Differential Responses of Adult Cardiac Fibroblasts to \textit{in vitro} Biaxial Strain Patterns

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Different patterns of extracellular matrix (ECM) remodeling in the heart are thought to be dependent on altered mechanical and chemical conditions and can contribute to cardiac dysfunction. Cardiac fibroblasts are the primary regulators of the ECM and may respond to mechanical factors \textit{in vitro}. We hypothesized that different types of \textit{in vitro} strains, e.g. tensile or compressive, can stimulate different functional responses in cultured adult rat cardiac fibroblasts. In this study, we first showed that a single step in strain applied by a uniaxial stretch system stimulated collagen III and fibronectin mRNA levels and transforming growth factor-\( \beta _1 \) (TGF-\( \beta _1 \)) activity in the adult phenotype of rat cardiac fibroblasts. Two-dimensional deformations were measured by tracking fluorescent microspheres attached to the substrate and cultured cells. For 10\% uniaxial strain, mean principal strains were 0.104 \( \pm \) 0.018 in the direction of stretch and \(-0.042\pm0.013\) in the perpendicular direction, verifying that the fibroblasts were simultaneously subjected to tensile (positive) and compressive (negative) strains. Furthermore, these cells were also subjected to area change and to shear. In order to examine the distinct effects of different types of deformation on cardiac fibroblasts, an equibiaxial stretch system was used to apply either pure tensile or compressive area strains, in the absence of shear. Magnitudes of equibiaxial strain were selected to apply local cell area changes identical to those applied in the uniaxial system. Results showed that pure tensile and compressive area strains induced divergent responses in ECM mRNA levels. TGF-\( \beta _1 \) activity was dependent on the magnitude of applied area strain regardless of the mode of deformation. These findings demonstrate that adult cardiac fibroblasts may respond differently to varied types of mechanical loading, suggesting that ECM remodeling may be locally regulated by specific mechanical stimuli in the heart.

\textbf{Introduction}

The cardiac fibroblast is the primary cell type responsible for the production of the extracellular matrix (ECM) proteins, such as fibrillar collagens type I and III and fibronectin, which provide essential structural and functional integrity to cardiac tissue (Booz and Baker, 1995; Villarreal and Kim, 1998). Cardiac fibroblasts also synthesize major growth factors in the heart, including transforming growth factor-\( \beta _1 \) (TGF-\( \beta _1 \)), which play key roles in the autocrine and paracrine regulation of tissue growth during normal development and in pathological remodeling (Long \textit{et al}., 1991; Eghbali-Webb, 1995). In studies of pressure-overload hypertrophy and scarring after infarction, changes in hemodynamic loading have been shown to modulate ECM laydown in the heart (Weber \textit{et al}., 1987; Villarreal \textit{et al}., 1992; Jugdutt and Amy, 1986). Moreover, varying
mechanical loading as seen during hypertension and infarction can yield diverse compositions and structural patterns in ECM remodeling, resulting in changes in myocardial function (Burgess et al., 1996; Holmes et al., 1997).

The mechanical regulation of cardiac fibroblasts in the heart may be complex since in vivo stresses and strains in the myocardium are multiaxial and nonuniform (Costa et al., 1997; Hunter and Arts, 1997). Several in vitro studies using fetal or neonatal rat cardiac fibroblasts cultured on elastomeric substrates have correlated stretch-induced increases in ECM synthesis with a single uniaxial estimate of mean substrate stretch (Carver et al., 1991; Butt et al., 1995). However, since the cellular deformation applied by these uniaxial devices is actually two-dimensional, or biaxial, with tensile, compressive, and shear components (Williams et al., 1992), it is not clear if the stretch-induced responses are regulated primarily by selected physical parameters and whether they are graded or all-or-none.

We hypothesized that different types of strains can stimulate changes in fibronectin, collagen type III gene expression and TGF-β₁ bioactivity in cultured adult rat cardiac fibroblasts. These markers of cardiac fibroblast function were selected as they are known to be upregulated early with ECM remodeling (Weber et al., 1987; Villarreal and Dillman, 1992). The objectives of the study were: (1) to determine whether uniaxial stretch stimulates fibronectin and collagen types I and III gene expression and TGF-β₁ activity in adult cardiac fibroblasts; (2) to identify and quantify the multiple strain components applied to these cells during uniaxial stretch; and (3) to use a well-defined equibiaxial stretch system to determine the distinct effects of uniform and isotropic tensile and compressive area strains on cardiac fibroblasts in the absence of shear. Our results showed that uniaxial stretch stimulated increases function collagen type III and fibronectin mRNA levels and TGF-β₁ activity in adult rat cardiac fibroblasts. Digital imaging and finite strain analysis were used to verify the transmission of tensile, compressive, and shear from the stretched substrate to the attached cells. Furthermore, we demonstrated that pure tensile and compressive area strains can stimulate different responses in collagen type III and fibronectin gene expression and TGF-β₁ activity in adult cardiac fibroblasts.

Materials and Methods

Mechanical cell stretch devices

As shown in Figure 1(a), the uniaxial stretch device, a modification of a design by Sadoshima et al. (1992), consists of a polycarbonate chamber, a transparent silicone elastic membrane (0.25 mm thick, gloss finish; Specialty Manufacturing, Inc., Saginaw, MI), and a pair of clamps with stainless steel rods and screws and rectangular walls made of acetal plastic. The clamped edges of the rectangular membrane (50 mm × 100 mm) are inserted into notches (for positions 0, 10 and 20% stretch) in the wall of the polycarbonate chamber so that the membrane rests at the bottom of the chamber.

The equibiaxial stretch device for cultured cells has been previously described (Lee et al., 1996). Briefly, it consists of three concentric polycarbonate cylinders (an inner indenter ring, a membrane holder, and an outer screw top) and a transparent elastic membrane which is attached to the membrane holder by a silicone rubber O-ring. The membrane forms the bottom of the cell culture chamber and the inner indenter ring (diameter 60.0 mm, height 46.5 mm) forms the wall. When the screw top is turned, a flange at its top pushes down the indenter ring. Indentation of the ring against the membrane results in a homogeneous, planar equibiaxial stretch of the membrane [Fig. 1(b)].

For both strain systems, before assembly of each device, the silicone elastic membrane was coated with a solution of 0.01% collagen type I (Sigma Chemical Co., St Louis, MO) in 0.1 M acetic acid by airbrushing (Badger Air-brush Co., Franklin Park, IL). The assembled devices were sterilized using ethanol and UV light and placed in standard plastic tissue culture dishes (Becton Dickinson and Co., Lincoln Park, NJ). The bottoms of both the uniaxial and equibiaxial devices are transparent to allow imaging of the substrate and the cultured cells using an inverted microscope.

Cell culture

Adult rat cardiac fibroblasts were isolated from three to four hearts from 7–8-week-old male Sprague–Dawley rats (200–250 g) according to a modification of a previously described protocol (Eghbali et al., 1991). Studies were conducted on cardiac fibroblasts (passages two to four) that were plated at subconfluence onto the collagen-coated membranes of the stretch devices (Lee et al., 1996). Cells were cultured in Dulbecco’s modified Eagle medium (DMEM) with antibiotics [penicillin, streptomycin and fungizone (PSF) and 10% fetal bovine serum (FBS) and then growth-arrested for 24 hours in a reduced serum medium (DMEM with PSF and 0.5%
Figure 1  (a) Schematic of the uniaxial stretch device for cultured cells, modified from Sadoshima et al. (1992). Cardiac fibroblasts were grown on a transparent silicone elastic membrane which was collagen-coated and clamped at each end. The clamped substrate was placed in a notched polycarbonate cell culture chamber and stretched 10% or 20% along the length of the frame. (b) Schematic of equibiaxial stretch device by Lee et al. (1996). Cells were cultured on the surface of the collagen-coated elastic membrane, which forms the bottom of the stretch device. Indentation of the membrane by the inner ring applies homogeneous, planar equibiaxial strain to the substrate and cultured cells.

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heat-inactivated FBS] before applying a single step in strain.

Quantification of two-dimensional substrate and cell strains in the cell stretch devices

The change in shape of a two-dimensional area can be described by the two “principal strains”, which represent the maximum and minimum changes in length along mutually perpendicular axes. Positive “tensile” strains describe an increase in length and negative “compressive” strains represent shortening. It is also convenient to express the strains in terms of the “extension ratio” of deformed to undeformed segment lengths.

Two-dimensional strain can also be separated into area change and “shear”. For example, in an equibiaxial stretch device (Schaffer et al., 1994) (Lee
et al., 1996), all segments change length equally so the principal strains are equal and there is no shear. Tensile strain in this device produces a pure area dilation and compressive strain produces a pure area contraction. The area ratio, defined as the ratio of the deformed area to the undeformed area, is equal to the product of the principal extension ratios. Conversely, in the case of uniaxial stretch (Sadoshima et al., 1992) (Williams et al., 1992), the principal strains are not equal to each other. The change in area reflects the combination of tensile strain in one direction and compression in the perpendicular direction. In addition to an area change, there is also significant shear. The maximum is defined as \( \frac{1}{2}(E_{\text{max}} - E_{\text{min}}) \) where \( E_{\text{max}} \) and \( E_{\text{min}} \) are the principal strains (Fung, 1993). In the equibiaxial case \( E_{\text{c}} = E_{\text{r}} \) and maximum shear is zero. Thus, two-dimensional strains may be tensile or compressive or a combination of both. Two-dimensional strains can also be considered to comprise a combination of “area change” and “shear” \( [\text{measured by maximum shear} = \frac{1}{2}(E_{\text{max}} - E_{\text{min}})] \). The maximum shear is computed from the difference between the principal strains. Thus, in the presence of homogeneous shear, circles becomes ellipses.

For both the uniaxial and equiaxial devices, the two-dimensional principal strains of the elastic membrane and the cells were determined by homogeneous finite strain analysis. The techniques and analysis methods have been described previously (Lee et al., 1996). Briefly, latex fluorescent microspheres (1 \( \mu \)m in diameter) were attached to the collagen-coated membrane and the cultured cells for use as markers of material displacement. In phase-contrast and epifluorescence images, segment lengths between triangular arrangements of markers were measured within two minutes after a single step in strain and used to calculate the symmetric two-dimensional Lagrangian strain tensor (Fung, 1993). In this study, mechanical deformation of the cells is described by this single-step measurement in applied strain. Biological responses to this deformation were measured 24 h after the acute application of strain, as described in the following sections.

In the uniaxial stretch system, the two-dimensional principal strains were expressed as \( E_1 \) and \( E_2 \) in rectangular Cartesian coordinates, where axes 1 and 2 are mutually perpendicular. For convenience, principal strains in the equibiaxial strain system were expressed as \( E_1 \) and \( E_2 \) in polar coordinates, where the circumferential (c) and radial (r) axes are mutually perpendicular. Area ratio, the ratio of the deformed area (A) to the undeformed area (\( A_0 \)), is equal to the product of the linear extension ratios, which are readily calculated from the two-dimensional strains (Lee et al., 1996).

### RNA isolation and Northern analysis

Twenty-four hours after the application of a single step in strain, cell cultures were harvested and total RNA was extracted from the lysate. UV spectrophotometry at 260 nm was used to quantitate the final RNA yield. Samples of total cellular RNA were size-fractionated by 1% agarose gel electrophoresis, transferred to nylon membranes, fixed to the membrane by exposing to UV light, and hybridized with cDNA probes labeled with \([^{32}P]\)dCTP (3000 Ci/mmol) by random priming (Villarreal and Dillman, 1992). The following probes were used for Northern blot analysis in this study: (1) fibronectin (FN); a rat cDNA for fibronectin containing 875 bp of the \( \beta \) coding region; (2) collagen III (cIII); a mouse collagen-\( \alpha_1 \) (III) probe of 500 bp for the coding region; (3) collagen I (cI); a rat collagen-\( \alpha_1 \) (I) probe of 900 bp for the 3\( \prime \) non-coding and coding regions; and (4) 28S ribosomal RNA: 29 bp DNA oligomer. The filters were washed and subsequently exposed to X-ray films. All data are expressed as the ratio of mRNA/28S signals as determined by quantitative image analysis.

### TGF-\( \beta_1 \) activity in fibroblast conditioned medium

The biological activity of TGF-\( \beta_1 \) was measured in fibroblast conditioned medium using a modified mink lung epithelial cell bioassay (Gibbons et al., 1992). Conditioned medium was collected from stretched and unstretched cardiac fibroblast cultures. CCL-64 mink lung epithelial cells (American Type Culture Collection, Rockville, MD) were plated in Eagle’s minimum essential medium supplemented with non-essential amino-acids, 10% heat-activated FBS, and antibiotics. Freshly plated subconfluent cells in 96-well plates were incubated in serum-containing medium for 4 to 5 h washed, and incubated with conditioned medium dilutions (up to 1:100) in serum-free medium. To determine DNA synthesis, these cells were pulsed with \([^{3}H]\)thymidine (2.3 \( \mu \)Ci/ml) at 18 h after treatment and harvested onto glass fiber filters (Whatman Paper) at 24 h for detection in a scintillation counter. TGF-\( \beta_1 \) (R&D Systems, Minneapolis, MN) was added to each assay plate to generate a standard curve. TGF-\( \beta_1 \) activity was...
assessed by its growth inhibitory effects in the bioassay and specificity of the response was determined by addition of the TGF-β1 neutralizing antibody (R&D Systems).

Statistical analysis

Results are expressed as mean ± s.d. and are representative of a minimum of three independent experiments, unless otherwise noted. One-way ANOVA was used with Bonferroni-Dunn corrections for multiple post-hoc comparisons between groups. Differences were regarded as statistically significant when P<0.05.

Results

Uniaxial stretch stimulates extracellular matrix mRNA levels and TGF-β1 activity in adult cardiac fibroblasts

A single application of 10% uniaxial strain resulted in a significant three-fold increase in both FN and cIII mRNA expression 24 h after stretch, as shown in Figure 2. In contrast, mean levels of FN and cIII mRNA decreased at 20% nominal strain when compared with the unstretched control (100%).

Levels of mRNA for cI showed a slight increase 24 h after application of 10% uniaxial strain (13.6.6% ± 14.9% compared with unstretched control) and no significant change at 20% strain (83% ± 8.4%).

To measure the biological activity of TGF-β1 released by cardiac fibroblasts, conditioned media was collected and diluted between 10- and 100-fold for detection using the mink lung epithelial cell bioassay. Conditioned media from fibroblasts stretched at 10% uniaxial strain showed significantly higher TGF-β1 activity as indicated by a greater inhibition of [3H]thymidine incorporation, as shown in Figure 3. 20% uniaxial stretch also elicited significantly increased TGF-β1 activity in conditioned media. Specificity of TGF-β1 in the diluted conditioned media was confirmed by incubation with 100 µg/ml of TGF-β1 antibody (Ab), using 100 µg/ml of non-specific immunoglobulin (Ig) in control comparisons. Inhibition of [3H]thymidine incorporation by active TGF-β1 for 10% uniaxial stretch (9.5 ± 0.8 kcpm for “10% × Ig” compared with 11.7 ± 0.8 kcpm for “0% × Ig”; P = 0.002) was neutralized by addition of the TGF-β1-specific antibody (12.4 ± 1.1 kcpm for “10% + Ab”). Data are mean ± s.d. and representative of four independent experiments.
Table 1 Summary of two-dimensional cell strain components (E1 and E2) for adult cardiac fibroblasts cultured on substrates that were stretched 10% and 20% along the length of the uniaxial device (n = 12 and n = 3, respectively).

<table>
<thead>
<tr>
<th>Nominal Stretch</th>
<th>E1</th>
<th>E2</th>
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<tbody>
<tr>
<td>10%</td>
<td>0.104 ± 0.018</td>
<td>-0.042 ± 0.013</td>
</tr>
<tr>
<td>20%</td>
<td>0.210 ± 0.019</td>
<td>-0.061 ± 0.024</td>
</tr>
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The strain measurements indicate that at any given location, cells are subjected to tensile (positive) and compressive (negative) strain components. Furthermore, cells are also subjected to shear, which is computed as the difference between principal strains.

Figure 4 Adult rat cardiac fibroblasts before (a) and after (b) stretching the silicone elastic substrate 10% along the length of the uniaxial stretch device. The principal strains, E1 and E2, were determined from the displacement of latex microspheres attached to the apical surface of the cells. Measurement bars in both views indicate a length of 40 μm.

Cells stretched in the uniaxial system are subjected to shear, tension and compression

Two-dimensional strains were determined for both substrate and cultured adult rat cardiac fibroblasts, which were stretched 10% or 20% along the length of the uniaxial stretch device. Figure 4 shows cultured fibroblasts, with microspheres attached to the apical cell surface, before and after application of 10% nominal strain. In the uniaxial stretch device, the substrate and cells were subjected to tensile (positive) strains along the axis of extension (E1) and compressive (negative) strain perpendicular to this axis (E2). Cell strain measurements are summarized in Table 1. Cell area ratios, A/A0 (deformed area: undeformed area), for 10% and 20% nominal strains were computed as 1.06 and 1.12, respectively. Shear distributions in the uniaxial stretch device were nonuniform due to the clamped edges of the membrane and computed as the nonzero difference of the principal strains.

The equibiaxial stretch system delivers uniform and isotropic tensile or compressive strains to cells in absence of shear

As reported in our previous study (Lee et al., 1996), equibiaxial stretch of the silicone elastic substrate and cultured cardiac fibroblasts produced mean circumferential strains (Ec) that were not significantly different from radial strains (Er). For example, for 6% nominal tensile strain, mean principal strains were Ec = 0.060 ± 0.003 and Er = 0.058 ± 0.002. Mean shear was negligible. Compressive cell strains were also not significantly different from substrate strains. The applied strains in this system have been confirmed as uniform (does not vary with position on the substrate) and isotropic (does not vary with axis orientation).

In this study, magnitudes of equibiaxial strain were selected by matching the cell area ratios, A/A0, applied by the uniaxial and equibiaxial stretch systems. Specifically, tensile equibiaxial strains of 3% and 6% yielded cell area ratios of 1.06 and 1.12, which were equivalent to the cell area changes produced by 10% and 20% uniaxial strain.

Pure tensile and compressive equibiaxial strains induce different responses in extracellular matrix mRNA levels

Tensile strains applied by the equibiaxial stretch device resulted in a differential mRNA expression in the cardiac fibroblasts, similar to the up- and downregulation responses observed for uniaxial strain. At 3% equibiaxial strain, mRNA levels for cIII and FN increased 1.5-fold, whereas at 6% equibiaxial strain, cIII levels decreased and FN levels
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Levels of TGF-β₁ activity are dependent on the magnitude of tensile or compressive equibiaxial strain

Both tensile and compressive equibiaxial strains elicited similar increases in TGF-β₁ activity in stretched cardiac fibroblasts, as shown in Figure 6. As described before, inhibition of [³H]-thymidine incorporation by active TGF-β₁ was neutralized by addition of the TGF-β₁-specific antibody (data not shown). While a lower magnitude of 3% strain (either tension or compression) did not increase TGF-β₁ activity in fibroblast conditioned medium, a higher strain 6% magnitude (either tension or compression) stimulated significantly greater TGF-β₁ activity in the stretched cells when compared with unstretched controls.

Discussion

In vitro uniaxial and biaxial cell stretch systems have rapidly become standard models for studying the effects of mechanical load in a number of cardiovascular cell types, including neonatal cardiac myocytes (Sadoshima et al., 1993; Kijima et al., 1996), neonatal or fetal cardiac fibroblasts (Curver et al., 1991; Butt and Bishop, 1997), vascular endothelial cells (Caille et al., 1998), and vascular smooth muscle cells (Barbee et al., 1994) (Inoue et al., 1998). While two of these studies have quantified the actual two-dimensional deformation in the stretched cells (Barbee et al., 1994; Caille et al., 1998), none have directly correlated different patterns of measured cell strain to specific functional responses. Since recent studies have indicated that the physical shape of a cell may provide critical control of its growth and function (Chen et al., 1997), one of the primary objectives of this study was to identify the multiple types of strain components that may be responsible for the adult cardiac fibroblast response to two-dimensional deformation applied in a uniaxial stretch system. Furthermore, we examined the differential effects of distinct types of deformation, such as tension or compression, on ECM gene expression and growth factor activity in cultured adult rat cardiac fibroblasts. Specifically, as a first step toward developing a
Our results showed that static application of uniaxial stretch at 10% and 20% stimulated FN and cIII mRNA levels and TGF-$\beta_1$ activity in adult rat cardiac fibroblasts at 24 h. However, cI mRNA levels were not markedly affected by uniaxial stretch during this time period, resulting in a slight increase only at 10% strain. These findings correlate well with results from previous studies of cardiac tissue remodeling. In an in vitro rat model of myocardial infarction, Cleutjens et al. (1995) reported that increases in type III procollagen mRNA preceded increases in type I mRNA and that collagen deposition in the infarct may be triggered by cyclic stretching of the wound. In other studies, FN was shown to increase in rat hearts 24 h after infarction, likely due to de novo synthesis by connective tissue cells (Casscells et al., 1990). The early and prominent roles of cIII and embryonic FN have also been observed in dermal wound healing (Kurkinen et al., 1980). These observations suggest that cIII and FN may be more relevant in response to acute strains, as reported in our studies, as they are in acute infarction and wound healing.

These findings have also correlated well with in vivo and in vitro studies of cardiac hypertrophy, which reported increased mRNA levels for collagens type I and III, FN, or TGF-$\beta_1$ (Villarreal and Dillmann, 1992; Lee et al., 1995). Similarly, stretch-induced stimulation of ECM proteins and TGF-$\beta_1$ have recently been shown in other cell types. In rat glomerular mesangial cells, nonuniform cyclic stretch increased levels of mRNA for collagen I, IV, and FN, and TGF-$\beta_1$ (Yasuda et al., 1996), with maximal increases for the ECM proteins occurring between 12 and 24 h. In rabbit aortic smooth muscle cells, stretch stimulated a two-fold increase in fibrillar collagen synthesis and increased secretion of active TGF-$\beta_1$ in the conditioned medium (Li et al., 1998).

We have for the first time described using finite strain analysis, the two-dimensional deformation imposed on mechanically stimulated cardiac fibroblasts. We demonstrate that in the uniaxial stretch system, these cells were subjected simultaneously to strains that were tensile (positive), compressive (negative), and shearing (i.e. twisting) in nature. While the intact myocardium undergoes a wide range of tension, compression, and shear, it is not clear which physical signal may direct the specific patterns of matrix remodeling produced by different mechanical conditions. In order to quantify the effects of different mechanical loading on cell function, the equibiaxial stretch system has been developed by several groups, including our laboratory, as the simplest system to apply a uniform and isotropic deformation to cultured cells (Hung and Williams, 1994; Schaffer et al., 1994; Lee et al., 1996). Specifically, in equibiaxial stretch, pure tensile or compressive cell area strains are applied to the attached cells, independent of cell orientation and in the absence of shear. To our current knowledge, our system is the only equibiaxial stretch device among these designs that allows in situ measurement of cell area strains for applied nominal strains up to 20% (Lee et al., 1996).

A number of studies have strongly indicated that local cell area, as controlled by degree of spreading, can govern vascular cell survival and growth, regardless of the type of matrix protein or integrin antibody added to mediate adhesion (Ingber et al., 1995; Mooney et al., 1995; Chen et al., 1997). The equibiaxial strain magnitudes for this study were thus selected to produce cell area ratios that matched those in the uniaxial system, as quantified by the calculated linear stretch ratios. As previously reported, adult rat cardiac fibroblasts that were stretched in our equibiaxial strain system remained attached at all applied strains (up to 6%) and no changes in cell number were observed (Lee et al., 1996; MacKenna et al., 1998). Additionally, Chen et al. (1996) have demonstrated that no cellular injury was detected in vascular cells that were stretched up to 30% equibiaxial strain. In our studies, the ECM components that were highly responsive to uniaxial stretch, namely FN, cIII, and TGF-$\beta_1$, were selected for subsequent testing in the equibiaxial strain system, whereas cI expression was not further examined due to its minimal response to uniaxial stretch for our fibroblast model.

Results derived from using our equibiaxial devices indicate that pure tensile and compressive area strains elicited different effects on FN and cIII gene expression in the cardiac fibroblasts. Specifically, we observe that pure tension was able to replicate the up and down pattern of FN and cIII mRNA expression, as observed with the uniaxial system. Thus, these results suggest that tensile signals may be a primary determinant of stretch-induced matrix synthesis in these cells. These initial findings further indicate that pure compression results in the down-regulation of mRNA levels for the FN and cIII
proteins. In contrast, the effects of local cell area strains on TGF-β₁ activity appear magnitude-dependent, stimulating increased bioactivity at higher magnitudes regardless of the mode of deformation, e.g. tension or compression. Based on our observations, shear and cell orientation effects, both present in the uniaxial system, may account for secondary differences in the fibroblast responses and remain to be examined in future studies.

The ability of cardiac fibroblasts to detect distinct magnitudes and patterns of biaxial strain may provide a significant type of signaling specificity that could contribute to a multitude of functional outcomes, especially when coupled with differentially regulated mechanotransducers. Using the equibiaxial stretch device and adult rat cardiac fibroblast model described here, two recent studies have demonstrated that pure tensile and/or compressive area strains can stimulate early mechanotransduction mechanisms, such as the extracellular signal-regulated kinase (ERK2) and c-Jun NH₂-terminal kinase (JNK1) pathways or G protein activation, within minutes of application of stretch (MacKenna et al., 1998; Gudi et al., 1998). In the study by MacKenna et al. (1998), static 4% tensile strain directly stimulated ERK and JNK1 activity in cardiac fibroblasts through different integrin pathways, with peak activation between 5 and 10 min. Similarly, Gudi et al. (1998) demonstrated that pure tension–compression loading cycles at maximum strains of 3% and 6% activated specific G protein subunits in these cells within 10–60 s of applied strain. In conjunction with the data presented here and by other in vitro stretch reports (Carter et al., 1992; Butt and Bishop, 1997), these studies indicate the potential diversity of mechanical signaling for adult cardiac fibroblasts. It should be noted, however, that there are clear limitations of extrapolating static in vitro data from selected time intervals (e.g. 24 h) to dynamic in vivo conditions. Moreover, the quantitative relationships between different types of strain components and the activation of linked mechanotransducers in cardiac fibroblasts remain to be determined for a wide variety of loading conditions, including the effects of dynamic equibiaxial strain (Yasuda et al., 1996; Sotoudeh et al., 1998) or synergy with chemical growth factors (Butt and Bishop, 1997). Finally, to fully understand the mechanical regulation of cardiac fibroblast function, it will be essential to extend the quantitative in vitro studies to include three-dimensional and time-dependent effects of stretch in these cell cultures. Observations derived from in vivo experimental models highlight the complexities of cardiac ECM regulation. Indeed, published results indicate that in vivo pressure and volume overload generate distinct patterns of myocardial ECM remodeling (Weber et al., 1987; Iimoto et al., 1992). Whereas in experimental animal models pressure overload typically results in extensive myocardial fibrosis, volume overload results in no notable increases in ECM proteins although collagen cross-linking is reported to increase (Iimoto et al., 1992). The molecular mechanisms that guide these pathophysiological responses are currently not well understood. In the simplest of analogies, passive mechanical stretch may more closely model volume overload in vivo rather than pressure overload; however, as discussed above, this oversimplification would suffer from significant experimental shortcomings.

In summary, we have demonstrated that known sensitive functional markers of cardiac tissue remodeling, such as fibrillar collagen, fibronectin, and TGF-β₁, may be differentially regulated in adult cardiac fibroblasts by specific mechanical strain components. The use of different cell stretch systems, as presented here, provide a systematic approach to developing a quantitative understanding of the mechanical signaling mechanisms that may critically regulate extracellular matrix remodeling in the heart.

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