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Rheological responses of cardiac fibroblasts to mechanical stretch

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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*.

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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,