Effects of Blocking Cell-Cell and Cell-Matrix Interactions on Cardiac Cell Mechanical Properties

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Statement of Purpose: Cardiovascular diseases like atherosclerosis are number one cause of death in United States. In an atherosclerotic artery, vascular smooth muscle cells undergo a phenotypic shift; enter the intima layer and deposit extracellular matrix. This makes the cellular environment very heterogeneous. There is a close association between cellular mechanics and diseases. Most tissue-level models developed over past two decades assume homogenous mechanical properties within a single cell type. However, measurements of cellular mechanical properties show a 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]. Our goal is to understand this heterogeneity and incorporate realistic levels in tissue level models. This will aid towards developing a cost effective method to predict behavior in both healthy and diseased tissue. Cell behavior is largely dependent on interactions with neighboring cells and extracellular matrix components. Based on our hypothesis, our lab studied the effects of blocking cell-cell (N-cadherin) and cell-matrix (integrin B1) on VSMC. The results showed that VSMCs under such conditions were less stiff, more relaxed, and took on a more synthetic phenotype after 5 days in culture. Our current study focuses on blocking cell-cell and cell-matrix interactions in cardiac cells (cardiomyocytes).

Methods: Cardiomyocytes used in this study are dissected from neonatal (day 3) rat hearts. Six well culture plates are used in this study. Coverslips used for cell culture are cleaned in 100% alcohol and distilled water, followed by overnight UV sterilization. The surface of the coverslips is than coated with 1mg/ml of fibronectin. Cardiomyocytes are seeded at the density of 50,000cells/cm^2 and cultured for 5 days. Throughout the culture period, 3 different media conditions are used: regular media (DMEM+10%FBS+1%anti/anti) (control), regular media with 50µg/ml integrin β1 antibody, and regular media with 50µg/ml of N-cadherin antibody. On day 5, Atomic Force Microscopy (AFM) (Asylum Research MFP-3D),

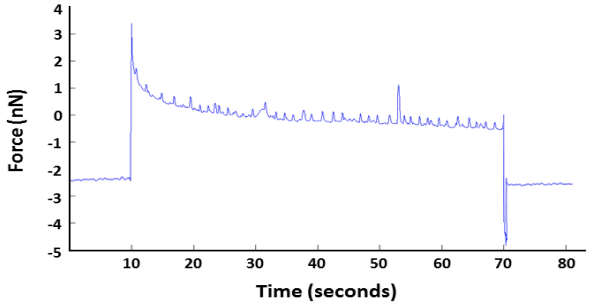

Figure 1: Sample cardiomyocyte stress relaxation curve. Spikes in data represent cellular contractions (beating). Raw data and the relaxation portion of the data after normalization with overlying QLV and SLS model fits

cytoindentation experiments are performed using a 5-µm diameter borosilicate spherical-tipped probe on a silicon-nitride cantilever (spring constant ~0.12 N/m). Twenty cells on each sample are indented 5 times to ~1 µm depth at 1µm/sec to get 5 force curves and 2 stress relaxation curves per cell. Immunofluorescence imaging is used to confirm antibody blocking (secondary antibodies to anti-N-cadherin 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: Blocking N-cadherin and integrin β1 interactions individually and in combination resulted in greatly reduced cellular (VSMC) elastic moduli measures (less stiff) and increased cellular percent relaxation measures (more viscous). The average cell-to cell elastic modulus coefficient of variation (COV) for the control conditions was 71.7%, very close to the average level of variation in VSMC elastic moduli measures in the previous study (70.3%). This measure dropped fairly uniformly from the control samples to the test (antibody) samples to an average of 44.8%. Currently, we are doing AFM mechanical testing on cardiomyocyte studies.

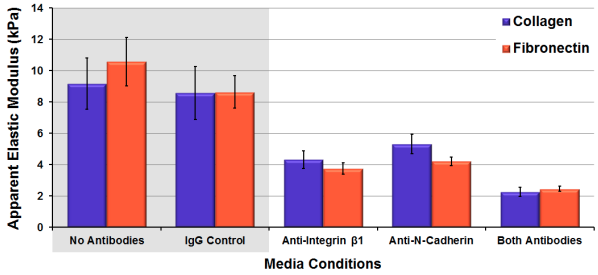

Figure 2:Apparent elastic moduli of day 5 VSMCs on collagen and fibronectin with different media conditions. Data presented as mean ± standard error

Conclusion: From VSMC studies we concluded that the cells under the test conditions were more homogeneous in their mechanical properties (elastic moduli), than the cells under the control conditions. This was the first significant drop in cellular mechanical heterogeneity within a sample population that was observed in this study. It suggests that varying cell-cell and cell-matrix interactions are at least in part responsible for the high level of heterogeneity that is commonly observed in 2D cell culture studies. The repeated point COV measures were much lower than the cell-to-cell COV measures, indicating that variations in the measurement technique could not account for the cell-to cell variations that were observed. We hope to observe similar results in cardiac cells studies to better understand cellular mechanical heterogeneity and incorporate them in tissue level models.

References: [1] Jaasma, MJ. Ann Biomed Engr. 2006;34:759-768.[2] Tse, J. Current Protocols in Cell Biology, 2010. 10(16). [3] Wang, YL. Mol. Motors and the Cytoskeleton. 1998: 489-496.