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Sub-cellular force microscopy in single normal and cancer cells

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ABSTRACT

This work investigates the biomechanical properties of sub-cellular structures of breast cells using atomic force microscopy (AFM). The cells are modeled as a triple-layered structure where the Generalized Maxwell model is applied to experimental data from AFM stress-relaxation tests to extract the elastic modulus, the apparent viscosity, and the relaxation time of sub-cellular structures. The triple-layered modeling results allow for determination and comparison of the biomechanical properties of the three major sub-cellular structures between normal and cancerous cells: the up plasma membrane/actin cortex, the mid cytoplasm/nucleus, and the low nuclear/integrin sub-domains. The results reveal that the sub-domains become stiffer and significantly more viscous with depth, regardless of cell type. In addition, there is a decreasing trend in the average elastic modulus and apparent viscosity of the all corresponding sub-cellular structures from normal to cancerous cells, which becomes most remarkable in the deeper sub-domain. The presented modeling in this work constitutes a unique AFM-based experimental framework to study the biomechanics of sub-cellular structures.

1. Introduction

Atomic force microscopy (AFM) is an established method for tissue-level and single-cell biomechanical characterization. Several studies have shown that transformed cells are generally softer and more deformable than their healthier counterparts which can facilitate their motility and metastasis [1]. Particularly for breast epithelial cells, AFM results have shown that the highly metastatic MDA-MB-231 cell line is significantly more deformable than the non-tumorigenic MCF10A cells [2,3]. Experimentally, agents that increase cell stiffness decrease breast cancer invasiveness, providing evidence for a direct relationship between the metastatic potential of cancer cells and their mechanical properties [4]. Several articles have also revealed that time-dependent viscoelastic properties can also serve as indicators of cell disease [5]. The determination of viscoelastic properties has been done by implementing stress-relaxation test using different techniques, particularly AFM [5,6]. Meanwhile, the network of cytoskeletal proteins and the nucleus have been found to play the most significant role in determining the biomechanical properties of cells [7].

To determine single cell representative biomechanical properties, the generated experimental data are typically analyzed through the Hertz model and its extension into the time domain, the Standard Linear Solid (SLS) model [8–10]. Different research groups have used this approach to introduce cell biomechanical properties as “label-free” biophysical markers to predict cancer risk, cancer progression from benign to aggressive stages [32], and treatment efficacy [33]. Despite promising results, the current trend in single-cell force microscopy does not provide the most comprehensive and information-rich picture of cell biomechanics. The use of the SLS model for quantification of cell biomechanical properties assumes that the cell is an entirely homogenous material, ignores the cell's complex multi-layered architecture, and neglects the cell's nonlinear depth-dependent elastic and viscoelastic properties [11–13,34]. Moreover, the indentation depth for cell measurements is typically kept below 10% of a cell thickness to keep the validity of the assumptions and to avoid possible error introduced by deformation nonlinearity and substrate contributions [14]. However, there is growing evidence in the literature that this limitation is too conservative and contributions from the substrate are negligible up to 50% of the cell thickness [15,16], overlooking detailed information about the cell mechanics in deeper layers of the cells [17].

The Generalized Maxwell (GM), a more advanced viscoelastic model has been successfully applied to relaxation measurements of

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biological samples [18–20]. This model incorporates the idea of multiple relaxation times, and therefore, it can provide insight about the transient response of sub-cellular structures to external mechanical stimuli by distributing the response across several time scales. Consequently, the model leads to more extensive parameter sets to characterize and analyze the biomechanical properties of cell components. There have been only a few studies based on the GM model for characterizing cell viscoelastic properties by AFM nano-indentation [21,22], however the comparison of the biomechanical characterization of the sub-cellular structures among normal and cancerous cells has been left untouched.

This paper reports the biomechanical properties of sub-cellular structures of breast carcinoma MDA-MB-231 and benign MCF10A cells. For this purpose, stress-relaxation AFM tests are conducted on single cells after applying a deep probe indentation (up to 50% of cell height), and the resulting experimental data are analyzed with the GM viscoelastic model.

2. Materials and methods

2.1. Cell culture and sample preparation

MCF10A and MDA-MB-231 breast cell lines representative of non-invasive and highly invasive breast cancer models, respectively, were purchased from the American Type Culture Collection (ATCC). The cells were maintained in plastic T-25 cm^2 culture flasks in standard cell culture medium presented in Refs. [2,3]. For the AFM tests, the cells were harvested and then seeded at a density of 1×10^5 cells per 12 mm^2 glass coverslips coated with 0.1 mg/mL collagen type IV (Sigma–Aldrich, St. Louis, MO) for 24–30 h at 37 °C in humidified 7% CO_2-93% air atmosphere prior to the AFM experiments to allow the cells to adhere. A buffered HEPES solution was then added to the coverslip samples (final concentration of 13.5 mM) to maintain a physiological pH of 7.2 during testing.

2.2. Scanning electron microscopy (SEM)

SEM was performed to investigate morphology and the height for both cell lines on the surface. The cells were fixed in 3.7% formaldehyde in PBS for 10 min, 24–30 h after plating on collagen-coated coverslips. The samples were critical-point-dried to eliminate changes in the cell morphology, sputter-coated with a thin layer of gold palladium, and mounted on an SEM sample holder using conductive tape prior to the SEM imaging. To approximate the cells height, the SEM images were acquired with 85° tilt of the sample stage to allow a side-view visualization. The images were obtained using a Leo ZEISS 1550 field-emission SEM instrument.

2.3. Atomic force microscopy (AFM)

The AFM experiments were performed with a Dimension Icon AFM with a closed loop controller (Bruker Corporation, Billerica, MA) integrated with an optical microscope. Olympus TR400PSA V-shaped SiNi cantilevers (Olympus, Tokyo, Japan) of ~200 μm length with approximate spring constant values of ~0.02 N/m were employed in all AFM experiments; exact spring constant values were measured via the thermal tuning method. The sharp probes were modified by attaching glass spheres (Duke Scientific, Waltham, MA) of ~10 μm diameter onto the cantilever free end with two-part epoxy (Miller Stephenson, Sylmar CA), which helped reduce damage to the cells due to contact. The exact diameter of the glass sphere and its attachment location were identified using a HIROX KH-7700 3D Digital Video Microscope. The measurements were carried out on single cells in their respective culture medium at room temperature (24 °C) using AFM contact mode. The indentations were done above each cell's nucleus under optical control. Stress-relaxation test corresponding to each cell response to a unit step displacement was implemented [10] to acquire stress-relaxation curves for a total period of 60 s at a 5 kHz sample rate and a constant high approach velocity of 5 μm/s to approximate a pure step displacement. A maximum force trigger of 30 nN was implemented for all curve acquisition, leading to a deep indentation of about 3 μm on the cells, which are sufficient to probe the membrane and the underlying cytoplasm and nucleus [23].

2.4. Generalized Maxwell viscoelastic model

One model that has been successfully applied to AFM stress-relaxation experiments on biological samples is the Generalized Maxwell (GM) model [18–20]. The GM model is similar in form to the Standard Linear Solid (SLS) model which was previously widely employed [8–10], but incorporates the concept of multiple relaxation times corresponding to a larger number of viscous elements. A helpful visualization is an arrangement of stacked viscous balls of increasing viscosity. As shown in Fig. 1A, an applied force would quickly deform the less viscous element, which would slowly relax. The more viscous elements, however, would quickly snap back to their initial shape due to the larger restoring force. The same is true for sub-structures of heterogeneous composition. A parallel representation of the elements effectively represents the same effect but distributes the response across the time domain. Fig. 1B shows the representative mechanical system of the GM model, where the subscript on the parameters denotes the branch in the parallel structure. The equations of motion for the system may be written compactly:

$$\sigma = \left(E_R + \frac{E_1\mu_1\mathscr{D}}{E_1+\mu_1\mathscr{D}} + \cdots + \frac{E_n\mu_n\mathscr{D}}{E_n+\mu_n\mathscr{D}}\right)\varepsilon\,, \tag{1}$$

where E_R is the relaxed modulus, E_n is the n-th elastic coefficient, μ_n is the n-th viscous coefficient, and $\mathscr{D}$ is the differential operator such that $\mathscr{D}^n(\cdot) = d^n/dt^n(\cdot)$. A transformation into the Laplace domain results in the ratio of stress to strain equation:

$$\frac{\overline{\sigma}}{\overline{\varepsilon}} = K(s) = \left(E_R + \frac{E_1\mu_1 s}{E_1+\mu_1 s} + \cdots + \frac{E_n\mu_n s}{E_n+\mu_n s}\right). \tag{2}$$

Through the analogous modulus of rigidity, a time-dependent elastic modulus may be expressed in the Laplace domain as:

$$\overline{E}(s) = (1+\nu)\,K(s). \tag{3}$$

The relation between an applied normal force, F, by a spherical body with radius, R, and a sample deformation, δ, with Poisson's ratio, ν and elastic modulus, E, can be expressed by the Hertz model [24] as follows:

$$F = \frac{4E\sqrt{R}}{3(1-\nu^2)}\delta^{\frac{3}{2}} \tag{4}$$

Assuming that the initial contact force is applied constantly throughout the relaxation, it may be modeled as a Heaviside function. Eq. (4) may then be extended into the Laplace domain by replacing elastic modulus with the result of Eq. (3):

$$\overline{F}(s) = \frac{4}{3}\frac{\sqrt{R\delta_0^3}}{(1-\nu)}\frac{K(s)}{s}, \tag{5}$$

where δ_0 is the maximum indentation resulting from the application of the contact force. Substitution of Eq. (2) into Eq. (5) and