A Dynamic Platform for Recapitulating Healthy and Diseased Cardiovascular Microenvironments

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Statement of Purpose: Cardiomyopathies are characterized by changes in tissue stiffness, changes in the alignment of cells[1] and extracellular matrix proteins, as well as changes in gene expression of the cells themselves[2] Traditional cell culture approaches have revealed valuable insights into the mechanisms and potential treatments of cardiomyopathies. However, a cell culture platform that replicates additional aspects of the in vivo cellular microenvironment would allow future studies that are currently intractable. Here, we describe a dynamic cell culture platform that can be used to pattern cardiac cells in specific orientations while allowing *in situ* changes in substrate stiffness. Neonatal rat ventricular myocytes were cultured on poly(ethylene glycol) (PEG) hydrogels in aligned and random orientations, and we measured cellular orientations as well as gene expression in response to dynamic changes in substrate stiffness. Cells cultured on substrates with micron-scaled channels align uniformly along the channels and form monolayers of aligned cells similar to the native myocardium. In addition, fetal gene activation was attenuated in cells cultured on hydrogel substrates when compared to cells cultured on plastic substrates.

Methods: Neonatal rat ventricular myocytes (NRVMs) were isolated from neonatal Sprague-Dawley rats as previously described[3]. A photolabile PEG crosslinker (PEGdiPDA) was synthesized as previously described[4]. Gelatin was mixed into the monomer solution to promote cell adhesion. Monomer solutions consisted of 8.2 wt% PEGdiPDA, 6.8 wt% PEG monoacrylate (FW = 400), 1 mg/mL gelatin, 0.1M ammonium persulfate, and 0.2M tetraethylmethylene-diamine (TEMED). Gels were formed by pipetting monomer solution on to a siliconized glass slide and covering the monomer droplet with a glass coverslip treated with an acrylated silane. Substrates with channeled features were formed by a 10 min exposure of a polymerized gel sample to 365nm UV light at 10 mW/cm^2 through a chrome photomask to spatially control the reaction *In s*itu substrate softening was achieved after treating the cells with phenylephrine (PE), a drug known to induce unhealthy, fetal gene expression. Quantitative polymerase chain reaction (qPCR) was used to measure expression of several fetal genes: atrial naturetic factor (ANF) and alpha and beta myosin heavy chain isoforms (αMHC, βMHC). RNA was collected two days after substrate softening, and cDNA was synthesized. Sarcomeres were visualized by staining F-actin using fluorescently labeled phalloidin. Cell size and aspect ratio were measured using ImageJ software, and cell and sarcomere alignment were measured using Fourier analysis and a custom Matlab script.

Results: Treatment with PE resulted in increased cell area on all substrates with a lower cell area on PEG hydrogels. Cell aspect ratio was higher on PEG hydrogels with a trend towards increasing aspect ratio with decreasing substrate modulus. Quantitative analysis of cell and sarcomere alignment reveals that culture on channeled PEG substrates direct alignment of NRVMs into the nearly uniformly aligned patterns characteristic of healthy myocardial tissue while NRVMs cultured smooth PEG substrates are arranged in nearly random orientations similar to hearts with myofibrillar disarray (Figure 1).

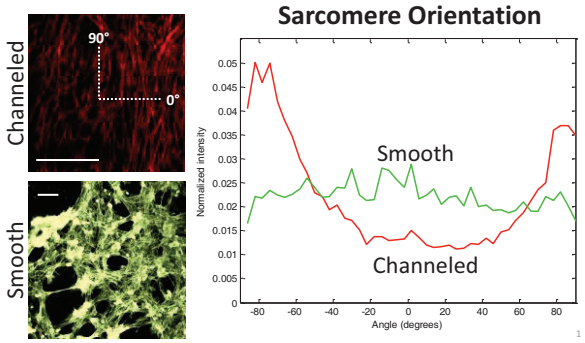

Figure 1. Cells cultured on patterned hydrogels form aligned sarcomeres that can be quantitatively analyzed.

The expected increase in ANF expression in response to PE treatment was attenuated in NRVMs cultured on PEG hydrogels with non-treated cells expressing a lower basal level of ANF. There was a trend for decreasing expression of αMHC with decreasing substrate modulus. The ratio of αMHC to βMHC expression was higher in cells cultured on PEG gels with a Young's modulus of 35kPa while cells cultured on 10kPa and substrates softened from 35 to 10kPa substrates had a lower ratio of αMHC to βMHC.

Conclusions: We engineered a dynamic cell culture platform for studying NRVMs that recapitulates aspects of the native healthy and diseased myocardium. Namely, the platform can be used to direct cell alignment, gene expression as well as substrate modulus. In addition, this platform facilitates analysis using both cell mechanics and molecular biology approaches. Gene expression results suggest that culturing cardiac myocytes on traditional plastic substrates results in an increase basal level of fetal gene activation that is attenuated by culturing on PEG hydrogels of varying substrate modulus. Work in progress includes embedding fluorescent beads for measuring cell traction forces and stresses, as well as *in situ* modulation of substrate topography mimicking other aspects of cardiomyopathies.

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