Effects of substrate composition and structure on the mechanical properties of cardiomyocytes in 2D culture

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Statement of Purpose: Cardiomyocyte phenotype changes significantly in 2D culture systems depending on the substrate composition (e.g. collagen type I vs fibronectin). [1, 2] Aligned matrices have been shown to promote in vivo-like myocyte phenotype in 2D cultures. [2] The changes in myocyte cytoskeleton can lead to differences in the cell stiffness that in turn can alter the cell's response to mechanical stresses. The goal of this study is to characterize the mechanical properties of cardiomyocytes in 2D culture on various substrates to better understand the variations in cardiomyocyte phenotype and function due to the diversified adhesion binding between the cardiac cells and their underlying substrate matrix.

Methods: Polystyrene dishes were coated with fibronectin or collagen (Invitrogen). 100 µl of collagen or fibronectin solution (10 µg/ml) was dropped onto the dishes and spread uniformly in a thin (~200 µm) layer and allowed to set for at least 15 min in an incubator at 37°C. The thin fibronectin or collagen layer on the surface had minimal effects on substrate mechanical properties. To align fibers, collagen was scraped in one direction prior to placing the dish in the incubator. This led to good alignment of fibrils in the center of the dish assessed by atomic force microscopy (AFM) imaging. Cardiomyocytes were harvested from day 3 neonatal rat hearts. The cells were seeded at 50,000 cells/cm^2 and cultured in DMEM +13% serum +cytosine β-D-arabino-furanoside+ aphotericin. At each time point, the dishes with media were placed in a Dimension 3100 AFM (Veeco). On each sample (fibronectin, unaligned, and aligned collagen), 15-20 cells were each indented 5x to ~1 µm depth at 5 µm/s using a borosilicate spherical probe (BioForce Nanosciences, spring constant, k~ 0.58 N/m, radius, R= 2.5 µm). After AFM indentation, the cells were fixed and F-actin was stained using phalloidin. Additional cells were cultured for 1 week on varying stiffness (7-75kPa) collagen coated acrylamide gels [3] before they were indented in the AFM. Elastic modulus, E, was estimated by fitting the Hertz model to the first 500 nm of indentation:

$$F = \frac{4}{3}\frac{E}{(1-\upsilon^2)} R^{\frac{1}{2}} \delta^{\frac{3}{2}}$$

where F is the measured force, υ is Poisson's ratio (0.5), δ is the indentation depth.

Results/Discussion: On all substrates, after only 1 day of culture, cardiomyocytes were found to have similar soft apparent elastic moduli of ~4-6 kPa (Figure 1). In addition, the elastic modulus of the cardiomyocytes was found to increase significantly during the first 5 days in culture on all substrate. The cells cultured on fibronectin were stiffer than those on collagen from day 3 onwards and they were observed to beat nearly 2x faster. The cells on aligned collagen were stiffer than those on unaligned collagen surfaces at all time points. No significant differences in hysterisis between the extend and retract curves were noticed at any of the time points or between any of the substrates although there was large variation between cells. This indicates that there were no significant changes in viscous properties of the cells on the different substrates. Even the stiffest cells in this study were softer than previously reported measurements on cardiomyocytes [4,5]. Cardiomyocyte elastic modulus was found to increase significantly with age [5] and so this difference is likely due to the very young (3 day) age of the rats used in this study. After 1 week in culture, cells on the softest acrylamide gels appeared to have a lower elastic modulus (Figure 2). However, the data is difficult to analyze using the standard Hertz model since it is difficult to decouple the movement of the underlying soft substrate from the indentation on the cell even for small indentation depth. The cells were stiffer on the stiffer gels. The modulus of the cardiomyocytes on the stiffest gel matched that of those cultured on the unaligned collagen polystyrene dish.

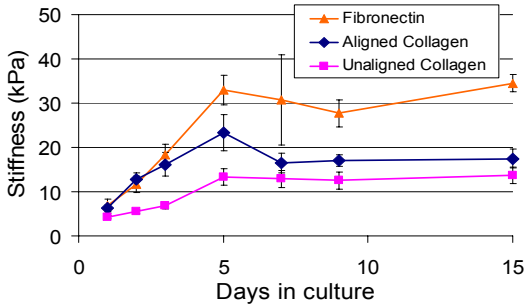
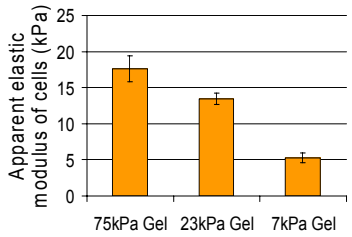


Figure 1. Average +/- s.e.m apparent elastic modulus as a function of time in culture of cardiomyocytes cultured on fibronectin, aligned, and unaligned collagen coated surfaces (n =15-20 cells).

Figure 2. Average +/- s.e.m apparent modulus of myocytes on collagen coated acrylamide gels of varying stiffnesses after 1 week in culture (n = 20 cells).

Conclusions: The compressive elastic stiffness of cardiomyocytes is dependant on both the composition as well as organization of the matrix they are cultured on. Underlying substrate stiffness also seems to have an effect. We are currently developing more complex theoretical models to analyze nanoindentation of cells on very soft underlying substrate to investigate this effect further.



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