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Stiffness *versus* prestress relationship at subcellular length scale

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Abstract

Local intracellular variations of cell mechanical properties, which are essential for vital cellular functions, have not been well characterized and are poorly understood. Here, we used results from our previous biomechanical imaging study to obtain relationships between intracellular shear modulus and prestress. We found that the subcellular shear modulus *vs.* prestress relationships exhibited positive linear correlations, consistent with previously observed behaviors at the whole cell and tissue levels. This, in turn, suggests that the prestress may be a unifying factor that determines material properties of living matter at different length scales.

1. Introduction

The ability of cells to regulate their vital functions demands that cells can easily adjust their mechanical properties both globally, at the whole cell level, and locally, at the subcellular level (Ingber 2003a). It has been shown that cell mechanical properties can be altered by modulating their endogenous contractile stress (prestress). In particular, it has been observed that cell stiffness increases approximately linearly with increasing prestress (Wang et al. 2001, 2002; Fernandez et al., 2006; Lam et al., 2012). These observations have been interpreted in terms of the cellular tensegrity model (Wang et al., 2001, 2002; Stamenovi and Ingber, 2002; Stamenovi, 2005; Baudriller et al., 2006). According to this model, stiffness is conferred to the cell by mechanical prestress borne by the cytoskeletal contractile actin network. The hallmark of such a system is that its stiffness must increase nearly proportionally with increasing prestress (Volokh 2011; Ingber et al., 2014). Ingber hypothesized that cells can use this tensegrity mechanism locally in order to regulate their

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Conflict of interest statement

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functions globally (Ingber 2003a, 2003b). If true, the linear relationship between stiffness and prestress must extend to subcellular variations. However, experimental evidence to support this claim is lacking since simultaneously generating detailed maps of subcellular prestress and stiffness distributions is a technically difficult task. Therefore, past attempts to map subcellular stiffness and prestress had to rely on *a priori* assumptions regarding the nature of their distributions (Park et al., 2010; Tambe et al., 2013).

We recently developed a technique, called biomechanical imaging, capable of generating spatial maps of subcellular shear modulus and prestress in living cells based on simultaneous measurements of cellular traction forces and intracellular displacements (Canovi et al., 2013). Measurements require up to 30 s of interrogation time and provide spatial resolution on the order of a few micrometers. Importantly, this technique requires no *a priori* assumptions about the distributions of either shear modulus or prestress. Here we used results from the biomechanical imaging study to obtain a relationship between intracellular shear modulus and prestress.

2. Materials and Methods

2.1. Biomechanical Imaging

Complete details about the theory of biomechanical imaging can be found in our earlier publication (Canovi et al., 2013). Briefly, we model the cell as a “thin”, incompressible, linearly elastic, isotropic, but highly inhomogeneous solid of variable thickness. The cell is in equilibrium with the substrate, and no external forces act on the cell except traction forces at the cell-substrate interface, $\mathbf{t}(\mathbf{x})$. We assume that the intracellular prestress distribution, $\boldsymbol{\sigma}(\mathbf{x})$, is entirely due to $\mathbf{t}(\mathbf{x})$, where $\mathbf{x}$ is a position vector in the plane of stress. Using a plane stress approximation, we obtained the equilibrium equation and the constitutive equation at the reference configuration as follows

$$\sigma_{ij,j} + t_i / \hat{h} = 0 \quad (1)$$

and

$$\sigma_{ij} = \mu(\mathbf{x}) [2(u_{k,k})\delta_{ij} + u_{i,j} + u_{j,i}], \quad (2)$$

respectively, where $\hat{h}$ is a reference uniform cell thickness, $\mu(\mathbf{x})$ is the intracellular shear modulus field, $\mathbf{u}(\mathbf{x})$ is the intracellular deformation (displacement) vector field associated with $\boldsymbol{\sigma}(\mathbf{x})$, and δ_{ij} is the Kronecker delta. All tensors and vectors in Eqs. (1) and (2) are two-dimensional and the Einstein's summation rule over $i, j = 1, 2$ applies.

Since we can measure $\mathbf{t}(\mathbf{x})$ but not $\mathbf{u}(\mathbf{x})$, without further information about $\mu(\mathbf{x})$, it is impossible to obtain $\boldsymbol{\sigma}(\mathbf{x})$ from Eqs. (1) and (2).

We next imposed a uniaxial planar strain to the substrate and measured a) an incremental cell displacement field, $\mathbf{u}(\mathbf{x})$, by tracking motion of microbeads internalized in the cell, and b) the incremental change in traction forces, $\mathbf{t}(\mathbf{x})$. The imposed strain resulted in an

increment of the intracellular stress, $\sigma(\mathbf{x})$. Thus, the equilibrium equation and the constitutive equation for this incremental deformation are

$$\Delta\sigma_{ij,j} + \Delta t_i / \hat{h} = 0 \quad (3)$$

and

$$\Delta\sigma_{ij} = \mu(\mathbf{x}) [2(\Delta u_{k,k})\delta_{ij} + \Delta u_{i,j} + \Delta u_{j,i}], \quad (4)$$

respectively.

From the combination of measured $\mathbf{u}(\mathbf{x})$ and $\mathbf{t}(\mathbf{x})$, we can now solve the inverse problem [Eqs. (3) and (4)] (Barbone and Oberai 2007) to obtain $\mu(\mathbf{x})$, and then use $\mu(\mathbf{x})$ to compute $\sigma(\mathbf{x})$ from Eqs. (1) and (2).

2.2. Experimental Procedures

Cell culture. NIH 3T3 (ATCC) fibroblasts were seeded sparsely on 0.7-mm thick polyacrylamide gels of elastic modulus of 3.6 or 7.6 kPa and Poisson's ratio of 0.445. The apical surface of the gel was micropatterned with an array of fluorescently labeled fibronectin 2- μm dots at 6 μm center-to-center spacing (Polio et al., 2012). The cells can adhere only to the fibronectin dots. Immediately after seeding the cells, 500 nm fluorescent microbeads were added to the media, which were phagocytosed by cells overnight to serve as fiducial markers for intracellular displacements.

Strain Application and Imaging. An in-plane homogenous, purely uniaxial strain field (8%) was applied to the gel surface *via* a parallel plate indenter. Images of the cell, micropatterned substrate, and internalized beads were taken immediately before and immediately after strain application. The time to take these measurements did not exceed 30 s. Images of the micropatterned substrate were then converted into traction forces and images of internalized microbeads into intracellular displacements. Cell boundaries were determined by tracing the outline of the cell as seen on brightfield images. Cell height distribution, $h(\mathbf{x})$, was determined by using height of internalized microbeads within the z -stack (Canovi et al., 2013).

2.3. Data Analysis

Shear Modulus and Prestress Computation. From measured $\mathbf{t}(\mathbf{x})$ and an initial uniform guess for $\mu(\mathbf{x})$, we predicted $\mathbf{u}(\mathbf{x})$ field *via* finite element analysis. The measured $\mathbf{t}(\mathbf{x})$ were typically out of equilibrium, and so were projected in a least-squares on the nearest equilibrium traction distribution. The added force correction was taken to be the 'force error'. This led to a force signal-to-noise ratio (SNR) varying from 0.87 to 8.4, with median 2.1. We compared predicted $\mathbf{u}(\mathbf{x})$ to the observed $\mathbf{u}(\mathbf{x})$. We then iteratively updated $\mu(\mathbf{x})$ seeking to minimize the difference between the predicted and measured values. Taking the final displacement mismatch as an indicator of displacement error allowed us to predict a displacement SNR for each cell, which ranged from 1.06 to 5.9, with median 1.57.

The map of $\mu(\mathbf{x})$ was then combined with the data for $\mathbf{t}(\mathbf{x})$ to obtain the map of $\sigma(\mathbf{x})$. Since $\mu(\mathbf{x})$ and $\sigma(\mathbf{x})$ were computed assuming a uniform cell thickness $\hat{h}$, we made corrections by multiplying computed values by $\hat{h}/h(\mathbf{x})$.

From the calculated σ , we computed a scalar metric of the prestress as the von Mises stress,

i.e., $P = \sqrt{\sigma_{11}^2 - \sigma_{11}\sigma_{22} + \sigma_{22}^2 - 3\sigma_{12}^2}$, where σ_{ij} are Cartesian components of σ .

Since our iterative scheme requires an initial guess of $\mu(\mathbf{x})$, regions of the cell that were scarcely populated with the microbeads tended to stay around this guessed value. Thus, we could solve the inverse problem using several different initial guesses in order to create an ensemble of different reconstructed $\mu(\mathbf{x})$. Within this ensemble, there were typically regions with lower variance, and other regions with higher variance. A threshold was chosen based on the variance in ‘beadless’ regions of the cell. We only considered cell regions below this variance threshold. Additional thresholding was performed to exclude outliers ($\mu > 5$ kPa and $P > 2$ kPa) that were arbitrarily high due to artifacts associated with the height correction (Canovi et al., 2013). We then used the thresholded data to obtain μ vs. P relationships for each cell.

Statistical Analysis. As a measure of correlation between μ and P , we calculated the Pearson’s correlation coefficient (R) for each cell. We considered the correlation to be strong if $|R| > 0.7$, moderate if $0.5 < |R| \leq 0.7$, weak if $0.2 < |R| \leq 0.5$, and no correlation if $|R| \leq 0.2$. We considered the correlation to be significant at ~ 0.05 level of significance if $|R| > 2/n^{1/2}$, where n is the number of data points. We fitted the μ vs. P relationships by linear regressions using the total (Deming) regression model since both μ and P were errors-in-variables.

3. Results and Discussion

Intracellular μ vs. P plots were obtained for $n = 18$ cells. In all cells the data exhibited scattering and positive and significant correlations. Those correlations were strong in 12 cells, moderate in 5 cells, and weak in 1 cell (Table 1). Representative plots for each case are shown in Fig. 1. The slopes of the linear regressions exhibited high cell-to-cell variability, ranging from ~ 2.24 to ~ 7.65 . On average, the μ vs. P slope of the strong correlations (3.31 ± 1.09) was smaller than the μ vs. P slope of the weak to moderate correlations (5.34 ± 1.95), and this difference was significant ($p = 0.011$). The intercepts were much smaller in the cells with strong correlations than in the cells with the weak to moderate correlations (Table 1). On average, the intercept associated with the strong correlations (-0.00585 ± 0.0114) was not significantly different from zero ($p = 0.104$), whereas the intercept associated with the weak to moderate correlations (-0.915 ± 0.62) was significantly different from zero ($p = 0.015$). Taken together, these results suggest that in 2/3 of the observed cells μ increased nearly proportionally with P .

A close association between the shear modulus and the prestress has been observed at different length scales, from the cell to the organ level (cf. Stamenovi 1990; Bursa et al., 2000; Wang et al., 2001, 2002; Fernandez et al., 2006; Lam et al., 2012), leading to the hypothesis that this relationship may reflect a common physical principle that extends across

length scales (Fredberg et al., 1998; Fernandez et al., 2006). Such principles are, for example, embodied in tensegrity architecture, which applies to a broad range of length scales in living organisms (Chen and Ingber 1999; Ingber 2003b). Our results, showing that this relationship extended to subcellular variations, further corroborated the idea of bio-tensegrity's length-scale invariance. This, in turn, suggests that in living organisms mechanical prestress may play a central and unifying role in regulating their mechanical responses and physiological functions. At the subcellular level, this means that the cell can tune its local stiffness *via* local prestress, which is important for regulation of global functions of the cell, including directionality and speed of migration and signal transduction along preferential pathways (Ingber 2003a). This finding is also consistent with Ingber's idea that the cytoskeleton is composed of multiple hierarchically organized tensegrity modules whose local structural stiffness is determined by local prestress (Chen and Ingber 1999; Ingber 2003b).

The distributions of μ and P were inferred from small measured bead displacements, and small differences in measured tractions. As difference measurements, these are highly susceptible to noise, which can lead to a high data variability. Of the 18 cells studied, for example, only 6 showed displacement SNR > 2 , with the median SNR = 1.57. Similar precision limits affect the traction measurements. The traction data for about half the cells had SNR < 2 , and two cells needed force corrections toward equilibrium larger than the mean magnitude of forces applied to those cells. Data from at least one cell showed a negative correlation between tractions and displacements. The strength of the μ vs. P correlation showed no obvious relation to the SNR measures of data quality.

At least one source of displacement measurement error was physiological in origin, and was due to the use of phagocytosed microbeads as fiducial markers. Some of the beads were presumably located within vesicles whose movement is driven by molecular motors rather than by an externally applied strain. Therefore, bead motion might not be entirely linked to cytoskeletal deformation. Indeed, our analysis showed that bead displacements were not always consistent with the direction of the applied traction field. Interestingly, we did not observe qualitative differences in the μ vs. P correlations between cells, regardless of whether the bead motion was or was not consistent with the traction data. Additional variability in the μ vs. P relation is possibly due to inhomogeneity in the distribution of actin and myosin in the cells, as observed previously (Park et al. 2009; Aratyn-Schaus et al. 2011).

In summary, the μ vs. P relationship at the subcellular level exhibited a positive linear correlation, as observed previously on the whole cell and tissue levels. This, in turn, suggests that prestress may be a unifying factor that determines material responses at different length scales, which is consistent with the hypothesis of bio-tensegrity's length-scale invariance.

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References

- Aratyn-Schaus Y, Oakes PW, Gardel ML. Dynamic and structural signatures of lamellar actomyosin force generation. *Molecular Biology of the Cell*. 2011; 22:1330–1339. [PubMed: 21307339]
- Barbone PE, Oberai AA. Elastic modulus imaging: some exact solutions of the compressible elastography inverse problem. *Physics in Medicine and Biology*. 2007; 52:1577–1593. [PubMed: 17327650]
- Baudriller H, Maurin B, Cañadas P, Montcourrier P, Parmeggiani A, Bettache N. Form-finding complex tensegrity structures: application to cell cytoskeletal modelling. *Comptes Rendus Mécanique*. 2006; 334:662–668.
- Bursa P, McGrath CV, Eisenberg SR, Stamenovi D. A microstructural model of elastostatic properties of articular cartilage in confined compression. *Journal of Biomechanical Engineering*. 2000; 122:347–353. [PubMed: 11036557]
- Canovi EP, Seidl DT, Polio SR, Oberai AA, Barbone EP, Stamenovi D, Smith ML. Biomechanical imaging of cell stiffness and prestress with subcellular resolution. *Biomechanics and Modeling in Mechanobiology*. 2013; 10:1007/s10237-013-0526-8
- Chen CS, Ingber DE. Tensegrity and mechanoregulation: from skeleton to cytoskeleton. *Osteoarthritis and Cartilage*. 1999; 7:81–94. [PubMed: 10367017]
- Fernández P, Pullarkat PA, Ott A. A master relation defines the nonlinear viscoelasticity of single fibroblasts. *Biophysical Journal*. 2006; 90:3796–3805.
- Fredberg JJ, Wang N, Stamenovi D, Ingber DE. Micromechanics of the lung: from the parenchyma to the cytoskeleton. In: Hlastala, MP.; Robertson, HT., editors. *Complexity in Structure and Function of the Lung*. Dekker; New York: 1998. p. 99-122. (Lung Biol. Health Dis. Ser.)
- Ingber DE. Mechanosensation through integrins: Cells act locally but think globally. *Proceedings of the National Academy of Sciences of the United States of America*. 2003a; 100:1472–1474. [PubMed: 12578965]
- Ingber DE. Tensegrity I. Cell structure and hierarchical systems. *Journal of Cell Science*. 2003b; 116:1157–1173. [PubMed: 12615960]
- Ingber DE, Wang N, Stamenovi D. Tensegrity, cellular biophysics, and the mechanics of living systems. *Reports on Progress in Physics*. 2014; 77:046603. [PubMed: 24695087]
- Lam RHW, Weng S, Lu W, Fu J. Live-cell subcellular measurement of cell stiffness using a microengineered stretchable micropost array membrane. *Integrative Biology*. 2012; 4:1289–1298. [PubMed: 22935822]
- Park CY, Tambe D, Alencar AM, Trepas X, Zhou EH, Millet E, Butler JP, Fredberg JJ. Mapping the cytoskeletal prestress. *American Journal of Physiology Cell Physiology*. 2010; 298:C1245–C1252. [PubMed: 20164383]
- Polio SR, Rothenberg KE, Stamenovi D, Smith ML. A micropatterning and image processing approach to simplify measurement of cellular traction forces. *Acta Biomaterialia*. 2012; 8:82–88. [PubMed: 21884832]
- Stamenovi D. Micromechanical foundations of pulmonary elasticity. *Physiological Reviews*. 1990; 70:1117–1134. [PubMed: 2217556]
- Stamenovi D. Effects of cytoskeletal prestress on cell rheological behavior. *Acta Biomaterialia*. 2005; 1:255–262. [PubMed: 16701804]
- Stamenovi D, Ingber DE. Models of cytoskeletal mechanics of adherent cells. *Biomechanics and Modeling in Mechanobiology*. 2002; 1:95–108. [PubMed: 14586710]
- Tambe DT, Croutelle U, Trepas X, Park CY, Kim JH, Millet E, Butler JP, Fredberg JJ. Monolayer stress microscopy: limitations, artifacts, and accuracy of recovered intercellular stresses. *PLoS One*. 2013; 8:e55172. [PubMed: 23468843]
- Volokh KY. On tensegrity in cell mechanics. *Molecular & Cellular Biomechanics*. 2011; 8:195–214. [PubMed: 21977516]
- Wang N, Naruse K, Stamenovi D, Fredberg JJ, Mijailovich SM, Toli-Nørrelykke IM, Polte T, Mannix R, Ingber DE. Mechanical behavior in living cells consistent with the tensegrity model. *Proceedings of the National Academy of Sciences of the United States of America*. 2001; 98:7765–7770. [PubMed: 11438729]

Wang N, Toli -Nørrelykke IM, Chen J, Mijailovich SM, Butler JP, Fredberg JJ, Stamenovi D. Cell prestress. I. Stiffness and prestress are closely associated in adherent contractile cells. *American Journal of Physiology Cell Physiology*. 2002; 282:C606–C616. [PubMed: 11832346]

Figure 1.

Representative plots of intracellular shear modulus vs. prestress data (dots) and the corresponding linear regressions (solid line) for A) a cell with a strong correlation (Cell #18 from Table 1), B) a cell with a strong correlation (Cell #3 from Table 1), C) a cell with a moderate correlation (Cell #6 from Table 1), and D) a cell with a weak correlation (Cell #5 from Table 1).

Table 1

Values for correlation coefficient R , the regression slope and the intercept.

Cell #	R	Slope	Intercept
1	0.825	2.300	-0.075
2	0.559	7.616	-1.248
3	0.805	6.145	-0.284
4	0.826	4.344	-0.040
5	0.262	7.651	-2.008
6	0.663	3.541	-0.376
7	0.886	2.623	-0.154
8	0.696	3.251	-0.426
9	0.512	5.603	-0.774
10	0.887	3.295	-0.115
11	0.550	4.404	-0.656
12	0.889	2.976	0.046
13	0.827	2.952	-0.162
14	0.840	2.236	-0.026
15	0.750	3.044	0.026
16	0.822	3.657	0.085
17	0.736	3.690	-0.109
18	0.918	2.458	0.106