Characterizing Effects of Microenvironments on Mechanical Properties of Cardiac Microtissues

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Statement of Purpose: Since the advent of routine cell culture more than 45 years ago, the most common cell culture approach has been two dimensional (2D) on cell culture polystyrene or glass surfaces. However, 2D culture is far too simplistic and overlooks many parameters important for tissue physiology including mechanical cues, communication between cell and its matrix and communication between adjacent cells. 3-Dimensional culture is a bridge to connect this gap of 2D cell culture and whole tissue. It gives a better representation of physiological conditions. So we plan to study mechanical properties on cells under 3D culture. Most tissue-level models assume homogeneous mechanical properties within a single cell type.[1-5] But, in reality large variability in whole-cell mechanical properties between cells from a single population exists[6-9]. The goal of our project is to understand causes of cellular mechanical heterogeneity experimentally in 2D & 3D and incorporate realistic levels in tissue-level models. To this end, one of our aims is to investigate effects of blocking intercellular and cell matrix interactions on cell mechanics in a 3D environment. For the purpose of this project, we use hanging drop spheroid culture technique for our 3D cell culture. The results obtained from our experimental data will be incorporated into multicellular mechanical model.

Methods: Cardiomyocytes were obtained from 3 day old neonatal rat hearts and cultured using hanging drop technique to form cardiac microtissues (spheroids) on a petri dish. Various density of cells from 200 cells/spheroid to 40,000 cells/spheroid were used to create spheroids and find the optimum density that gives most spherical microtissue that is viable over time in culture. A live-dead assay was also performed to confirm the viability of spheroids overtime. To study mechanical properties (elastic modulus), Atomic Force Microscopy (AFM) nanoindentation experiments were performed various time points to measure the elastic modulus of cardiac microtissues. AFM was perfomed using silicon nitride cantiliver with borosilicate probe with 5µm diameter. Data obtained from AFM was fit to Hertz model to obtain elastic modulus. Stres relaxation reponse of cells were also measured using AFM. Quasilinear Viscoelastic (QLV) model was fit to estimate percentage relaxation during 60sec hold. Spheroids were also treated with media containing a 10 mM solution of proliferation inhibitor (ara-C) to limit fibroblast growth and achieve a relatively pure myocyte culture even at late culture time points. Cells were also cultured in 96 well plates for 12 days and proliferation assay was performed overtime to confirm fibroblast inhibition. To study the effects of microenvironments on cell mechanics, cell matrix (integrin β1) and cell cell interactions (connexin 43) were blocked using anti-integrin β1 antibody and anti connexins 43 antibody respectively.

Results: It was observed that cells start to aggregate and form spheroids on an average by 3 days in culture. Lower density spheroids are very small and do not give a microtissue where as higher density spheroids do not aggregate very well and have a disintegrated layer of cells around a central sphere. Spheroids were observed to be viable over a period of 12 days in culture from live/dead analysis. It was also observed that 10mM concentration of ara-C helps inhibit fibroblast proliferation in culture. As far as mechanical studies are concerned, we are still analyzing AFM data. However, from previous studies done in our lab on VSMC, it was concluded that the cells under the test conditions (blocking cell-cell and cell-matrix interactions) 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. 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. We except similar results with cardiac cells as well.

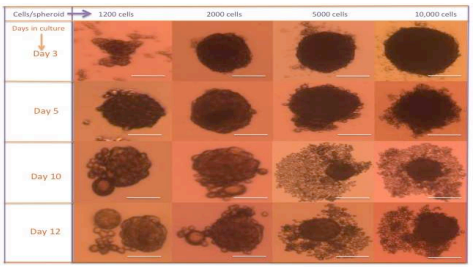

Figure 1: Cardiac microtissuecultured in hanging drop at various time points

Conclusions: Previous study in our lab with Vascular Smooth Muscle Cells in 2D has shown that blocking N-cadherin and integrin β1 interactions individually and in combination resulted in more synthetic-looking vascular smooth muscle cells with reduced elastic moduli and increased percent relaxation measures. We hope to see similar trend with our 2D and 3D studies on cardiac cells. Finally, our goal with this project is to develop a multicellular model that can predict the mechanical behavior of cells in response to stimuli. This can aid in increasing the speed and decreasing the cost of drug development, tissue engineering, and regenerative medicine therapies, thereby possibly increasing the quality and longevity for many people.

References: [1] Vonmaltzahn, Journal of Biomechanics, 1984[2] Kilpatrick, Journal of Biomechanical Engineering, 2001[3] Holzapfel, European Journal of Mechanics, 2002[4] Wuyts, Physics in Medicine and Biology, 1995[5] Armentano, Circulation Research, 1995[6] Charras, Biophysical Journal, 2002 [7] Hemmer, Proc. Institution of Mech. Engineers Part H-Journal of Engineering in Medicine, 2008[8] Jaasma, Annals of Biomedical Engineering, 2006[9] Jones, Journal of Biomechanics, 1999