D cultures, although admittedly mechanical strains can be more complex to apply in 3D [90]. In the future, 3D cultures may become more widely used to characterize cellular responses to mechanical strains.

The effect of cyclic strains on ECM synthesis appears to be specific to the different cell types in cardiovascular tissues. For example, collagen synthesis increased with strain in SMCs and cardiac fibroblasts, whereas the body of responses in ECs is less uniform. Another cardiovascular cell type is heart valve interstitial cells, which resemble myofibroblasts [91]. Heart valve cells’ responses to mechanical strains have only recently been explored in 2D and 3D culture systems [92,93] although no study has focused exclusively on ECM modulation. Porcine aortic valve interstitial cells produced more protein and GAGs than porcine aortic SMCs in 3D culture [93]; clearly, valve cells require further investigation as they are responsible for valvular remodeling and disease. Among all the reports of cardiovascular cells, inconsistencies regarding the matrix production elicited by mechanical stimuli may be due to different breeds, species, and ages of animals from which the cells were derived. Furthermore, variations in ECM gene expression with stretch may be due to the effects of pure tensile or compressive strains imposed by different stretch devices. In addition to the stretch applied to the cultured cells, their metabolic state, culture conditions, and accumulation of secretory products likely also affect their synthesis of matrix components and matrix mediators.

While the role of integrins in mechanotransduction is well known [48], other membrane-bound proteins such as the ADAMs (a disintegrin and metalloproteinase domain) also support integrin-mediated cell adhesion because they can cleave ECM proteins by their metalloproteinase domains [94]. Recently, a new mechanism of mechanotransduction was proposed in which mechanical stimulation encourages growth factor shedding into the ECM [95]. Because ADAMs have the ability to shed many cell-adhesion molecules and cell-surface proteins including cytokines and growth factors, they may be key regulators in mechanotransduction signaling pathways and their synthesis by cardiovascular cells certainly merits further investigation. The relevant intracellular signaling pathways, however, may differ for various types of strains. Numerous mechanotransduction pathways (most involving MAP kinase) for specific cell types have been
proposed [15,16,20,32–35,48,49], but only a few reports have indicated which pathways are specifically activated by static stresses. The application of static strains to cardiac fibroblasts, for example, activated G protein subunits [96] and the ERK2 or JNK1 pathways [97], whereas release of endothelin-1 from cardiomyocytes under static strains activated MAP kinases and Raf-1 in stretch-induced cardiac hypertrophy [45]. It has yet to be determined which particular pathway is required for regulation of the synthesis of each of the ECM molecules under the diversity of mechanical stimuli experienced in vivo. Overall, determining the precise role of static and cyclic mechanical stimulations on the regulated synthesis of various cardiovascular matrix macromolecules should remain an intriguing area of research for many years to come.

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References


