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Single-cell mechanics – An experimental–computational method for quantifying the membrane–cytoskeleton elasticity of cells

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ABSTRACT

The membrane–cytoskeleton system plays a major role in cell adhesion, growth, migration, and differentiation. F-actin filaments, cross-linkers, binding proteins that bundle F-actin filaments to form the actin cytoskeleton, and integrins that connect the actin cytoskeleton network to the cell plasma membrane and extracellular matrix are major cytoskeleton constituents. Thus, the cell cytoskeleton is a complex composite that can assume different shapes. Atomic force microscopy (AFM)-based techniques have been used to measure cytoskeleton material properties without much attention to cell shape. A recently developed surface chemical patterning method for long-term single-cell culture was used to seed individual cells on circular patterns. A continuum-based cell model, which uses as input the force–displacement response obtained with a modified AFM setup and relates the membrane–cytoskeleton elastic behavior to the cell geometry, while treating all other subcellular components suspended in the cytoplasmic liquid (gel) as an incompressible fluid, is presented and validated by experimental results. The developed analytical–experimental methodology establishes a framework for quantifying the membrane–cytoskeleton elasticity of live cells. This capability may have immense implications in cell biology, particularly in studies seeking to establish correlations between membrane–cytoskeleton elasticity and cell disease, mortality, differentiation, and migration, and provide insight into cell infiltration through nonwoven fibrous scaffolds. The present method can be further extended to analyze membrane–cytoskeleton viscoelasticity, examine the role of other subcellular components (e.g., nucleus envelope) in cell elasticity, and elucidate the effects of mechanical stimuli on cell differentiation and motility.

Statement of Significance

This is the first study to decouple the membrane–cytoskeleton elasticity from cell stiffness and introduce an effective approach for measuring the elastic modulus. The novelty of this study is the development of new technology for quantifying the elastic stiffness of the membrane–cytoskeleton system of cells. This capability could have immense implications in cell biology, particularly in establishing correlations between various cell diseases, mortality, and differentiation with membrane–cytoskeleton elasticity, examining through-tissue cell migration, and understanding cell infiltration in porous scaffolds. The present method can be further extended to analyze membrane–cytoskeleton viscous behavior, identify the contribution of other subcellular components (e.g., nucleus envelope) to load sharing, and elucidate mechanotransduction effects due to repetitive compressive loading and unloading on cell differentiation and motility.

1. Introduction

The response of eukaryotic cells to mechanical stimuli plays an important role in cellular behavior, including cell adhesion, growth, migration, and differentiation. Mitrossilis et al. [1] reported that live cells can sense the rigidity of a substrate, align in the direction of maximum stiffness, and migrate toward stiffer regions. Engler et al. [2] prompted human mesenchymal stem cells (hMSCs) to specify lineage and commit to phenotypes with extreme sensitivity to tissue elasticity and reported that relatively soft, stiff, and rigid matrices mimicking brain, muscle, and

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collagenous bone are neurogenic, myogenic, and osteogenic, respectively. Although these experiments reveal a substrate stiffness effect on cell fate, other studies have shown distinctly different responses between healthy and diseased cells subjected to mechanical stimuli. For example, Suresh [3] studied the elasticity of red blood cells (RBCs) and discovered that RBCs infected by malaria-inducing parasite P exhibit higher stiffness than healthy RBCs. These studies and many others demonstrate that in-depth knowledge of cell elasticity is critical for recognizing, quantifying, and classifying cells at different stages of disease.

Despite important advances in cell property measurement and modeling, cell material characterization continues to attract significant attention. The mechanical response of live cells is strongly affected by their complex and dynamic internal structure. Bao and Suresh [4] and Lim et al. [5] presented extensive reviews of common experimental techniques for cell characterization and mathematical models of cellular and subcellular material behavior. However, the highly dynamic nature of live cells makes modeling of their response to different mechanical stimuli extremely difficult. Consequently, the majority of material parameters do not reflect the true mechanical properties of the cell and/or subcellular components. While material parameters may be used for qualitative comparisons, recent studies [6,7] have introduced techniques accounting for active remodeling of the actin cytoskeleton (AC) and providing further motivation for the development of more realistic mechanical models of live cells.

Accurate force–displacement measurements obtained by Kang et al. [8] with an atomic force microscope (AFM) have revealed the potential of AFM to probe the mechanical behavior of cells. Various analytical and numerical models have been used to correlate AFM measurements with cellular response. Kirmizis and Logothetidis [9] presented an extensive review of AFM applications in cell mechanics. Analytical approaches in previous cell mechanics studies [9,10] have been based on Hertz contact theory of blunt conical [11] and spherical [12] indenters. Lulevich et al. [13] developed a Hertzian contact model of a balloon-like cell consisting of two isotropic elastic outer layers representing the plasma membrane and cytoskeleton. Wottawah et al. [14] used an optical stretcher to investigate the passive relaxation of transiently crosslinked actin cortex of suspended fibroblasts. Nawaz et al. [15] performed optical trapping indentation experiments with fibroblasts and reported a fully elastic response independent of deformation rate for shallow indentations and the dependence of the elastic modulus predominantly on the actin cortex. Schlosser et al. [16] used optical tweezers to mechanically stimulate suspended fibroblasts and observed that the cortical acto-myosin network is a key subcellular component mostly contributing to the cytoskeleton strength, whereas the contribution of the microtubule network is secondary.

Although the previous studies have provided important insight into the mechanical response of different cells, simplifying assumptions about the cell shape and cytoskeleton thickness and compressibility cast doubt about the validity of the reported results. Eukaryotic cells typically contain several heterogeneous active polymer subcellular components, such as AC, intermediate filaments, microtubules, and nucleolus. In addition, the AC concentration, intermediate filaments, and microtubule structures continuously change as these components undergo active polymerization and depolymerization. Although Hertz-type contact models have been used to estimate the cell stiffness, these models do not account for several important effects, such as cell geometry, cytoplasmic fluid interaction, and load sharing by subcellular components. Cell remodeling and reorganization due to mechanical stimulus further increase subcellular complexity and could also lead to cell reshaping. Thus, it is necessary to consider more sophisticated models, which can account for the effects of active remodeling and reorganization of cell substructures on both material properties and shape (geometry) of mechanically probed live cells. Although, passive material characterization of composite media has been the focus of numerous studies, the complexity and active remodeling of subcellular structures have been major obstacles in quantifying cellular material properties.

The main objective of this study is to introduce a novel analytical–experimental methodology, which yields reliable estimates of the membrane–cytoskeleton equivalent elastic modulus by solving an inverse problem, using as input force–distance measurements obtained with an AFM-confocal microscope (CM) setup specifically designed to preform displacement-control compression tests of single cells. Experimental results are used to correlate the membrane–cytoskeleton equivalent elastic modulus to the cell shape and dimensions, which are controlled by special arrays of single-cell circular patterns [17,18]. The cell cytoskeleton is modeled as an AC assembly [19], whereas all other subcellular components suspended in the cell cytoplasmic gel (e.g., organelles and nucleus envelope) are modeled as an incompressible fluid. A statistical mechanics method commonly applied to network polymers is used to obtain a lower bound of the AC elastic modulus and, in turn, the equivalent elastic modulus of the membrane–cytoskeleton system. A numerical method for studying the plasma membrane–cytoskeleton elasticity is derived from a parametric finite element analysis (FEA). The numerical technique is then applied to actual experimental data of fixed and live hMSCs to obtain estimates of the membrane–cytoskeleton elastic modulus, which are compared with previously reported experimental results to validate the developed method.

2. Experimental methods

2.1. Single-cell patterning of culture dishes

Single hMSCs were attached onto circular patterns of partially exposed glass dishes (MatTek, Ashland, MA) coated with a nonfouling polyethylene glycol (PEG)-like film, as described previously [17]. Briefly, PEG-like films were grafted onto glass culture dishes by plasma polymerization of 99.9% pure diethylene glycol dimethyl ether vapor (Sigma–Aldrich, St. Louis, MO). The coated dishes were then covered by a soft shadow mask of polydimethylsiloxane (PDMS) with circular windows, and the PEG-like film was locally removed by oxygen plasma etching through the mask windows. Finally, the PDMS mask was carefully removed to prevent the detachment of the PEG-like film from the dish.

A total of 81 circular patterns of 50 μm in diameter (i.e., 2000 μm^2 circular pattern areas) were produced on glass culture dishes by the aforementioned process. The pattern yield was about 90%. The number of single cells attached to circular patterns depended on cell concentration, incubation time, and quality of nonfouling film. Hence, the number of single cells seeded on circular glass areas was always less than the number of circular patterns on the culture dishes.

2.2. Single cell culture and staining

A vial of frozen hMSCs in passage 7 obtained from a healthy 19-year old male of unknown race were isolated by Lonza (Walkersville, MD), thawed, cultured, and incubated at 37 °C with 5% CO_2 for 2 weeks in mesenchymal stem cell basal medium (MSCBM) containing 50 mL of mesenchymal cell growth supplement, 10 mL of L-glutamine, and 0.5 mL of GA-1000 (Lonza, Walkersville, MD). After incubation, the cells were passed onto four dishes with 2000 μm^2 circular patterns. The cells seeded on two of the dishes were fixed with 4% paraformaldehyde,

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