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Short communication

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.

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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; Fernández et al., 2006; Lam et al., 2012). These observations have been interpreted in terms of the cellular tensegrity model (Wang et al., 2001, 2002; Stamenović and Ingber, 2002; Stamenović, 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 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 (Canović et al., 2014). 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 (Canović et al., 2014). 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

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

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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, $\Delta\mathbf{u}(\mathbf{x})$, by tracking motion of microbeads internalized in the cell and (b) the incremental change in traction forces, $\Delta\mathbf{t}(\mathbf{x})$. The imposed strain resulted in an increment of the intracellular stress, $\Delta\boldsymbol{\sigma}(\mathbf{x})$. 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 $\Delta\mathbf{u}(\mathbf{x})$ and $\Delta\mathbf{t}(\mathbf{x})$, 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 $\boldsymbol{\sigma}(\mathbf{x})$ from Eqs. (1) and (2).

2.2. Experimental procedures

2.2.1. 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.

2.2.2. 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 (Canović et al., 2014).

2.3. Data analysis

2.3.1. Shear modulus and prestress computation

From measured $\Delta\mathbf{t}(\mathbf{x})$ and an initial uniform guess for $\mu(\mathbf{x})$, we predicted $\Delta\mathbf{u}(\mathbf{x})$ field *via* finite element analysis. The measured $\Delta\mathbf{t}(\mathbf{x})$ was typically out of equilibrium and so projected in 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 $\Delta\mathbf{u}(\mathbf{x})$ to the observed $\Delta\mathbf{u}(\mathbf{x})$. 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 $\boldsymbol{\sigma}(\mathbf{x})$. Since $\mu(\mathbf{x})$ and $\boldsymbol{\sigma}(\mathbf{x})$ 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 $\boldsymbol{\sigma}$, 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 $\boldsymbol{\sigma}$.

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 (Canović et al., 2014). We then used the thresholded data to obtain μ *vs.* P relationships for each cell.

2.3.2. 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 two-third 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.* Stamenović, 1990; Bursać et al., 2000; Wang et al., 2001, 2002; Fernández 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; Fernández 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 biotensegrity's length-scale invariance. This, in turn, suggests that in living organisms mechanical prestress may

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

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