A Computational Framework for Predicting Cell-Specific Nucleo-Cytoskeletal Forces in Mesenchymal Stem Cells

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INTRODUCTION: Understanding the influence of mechanical forces on cell function and fate is crucial in unraveling the intricate mechanisms that govern cellular behavior. The cytoskeleton, a dynamic network of protein filaments, plays a pivotal role in sensing and transmitting mechanical cues within cells. The nucleus relies on cytoskeletal mechanical input through nuclear envelope adaptor proteins to sense external stimuli and respond by regulating intra-nuclear chromatin organization. This research provides a means for examining the interplay between mechanical forces and the cytoskeleton in regulating various cellular processes, including cell adhesion, migration, division, and differentiation. Through studying and simulating the cellular response to mechanical forces, this research aims to bridge the gap between mechanics and biology, uncovering the interrelation between physical forces and biochemical signaling. The developed computational framework reliably reconstructs nucleo-cytoskeletal morphology and computes cytoskeletal force on the nuclear surface via finite element (FE) analyses. Utilizing both manual and automated tracking techniques, the 3D nuclear geometry and relative location of F-actin stress fibers were extracted from confocal microscope images. This novel approach generates cell-specific FE models that incorporate i) F-actin configuration around the perinuclear region and ii) nuclear morphology to estimate nuclear envelope tension produced by F-actin cytoskeleton.

METHODS: Confocal Z-stack images of MSCs were taken for two cell states: Intact MSCs and Isolated Nuclei. The nuclear morphology was analyzed for the confocal image stacks and nuclear height, width, and length were computed for corresponding image planes top (X-Y), side (X-Z), side (Y-Z). The isolated nuclear geometry was computed to be ellipsoidal. The confocal images of Intact MSCs were then used for an uncoupled analysis of cytoskeletal organization across the apical surface of the nucleus or "actin cap". An emphasis was placed on tracking apical stress fibers, as it is the location in which the cytoskeleton and nucleus become mechanically coupled[1]. The XY coordinates for each fiber across the center plane of the nuclear geometry, where x was assumed to be equal to zero, were recorded for subsequent vertical projection onto the ellipsoidal nuclear surface.

This study computationally depicts the composition of MSCs through three primary components: the Nuclei, F-actin Stress Fibers, and scaffold. The cellular model components were reconstructed using a combination of computational resources, such as Amira, Hypermesh, and MATLAB. The intricate cytoskeletal arrangement around the nucleus was simplified into parallelly aligned fibers that span the y-axis. The geometric surface for the actin stress fibers were aligned in parallel across the y-axis and ran in tangency along the surface of the sphere. The fibers were 0.2 micrometer wide and spanned the top 140 degrees of nuclei. After manually recreating the idealized cellular geometry, the model components were imported into Abaqus/ CAE 2021 to be parameterized for optimal performance. A height and shape comparison of the intact nuclear model and the isolated nuclear model after being deformed was used to verify the validity of the model behavior.

RESULTS: The model behavior was evaluated in relation to Young's Modulus, Poisson's ratio, and nuclear volume. The reconstructed cellular model was examined against the control conditions at +/- 50% of the control parameters. The most significant effect on model behavior, and subsequent nuclear deformation, was due to change in Young's Moduli. There was a significant decrease in force needed to distort the nucleus for elastic moduli's between 0.5 to 1 Kpa (**Figure 1)**. Contrarily, changes in Poisson's ratio proved to be least impactful to the system. Deforming the initial nuclear model to a target of apical intact height of 9.9 µm required a uniform force of 1.498 µN applied to 15 modeled actin stress (**Figure 2**). The next step of this study aims to create a model capable of tracking forces unique to individual actin fibers, which will in turn promote more accurate correspondence to the intact nuclear geometry.

DISCUSSION: In this study we have developed a means of automated tracking of apical cytoskeletal arrangement from confocal images and producing cell-specific finite element models of nuclei, analyzed *in silico* to determine cytoskeletal forces on the nuclear surface. Assumptions made for modeling may limit the physiological accuracy of the data. However, this innovation provides a generalized method for tracking complex forces that are largely unknown and rarely investigated due to specialized experimental setup requirements.

SIGNIFICANCE/CLINICAL RELEVANCE: Mechanical signals have been acknowledged as influential in various physiological and pathological processes, such as tissue development, wound healing, immune response, and cancer progression. Yet still, current research is limited in its ability to quantify and predict internal forces without the use of expensive experimental setup, of which can only produce static results. Imaging techniques such as super-resolution fluorescence microscopy and electron microscopy have revealed the complex architecture and dynamics of actin structures, however reconstructing the interconnected actin stress fibers of the actin cap remains a technical barrier. Here, we provide a framework for computational reconstruction of cell-specific morphology and actin cytoskeleton configuration that utilizes a novel machine learning algorithm for cell-specific data extraction. This is a novel tool, enabling our ability to make cell-specific cytoskeletal force predictions and improve targeted tissue development down the line.

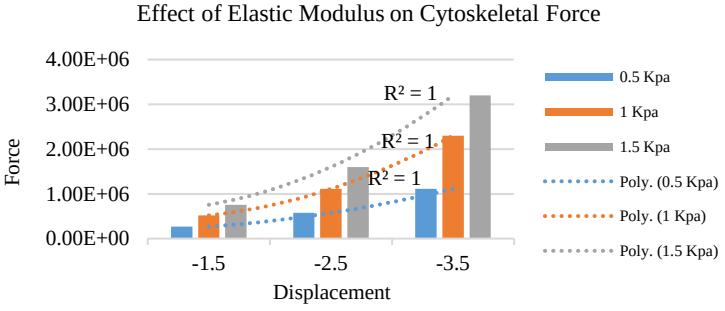

Fig.1 Effect of Nuclear Elastic Modulus on Actin Stress Fiber force input needed to displace the unloaded nuclei to the target Intact geometry height and profile.

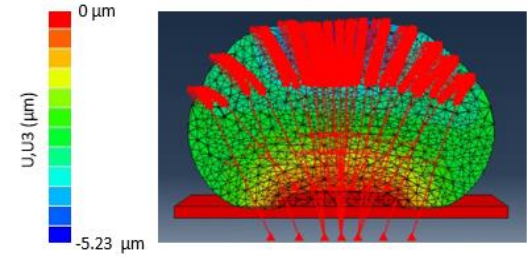

Fig.2 Cross-sectional view of Z-displacement in ABAQUS. FE model displaced 5.23 µm to reach the measured target nuclear height of 9.9 µm.

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