Polyacrylamide Gels with Various Stiffness for the Study of the Cardiac Fibroblast Migration

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Statement of Purpose:

Cardiac cells, which mainly include myocytes and fibroblasts, have been shown in many studies to be sensitive to their mechanical environment [1]. Our lab is in the progress of developing a spatially and temporally controlled traction force imaging system. The purpose of such a system is to create accurate patterns of cells for the analysis of myocyte beating force as well as cell-cell and cell-matrix mechanical coupling. In this study, we characterize the time dependent mechanical properties of our acrylamide gels to accurately model the mechanics of the dynamic cardiac cellular environment in vitro. In addition, our secondary goal is to assess the changes in migratory force of cardiac fibroblasts on gels of various moduli. Cardiac fibroblast mechanical coupling is important during cardiac repair and by using substrates of various moduli we can begin to build a more accurate in vitro model for the cardiac cell microenvironment [2].

Methods:

Polyacrylamide gels were prepared on #1 glass coverslips. After cleaning the surface with NaOH and washing with distilled water, 3-aminopropyltrimethoxy silane was applied to the surface for 5 min and then washed in a shaker for 1 hour. After this, the slips were placed on a test tube rack and 0.5% gluteraldehyde was added until it covered the surface and allowed to sit for 30 min. After which the slips were cleaned in distilled water in a shaker for 1 hour yielding activated slips.

Acrylamide gels were prepared according to Table 1:

Final Acryl/Bis	40% Acrylamide (µl)	2% Bis (µl)	Young's Modulus (kPa)
8%/0.08%	1000	200	75
8%/0.05%	1000	125	23
5%/0.025%	625	63	7

Table 1. Composition of acrylamide gels and their Young's Modulus

For each sample contains 20µl of 0.2 µl fluorescent beads, which is used for traction mapping. 20 µl of each gel solution was added to an activated slip and sandwiched with another clean non-activated slip. After removal of this top slip, the gels were gently washed in 50mM HEPES. A sulfo-SANPAH solution was prepared and applied to the gels, which allows the attachment of a Vitrogen 100© collagen solution.

Testing of Young's Modulus was done by the use of AFM. The gels were then placed in a culture dish and day 3 neonatal cardiac fibroblasts were deposited on the surface at 5000 cells/gel. The gels were then placed into the onstage incubator of a Zeiss 200M Axiovert microscope and images were taken every minute for 2 hours.

Results/Discussion:

Figure 1 shows the results of testing the depression depth of the AFM tip in gels of varying stiffness.

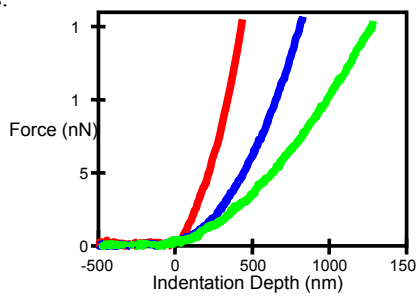

Figure 1. Force curve showing AFM tip depression in gels of various elastic moduli. (75 kPa – Red, 23 kPa – Blue, 7kPa – Green)

Each gel showed little variation in the elastic modulus at different points on the surface indicating good uniformity. The polyacrylamide gels were found to be nearly linearly elastic for the small deformations relevant to cell migration.

Figure 2 shows a typical image obtained using traction force microscopy:

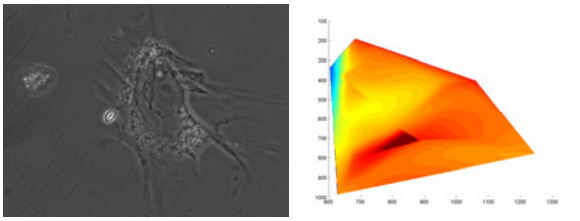

Figure 2. Images of a cardiac fibroblast and its traction force map

Typical tractions of migratory fibroblasts on the 75 kPa gels were found to be ~ 65µPa. Images also indicated that the area of traction on all gels was larger than the area of the cell indicating that these traction forces may play a part in cell-cell or cell-matrix signaling.

Conclusion:

Cardiac fibroblasts were found to exert forces large enough to potentially affect proximal cells. This agrees with in vivo studies hinting that mechanical coupling of fibroblasts in the heart both between cells and through the extra-cellular environment could propagate over larger distances [3]. Our current on-going work focuses on the effect of substrate modulus on cardiomyocyte pulsatile forces and mechanical coupling.

References:

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