Biochemical and Biophysical Research Communications 430 (2013) 1028–1033

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Rheological responses of cardiac fibroblasts to mechanical stretch

Min Ye Shen, Jarett Michaelson, Hayden Huang *

351 Engineering Terrace, 500 W 120th Street, Columbia University, New York, NY 10027, USA

ARTICLE INFO

Article history:
Received 6 December 2012
Available online 19 December 2012

Keywords:
Microrheology
Complex modulus
Mechanical stretch
Fibroblast
Actin

ABSTRACT

Rheological characterization of cells using passive particle tracking techniques can yield substantial information regarding local cellular material properties. However, limited work has been done to establish the changes in material properties of mechanically-responsive cells that experience external stimuli. In this study, cardiac fibroblasts plated on either fibronectin or collagen were treated with cytochalasin, mechanically stretched, or both, and their trajectories and complex moduli were extracted. Results demonstrate that both solid and fluid components were altered by such treatments in a receptor-dependent manner, and that, interestingly, cells treated with cytochalasin were still capable of stiffening in response to mechanical stimuli despite gross stress fiber disruption. These results suggest that the material properties of cells are dependent on a variety of environmental cues and can provide insight into physiological and disease processes.

1. Introduction

Characterizing the physical properties of biological cells is crucial for understanding and modeling cellular and tissue response to mechanical stimuli. Since cells can be considered glass-like materials, rheological analysis characterizes cells based on their fluid- and solid-like characteristics. Most work on rheological characterization of cells uses external probes, such as AFM [1–3], magnetic twisting [4–6] or micromanipulation [7–11], or optical stretching [12,13]. These techniques have various advantages, but the results can be overrepresentative of the cortical cytoskeleton and cell membrane and not of the cell interior. These methods also suffer the disadvantage that interpretation of mechanoresponses can be ambiguous because these techniques invariably impose mechanical stresses to the cells to measure their deformation.

Particle-tracking microrheology (PTM) is a technique developed to characterize mechanical properties of cell interiors, based on tracking small objects, such as beads. PTM yields the bead mean-squared displacement (MSD) over a range of lag times, and by fitting an appropriate model to the MSD, the complex modulus can be determined [14–20]. PTM is advantageous because after the beads are introduced, there are no further mechanical stimuli necessary for readout; thus this technique is ideally suited for examining the changes in the properties of living cells in response to mechanical stimuli.

PTM has been used in a variety of cell studies, including characterization of nuclear connections by the cytoskeleton [21], determination of prestress development [22], measurements of responses to cell–cell adhesion [23], characterization of 3D-matrix-embedded cells [14], and distinguishing primary from stem cells [24]. However, despite the importance of cell properties in response to physical forces, few studies have used PTM to characterize such responses. One study determined that cytoplasmic stiffening occurs in response to fluid shear in 3T3 cells [25], and another study established that alveolar epithelial cells exhibit diminished stiffness when stretched [26]. Many stretch sensitive cells, such as cardiac fibroblasts, have not been well-examined using PTM. While the molecular mechanotransduction of these cells is more extensively studied [27–29], the physical properties of the cells themselves are still not well-characterized. Cardiac fibroblast mechanotransduction is crucial for regulating heart properties, especially in remodeling in response to cardiovascular diseases. Thus, it is essential to understand how cell properties may be influenced by external perturbations.

Because actin is essential for regulating cellular rheological properties, we hypothesize that cardiac fibroblasts would exhibit diminished MSD in response to mechanical stretch application. Further, increases in MSD would accompany cytochalasin treatment due to disruption of the actin network. Finally, combining cytochalasin and stretch would be materially similar to cytochalasin treatment alone, given that a good deal of mechanoresponse is governed by intact actin networks. In this study, we quantitatively characterize rheological responses in primary cardiac fibroblasts to test these hypotheses and further determine whether there is receptor-dependence on such responses, since cardiac fibroblasts engage more than one type of adhesion molecule *in vivo*.

* Corresponding author.
E-mail address: hayden.huang@columbia.edu (H. Huang).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.12.044

2. Methods

2.1. Cell culture

Cardiac fibroblasts were isolated from neonatal rat pups and maintained in high glucose Dulbecco's Modified Eagle's Medium (Sigma, St. Louis, MO) supplemented with 10% FBS and penicillin/streptomycin. Fibroblasts were maintained at 37 °C in a 5% CO_2 environment. Cells were plated on either plastic cell culture dishes, or flexible silicone membranes, the latter coated with 5 ug/ml fibronectin (Life Technologies, Grand Island, NY) or 0.2 mg/ml collagen (Sigma). Cells were assayed at 70–80% confluence, one to two days post plating. All animal work was done with the approval of the Institute of Comparative Medicine.

Actin was disrupted using cytochalasin D (cytD, Life Technologies) at 1 μM for an hour. The results from cytD disruption were compared to a DMSO-treated vehicle control.

2.2. Bead introduction

0.5 μm diameter fluorescent carboxylate-modified microspheres (Life Technologies) were ballistically injected into cells following the Helios Gene Gun system protocol (Bio-Rad, Hercules, CA). Cartridges coated with beads were inserted into the Gene Gun system and pressurized helium was used to propel the beads at the cells. The pressure was chosen such that beads become embedded within the cells while preserving viability (~250 psi). Cells were then washed with HBSS and left to recover before imaging. For membrane experiments, cells were trypsinized and replated on flexible silicone membranes after recovery.

2.3. Actin stain

Cells were fixed with a 4% paraformaldehyde solution and permeabilized with 0.1% Triton-X-100. Actin was stained using Alexa Fluor 488 phalloidin (Life Technologies) based on manufacturer's protocol. Images were acquired under identical imaging conditions and were brightness/contrast enhanced together for clarity.

2.4. Cell stretch

A custom-built stretch device was used to stretch cells plated on silicone membranes at a rate of 1 Hz with a 5% applied uniform biaxial strain, inside the cell culture incubator. Cardiac fibroblasts were stretched for either one or 24 h, after which the cells were immediately assayed. For both stretch and actin disruption, cells were stretched and dosed with cytD for an hour for short-term experiments, and stretched for 23 h and then stretched and dosed with cytD for the last hour for long-term experiments.

2.5. Microrheological analysis

The beads within cells were imaged using an inverted fluorescence microscope (Olympus, Center Valley, PA) using a 40× NA 0.6 objective, and videos of the cells were taken at a frame rate of 15 frames/s for 1000 frames. The beads were tracked using a custom MATLAB script. The trajectory was used to calculate the mean squared displacement (MSD) using Eq. (1).

$$\mathrm{MSD} = \langle \Delta r^2(\tau) \rangle = \langle (r(t+\tau) - r(t))^2 \rangle \quad (1)$$

Noise resulting from stage shift during video acquisition was attenuated by subtracting average probe movement throughout the image sequence. To eliminate vibration-induced noise, MSDs were fit to a second-degree curve. This noise was generally not significant, as the R^2 of the fit exceeded 0.98 in all cases. These smoothing algorithms eliminated small fluctuations that occasionally resulted in a negative MSD-versus-lag time slope. At frequencies where G' or G'' was non-physical, the solid or loss modulus was taken to be zero, respectively.

To compare the complex moduli, the MSD standard errors were processed through the GSER model [16,30]. Plots showing MSD depict error bars showing standard errors associated with MSD. G' and G'' results are presented at 1 Hz as the mean plus a range: that is, as G' (G'_{low}, G'_{high}) and G'' (G''_{low}, G''_{high}) corresponding to the range based on the MSD standard errors. Statistical testing was done via t-test on the MSDs at 1 Hz.

3. Results

3.1. Effects of receptor-specific actin disruption

Incubating fibroblasts plated on cell-culture dishes in 1 μM cytochalasin D for an hour resulted in a higher bead MSD compared to that for cells incubated in DMSO, at all frequencies ($p < 0.0001$ at 1 Hz, Fig. 1A). G' was zero at all analyzed frequencies for cytD-treated cells, but was 0.1 Pa (0.04, 0.13) for the DMSO-treated cells (at 1 Hz), indicating that actin disruption leads to a decrease in solid-like behavior of the cells. However, cell viscous properties was also altered by cytD treatment; G'' decreased from 6.4 Pa (5.6, 7.5) to 1.3 Pa (1.2, 1.4) with actin disruption.

Because we wanted to assess the stretch response of these cells, we also plated fibroblasts on flexible silicone membranes and treated them with DMSO and cytD. With flexible silicone membranes, we engaged primarily collagen or fibronectin receptors via membrane coating. Thus, these experiments provide an opportunity to assess receptor-dependent changes in cell properties and responses to actin disruption.

With DMSO treatment, we found that collagen-plated cells exhibited higher MSDs at all frequencies, compared to fibronectin-coated cells ($p < 0.0001$ at 1 Hz, Fig. 1B). Both collagen- and fibronectin-plated cells exhibited low G', with $G' = 0$ for fibronectin-plated cells and 0.2 Pa (0.2, 0.3) for collagen-plated cells. These values are comparable to G' for cells plated in the plastic dishes. When fibronectin-plated cells were treated with cytD, their MSDs increased across all frequencies ($p < 0.0005$ at 1 Hz). However, collagen-plated cells that were treated with cytD exhibited mild changes in MSD mostly at the lower time lags, where the MSD dropped, and were mostly unchanged at moderate and higher time lags ($p > 0.05$ at 1 Hz). For both adhesion molecules, cell G' was zero with cytD treatment. For collagen-plated cells, the range of G'' shifted slightly from 3.3 Pa (3.1, 3.6) to 3.3 Pa (2.9, 3.7), indicating little change in viscous properties. On fibronectin, however, cell G'' dropped from 7.0 Pa (6.3, 7.8) to 1.2 Pa (1.0, 1.6) with cytD treatment.

These data demonstrate that rheological properties of cells exhibit receptor-dependencies, and further, that the response of cells to actin disruption also depends on the receptors being engaged. It appears that engaging collagen renders the fibroblasts somewhat insensitive to cytD treatment. These data also show that both solid and fluid components are affected by cytD treatment.

3.2. Cellular response to mechanical stretching

The fluid dominance of cardiac fibroblasts observed thus far may result from the absence of mechanical stimuli these cells experience *in vivo*. In hearts, cardiac fibroblasts are under persistent stretch and thus the static *in vitro* characterization of these cells may not be representative of their *in vivo* state. To determine if cellular properties change in response to mechanical stretch,

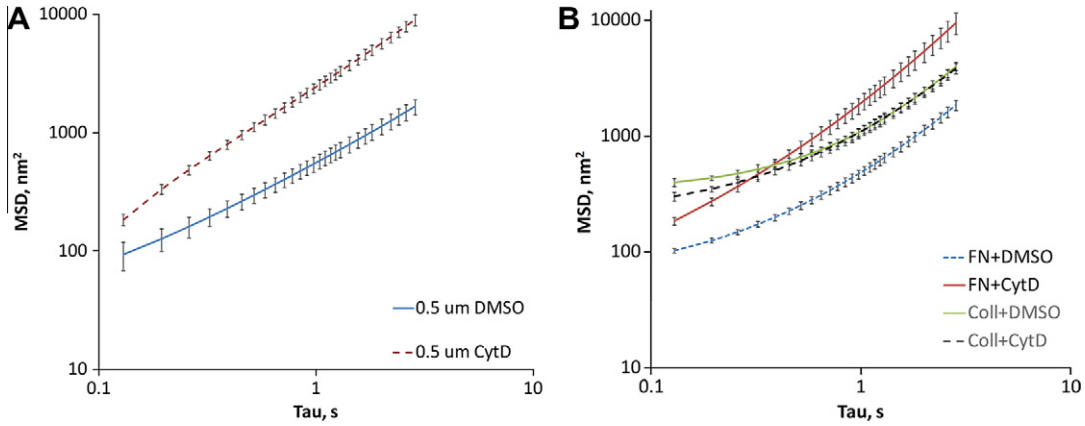

Fig. 1. (A) Cardiac fibroblasts plated on plastic cell culture dishes exhibit a significant increase in MSD under cytochalasin D (cytD) treatment. (B) Cells plated on fibronectin-coated (FN) silicone membranes exhibit significant increases in bead MSD upon cytD treatment. However, cells plated on collagen-coated (Coll) membranes exhibited more subtle changes in bead MSD. These results suggest that cell rheology is receptor-dependent.

cardiac fibroblasts were plated on both fibronectin and collagen coated membranes and stretched at 5% strain for one hour (Fig. 2A). For fibronectin-plated cells, MSD did not change significantly with 1 h of stretch ($p > 0.05$ at 1 Hz). G' was zero both at baseline and after one hour of stretch. G'' shifted from 7.0 Pa (6.3, 7.8) to 7.2 Pa (5.6, 10.0). Collagen-plated cells, however, exhibited more dramatic changes in response to stretch, with a large shift in MSD ($p < 0.0001$ at 1 Hz), accompanied by an increase in G' from 0.2 Pa (0.2, 0.3) to 2.3 Pa (1.9, 2.8). G'' also increased from 3.3 Pa (3.1, 3.6) to 7.8 Pa (7.1, 8.7). Thus, cell material mechanoresponse also exhibits strong receptor dependency.

We next stretched cells for 24 h, to assess whether alterations in material properties would continue to develop. Collagen-plated cells rounded up and were mostly unusable for rheological analysis. Fibronectin-plated cells exhibit significant decreases in MSD with 24 h of stretch ($p < 0.0001$ at 1 Hz, Fig. 2B). Consistent with this change in MSD, G' increased from 0 to 6.6 Pa (6, 7.3) with 24 h of stretch; G'' increased from 7 Pa (6.3, 7.8) to 15.6 Pa (14.5, 16.8). These results show that for fibronectin-plated cells, increased stretch duration leads to an increase in both solid and loss moduli.

3.3. Actin-disrupted response to mechanical stretching

Because the actin cytoskeleton is a crucial part of both the cell's material properties and its capacity of response to mechanical stimuli, disruption of actin using cytD can be expected to attenuate the observed increase in G^* in response to applied stretch. It is thus a reasonable hypothesis that if cells were treated with cytD concurrently with a single hour of stretch, that the cells would appear similar to cells treated with cytD without stretch. Further, if cells were stretched for 24 h and dosed with cytD for the last hour of stretch (from hours 23 to 24), that much of the mechanically-induced reinforcement of the cells would be lost, resulting in cells that were similar to unstretched cells or to cytD-treated cells.

To assess the effect of stretch and/or cytD treatment on stress fiber organization, we performed actin fluorescence labeling using phalloidin (Fig. 3). Regardless of membrane coating adhesion molecule, stretching the cells for either one or 24 h (the latter for fibronectin-plated cells only) led to reinforcement of actin, with slightly elevated intensities and increased alignment. Although there was no preferred fiber direction among different cells, each cell exhibited more uniform fiber alignment. Disruption with cytD resulted in global loss of fibers and the presence of focal deposits. Cells that were treated with cytD and stretched did not show any recovery of the stress fibers. These results indicate that cells do not exhibit large-scale stress fiber recovery, even with stretch, lending support to our hypothesis.

We next performed microrheological characterization of the same conditions. Collagen-plated cells treated with cytD concurrently with one hour of stretch exhibited an MSD similar to that of cells that were stretched (with DMSO), with some frequency-dependent differences ($p > 0.05$ at 1 Hz, Fig. 4A). Compared to unstretched, cytD-treated collagen-plated cells, G' was unchanged at

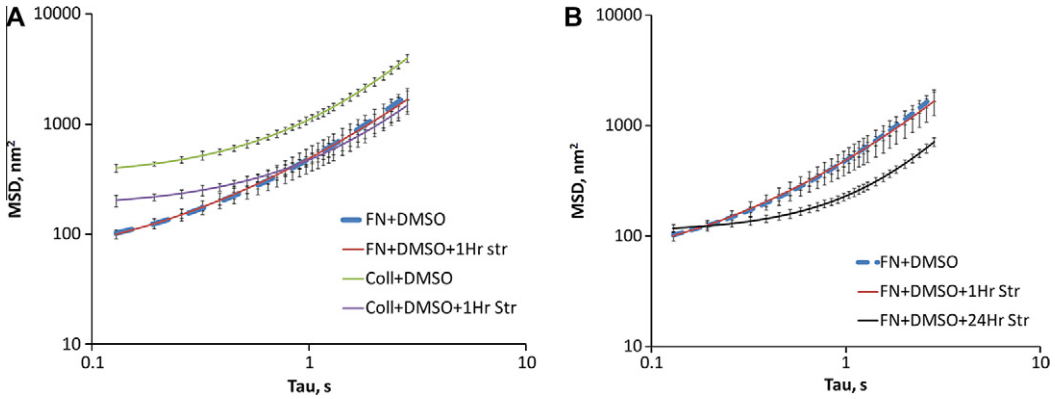

Fig. 2. (A) Stretching cardiac fibroblasts for one hour on collagen coated membranes results in a consistent decrease in bead MSD. However, for fibronectin-plated cells, there was no obvious change in MSD with the application of one hour of stretch. (B) Stretching cardiac fibroblasts for 24 h on fibronectin-coated membranes results in a significant frequency-dependent decrease in bead MSD, compared to either unstretched or 1 h-stretched cells.

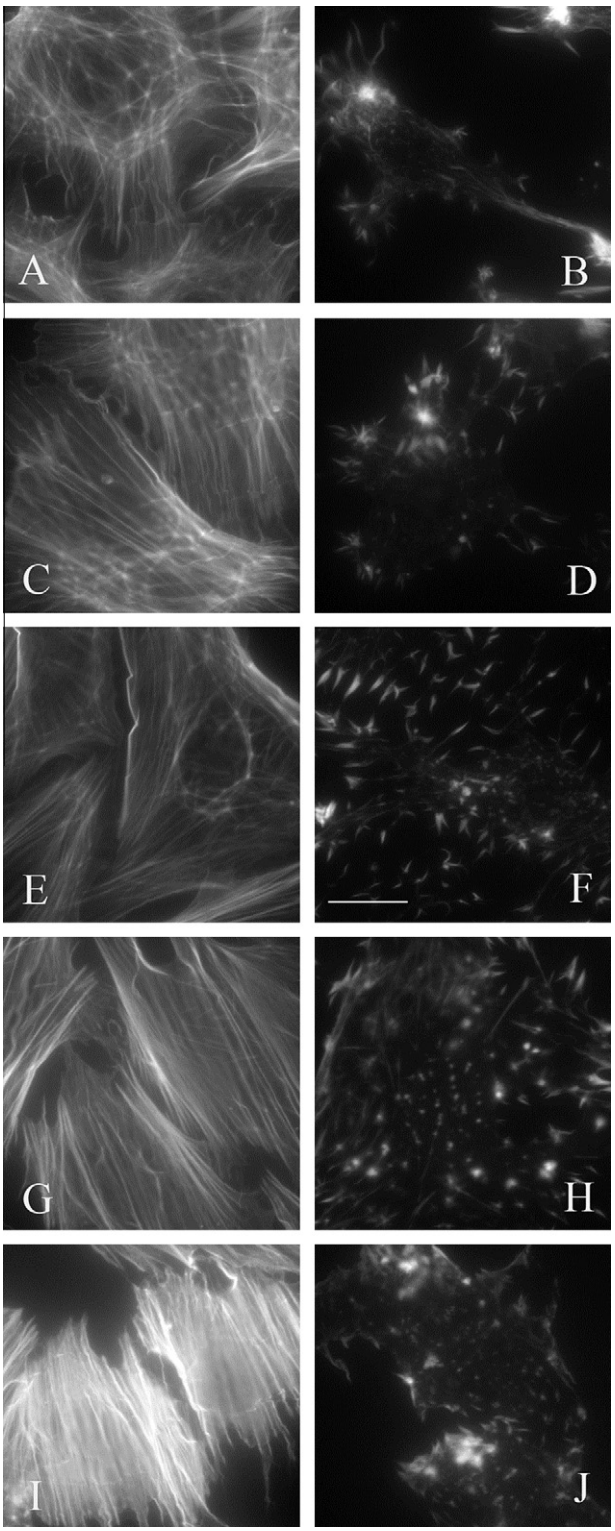

Fig. 3. Actin stain of cardiac fibroblasts plated with collagen and treated with (A) DMSO only, (B) cytD, (C) one hour of stretch, and (D) one hour of stretch with cytD. In the control (A), stress fibers exhibit polygonal distribution and varying fiber angles. Under applied stretch (C), stress fibers appear more aligned and the polygonal distribution appears attenuated. With cytD treatment (B,D) no fibers are observed; only focal deposits scattered throughout the cell. Similar staining of actin of cardiac fibroblasts plated on fibronectin and treated with (E) DMSO, (F) cytD, (G) one hour of stretch, (H) one hour of stretch with cytD, (I) 24 h of stretch, and (J) 24 h of stretch with cytD treatment during the last hour show similar patterns as for collagen-plated cells. Strong actin fiber alignment and reinforcement is observed in the 24 h stretched cells, which disappear with cytD treatment. Scale bar shown in panel $F = 20\ \mu m$.

0 Pa, but G'' increased from 3.3 Pa (3.0, 3.7) to 5.7 Pa (5.0, 6.6) with one hour stretch with cytD treatment.

Fibronectin-plated cells treated with cytD concurrently with one hour of stretch exhibited a bead MSD plot comparable in shape (close to a straight line) to cells treated with cytD alone, only shifted down ($p < 0.05$ at 1 Hz, Fig. 4B). Compared to unstretched, cytD-treated fibronectin-plated cells, G' remained unchanged at 0 Pa, but G'' increased from 1.2 Pa (1, 1.6) to 3 Pa (2.5, 3.8) when stretched with cytD for an hour. The 1 h collagen and fibronectin-plated cell data strongly suggest that treatment with cytD, while disruptive, does not completely eliminate the cellular reinforcement response to mechanical stretch.

To assess whether disruption of the actin cytoskeleton can attenuate or eliminate existing mechano-reinforcement of actin, cells (plated on fibronectin) were stretched for 24 h, with cytD added during the last hour of stretch. Cells treated this way exhibited comparable bead MSDs to cells that were stretched for 24 h without actin disruption, with some frequency-dependent differences ($p > 0.05$ at 1 Hz, Fig. 4C). When comparing unstretched, cytD-treated cells to stretched, cytD-treated cells, G' increased from 0 to 7 Pa (5.8, 8.9) and G'', from 1.2 Pa (1.0, 1.6) to 10.3 Pa (8.2, 14.0). In fact, cells stretched for 24 h with the last hour in cytD were more stiff but less viscous compared to cells stretched for 24 h with the last hour in DMSO, the latter of which had G' of 6.6 Pa (6, 7.3) and G'' of 15.6 Pa (14.5, 16.8).

4. Discussion

The major conclusions of this study are that (1) cell rheological properties are receptor and mechanical-stimuli dependent, (2) both solid-like and fluid-like characteristics change in response to external stimuli, and (3) despite global actin disruption that clearly eliminates visible stress fibers, local cell properties at intermediate-to-long time frames are still sensitive to mechanical stimuli. Regarding the third point, the significance of these results is that mechanically conditioning cells may exert some level of local protection against global disruption. Thus, even when stress fibers are disrupted, some level of cell mechanosignaling can still occur, and there is still microscale reinforcement.

If we use the actin 'cage' model for examining microrheology, we hypothesize that there are different actin subnetworks in cells. When baseline networks are disrupted, cells appear softer and less viscous across a wide frequency range, due to enhanced bead mobility. By itself, mechanical stimuli reinforces the baseline networks, leading to diminished bead mobility. For short durations of applied stimuli, these reinforcements are vulnerable to cytD disruption. Thus, the MSD-time lag relationship for fibronectin-plated cells changes from a curve to something closer to a straight line with cytD treatment. With short-term mechanical stretch concurrent with cytD treatment, the MSD shifts lower but remains closer to a straight line, suggesting that the actin 'cage' is still mostly missing (Fig. 4B). When mechanical stimuli are used to precondition cells prior to actin disruption, however, new subnetworks may be generated that appear to be less prone to disruption by cytD, thereby leading to maintenance of a curved MSD-time lag relationship (Fig. 4C). It is clear, then, that the material behavior of cells is considerably more sophisticated than a passive glassy material in the presence of external stimuli.

Physiologically, the results from this study may help explain certain responses in cardiac remodeling. In cases where increased loading occurs, such as those that follow myocardial infarction or induced pressure overload, our results suggest that the short-term response of fibroblasts via collagen is greater than when compared to fibronectin. However, extended loading when engaging the collagen receptors may be inimical to fibroblast health, as

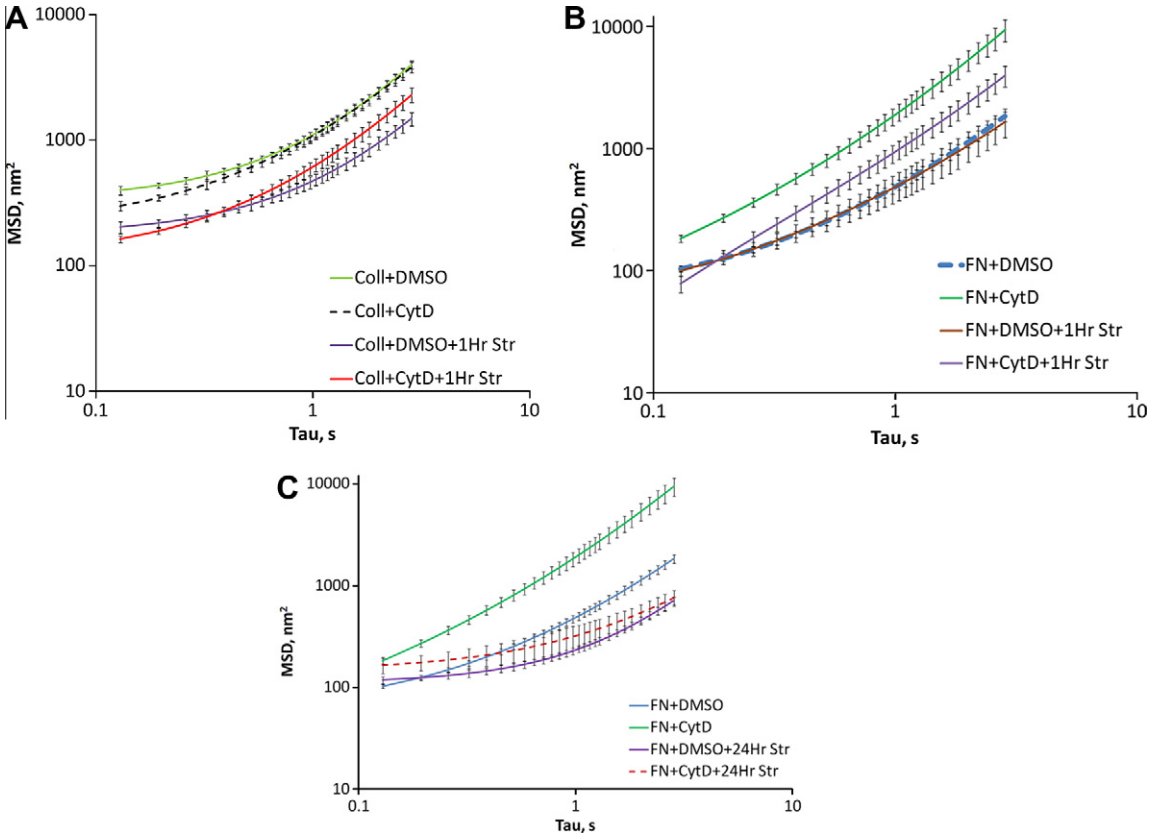

Fig. 4. (A) Cardiac fibroblasts plated on collagen and treated with CytD during one hour of stretch exhibited a bead MSD comparable to DMSO-treated controls, with some frequency dependence. There was a significant decrease in bead MSD compared to CytD treated cells, indicating that actin disruption did not eliminate the capacity for cells to reinforce in response to mechanical stretch. (B) Cells plated on fibronectin-coated membranes and treated with CytD during one hour of stretch also exhibited bead MSD decrease compared to CytD treatment alone. (C) Cells plated on fibronectin-coated membranes and stretched for 24 h and treated with CytD during the last hour of stretch exhibited an extension of the 1 h trends; showing a decrease in MSD to levels comparable to DMSO-treated control cells, with some frequency-dependent differences.

demonstrated by the altered cell morphology and detachment when loaded for 24 h. Thus, one would expect that fibroblasts would tend to favor a more fibronectin-based environment over longer times, which is consistent with both increased fibronectin in general tissue remodeling and earlier upregulation of fibronectin compared to collagen in cardiac cells [31–34]. These results thus suggest that collagen receptors may engage short-term responses, whereas fibronectin receptors engage longer-term remodeling behavior. Interestingly, collagen-plated cells appear to be more insensitive to cytD disruption, perhaps because cells plated on collagen had low stiffness at baseline. It may be that the local actin cages are not well-formed when only collagen is engaged, thus rendering the cell less vulnerable to cytD effects but capable of greater reinforcement in response to mechanical stimuli. Another significant conclusion from this study is that changes at the microscale can be very dramatic without any obvious changes at the gross actin-labeling level, underscoring the need for techniques such as PTM for examining these changes.

Left unresolved in this study is the precise role of the fluid-like component of cell properties. The changes in the loss modulus may be indicative of alterations to cell cytoplasmic viscosity; the mechanism by which the cell alters this property is not well-characterized. In this study, we limited statistical analyses to properties at 1 Hz. A wider frequency range would be helpful in determining potential components that may contribute to cellular properties. Further work assessing the effects of a combination of ligands, different strains or shear stresses may also be illuminating.

We demonstrated that cell properties are sensitive to a variety of plating conditions and external stimuli, and that mechanically conditioning cells may impart some form of enhanced mechanical state that persists despite gross actin disruption. Such changes to cell properties underscore the need for more systematic examination of cellular material responses and creates the opportunity for linking cellular properties to physiological and disease processes.

Acknowledgments

This work was supported in part by NSF CMMI-1130376.

References

[1] K.D. Costa, Single-cell elastography: probing for disease with the atomic force microscope, Dis. Markers 19 (2003) 139–154.
[2] C.M. Hale, S.X. Sun, D. Wirtz, Resolving the role of actoymyosin contractility in cell microrheology, PLoS One 4 (2009) e7054.
[3] X. Xiong, S. Guo, Z. Xu, P. Sheng, P. Tong, Development of an atomic-force-microscope-based hanging-fiber rheometer for interfacial microrheology, Phys. Rev. E: Stat. Nonlinear, Soft Matter Phys. 80 (2009) 061604.
[4] S. Hu, L. Eberhard, J. Chen, J.C. Love, J.P. Butler, J.J. Fredberg, G.M. Whitesides, N. Wang, Mechanical anisotropy of adherent cells probed by a three-dimensional magnetic twisting device, Am. J. Physiol. Cell Physiol. 287 (2004) C1184–C1191.
[5] S.S. An, B. Fabry, X. Trepat, N. Wang, J.J. Fredberg, Do biophysical properties of the airway smooth muscle in culture predict airway hyperresponsiveness?, Am J. Respir. Cell Mol. Biol. 35 (2006) 55–64.
[6] N. Wang, S. Hu, J.P. Butler, Imaging stress propagation in the cytoplasm of a living cell, Methods Cell Biol. 83 (2007) 179–198.
[7] B. Fabry, G.N. Maksym, S.A. Shore, P.E. Moore, R.A. Panettieri Jr., J.P. Butler, J.J. Fredberg, Signal transduction in smooth muscle. Selected contribution: time course and heterogeneity of contractile responses in cultured human airway smooth muscle cells, J. Appl. Physiol. 91 (2001) 986–994.

[8] H. Huang, R.D. Kamm, P.T. So, R.T. Lee, Receptor-based differences in human aortic smooth muscle cell membrane stiffness, Hypertension 38 (2001) 1158–1161.
[9] H. Huang, J. Sylvan, M. Jonas, R. Barresi, P.T. So, K.P. Campbell, R.T. Lee, Cell stiffness and receptors: evidence for cytoskeletal subnetworks, Am. J. Physiol. Cell Physiol. 288 (2005) C72–C80.
[10] G. Massiera, K.M. Van Citters, P.L. Biancaniello, J.C. Crocker, Mechanics of single cells: rheology, time dependence, and fluctuations, Biophys. J. 93 (2007) 3703–3713.
[11] Y.C. Poh, S. Na, F. Chowdhury, M. Ouyang, Y. Wang, N. Wang, Rapid activation of rac GTPase in living cells by force is independent of src, PLoS One 4 (2009) e7886.
[12] S.C. Kuo, Using optics to measure biological forces and mechanics, Traffic 2 (2001) 757–763.
[13] M. Tassieri, G.M. Gibson, R.M. Evans, A.M. Yao, R. Warren, M.J. Padgett, J.M. Cooper, Measuring storage and loss moduli using optical tweezers: broadband microrheology, Phys. Rev. E: Stat. Nonlinear, Soft Matter Phys. 81 (2010) 026308.
[14] P. Panorchan, J.S. Lee, T.P. Kole, Y. Tseng, D. Wirtz, Microrheology and ROCK signaling of human endothelial cells embedded in a 3D matrix, Biophys. J. 91 (2006) 3499–3507.
[15] C. Hohenegger, M.G. Forest, Two-bead microrheology: modeling protocols, Phys. Rev. E: Stat. Nonlinear, Soft Matter Phys. 78 (2008) 031501.
[16] M. Jonas, H. Huang, R.D. Kamm, P.T. So, Fast fluorescence laser tracking microrheometry, II: quantitative studies of cytoskeletal mechanotransduction, Biophys. J. 95 (2008) 895–909.
[17] D. Wirtz, Particle-tracking microrheology of living cells: principles and applications, Annu. Rev. Biophys. 38 (2009) 301–326.
[18] L. Selvaggi, M. Salemme, C. Vaccaro, G. Pesce, G. Rusciano, A. Sasso, C. Campanella, R. Carotenuto, Multiple-particle-tracking to investigate viscoelastic properties in living cells, Methods 51 (2010) 20–26.
[19] J.C. Crocker, M.T. Valentine, E.R. Weeks, T. Gisler, P.D. Kaplan, A.G. Yodh, D.A. Weitz, Two-point microrheology of inhomogeneous soft materials, Phys. Rev. Lett. 85 (2000) 888–891.
[20] T.G. Mason, K. Ganesan, J. van Zanten, D. Wirtz, S.C. Kuo, Particle tracking microrheology of complex fluids, Phys. Rev. Lett. 79 (1997) 3282–3285.
[21] C.M. Hale, A.L. Shrestha, S.B. Khatau, P.J. Stewart-Hutchinson, L. Hernandez, C.L. Stewart, D. Hodzic, D. Wirtz, Dysfunctional connections between the nucleus and the actin and microtubule networks in laminopathic models, Biophys. J. 95 (2008) 5462–5475.
[22] A.W. Lau, B.D. Hoffman, A. Davies, J.C. Crocker, T.C. Lubensky, Microrheology, stress fluctuations, and active behavior of living cells, Phys. Rev. Lett. 91 (2003) 198101.
[23] A. Pai, P. Sundd, D.F. Tees, In situ microrheological determination of neutrophil stiffening following adhesion in a model capillary, Ann. Biomed. Eng. 36 (2008) 596–603.
[24] B.R. Daniels, C.M. Hale, S.B. Khatau, S. Kusuma, T.M. Dobrowsky, S. Gerecht, D. Wirtz, Differences in the microrheology of human embryonic stem cells and human induced pluripotent stem cells, Biophys. J. 99 (2010) 3563–3570.
[25] J.S. Lee, P. Panorchan, C.M. Hale, S.B. Khatau, T.P. Kole, Y. Tseng, D. Wirtz, Ballistic intracellular nanorheology reveals ROCK-hard cytoplasmic stiffening response to fluid flow, J. Cell Sci. 119 (2006) 1760–1768.
[26] M. Eldib, D.A. Dean, Cyclic stretch of alveolar epithelial cells alters cytoskeletal micromechanics, Biotechnol. Bioeng. 108 (2011) 446–453.
[27] B.S. Hu, L.K. Landeen, N. Aroonsakool, W.R. Giles, An analysis of the effects of stretch on IGF-I secretion from rat ventricular fibroblasts, Am. J. Physiol. Heart Circ. Physiol. 293 (2007) H677–H683.
[28] B. Husse, W. Briest, L. Homagk, G. Isenberg, M. Gekle, Cyclical mechanical stretch modulates expression of collagen I and collagen III by PKC and tyrosine kinase in cardiac fibroblasts, Am. J. Physiol. Regul. Integr. Comp Physiol. 293 (2007) R1898–R1907.
[29] A.A. Lee, T. Delhaas, A.D. McCulloch, F.J. Villarreal, Differential responses of adult cardiac fibroblasts to in vitro biaxial strain patterns, J. Mol. Cell. Cardiol. 31 (1999) 1833–1843.
[30] M. Jonas, H. Huang, R.D. Kamm, P.T. So, Fast fluorescence laser tracking microrheometry. I: instrument development, Biophys. J. 94 (2008) 1459–1469.
[31] B.C. Berk, K. Fujiwara, S. Lehoux, ECM remodeling in hypertensive heart disease, J Clin. Invest. 117 (2007) 568–575.
[32] M.M. Ulrich, A.M. Janssen, M.J. Daemen, L. Rappaport, J.L. Samuel, F. Contard, J.F. Smits, J.P. Cleutjens, Increased expression of fibronectin isoforms after myocardial infarction in rats, J. Mol. Cell. Cardiol. 29 (1997) 2533–2543.
[33] F.J. Villarreal, W.H. Dillmann, Cardiac hypertrophy-induced changes in mRNA levels for TGF-beta 1, fibronectin, and collagen, Am. J. Physiol. 262 (1992) H1861–H1866.
[34] S.D. Zimmerman, D.P. Thomas, S.G. Velleman, X. Li, T.R. Hansen, R.J. McCormick, Time course of collagen and decorin changes in rat cardiac and skeletal muscle post-MI, Am. J. Physiol. Heart Circ. Physiol. 281 (2001) H1816–H1822.