A new microrheometric approach reveals individual and cooperative roles for TGF-β1 and IL-1β in fibroblast-mediated stiffening of collagen gels

Lester Y. Leung,* David Tian,* Clifford P. Brangwynne,† David A. Weitz,† and Daniel J. Tschumperlin*,[1]

*Program in Molecular and Integrative Physiological Sciences, Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA; and †Division of Engineering and Applied Sciences and Department of Physics, Harvard University, Cambridge, Massachusetts, USA

ABSTRACT **The stiffness of the extracellular matrix can profoundly influence cell and tissue behaviors. Thus there is an emerging emphasis on understanding how matrix mechanical environments are established, regulated, and modified. Here we develop a microrheometric assay to measure the mechanical properties of a model extracellular matrix (type I collagen gel) and use it to explore cytokine-induced, cell-mediated changes in matrix mechanical properties. The microrheometric assay uses micron-scale ferrimagnetic beads embedded within collagen gels during fibrillogenesis. The beads are magnetized, then subjected to a twisting field, with the aggregate rotation of the beads measured by a magnetometer. The degree of bead rotation reflects the stiffness of the surrounding matrix. We show that the microscale assay provides stiffness measures for collagen gels comparable to those obtained with standard macroscale rheometry. To demonstrate the utility of the assay for biological discovery, we measure stiffness changes in fibroblast-populated collagen gels exposed to three concentrations of six cytokines over 2 to 14 days. Among the cytokines tested, transforming growth factor-β1 and interleukin-1β enhanced matrix stiffness, and together exerted cooperative effects on cellular modulation of matrix mechanics. The microrheometry approach developed here should accelerate the discovery of biological pathways orchestrating cellular modulation of matrix mechanics.—Leung, L. Y., Tian, D., Brangwynne, C. P., Weitz, D. A., Tschumperlin, D. J. A new microrheometric approach reveals individual and cooperative roles for TGF-β1 and IL-1β in fibroblast-mediated stiffening of collagen gels. *FASEB J.* 21, 2064–2073 (2007)**

Key Words: compliance · extracellular matrix · remodeling · myofibroblast · fibrosis

The extracellular matrix (ECM) provides structural scaffolding for tissue organization and endows tissues with much of their mechanical strength and integrity (1). The ECM also provides a rich signaling environment, contributing biochemical and mechanical cues important to morphogenesis, wound healing, and disease processes (1–3). Thus the mechanical properties of a tissue's ECM are optimized for proper organ function, and these same properties strongly influence the functional behaviors of resident cells (4–7). When pathophysiological changes in tissue mechanics develop (2, 3, 5, 6), therapeutic approaches require a mechanistic understanding of the signals and cellular processes that regulate matrix mechanics (2). Although sophisticated tools are available to study the global and local changes in matrix architecture mediated by cell-matrix interactions (2, 8–10), fewer options exist to quickly and efficiently measure cell-mediated changes in matrix mechanical properties. The objectives of this study are to validate a new method for measuring the mechanics of a simplified matrix (type I collagen gels) undergoing cell-mediated remodeling and to demonstrate the assay's unique screening capabilities by measuring changes in matrix stiffness driven by a panel of cytokines implicated in fibrotic disorders.

Previous investigations of cell-mediated matrix remodeling have relied extensively on fibroblasts, the primary matrix regulatory cell, embedded within 3-dimensional scaffolds of purified reconstituted extracellular matrix proteins. Such models have underscored the distinct ways cells interact with 3-dimensional matrices as opposed to traditional 2-dimensional tissue culture surfaces (11). The 3-dimensional models have been instrumental in the discovery and mechanistic understanding of cell-mediated matrix compaction, and the influence of an array of soluble stimuli on this process (reviewed by Grinnell, ref. 10). Moreover, these 3-dimensional models have demonstrated important roles for matrix dimensionality (12) and mechanics (13) in regulating gene expression, particularly expression of matrix constituents, emphasizing the potential for cells to sense and change the mechanics of their environment. However, measuring changes in gene

[1] Correspondence: HSPH-Environmental Health, 665 Huntington Ave., Boston, MA 02115, USA. E-mail: dtschump@hsph.harvard.edu.

doi: 10.1096/fj.06-7510com

0892-6638/07/0021-2064 © FASEB

and protein expression, even those strongly implicated in matrix biology, is likely to be only a rough proxy for measuring cell-mediated changes in matrix mechanical properties.

To directly assess the mechanical properties of extracellular matrix preparations, traditional engineering methods such as rotational shear (14, 15), confined compression (16), and uniaxial tension (17, 18) testing have been used. While these methods allow comprehensive testing of the matrix, they are challenging to adapt for cell-matrix constructs, especially for the incubation times needed to observe cell-mediated matrix remodeling (*e.g.*, matrix compaction, fibril reorganization, collagen synthesis; refs. 2, 9, 10, 19–21). At the macroscale, these challenges have been overcome using cell-matrix constructs cultured in tubular geometries and mechanically characterized under defined mechanical and biological conditions (19, 22, 23). The resource costs associated with these experiments have so far largely precluded exploration of the effects of soluble mediators, small molecule inhibitors, and genetic modifications outside the known fibro-proliferative molecular pathways.

A complementary approach that could reduce resource costs and enhance discovery efforts is to use micron-scale particles embedded within the matrix as mechanical probes. The approach is similar to microrheometric methods for tracking spontaneous (24–27) or forced (28–31) motions of micron-scale particles within or on the surface of polymer, protein, and cellular preparations. Velegol and Lanni (29) first explored the tracking of forced bead oscillations as a method to mechanically characterize collagen gels. Though successful, the method relied on laser trapping and oscillating individual beads dispersed throughout a gel and calculating local mechanical properties, a cumbersome task given the regional heterogeneity of bead behavior within the collagen gels. To overcome this limitation, we adapted a previously developed method (28, 32) to simultaneously measure the collective behavior of a population of beads embedded within a type I collagen matrix; the method provides a single robust readout of matrix mechanics suitable for screening responses to a variety of interventions. Here we validate this approach in collagen gels, extend it to matrix constructs undergoing cell-mediated remodeling, and use the assay to screen the effects of selected cytokines on cell-mediated changes in matrix mechanical properties. We also measure changes in expression of four matrix remodeling-associated genes under identical treatment conditions for comparison with changes in matrix mechanical properties.

MATERIALS AND METHODS

Materials

Adult human lung fibroblasts (LL-24) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Recombinant human transforming growth factor-β1 (TGF-β1), platelet-derived growth factor-BB (PDGF-BB), and interleukin (IL) -1β, IL-4, and IL-13 were obtained from R&D Systems (Minneapolis, MN, USA). Human endothelin-1 (Et-1) was obtained from Calbiochem (San Diego, CA, USA). Porous polyethylene sheets were obtained from Small Parts, Inc. (Miami Lakes, FL, USA). Collagen type I solution (Vitrogen 100) was from Cohesion Tech (Palo Alto, CA, USA).

Cell culture

LL-24 adult human fibroblasts were grown in a monolayer culture at 37°C and 5% CO_2 in F12K plus 15% fetal bovine serum (FBS), as recommended by ATCC. The medium was supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin. Cells between passages 9 and 11 were used for all experiments. Adult lung fibroblasts were used in this study based on their involvement in pulmonary fibrosis and other matrix disorders of the adult lung (3, 33).

Collagen gel preparations

Vitrogen 100 was mixed with 10× phosphate-buffered saline (PBS) and 0.1 M NaOH in a ratio of 8:1:1 to prepare isotonic solutions at pH of 7.4 ± 0.2 and a final collagen concentration of 2.0 mg/ml. Ferrimagnetic beads (4.5±0.45 μm diameter) in several batches were produced and characterized at the Harvard School of Public Health. Unless otherwise indicated, beads were used at a final mass concentration of 12.5 μg per 100 μl of final collagen solution. Beads were equilibrated in 1× PBS before addition to collagen mixtures. The method used here for embedding beads within collagen gels is equivalent to a previously described method for passively adsorbing a collagen coating onto ferrimagnetic beads (34). The protocol described above thus results in passive adsorption of collagen onto the surface of the beads during fibrillogenesis and integration of the beads into the matrix of the collagen gel.

To create cell-matrix constructs, the neutralized dilute collagen and ferrimagnetic beads were mixed with LL-24 fibroblasts suspended in F12K medium supplemented with 5% FBS to achieve the desired final cell concentration. To prepare constructs that maintain their radial dimension during extended culture periods, 200 μl of the combined solution was aliquoted into porous polyethylene tubes punched and hollowed from porous polyethylene sheets (average pore size of 80–120 μm, dimensions of 10 mm external diameter, 6 mm internal diameter, and length of 6 mm). The bottoms and outer sides of the polyethylene cylinders were covered in Parafilm to prevent collagen leakage during fibrillogenesis and each cylinder was placed in a well of a 24-well plate. The constructs were incubated for 45 min at 37°C in room air to facilitate fibrillogenesis. When fibrillogenesis was complete, the Parafilm was removed from each cylinder and 500 μl of F12K plus 5% FBS was added to each well; this serum concentration was chosen to maximize cellular responsiveness to subsequent cytokine stimulation (35).

Constructs were stimulated with TGF-β1, PDGF-BB, Et-1, IL-1β, IL-4, and IL-13 at concentrations of 2, 20, and 50 ng/ml, covering the typical range of biological activity for these cytokines (35–47). Cytokine stimulation was initiated when the cells were fed 1 day after fibrillogenesis. Cells were then fed every 2 days with F12K plus 5% FBS and the indicated cytokines, alone or in combination. Time course experiments were continued for up to 14 days, which allowed sufficient time for slow-developing cellular processes such as matrix neosynthesis (2, 9, 10, 19–21) to occur and their effects on matrix mechanics to be included in the analysis.

Microrheometry of collagen gels

Magnetic bead twisting analysis of collagen gels was performed using a described device (28, 32, 48). The device exposes matrix constructs to a brief magnetic pulse, which uniformly magnetizes the beads embedded within the matrix (**Fig. 1*A***). The device then exposes constructs to either a constant twisting field (10, 20, or 40 Gauss) for 60 s or a sinusoidally varying twisting field applied at one of two frequencies (0.05 and 0.2 Hz) for 10 cycles. The twisting field reorients the beads, and the ensemble average rotation of the beads is calculated by measuring the change in the remanent magnetic field of the beads in the direction orthogonal to the twisting field. The remanent field strength can decay slowly due to random bead motions, so this slow decay is accounted for by measuring the decrease in magnetic field strength in time-matched control experiments. Changes in the corrected remanent field strength are the sole result of bead rotation in the twisting field (Fig. 1*B*).

The extent of bead rotation in response to a twisting field reflects the properties of the material in which the beads are embedded; the stiffer the material, the smaller the rotation. For constant twist experiments, the material is characterized by an "apparent stiffness," which is computed from the applied torque and the resulting ensemble angular rotation of the beads. The applied torque is a product of the magnitude of the twisting field applied, and the magnetic content of the beads determined from bead rotations in a fluid of known viscosity (32, 49). The measurement of cumulative bead rotation after 60 s in a constant twisting field is analogous to a shear-creep test. The apparent stiffness calculated in this manner is closely related to the material shear modulus (G) for beads that are uniformly and fully embedded within a linear isotropic material (50).

For oscillatory twist experiments, the twisting field $H_z(t)$ applied to the beads is a sinusoidal function with amplitude H_a and frequency f: $H_z(t) = H_a \sin(2\pi f t)$. The time-varying angle of bead rotation $\Phi(t)$ produced by the twisting field is computed by comparing the measured bead field $B(t)$ to a reference (before twist) field B_0: $\Phi(t) = \cos^{-1}[B(t)/B_0]$. The specific torque present at the bead surface $T(t)$ is then: $T(t) = c\, H_z(t) \cos[\Phi(t)]$, where c is a bead calibration constant incorporating the magnetic content of the beads (49). The complex modulus G for the material at any frequency is defined as: $G(f) = T(f)/\Phi(f)$. This can be represented as $G(f) = G'(f) + i\, G''(f)$, where i is sqrt(-1), G' is the storage modulus, and G" is the loss modulus (48).

Macrorheometry of collagen gels

The bulk elastic modulus of collagen gels was measured using a rheometer (CVOR, Bohlin Instruments, East Brunswick, NJ, USA) with a 4°, 40 mm cone and plate geometry. The rheometer is equipped with a heating unit, and samples were maintained at 37°C during testing. The elastic modulus, $G'(\omega)$, and loss modulus, $G''(\omega)$, were measured as a function of frequency over the range of ω = 0.15–5 Hz in a stress-controlled mode. The maximum applied stress was 0.1 Pa. This led to a predominantly in-phase (elastic) strain with a maximum of ~0.09%. We verified that the frequency-dependent modulus did not depend on the magnitude of the applied stress until it was increased to larger than ~1 Pa (with a corresponding maximum strain of ~1%), and thus our measurements were in the linear regime.

Fluorescent staining of fibroblasts

Constructs were fixed with 3% paraformaldehyde, permeabilized with 1% triton X-100, and stained with Alexa Fluor 488 phalloidin and DAPI (Molecular Probes, Carlsbad, CA, USA). Filamentous actin cytoskeletal structures and nuclei were imaged using an inverted fluorescence microscope (Nikon) connected to a cooled CCD camera (Roper Scientific, Duluth, GA, USA).

Reverse transcription and real-time polymerase chain reaction (RT-PCR)

Cell-matrix constructs were freed from porous polyethylene rings and minced with forceps, then digested in collagenase solution (3 mg/ml, Invitrogen) at 37°C for 10 min. The crude digests from 8–10 constructs per condition were pooled, the fibroblasts collected by centrifugation, and total RNA was purified with a commercial kit (Rneasy, Qiagen, Valencia, CA, USA). Equal amounts of RNA (1 μg) were reverse transcribed with Ready-to-Go RT-PCR beads (Amersham, Arlington Heights, IL, USA). PCR primers (Invitrogen) targeting alpha-smooth muscle actin (α-SMA), collagen αI(I), collagen αI(III), connective tissue growth factor (CTGF), and glycer-

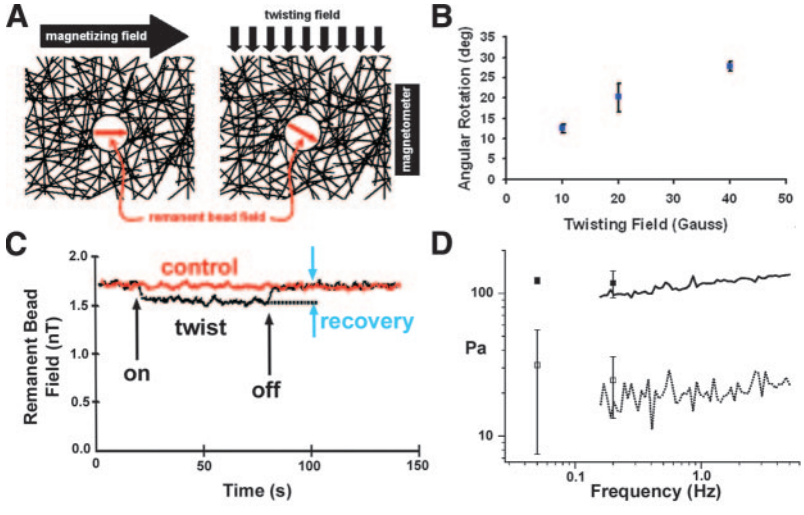

Figure 1. Validation of bead twisting for mechanical characterization of collagen type I gel. *A)* Schematic of magnetic twisting approach showing a matrix-embedded bead exposed to a magnetizing field, resulting in a remanent magnetic field associated with the bead. Exposure to a transverse magnetic field causes twisting of the bead, reorienting it toward the twisting field. The reorientation of the bead is detected by a magnetometer that measures the change in the remanent bead field transverse to the twisting field. *B)* The ensemble average angular rotation of beads (calculated from the change in bead remanent field) induced by varying strength twisting fields. *C)* The remanent magnetic field associated with beads during static twist and control experiments. *D)* Oscillatory bead twisting allows measurement of the matrix complex modulus at 0.05 and 0.2 Hz. The complex modulus is decomposed into storage (G', filled squares) and loss moduli (G", open squares). Macroscale rheometric characterization of collagen gel properties over 0.15–5 Hz showing similar results for G' (solid line) and G" (dashed line).

aldehyde phosphate dehydrogenase (GAPDH) were designed using Primer Express software (Applied Biosystems, Foster City, CA, USA) with similar melting point temperatures, primer lengths, and amplicon lengths in order to obtain similar PCR efficiency. Real-time PCR reactions used SYBR Green Master Mix (Bio-Rad, Hercules, CA, USA) to detect and quantify PCR products. The fold change in the expression of each gene in response to each treatment was calculated relative to untreated controls using the delta-delta Ct method with normalization to GAPDH (51).

Statistical analysis

Results from step twist experiments are presented as percent changes in apparent stiffness normalized to internal controls for each experiment. Results from cytokine stimulation dose- and time-response experiments were analyzed by Student's *t* test, comparing the cytokine-induced change in apparent stiffness at each time and dose to untreated time-matched controls. The modulation of TGF-β1 effects by other cytokines was analyzed by 1-way ANOVA with a Bonferroni correction applied for multiple comparisons. *P* values <0.05 were considered significant.

RESULTS

Magnetic bead twisting for mechanical characterization of collagen gels

To confirm that the beads embedded during the casting of collagen gels were firmly coupled to the matrix, we compared the rotation of beads during and after a step twist experiment. As shown in Fig. 1*C*, the remanent magnetic field associated with the beads, measured transverse to the twisting field, declines as the beads rotate toward alignment with the applied twisting field. Upon removal of the twisting field, the detected remanent field recovers to the level of no-twist controls (Fig. 1*C*), indicating that the beads rotate quickly back to their starting orientation. If the beads were not coupled to the matrix, some residual bead rotation would remain. These results demonstrate strong coupling of the beads to the collagen gel and a no-slip interaction at the bead-matrix interface.

Using the step twist microrheometry method, the apparent stiffness measured in a 20G twisting field averaged 38.7 ± 5.3 Pa in collagen gels ranging in volume from 40 to 100 μl (2 to 5 mm in thickness), with bead concentrations ranging from 20 to 100 mg/well (data not shown). Despite the relatively high mass concentration of beads in the constructs, the volume fraction of beads in the constructs was low (~0.005%). The apparent stiffness of the collagen gels measured here is within the range reported for the shear storage modulus of similar concentration collagen preparations (52). Moreover, the robustness of apparent stiffness measures reported above, which were obtained from gels over a range of different gel volumes and bead densities, alleviated concerns that the assay might be influenced by mechanical interactions between particles or between particles and the rigid walls of the wells.

Because biological materials frequently exhibit rate-dependent (viscoelastic) mechanical behavior, we also characterized the viscoelastic properties of the collagen gels using the same microrheometry system. Collagen preparations with embedded beads were exposed to an oscillatory twisting field at one of two frequencies (0.05 and 0.2 Hz) for 10 cycles, and the resulting complex modulus was decomposed into storage (G') and loss (G") moduli (48). While the measured loss modulus exhibited greater variability than did the storage modulus, both were independent of frequency within the limited range measured (Fig. 1*D*). The storage modulus averaged 121 ± 18 Pa, substantially higher than the apparent stiffness measured in static twist experiments. This difference likely reflects the fact that a finite viscous loss modulus (G") corresponds to a shear modulus that decreases slowly in time in the static twist measurement, and thus produces less apparent stiffness. However, when we exposed the same collagen preparations to static and oscillatory twist measurement techniques, the difference in measures of stiffness was much smaller, indicating that variability in collagen preparations also plays a role (data not shown).

To validate the microscale magnetic bead twisting approach, we compared it to a traditional macrorheometric method. Similar collagen preparations (1.5 mg/ml) were cast in a cone and plate rheometer and exposed to oscillatory shear in a stress-controlled mode over a wide frequency spectrum (53). Due to differences in the two different mechanical characterization methods, we were only able to directly compare the mechanical properties at a single frequency. Nevertheless, the magnitudes of the storage and loss moduli and their relative ratios were similar in the bead twisting and macrorheometry approaches (Fig. 1*D*), and both yielded results within the range of reported values for collagen preparations of similar concentration (52, 54). The highly sensitive macrorheometric technique was able to detect the subtle frequency-dependent increase in G' and G" (Fig. 1*D*), but as with the bead twisting approach, the macrorheometric technique appeared to display increasing variability in G" measurements with declining frequencies.

Measuring cell-mediated changes in matrix mechanics

To study fibroblast-collagen constructs in a relatively constant geometry for extended culture periods, we carried out fibrillogenesis inside short hollow tubes machined from porous polyethylene (**Fig. 2*A***). The intercalation of fibrils with the porous microstructure of the polyethylene prevented radial compaction of the constructs during the 2 wk of culture we studied. The constructs were left free to compact in the axial directions; this geometry facilitated microscopic visualization of the cells (and beads) during and after extended culture (Fig. 2*B*).

Over 7 days in culture, the cell-matrix constructs underwent cell-dependent changes in matrix storage modulus, as measured by the microscale bead twisting

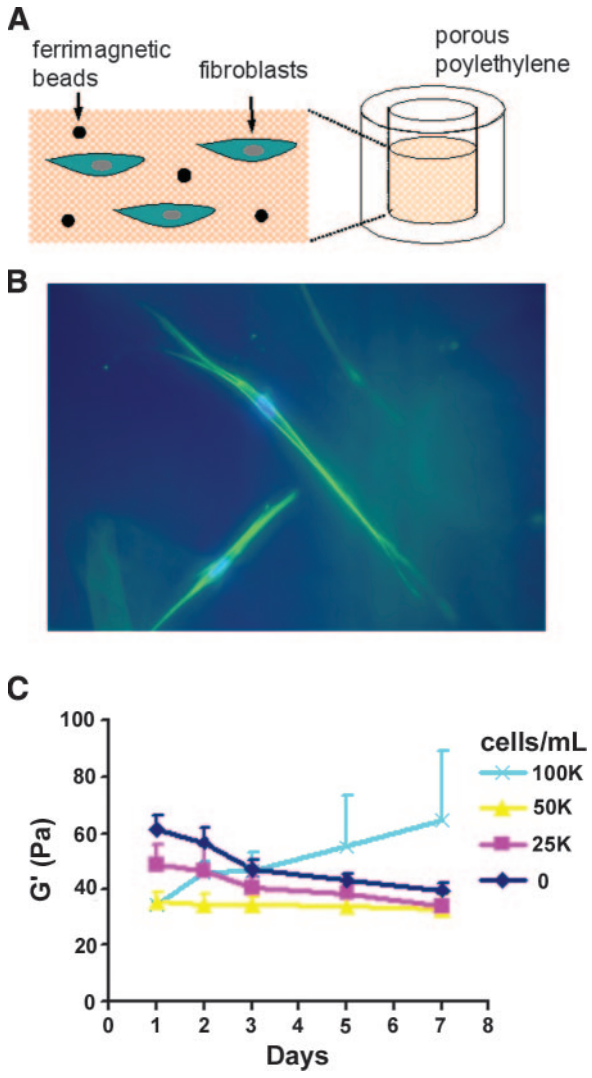

Figure 2. Measuring matrix mechanics in fibroblast-seeded collagen gels. *A)* Schematic of the bead-cell matrix constructs cast in a porous polyethylene cylinder to resist radial compaction of the matrix. *B)* Fibroblasts in collagen gel construct treated with TGF-β1 (20 ng/ml), fixed and stained at day 8 with Alexa 488 phalloidin (green) and DAPI (blue). *C)* Time- and cell density-dependent changes in storage modulus (G') of collagen matrix measured by microrheometry. Data are mean ± SE, n = 3–4.

assay in oscillatory mode (0.2 Hz frequency). In the absence of cells, the measured stiffness of the matrices underwent a gradual decline over 7 days (Fig. 2*C*). In the presence of human lung fibroblasts, the stiffness measured 1 day after seeding varied inversely with cell density. Over time, the cells increased the gel stiffness relative to no-cell controls, and attenuated or completely reversed the cell-free decline in stiffness (Fig. 2*C*). The strongest response was observed at the highest cell density (100,000 cells/ml). Although this cell density is at the low end of those used in other matrix remodeling assays (13, 20–23, 38, 55), higher cell densities sometimes led to detachment of gels from the porous polyethylene supports. Using a rough approximation for average cell volume of 1000 μm^3, the starting volume fraction of cells in the constructs was ~0.01% at the highest cell density used, highlighting the sparseness of the cells in the constructs. Keeping cell (and bead) densities low minimized potential bead-cell interactions and ensured that the mechanical measurements reflected matrix, not cellular, mechanical properties.

Cytokine-induced changes in matrix mechanics

We used the assay in step twisting mode to screen the effects of cytokines and growth factors on cell-mediated changes in matrix stiffness. While collagen gel mechanical properties were consistent within each preparation, they exhibited variability between preparations. Therefore, all experiments to measure cell-mediated changes in matrix stiffness were performed with suitable internal controls (cell-matrix constructs without exogenous soluble mediators) to normalize changes in matrix stiffness across experiments and conditions. We compared the effect of six different cytokines and growth factors implicated in fibrotic processes in the lung: TGF-β1, PDGF-BB, Et-1, IL-1β, IL-4, and IL-13 (3, 33, 36, 46, 47, 56–59). Each was administered to cell-matrix constructs 1 and 3 days after fibrillogenesis, and the apparent stiffness of cell-matrix constructs was measured at 4 days (**Fig. 3*A***). TGF-β1, the most widely studied profibrotic mediator, increased cell-matrix construct stiffness in a concentration-dependent fashion, reaching statistical significance at 50 ng/ml (P=0.048). IL-1β appeared to be the second most potent factor tested, but its effectiveness peaked at the intermediate concentration of 20 ng/ml; this effect did not reach statistical significance in this preliminary experiment. Contrary to expectations, PDGF-BB did not significantly enhance the stiffness of the cell-matrix constructs at any of the three concentrations. Et-1, IL-4, and IL-13 also exhibited statistically negligible effects on matrix stiffness at all three concentrations tested.

To confirm and extend these results, we measured time-dependent changes in cell-matrix construct stiffness at 2, 4, and 8 days for all cytokines and at 14 days for a subset, using the 20 ng/ml concentration for each soluble factor (Fig. 3*B*). This more extensive time course study, which used separate collagen preparations for each measurement group, produced consistent trends across and within treatments. As in the previous experiment, TGF-β1 and IL-1β exhibited the most potent enhancing effects, with statistically significant positive contributions to matrix stiffness for TGF-β1 at days 2 (P=0.043), 8 (P=0.004), and 14 (P=0.006), and IL-1β at days 4 (P=0.020) and 8 (P=0.017). Whereas PDGF-BB increased matrix stiffness at day 2 (P=0.0005), it decreased stiffness at days 4 (P=0.011), 8 (P=0.002), and 14 (P=0.035). The remaining cytokines exhibited more modest and variable effects on matrix stiffness, with statistically significant increases in matrix stiffness for Et-1 at day 2 (P=0.0007), IL-4 at day 8 (P=0.026), and IL-13 at day 2 (P=0.012).

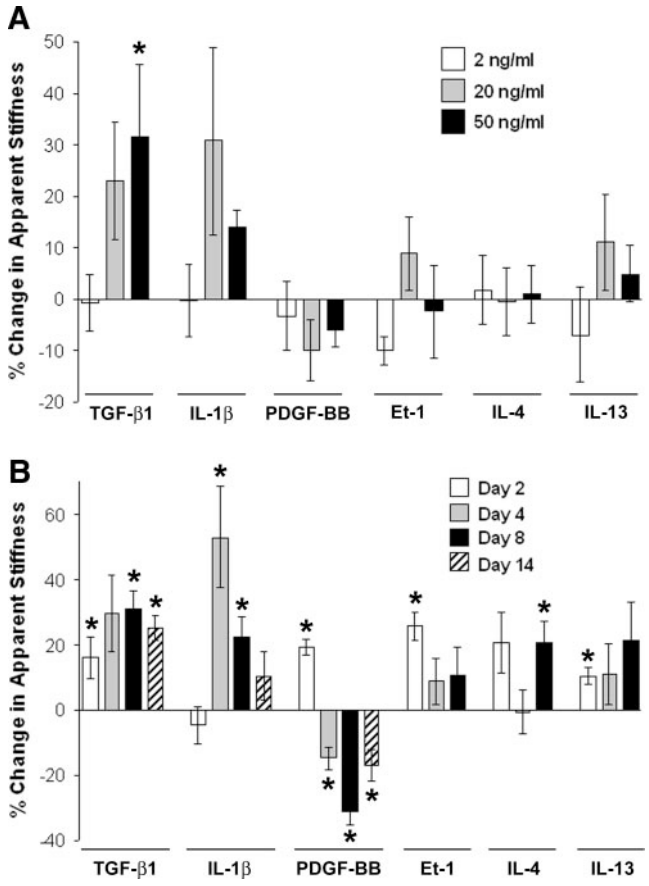

Figure 3. Screening cytokine and growth factor modulation of cell-matrix construct stiffness. *A)* Concentration-dependent effects of cytokines on apparent stiffness of matrix constructs measured by microrheometry 4 days after fibrillogenesis. *B)* Time-dependent changes in matrix apparent stiffness induced by cytokine and growth factor treatment at 20 ng/ml. In both panels data are mean ± SE, $n = 4–6$ for each group, $^*P < 0.05$ relative to no cytokine treatment.

Given the strong effect of TGF-β1 on cell-matrix construct stiffness and the frequency with which inflammatory and fibrotic processes develop together, we wondered whether any of the mediators, which by themselves had only modest effects, would augment or modulate TGF-β1's stiffening effects. At 8 days, Et-1 and IL-13 did not significantly modify the stiffening response of cell-matrix constructs to TGF-β1 (**Fig. 4*A***). In contrast, IL-1β did augment the TGF-β1-induced stiffening response ($P=0.029$, Fig. 4*A*). In separate experiments, the combination of TGF-β1 and IL-1β exhibited cooperative to synergistic effects on matrix stiffness at days 8 and 14 (Fig. 4*B*).

To begin the process of delineating how IL-1β and TGF-β1 could produce an augmented stiffening response, we tested for cooperative effects of these cytokines on the expression of four signature genes implicated in matrix remodeling processes: α-SMA, collagen types I and III, and CTGF. We treated cell-matrix constructs with 20 ng/ml IL-1β, TGF-β1, or both cytokines at 1 and 3 days after fibrillogenesis, then at 4 days collected and pooled mRNA from 8–10 constructs from each cytokine treatment group (and a time-

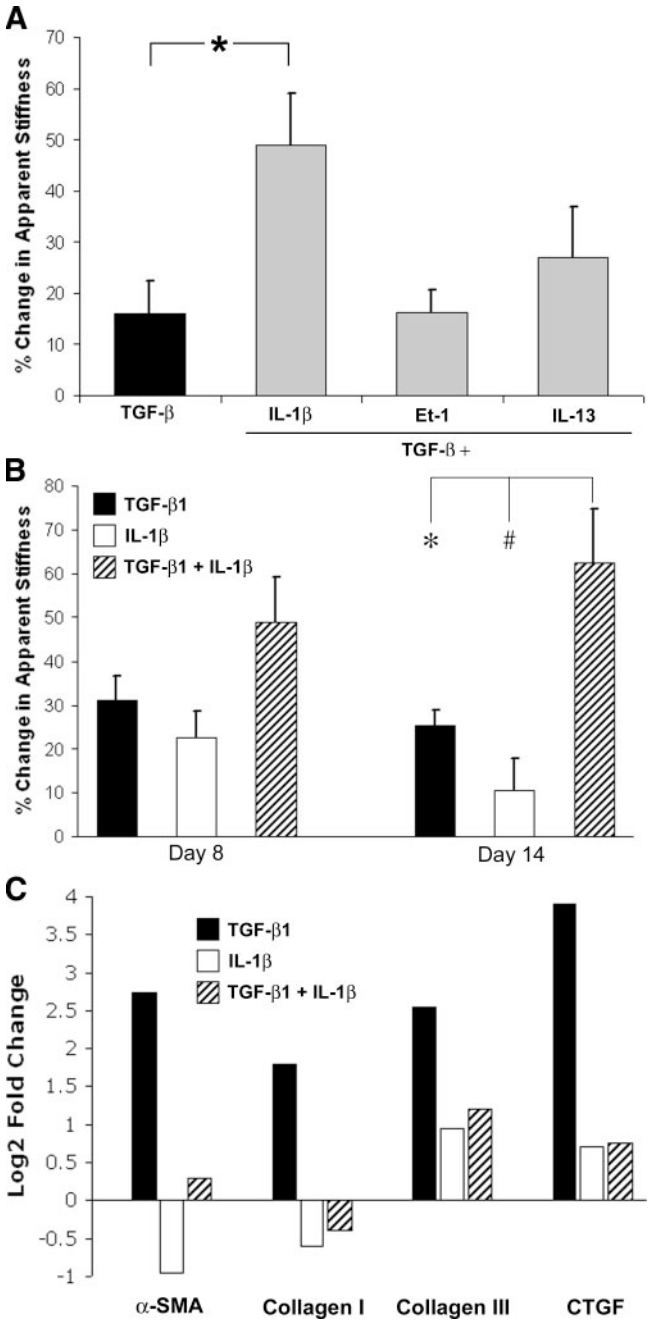

Figure 4. Augmentation of the TGF-β1 stiffening response by IL-1β. *A)* As measured at day 8, the stiffening response to TGF-β1 alone was unaffected by simultaneous treatment with Et-1 and IL-13 but was significantly enhanced by IL-1β ($^*P=0.029$). *B)* In separate experiments, at day 8 the combination of TGF-β1 and IL-1β induced a cooperative effect on matrix stiffness that was not statistically different from each alone, whereas at 14 days the combination of TGF-β1 and IL-1β induced greater stiffening of the matrix than either alone ($^*P=0.028$, $^{\#}P=0.003$). *A, B)* All cytokines at 20 ng/mL Data are mean ± SE, $n = 4–6$ for each group. *C)* Change in expression of matrix remodeling signature genes from 4 day constructs stimulated at 24 and 72 h by TGF-β1, IL-1β, or both. Cells from 8–10 constructs per group were pooled, their RNA extracted and gene expression was analyzed by quantitative real-time RT-PCR. Fold change in expression relative to unstimulated time-matched controls was calculated and the log2 transform is plotted.

matched control group). Treatment with TGF-β1 had the expected stimulatory effect on expression of all four remodeling signature genes by quantitative real-time PCR (Fig. 4*C*). In contrast, IL-1β had only a modest positive effect on collagen III and CTGF expression, and a modest negative effect on α-SMA and collagen I expression. None of the genes we examined exhibited augmented changes in expression when cells were simultaneously stimulated with both IL-1β and TGF-β1.

DISCUSSION

Ongoing efforts to visualize the architecture of extracellular matrices and model their mechanical behavior are providing new insight into the structural basis for matrix mechanics (9, 60–63). In contrast, parallel efforts to delineate the biological mechanisms and molecular pathways by which cells modulate matrix mechanics are limited by the dearth of tools optimized for the job. The assay described here is designed to address this need and accelerate systematic exploration of the stimuli and cellular programs that orchestrate resident cell modulation of matrix mechanics. The microscale bead twisting assay is shown here to efficiently measure collagen gel mechanical properties, extracting matrix stiffness measurements comparable to a traditional engineering approach (Fig. 1*D*). In contrast to traditional approaches, the microscale assay is well suited to follow cell-mediated changes in matrix mechanics over extended durations; due to the small sample volumes and short durations required for each measurement, the assay can be used to efficiently explore the effects of a variety of molecular interventions on the integrated functional outcome of matrix stiffness.

As a demonstration of the assay's strengths, we examined the effects of six cytokines over three concentrations and four different durations, and performed follow-up analyses of the interactions between a subset of the mediators. The cytokines we examined were chosen for their known or suspected association with tissue remodeling and fibrosis (3, 33). Though much is known about the biological effects of these cytokines, surprisingly little is known about their direct effects on matrix mechanical properties, a potentially crucial functional role that has been largely overlooked. Our results demonstrate early and sustained increases in matrix stiffness in response to TGF-β1, a more transient stiffening response to IL-1β, and a decrease in matrix stiffness in response to PDGF-BB (Fig. 3*B*). While the matrix stiffening response to TGF-β1 concurs with findings in cell-mediated strengthening of fibrin gels (17), the unexpected responses to PDGF-BB and IL-1β demonstrate the utility of the assay in uncovering new links between biological signaling and cellular modulation of matrix mechanics.

The responses to PDGF-BB and IL-1β were striking because they contradict the expected relationship between fibro-modulatory stimuli and changes in matrix stiffness. PDGF-BB is regarded as a profibrotic peptide in the lung; it induces collagen expression in cultured monolayer fibroblasts (33, 64), and inhibition of PDGF signaling attenuates pulmonary fibrosis (56). In our experimental system, the biological effects of PDGF-BB on human lung fibroblasts produced a decrease in the stiffness of collagen gels from 4 to 14 days. The decrease in stiffness caused by PDGF-BB may relate to the findings of Shreiber *et al.* (38), who observed that PDGF-BB decreases fibroblast traction forces while increasing migratory behavior in stressed collagen gels. These behaviors are opposite from the enhancement of traction forces by TGF-β1 reviewed by Tomasek *et al.* (2) and the increase in matrix stiffness that TGF-β1 produced in our system (Fig. 3*A*).

In contrast to PDGF-BB, the consensus view of IL-1β is more nuanced. IL-1β applied directly to lung fibroblasts can cause either proliferation (65) or apoptosis (36), but in general IL-1β is thought to decrease collagen synthesis by fibroblasts (33). These direct cellular responses to IL-1β should not be confused with systemic effects of IL-1β on inflammation and subsequent matrix remodeling (59). In our experimental system, IL-1β stimulated human lung fibroblasts to enhance matrix stiffness and augmented TGF-β1-induced stiffening. In contrast, neither Et-1 nor IL-13 significantly enhanced the TGF-β1 stiffening response, even though evidence exists for cooperative effects of these cytokine combinations in other scenarios (37, 40, 66). Although IL-1β and TGF-β1 have been shown to cooperate in stimulating production of fibronectin (42), prostaglandin E2 (35), and cyclooxygenase-2 (35, 39), they have opposite or antagonistic effects on other key fibroblast behaviors, including apoptosis, myofibroblast differentiation, and collagen synthesis (2, 36, 47, 58, 67, 68). Together the complex and unexpected stiffening responses to these cytokines and cytokine combinations reinforce the notion that effects on matrix mechanics cannot be predicted by extrapolating from known effects on cell signaling, proliferation, or matrix synthesis.

Our analysis of fibroblast gene expression further underscores the disconnect between biochemical and mechanical outcomes. While our analysis was limited to four matrix remodeling-associated genes, in no case did the relative change in gene expression induced by TGF-β1, IL-1β, or their combination parallel the relative change in matrix stiffness induced under identical conditions. The opposing effects of TGF-β1 and IL-1β on α-SMA and collagen I expression reported here are supported by similar findings from cytokine stimulation of traditional monolayer fibroblast cultures (36, 58, 67, 68).

The absence of correlation between matrix remodeling-associated gene expression and changes in matrix stiffness highlights the need for a comprehensive examination of the cellular, molecular, and microstructural processes by which cells and cytokines modulate matrix stiffness. A number of candidate processes could

be involved, including cellular proliferation, migration and traction force generation, matrix reorganization and cross-linking, and synthesis or destruction of matrix constituents. All of these processes likely contribute to changes in matrix stiffness, with their relative contributions evolving over time and depending on the cytokine(s) involved. The experimental tools to detect these processes are available or in development, and the theoretical models for assessing their contributions to matrix stiffness are improving rapidly (8, 9, 18, 53, 54, 60–62). A unique strength of the microrheometric approach developed here is identifying the biological stimuli, both expected and unexpected, that generate dramatic changes in matrix stiffness most suitable for these types of detailed analyses. In the case of IL-1β and its cooperative effect with TGF-β1, such a detailed approach seems warranted. Intriguingly, although many effects of TGF-β1 and IL-1β are antagonistic, both are known to stimulate fibroblast production of glycosaminoglycans (41, 44) which can alter the mechanical behavior of collagen gels (14, 15). Further analyses using this and other methods are needed to delineate the molecular and cellular processes that underlie cytokine-induced changes in matrix stiffness. The assay described here can play a role in these mechanistic studies by providing an efficient means to test molecular and genetic strategies targeting predicted regulators of matrix stiffness.

The approach we describe provides an efficient means to probe cellular modulation of matrix stiffness, but it is not without limitations. The experimental system of fibroblasts in a collagen gel, though widely used because it retains essential elements of cell-matrix 3-dimensional interactions, omits much of the structural and molecular complexity present *in situ*. Moreover, the concentration of cells and collagen in our system is low relative to most biological tissues, leading to microscale stiffness measurements that are lower than most macroscale measures of biological tissue stiffness (52). An open question thus remains as to whether the model system used here faithfully recapitulates the *in vivo* conversion of cellular signaling into matrix stiffness changes. Nevertheless, the multitude of cellular processes that must be integrated and coordinated to modulate tissue mechanics *in vivo* supports the use of simplified *in vitro* systems to help identify key candidate molecular and cellular programs.

Given the profound effects that extracellular matrix mechanical properties exert on tissue functionality and cellular behavior (2, 4–6, 13, 69), there is a great need to understand how cells actively modulate tissue mechanics. The assay described here provides a new approach that should facilitate systematic investigation in this critical area. When combined with imaging and theoretical and computational approaches, a better understanding of matrix mechanics and the cellular programs that modulate them will emerge. Together these approaches and the insights they provide will bolster attempts to engineer optimal replacement tissues and improve our understanding of how cellular control of tissue mechanics might be modulated to treat a variety of pathologies. FJ

We thank J. Cheng and E. Millet for instruction and invaluable assistance with the magnetic twisting device. Funding was provided by the National Institutes of Health (HL33009 and GM73628) and the American Lung Association.

REFERENCES

1. Hubbell, J. A. (2003) Materials as morphogenetic guides in tissue engineering. *Curr. Opin. Biotechnol.* **14,** 551–558
2. Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C., and Brown, R. A. (2002) Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* **3,** 349–363
3. Chapman, H. A. (2004) Disorders of lung matrix remodeling. *J. Clin. Invest.* **113,** 148–157
4. Discher, D. E., Janmey, P., and Wang, Y. L. (2005) Tissue cells feel and respond to the stiffness of their substrate. *Science* **310,** 1139–1143
5. Engler, A. J., Griffin, M. A., Sen, S., Bonnemann, C. G., Sweeney, H. L., and Discher, D. E. (2004) Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *J. Cell Biol.* **166,** 877–887
6. Paszek, M. J., Zahir, N., Johnson, K. R., Lakins, J. N., Rozenberg, G. I., Gefen, A., Reinhart-King, C. A., Margulies, S. S., Dembo, M., Boettiger, D., *et al.* (2005) Tensional homeostasis and the malignant phenotype. *Cancer Cell* **8,** 241–254
7. Engler, A. J., Sen, S., Sweeney, H. L., and Discher, D. E. (2006) Matrix elasticity directs stem cell lineage specification. *Cell* **126,** 677–689
8. Meshel, A. S., Wei, Q., Adelstein, R. S., and Sheetz, M. P. (2005) Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. *Nat. Cell Biol.* **7,** 157–164
9. Sawhney, R. K., and Howard, J. (2002) Slow local movements of collagen fibers by fibroblasts drive the rapid global self-organization of collagen gels. *J. Cell Biol.* **157,** 1083–1091
10. Grinnell, F. (2000) Fibroblast-collagen-matrix contraction: growth-factor signalling and mechanical loading. *Trends Cell Biol.* **10,** 362–365
11. Cukierman, E., Pankov, R., Stevens, D. R., and Yamada, K. M. (2001) Taking cell-matrix adhesions to the third dimension. *Science* **294,** 1708–1712
12. Lee, P. H., Trowbridge, J. M., Taylor, K. R., Morhenn, V. B., and Gallo, R. L. (2004) Dermatan sulfate proteoglycan and glycosaminoglycan synthesis is induced in fibroblasts by transfer to a three-dimensional extracellular environment. *J. Biol. Chem.* **279,** 48640–48646
13. Kessler, D., Dethlefsen, S., Haase, I., Plomann, M., Hirche, F., Krieg, T., and Eckes, B. (2001) Fibroblasts in mechanically stressed collagen lattices assume a "synthetic" phenotype. *J. Biol. Chem.* **276,** 36575–36585
14. Hsu, S., Jamieson, A. M., and Blackwell, J. (1994) Viscoelastic studies of extracellular matrix interactions in a model native collagen gel system. *Biorheology* **31,** 21–36
15. Zhu, W., Iatridis, J. C., Hlibczuk, V., Ratcliffe, A., and Mow, V. C. (1996) Determination of collagen-proteoglycan interactions in vitro. *J. Biomech.* **29,** 773–783
16. Knapp, D. M., Barocas, V. H., Moon, A. G., Yoo, K., Petzold, L. R., and Tranquillo, R. T. (1997) Rheology of reconstituted type I collagen gel in confined compression. *J. Rheology* **41,** 971–993
17. Neidert, M. R., Lee, E. S., Oegema, T. R., and Tranquillo, R. T. (2002) Enhanced fibrin remodeling in vitro with TGF-beta1, insulin and plasmin for improved tissue-equivalents. *Biomaterials* **23,** 3717–3731
18. Roeder, B. A., Kokini, K., Sturgis, J. E., Robinson, J. P., and Voytik-Harbin, S. L. (2002) Tensile mechanical properties of three-dimensional type I collagen extracellular matrices with varied microstructure. *J. Biomech. Eng.* **124,** 214–222
19. Girton, T. S., Oegema, T. R., Grassl, E. D., Isenberg, B. C., and Tranquillo, R. T. (2000) Mechanisms of stiffening and strength-

ening in media-equivalents fabricated using glycation. *J. Biomech. Eng.* **122,** 216–223
20. Guidry, C., and Grinnell, F. (1985) Studies on the mechanism of hydrated collagen gel reorganization by human skin fibroblasts. *J. Cell Sci.* **79,** 67–81
21. Nakagawa, S., Pawelek, P., and Grinnell, F. (1989) Long-term culture of fibroblasts in contracted collagen gels: effects on cell growth and biosynthetic activity. *J. Invest. Dermatol.* **93,** 792–798
22. Ogle, B. M., and Mooradian, D. L. (2002) Manipulation of remodeling pathways to enhance the mechanical properties of a tissue engineered blood vessel. *J. Biomech. Eng.* **124,** 724–733
23. Wakatsuki, T., Kolodney, M. S., Zahalak, G. I., and Elson, E. L. (2000) Cell mechanics studied by a reconstituted model tissue. *Biophys. J.* **79,** 2353–2368
24. Bausch, A. R., Moller, W., and Sackmann, E. (1999) Measurement of local viscoelasticity and forces in living cells by magnetic tweezers. *Biophys. J.* **76,** 573–579
25. Crocker, J. C., Valentine, M. T., Weeks, E. R., Gisler, T., Kaplan, P. D., Yodh, A. G., and Weitz, D. A. (2000) Two-point microrheology of inhomogeneous soft materials. *Phys. Rev. Lett.* **85,** 888–891
26. Tseng, Y., Kole, T. P., and Wirtz, D. (2002) Micromechanical mapping of live cells by multiple-particle-tracking microrheology. *Biophys. J.* **83,** 3162–3176
27. Yamada, S., Wirtz, D., and Kuo, S. C. (2000) Mechanics of living cells measured by laser tracking microrheology. *Biophys. J.* **78,** 1736–1747
28. Wang, N., Butler, J. P., and Ingber, D. E. (1993) Mechanotransduction across the cell surface and through the cytoskeleton. *Science* **260,** 1124–1127
29. Velegol, D., and Lanni, F. (2001) Cell traction forces on soft biomaterials. I. Microrheology of type I collagen gels. *Biophys. J.* **81,** 1786–1792
30. Valberg, P. A., and Feldman, H. A. (1987) Magnetic particle motions within living cells. Measurement of cytoplasmic viscosity and motile activity. *Biophys. J.* **52,** 551–561
31. Fabry, B., Maksym, G. N., Butler, J. P., Glogauer, M., Navajas, D., and Fredberg, J. J. (2001) Scaling the microrheology of living cells. *Phys. Rev. Lett.* **87,** 148102
32. Valberg, P. A., and Butler, J. P. (1987) Magnetic particle motions within living cells. Physical theory and techniques. *Biophys. J.* **52,** 537–550
33. Atamas, S. P. (2002) Complex cytokine regulation of tissue fibrosis. *Life. Sci.* **72,** 631–643
34. Glogauer, M., Ferrier, J., and McCulloch, C. A. (1995) Magnetic fields applied to collagen-coated ferric oxide beads induce stretch-activated Ca^{2+} flux in fibroblasts. *Am. J. Physiol.* **269,** C1093–C1104
35. Diaz, A., Chepenik, K. P., Korn, J. H., Reginato, A. M., and Jimenez, S. A. (1998) Differential regulation of cyclooxygenases 1 and 2 by interleukin-1 beta, tumor necrosis factor-alpha, and transforming growth factor-beta 1 in human lung fibroblasts. *Exp. Cell Res.* **241,** 222–229
36. Zhang, H. Y., Gharaee-Kermani, M., and Phan, S. H. (1997) Regulation of lung fibroblast alpha-smooth muscle actin expression, contractile phenotype, and apoptosis by IL-1beta. *J. Immunol.* **158,** 1392–1399
37. Tschumperlin, D. J., Shively, J. D., Kikuchi, T., and Drazen, J. M. (2003) Mechanical stress triggers selective release of fibrotic mediators from bronchial epithelium. *Am. J. Respir. Cell Mol. Biol.* **28,** 142–149
38. Shreiber, D. I., Enever, P. A., and Tranquillo, R. T. (2001) Effects of pdgf-bb on rat dermal fibroblast behavior in mechanically stressed and unstressed collagen and fibrin gels. *Exp. Cell Res.* **266,** 155–166
39. Roy, R., Polgar, P., Wang, Y., Goldstein, R. H., Taylor, L., and Kagan, H. M. (1996) Regulation of lysyl oxidase and cyclooxygenase expression in human lung fibroblasts: interactions among TGF-beta, IL-1 beta, and prostaglandin E. *J. Cell Biochem.* **62,** 411–417
40. Dube, J., Chakir, J., Dube, C., Grimard, Y., Laviolette, M., and Boulet, L. P. (2000) Synergistic action of endothelin (ET)-1 on the activation of bronchial fibroblast isolated from normal and asthmatic subjects. *Int. J. Exp. Pathol.* **81,** 429–437
41. Korducki, J. M., Loftus, S. J., and Bahn, R. S. (1992) Stimulation of glycosaminoglycan production in cultured human retroocular fibroblasts. *Invest. Ophthalmol. Vis. Sci.* **33,** 2037–2042
42. Pawluczyk, I. Z., and Harris, K. P. (1998) Cytokine interactions promote synergistic fibronectin accumulation by mesangial cells. *Kidney Int.* **54,** 62–70
43. Jinnin, M., Ihn, H., Yamane, K., and Tamaki, K. (2004) Interleukin-13 stimulates the transcription of the human alpha2(I) collagen gene in human dermal fibroblasts. *J. Biol. Chem.* **279,** 41783–41791
44. Imai, Y., Ibaraki, K., Odajima, R., and Shishiba, Y. (1994) Analysis of proteoglycan synthesis by retro-ocular tissue fibroblasts under the influence of interleukin 1 beta and transforming growth factor-beta. *Eur. J. Endocrinol.* **131,** 630–638
45. Doucet, C., Brouty-Boye, D., Pottin-Clemenceau, C., Canonica, G. W., Jasmin, C., and Azzarone, B. (1998) Interleukin (IL) 4 and IL-13 act on human lung fibroblasts. Implication in asthma. *J. Clin. Invest* **101,** 2129–2139
46. Hashimoto, S., Gon, Y., Takeshita, I., Maruoka, S., and Horie, T. (2001) IL-4 and IL-13 induce myofibroblastic phenotype of human lung fibroblasts through c-Jun NH2-terminal kinase-dependent pathway. *J. Allergy Clin. Immunol.* **107,** 1001–1008
47. Hashimoto, S., Gon, Y., Takeshita, I., Matsumoto, K., Maruoka, S., and Horie, T. (2001) Transforming growth Factor-beta1 induces phenotypic modulation of human lung fibroblasts to myofibroblast through a c-Jun-NH2-terminal kinase-dependent pathway. *Am. J. Respir. Crit. Care Med.* **163,** 152–157
48. Maksym, G. N., Fabry, B., Butler, J. P., Navajas, D., Tschumperlin, D. J., Laporte, J. D., and Fredberg, J. J. (2000) Mechanical properties of cultured human airway smooth muscle cells from 0.05 to 0.4 Hz. *J. Appl. Physiol.* **89,** 1619–1632
49. Wang, N., and Ingber, D. E. (1994) Control of cytoskeletal mechanics by extracellular matrix, cell shape, and mechanical tension. *Biophys. J.* **66,** 2181–2189
50. Mijailovich, S. M., Kojic, M., Zivkovic, M., Fabry, B., and Fredberg, J. J. (2002) A finite element model of cell deformation during magnetic bead twisting. *J. Appl. Physiol.* **93,** 1429–1436
51. Livak, K. J., and Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. *Methods* **25,** 402–408
52. Pedersen, J. A., and Swartz, M. A. (2005) Mechanobiology in the third dimension. *Ann. Biomed. Eng.* **33,** 1469–1490
53. Kaufman, L. J., Brangwynne, C. P., Kasza, K. E., Filippidi, E., Gordon, V. D., Deisboeck, T. S., and Weitz, D. A. (2005) Glioma expansion in collagen I matrices: analyzing collagen concentration-dependent growth and motility patterns. *Biophys. J.* **89,** 635–650
54. Storm, C., Pastore, J. J., MacKintosh, F. C., Lubensky, T. C., and Janmey, P. A. (2005) Nonlinear elasticity in biological gels. *Nature* **435,** 191–194
55. Grinnell, F., Ho, C. H., Lin, Y. C., and Skuta, G. (1999) Differences in the regulation of fibroblast contraction of floating versus stressed collagen matrices. *J. Biol. Chem.* **274,** 918–923
56. Abdollahi, A., Li, M., Ping, G., Plathow, C., Domhan, S., Kiessling, F., Lee, L. B., McMahon, G., Grone, H. J., Lipson, K. E., and Huber, P. E. (2005) Inhibition of platelet-derived growth factor signaling attenuates pulmonary fibrosis. *J. Exp. Med.* **201,** 925–935
57. Atamas, S. P., and White, B. (2003) Cytokine regulation of pulmonary fibrosis in scleroderma. *Cytokine Growth Factor Rev.* **14,** 537–550
58. Zhang, H. Y., and Phan, S. H. (1999) Inhibition of myofibroblast apoptosis by transforming growth factor beta(1). *Am. J. Respir. Cell Mol. Biol.* **21,** 658–665
59. Kolb, M., Margetts, P. J., Anthony, D. C., Pitossi, F., and Gauldie, J. (2001) Transient expression of IL-1beta induces acute lung injury and chronic repair leading to pulmonary fibrosis. *J. Clin. Invest* **107,** 1529–1536
60. Agoram, B., and Barocas, V. H. (2001) Coupled macroscopic and microscopic scale modeling of fibrillar tissues and tissue equivalents. *J. Biomech. Eng.* **123,** 362–369
61. Chandran, P. L., and Barocas, V. H. (2004) Microstructural mechanics of collagen gels in confined compression: poroelasticity, viscoelasticity, and collapse. *J. Biomech. Eng.* **126,** 152–166
62. Chandran, P. L., and Barocas, V. H. (2006) Affine versus non-affine fibril kinematics in collagen networks: theoretical studies of network behavior. *J. Biomech. Eng.* **128,** 259–270
63. Pizzo, A. M., Kokini, K., Vaughn, L. C., Waisner, B. Z., and Voytik-Harbin, S. L. (2005) Extracellular matrix (ECM) micro-

structural composition regulates local cell-ECM biomechanics and fundamental fibroblast behavior: a multidimensional perspective. *J. Appl. Physiol.* **98,** 1909–1921
64. Lewis, C. C., Chu, H. W., Westcott, J. Y., Tucker, A., Langmack, E. L., Sutherland, E. R., and Kraft, M. (2005) Airway fibroblasts exhibit a synthetic phenotype in severe asthma. *J. Allergy Clin. Immunol.* **115,** 534–540
65. Ingram, J. L., Rice, A. B., Geisenhoffer, K., Madtes, D. K., and Bonner, J. C. (2004) IL-13 and IL-1beta promote lung fibroblast growth through coordinated up-regulation of PDGF-AA and PDGF-Ralpha. *FASEB J.* **18,** 1132–1134
66. Zhou, X., Trudeau, J. B., Schoonover, K. J., Lundin, J. I., Barnes, S. M., Cundall, M. J., and Wenzel, S. E. (2005) Interleukin-13 augments transforming growth factor-beta1-induced tissue inhibitor of metalloproteinase-1 expression in primary human airway fibroblasts. *Am. J. Physiol.* **288,** C435–C442
67. Goldring, M. B., and Krane, S. M. (1987) Modulation by recombinant interleukin 1 of synthesis of types I and III collagens and associated procollagen mRNA levels in cultured human cells. *J. Biol. Chem.* **262,** 16724–16729
68. Qi, J., Chi, L., Maloney, M., Yang, X., Bynum, D., and Banes, A. J. (2006) Interleukin-1beta increases elasticity of human bioartificial tendons. *Tissue Eng.* **12,** 2913–2925
69. Zaman, M. H., Trapani, L. M., Siemeski, A., Mackellar, D., Gong, H., Kamm, R. D., Wells, A., Lauffenburger, D. A., and Matsudaira, P. (2006) Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. *Proc. Natl. Acad. Sci. U. S. A.* **103,** 10889–10894

Received for publication October 20, 2006.
Accepted for publication January 25, 2007.