Effects of Cell-Cell and Cell-Matrix Interactions on Vascular Smooth Muscle Cell Mechanical Properties

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Statement of Purpose: Most tissue-level mechanical models assume homogeneous mechanical properties within a single cell type. However, measurements of cellular mechanical properties show large variability in whole-cell mechanical properties between cells from a single population. This heterogeneity has been observed in many cell populations and with several measurement techniques but the sources are not yet fully understood [1]. It is important to account for this heterogeneity before incorporating realistic levels of variability for *in vivo* conditions in a multi-cell tissue-level model.

We hypothesize that this cellular mechanical heterogeneity is due to varying local microenvironment conditions (more neighboring cells or matrix components in one location than another). Cells physically couple their tensed cytoskeletal filaments to underlying matrix scaffolds through focal adhesion complexes (clusters of integrins) and to neighboring cells through adherens junctions. Integrin β1 mediates cellular interactions with collagen through α2β1 and fibronectin through α5β1. N-cadherin-based adherens junctions help to physically couple the cytoskeleton of one cell to that of its neighbor. In this study, antibodies will be added to the media to block N-cadherin and integrin β1 interactions. These antibodies will limit cell-cell and cell-matrix interactions and allow us to investigate diversified cellular interactions as the source of mechanical heterogeneity that has been observed in previous studies.

Methods: Glass coverslips were coated with thin layers of 1 mg/ml solutions of collagen and fibronectin. Vascular smooth muscle cells (VSMCs) (between passages 5 & 8) were seeded on the coverslips at 30,000 cells/cm^2 (subconfluent layer). For the test groups, the VSMC media was supplemented with 50 µg/ml anti-Ncadherin, 50 µg/ml integrin β1, or 50 µg/ml of both antibodies to block cell-cell and/or cell-matrix interactions. The cells were cultured under standard conditions, with the specific media exchanged every 48 hours. On day 5, AFM (Asylum Research MFP-3D) cytoindentation experiments were performed using a 5 µm diameter borosilicate spherical-tipped probe on a silicon-nitride cantilever (spring constant ~0.12 N/m). The cells remained on their matrix-coated coverslips throughout the study, with warm (37ºC) media exchanged every 30 minutes. Twenty cells on each sample were indented 5 times to ~1 µm depth at 1 µm/sec. The elastic modulus was estimated by fitting the Hertz model to the first 300 nm of indentation. Each cell was also subjected to two 1 µm step indentation and 60 second hold (stress relaxation) experiments. The Quasilinear Viscoelastic Model (QLV) was fit to the relaxation data to give a measure of the percentage of relaxation during the hold. Immunofluorescence imaging was used to confirm antibody blocking (secondary antibodies to anti-N-caherin and anti-integrin β1) and to visualize the cytoskeletal arrangement within the cells (Alexa Fluor 488 phalloidin to label filamentous actin, rhodamine anti-α-tubulin to label microtubules).

Results: For the VSMCs cultured on collagen, the elastic modulus measures decreased for cells with antibodies in the media in comparison to cells with regular media (Figure 1). The cells with both antibodies in the media were the least stiff of all samples. This was expected as cells that are allowed to adhere to matrix proteins and neighboring cells can form a more complex cytoskeleton and in turn become stiffer. The cell-to-cell variation in mechanical properties remained high for the control cells (for both elastic modulus and percentage relaxation measures). The average coefficient of variation (COV = standard deviation/mean) measure for samples without antibodies was 115.86% (consistent with variability seen in other AFM studies). The average cell-to-cell COV measure for samples with antibodies decreased substantially to 57.28%. By limiting the cellular interactions, the microenvironment variability within the sample decreased and, in turn, the cells exhibited decreased heterogeneity in their mechanical properties.

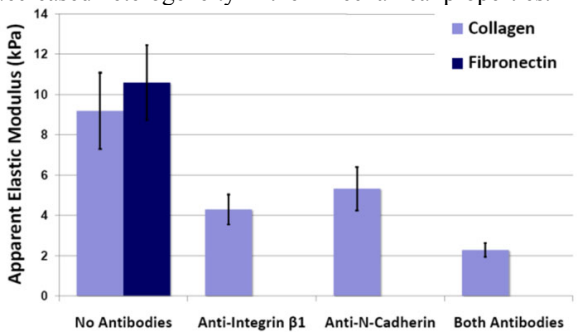

Figure 1. Apparent elastic moduli of day 5 VSMCs on collagen and fibronectin with different media conditions. Data presented as mean ± standard error.

Conclusions: Blocking cell-cell and cell-matrix interactions inhibited physical coupling between the cell cytoskeleton and extracellular components, resulting in decreased elastic moduli measures on collagen substrates. We are currently investigating the effects of the antibodies on VSMCs on fibronectin substrates. We anticipate that the VSMCs with antibodies on fibronectin will be stiffer than those on collagen, as cells bind to fibronectin through integrin β3 in addition to integrin β1. The reduction in mechanical variability among cells under blocking conditions supports our hypothesis that microenvironment variability is to blame for the variability that is observed in mechanical properties among cells from a single population. Researchers may use these results to consider heterogeneity in the cellular microenvironment *in vivo* and incorporate realistic levels of cellular heterogeneity in tissue-level mechanical models. Such models may help to better understand tissue behavior in both health and disease.

References: [1] Jaasma, MJ. Ann Biomed Engr. 2006;34:759-768.