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Review

# Role of mechanical factors in modulating cardiac fibroblast function and extracellular matrix synthesis

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**Abstract**

The cardiac fibroblast is the most abundant cell type present in the myocardium and is mainly responsible for the deposition of extracellular matrix (ECM). Important components of cardiac ECM include structural and adhesive proteins such as collagen and fibronectin. Excess deposition of cardiac ECM (fibrosis) has been associated with the pathophysiological mechanical overload of the heart. Therefore, the role of cardiac fibroblasts in ''sensing'', ''integrating'' and ''responding'' to mechanical stimuli is of great interest. The development of in vitro strain apparatuses has allowed scientists to investigate the effects of mechanical stimuli on cardiac fibroblast function. Cardiac fibroblasts express ECM receptors (integrins) which couple mechanical stimuli to functional responses. Mechanical stimulation of cardiac fibroblasts has been shown to result in activation of various signal transduction pathways. The application of defined mechanical stimuli to cultured cardiac fibroblasts has been associated with ECM gene expression, growth factor production, release and/or bioactivity as well as collagenase activity. Ultimately, for fibrosis to develop the overproduction of ECM must overcome any associated increases in collagenase activity. Mechanically induced upregulation of ECM production may follow direct or indirect pathways through the autocrine or paracrine action of growth factors. Given the complex nature of the interstitial milieu of the working heart, additional research is needed to further our understanding of the roles that mechanical stimuli play in excess deposition of myocardial ECM. 

*Keywords:* Angiotensin; Cell culture/isolation; Extracellular matrix; Fibrosis; Mechanotransduction

## 1. Deformations in the intact heart and deposition of cardiac extracellular matrix

The nature of mechanical deformations in the intact working heart are complex. Their complexity arises from their three-dimensionality, nonuniformity across different depths and variability across different regions of the ventricle [1]. Furthermore, deformations in the heart arise from both diastolic (passive) and systolic (contractile) functions. Mechanical deformations in the myocardium give rise to changes in ventricular wall stress. The estimation of ventricular wall stresses results from the use of mathematical algorithms which derive significant inherent limitations as to their possible ''real value'' interpretations [2]. So far, it has been difficult to establish if actual deformations and/or wall stress are directly responsible for altered deposition of cardiac extracellular matrix (ECM) proteins. Indirect evidence for a possible role of mechanical stimulation in the regulation of cardiac collagen deposition during development comes from studies that show relative changes in chamber collagen concentration following birth, as well as spatial differences in collagen mRNAs within the heart [3]. Excess deposition of ECM proteins has also been associated with many cardiac pathological entities which entail various degrees and types of mechanical overload [4]. However, in the majority of these cases mechanical overload is associated with the activation of humoral systems such as the renin angiotensin aldosterone system (RAAS) [4]. Furthermore, there is emerging evidence that indicates that the myocardial

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RAAS can also be modulated in response to mechanical stimulation [5]. Evidence derived from studies in other tissues (e.g. ligament, bone) indicate that fibroblasts indeed, have a capacity to ''sense'', ''integrate'' and ''functionally respond'' to mechanical stimuli [6,7] ultimately yielding alterations in ECM deposition. For fibrosis to ultimately develop it will have to overcome in time, any associated increases in collagenase mediated ECM degradation.

## 2. Synthesis of ECM by cardiac fibroblasts

About two thirds of myocardial cells are fibroblasts [8]. As illustrated in Fig. 1, cardiac fibroblasts (CF) grow in a complex tissue environment and are responsible for the production and deposition of the majority of ECM proteins in the cardiac interstitium [9]. A recollection of studies published on CF since 1990 illustrates an emerging functional profile for these cells. CF appear to be functionally responsive to a variety of hormones and growth factors [10]. Conversely, CF can synthesize and release many growth factors which are thought to play important roles in the remodeled heart. Indeed, evidence implicates CF as a source of autocrine/paracrine factors within the myocardium [11]. Examples of such factors include endothelin-1, tumor necrosis factor-α and angiotensin II among others [10]. CF are known to synthesize fibronectins, vitronectin, collagen types I, III and V, collagenases, among many other ECM and ECM related proteins [12,13]. Type I collagen is the major collagenous product of these cells representing 80% of total newly synthesized collagen [13] which is secreted into the culture medium as procollagens. About 20% of the total collagen synthesized is type III collagen and a small proportion is type V collagen (less than 5%). FN is the most abundant non-collagenous protein synthesized by these cells [13] and plays important roles in development, adhesion and wound healing and is upregulated in the heart with mechanical overload [14].

## 3. The regulation of cardiac fibroblast function by defined mechanical stimuli

The use of in vitro culture systems to mechanically stimulate cardiac cells allows for the systematic examination of mechanical regulation of cardiac fibroblast function in the absence of systemic effects. However, there are significant limitations that derive from using such systems. Limitations include the fact that in vitro systems cannot model either the complex myocardial tissue milieu, hormonal and growth factor environment or the three dimensional nature of the deformations. Thus, caution needs to be exercised when interpreting results derived from studies using in vitro strain systems. Furthermore, there are inherent phenotypic differences that result from the species used as source of the cells and their developmental stages.

For many years, several variations of a uniaxial stretch device has been used to investigate cellular and molecular responses to static and cyclic mechanical loading of cardiac cells [15]. Uniaxial stretch devices were initially used to study the functional responses of cultured neonatal rat cardiac myocytes to mechanical load [15–17]. More

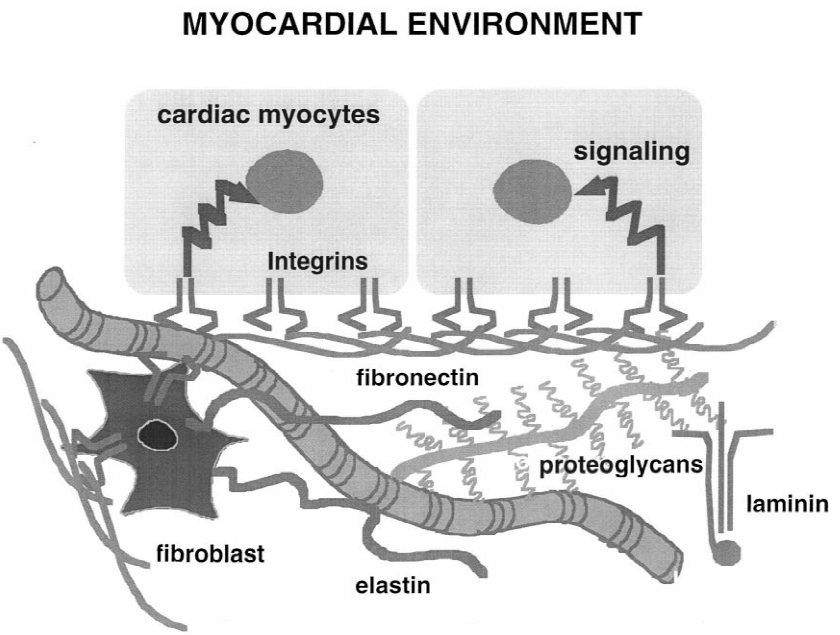


Fig. 1. Illustration of the complex tissue microenvironment in which cardiac fibroblasts grow. Many of the extracellular proteins described are produced by cardiac fibroblasts and serve to developmentally organize the myocardium ultimately yielding a load bearing structure.

recently such devices have been used in cultures of CF. In a study performed by Carver et al., neonatal rat cardiac fibroblast ECM gene expression was studied using uniaxial static and cyclic stimulation [18]. Results from static and cyclic studies show that the ratio of collagen type III to collagen type I protein increases in mechanically stimulated neonatal rat CF. The change in collagen ratio appears to mirror similar changes to those that occur in the early phases of dilated cardiomyopathy [19]. Type III procollagen gene expression is also increased in response to cyclic stretch for durations as short as 12 h whereas type I mRNA levels were unchanged.

In a study performed by Sadoshima et al. [15] it was demonstrated that static 20% stretch of neonatal rat CF increased phenylalanine and thymidine incorporation by 24 h yielding increased cell counts at 48 h. Enhanced cardiac fibroblast proliferation has been correlated with the development of myocardial fibrosis [20].

Although uniaxial stretch devices apply force only in one direction, there is also compression transverse to the axis of stretch with shearing forces present near the clamped edges of the elastic substrate [21]. A number of biaxial stretch devices have been designed which deform a flexible circular substrate. However, many of these devices also have similar limitations as uniaxial apparatuses due to the generation of nonhomogeneous strain fields. The generation of verifiable and uniform strains on cells should be prerequisites for experiments that attempt to define the role that specific mechanical stimuli have on cell function.

A new generation of deformable biaxial strain devices has been recently designed in our laboratories [21] which generate passive or dynamic equibiaxial strains of 0–10% or more. We recently examined whether the application of tensile and compressive strains to subconfluent cultures of adult rat CF grown on rat type I collagen yielded varying functional responses. Results indicate that a single 10% stretch applied by the uniaxial device stimulated increases in CF ECM gene expression. Interestingly, when CF were uniaxially stretched for 20% an inhibition of ECM mRNA's was observed. The inhibition of ECM mRNA's by 20% stretch may reflect among other possibilities either a true ''turn off'' of gene expression or the response of the cells to stressful stimuli. The application of larger magnitudes of mechanical stimuli to CF may lead to divergent and opposite functional responses. Decreased levels of collagen proteins are indeed observed early with ventricular remodeling in animal models of dilated cardiomyopathy [22] a process that is accompanied by collagenase activation.

In order to examine the effects of different types of deformation on CF, we used our equibiaxial stretch system to apply either pure tensile or compressive single step area strains, in the absence of shear. Estimation of cell area changes indicate that 3 and 6% equibiaxial stretches are equivalent to 10 and 20% uniaxial. The application of tensile strains (3 and 6%) resulted in a differential ECM mRNA expression in CF, similar to the up- and downregulation responses observed for 10 and 20% uniaxial stretches. For 3% equibiaxial strain, mRNA levels for collagen III and FN increased whereas at 6% equibiaxial strain, collagen III levels decreased and FN levels remained unchanged, compared with unstretched controls. In contrast, compressive equibiaxial strains produced decreases at −3% and −6% equibiaxial strains. Effects of compressive strains on mRNA levels for collagen III showed similar patterns of changes.

An emerging body of scientific evidence also indicates that the mechanical stimulation of cardiac cells may result in the release of growth factors, which in an autocrine or paracrine fashion may modulate cell function. Of particular interest are those factors known to be associated with the modulation of cardiac remodeling including, the angiotensin substrate angiotensinogen, transforming growth factor-β1 (TGF-β1), endothelin-1 and tumor necrosis factor-α (TNF-α) [11,14,23,24].

TGF-β1 is a humoral factor known for its ability to strongly and ubiquitously stimulate the production and deposition of ECM proteins. High levels of this growth factor occur with cardiac fibrosis [14,25]. The addition of TGF-β1 to cultured CF increases ECM mRNAs [26]. In our study [21] we determined that TGF-β1 bioactivity was modulated by mechanical strain and was dependent on the magnitude of applied area strain regardless of the mode of deformation (uni or equibiaxial, stretch or compression). However, there was no apparent correlation between levels of ECM gene expression and TGF-β1 bioactivity. Thus, it is still unclear if changes in collagen mRNA's which follow mechanical stimulation of CF is a direct result of the activation of signal transduction mechanisms that couple to the control of ECM gene expression or via the release of humoral factor(s) or a combination of both.

A recent study performed by Butt et al. [27] explored the issue of the interaction between cyclic mechanical load and humoral factors in cultured CF. In this study, rat embryonal CF were cultured onto elastin coated (Flex I) culture plates and tested with or without 10% fetal bovine serum or individually with angiotensin II, TGF-β1 or insulin growth factor-1 (IGF-1). During mechanical stimulation cells were loaded using 20% radial strain at a frequency of 1.5 Hz for 24 or 48 h. Results from their experiments indicate that stimulation of fetal CF with pure mechanical stimuli failed to yield increases in either procollagen synthesis or degradation at 24 or 48 h. However, in the presence of 10% FBS, procollagen synthesis was increased, with total amounts of procollagen degraded also slightly increasing. Results also indicated increases in procollagen (α1) I mRNA with serum at 48 h. The selective addition of either TGF-β1 or IGF-1 in the absence of serum led to further increases in procollagen mRNAs. Addition of angiotensin II failed to generate any significant changes in mRNA levels. No changes in cell proliferation were observed under the protocols used.

Thus, results from these experiments strongly suggest an interdependence between the mechanical stimulation of CF and the action of growth factors.

Mechanical modulation of cardiac fibroblast ECM degradation has also been recently examined. Several forms of latent matrix metalloproteinases (MMPs), are produced in the myocardium. A study by Tyagi et al. [12] examined if an activator of latent MMP is induced by mechanical stimulation. Human CF were stretched at 25 cycles/min in serum-free media or media with 5% serum. Results suggested that stretch induced a membrane MMP in CF that was similar to the MMP induced in ischemic hearts. Furthermore, tissue plasminogen activator of latent MMPs secretion was also elevated in the stretched cells. These results suggest that, under stretched conditions MMPs are induced in CF. If these phenomena indeed, occur in vivo under conditions of chronic mechanical overload it may lead to the development of adverse cardiac remodeling and failure.

TNF-α is a pleiotropic cytokine that is known to play important roles in the response to tissue injury and wound healing. Increased levels of TNF-α have been demonstrated in patients with dilated or ischemic cardiomyopathy [28,29]. Significant increases in TNF-α also occur in animal models of myocardial infarction [30]. In a recent study by Yokoyama et al. [31], it was demonstrated that mechanical stretching of neonatal rat CF stimulated the production and release of TNF-α. These results contrast to those derived from mechanical stimulation of neonatal rat myocytes where TNF-α release was unaltered. We have recently demonstrated that the addition of TNF-α to cultured neonatal rat CF results in a significant induction of angiotensin II AT1 receptors [23]. These observations suggest the existence of positive feedback loops where mechanical overload can induce the production and release of TNF-α which in turn (via the upregulation of $AT_1$ receptors) can reinforce a pro-fibrotic response to angiotensin II.

In pathological ventricular remodeling the enlargement of myocytes and myocardial fibrosis is typically observed simultaneously, implicating a possible functional interaction between cardiac myocytes and CF. In a study by Harada et al. [11], it was observed that when neonatal myocytes were co-cultured with CF, the size of myocytes and natriuretic peptide (ANP/BNP) secretion were significantly increased. This hypertrophic change of myocytes in the co-culture was significantly suppressed by BQ-123, an endothelin-A receptor antagonist. Cyclic stretch further increased ANP/BNP production in co-cultures. This increase in ANP/BNP production in the co-culture was suppressed by an angiotensin II type 1 receptor antagonist. Thus, this study raised the possibility that CF may modulate myocyte hypertrophy via secretion of endothelin-1 and that angiotesin II is involved in the interaction between myocytes and CF observed with mechanical stretch. Signaling between cardiac cells may indeed represent a viable event given that CF exist in high density in close apposition to muscle tissue.

## 4. Integrins in cardiac fibroblasts

CF, like other cell types respond to mechanical stimuli by changing patterns of gene expression through several modes of signal transduction. The mechanism by which CF ''sense'' mechanical stimuli is not clear. Forces are transmitted via cell surface/ECM connections. Integrins are one such class of molecules that mediate cell adhesion [32,33] accumulating at focal adhesion complexes and connecting to the actin cytoskeleton through adapter proteins [34–36]. Ingber and colleagues [37] have proposed the integrin-focal adhesion-cytoskeleton as a mechanically sensitive signaling organelle.

Integrins are heterodimeric cell surface molecules with long extracellular domains and short cytoplasmic tails with no known signaling motifs. The α/β combinations provide ligand specificity. Adult rat CF express a wide range of integrins including α1β1 (most predominant), α2β1, α5β1, αvβ3, αvβ1 and others (perhaps α4β1, α3β1, αvβ5, and αvβ6) [38]. In many cell signaling systems, specific integrin subtypes can mediate the activation of specific intracellular signals [39,40]. While this level of molecular detail has not yet been explored for mechanically stimulated CF, it may be important since as described below, an essential role for integrin substrate attachment specificity has been implied [38].

We explored integrin and ECM specificity using extracellular signal regulated kinase (ERK) and c-jun N-terminal kinase (JNK) as short term endpoints [38]. These two mitogenic stimuli are known to be activated transiently in mechanically stimulated cells [41–46]. Using the static equibiaxial stretch system in which quiescent CF were grown on specific ECM proteins and stretched by 4%, ERK and JNK are stimulated several fold in the first 5–10 min and then gradually decrease over time. To identify whether integrins mediated ERK/JNK stimulation, we plated CF on different ECM substrates prior to stretch. Interestingly, CF spread most rapidly and avidly on collagen I, but had no activation of ERK or JNK in response to stretch. Plating cells on fibronectin prior to stretching lead to activation of both ERK and JNK while plating on laminin or vitronecin only lead to activation of JNK. Results derived from the use of anti-integrin antibodies indicated that substrate dependencies were indeed linked to integrin specificity and distinctly to the ERK and JNK pathways. Wilson et al. [47] have found matrix induced differences in smooth muscle cell proliferation that were distinct from those found in the CF suggesting that there may also be tissue specificity to the regulation of integrin signaling. One potential explanation for a lack of mechanosensitivity through collagen may be that the endogenous cardiac matrix in the ventricles will normally

carry load and apply forces to the cells throughout the normal cardiac cycle. It would not be advantageous to have the CF consider every cycle as a stimulus. Perhaps fibronectin, vitronectin, and laminin connections only bear load in deleterious conditions such as hypertension or ischemic injury. We already know that fibronectin upregulation is a consequence of altered mechanical environment both in vitro and in vivo [3,10,14]. Therefore, it seems likely that CF ''sense'' changes through these connections responding first by producing more fibronectin, then more collagen fibers to bear the increased loads [48].

## 5. Mechanical activation of signal transduction/ second messengers in cardiac fibroblasts

Many of the new signaling paradigms that have evolved use existing pathways as their backbone. This is true for mechanotransduction in CF. Results from our studies evidence the activation of ERK and JNK following 4% stretch of CF [38]. Preliminary studies from our laboratories also indicate that with stretch the magnitude of ERK activation in CF is dose dependent. In contrast, we have observed a lack of ERK activation with compression. We have also noted that p38 does not respond to mechanical stimulation in CF [38]. While several stimuli have been shown to activate p38 without activating JNK, few have shown that JNK can be activated without p38 [22]. Both JNK and p38 are activated in ventricular tissue extracts in response to myocardial ischemia-reperfusion suggesting that p38 is either activated by a non-stretch dependent mechanism [23], or p38 activation occurs in the cardiac myocytes rather than in CF. It follows that in the control of gene expression the balance between the activation of the different signaling pathways and their duration (i.e. signal integration) must be essential for determining which genes should be activated or deactivated.

The involvement of second messengers such as cGMP, calcium and cAMP have not yet been explored in detail for mechanically stimulated CF. However, Gudi et al. [49] recently showed that the heterotrimeric G-proteins $G_{i1}$ and $G_q$, but not $G_{i3}$ are activated as a function of strain rate in mechanically stimulated CF. However, the downstream consequences of G protein activation are as of yet unclear. Clearly, many more of the established signal transduction/ signaling paradigms are likely to be involved in mechanical signaling in CF but remain to be explored.

## 6. Summary and future directions

Mechanical factors appear to modulate the deposition of cardiac ECM. However, the myocardial milieu in which the different cell types grow and function represents a complex interactive junction of electrical activities, mechanical forces and humoral signals (Fig. 2). Adding to this complexity is the fact that many of the humoral systems respond to mechanical stimuli. Thus, it is difficult to ascertain the precise in situ role that mechanical forces have on regulating CF function and ECM deposition. Recently in vitro studies have been performed to examine for the mechanical regulation of cardiac ECM production using cultures of CF. Results indicate that CF can ''sense and respond'' to mechanical stimuli by altering cell proliferation, ECM gene expression, collagenase activity and growth factor release. Thus, it is likely that CF

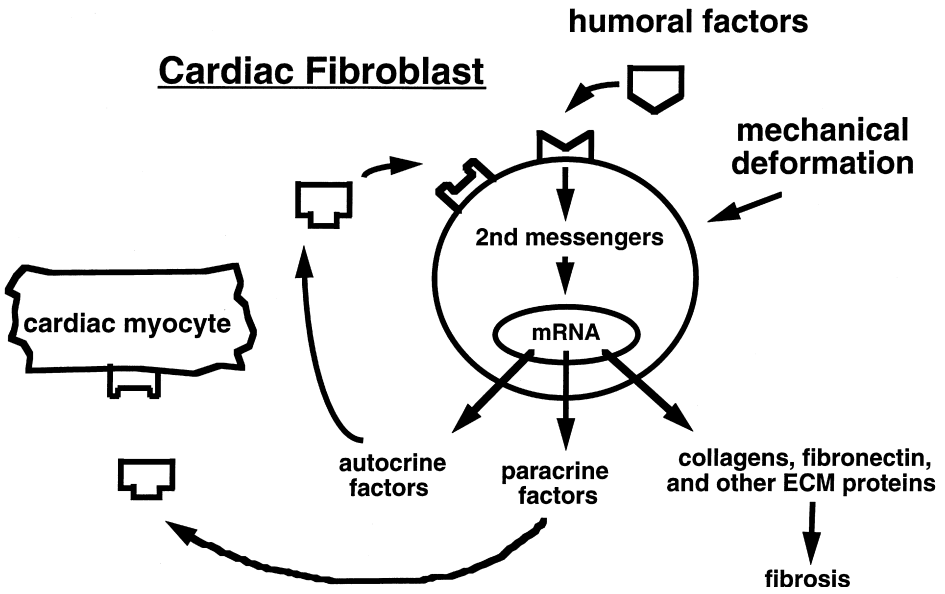


Fig. 2. Simplified model of cellular interaction for the cardiac fibroblast. The cell possesses a variety of receptors for humoral factors and can functionally respond to mechanical stimuli. Chemical and mechanical stimuli may directly couple to functional responses such as changes in cell proliferation, growth factor release or gene expression. Growth factors may then act in an autocrine or paracrine fashion to ''potentiate'' the mechanical stimulus.

''integrate'' via the activation of intracellular signal transduction pathways numerous signals to establish which genetic programs to regulate. Future studies will undoubtedly address many of these exciting questions.

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