Cardiomyocyte Response to Ultra Stiff Hydrogel Substrates

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Statement of Purpose: Substrate stiffness is known to have pronounced effects on the behavior of many cell types including cardiomyocytes, the beating cells of the heart.[1-3] While the native rat heart has a modulus of ~10-20 kPa[4], substantially stiffer substrates have been employed to study cardiomyocytes *in vitro* (e.g., TCPS with a modulus of ~1 GPa[5])[6] and have been successfully employed as cardiac patches in rat infarct models showing improved contractility.[7] The objective for the present study was to investigate whether cardiomyocytes isolated from neonatal rat ventricles would mature when cultured on ultra stiff substrates. A poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel system was used, which can readily be tuned to a range of moduli, through changes in crosslinking density, while maintaining the hydrogel chemistry and protein attachment. As a first step, cardiomyocyte maturation was evaluated through expression of genes that are known to change during maturation: the ratio of α- to β-myosin heavy chain (MHC), atrial natriuretic protein (ANP), and sarco(endo)plasmic reticulum Ca^{2+}ATPase (SERCA2a).

Methods: A solution of 80% (v/v) 2-hydroxyethyl methacrylate (HEMA) (Polysciences, Inc), either 0.1, 0.02, 0.0002 or 0.00002 (mol: 1 mol HEMA) tetraethylene glycol dimethacrylate (TEGDMA) crosslinker (Polysciences, Inc), and photoinitiator were polymerized under 320-500 nm UV light (5 mW/cm^2) for 6 min, then punched into discs (5 mm diameter, 0.8 mm thick). Collagen I was covalently bound to pHEMA via a 1,1-carbonyldiimidazole intermediate.[8] The tangent modulus in compression mode (15% /min to 40% strain) was determined on hydrated cylindrical gels (5mm diameter/5mm thick).

Neonatal rat ventricular myocytes were isolated from 1-3 day old Sprague-Dawley rats by trypsin digestion and pre-plated for 45 min to enrich for cardiomyocytes. Cardiomyocytes were seeded onto pre-soaked scaffolds at a density of 45,000 cells/cm^2. After 24 h, medium was exchanged, and was changed every 48 hours thereafter. Gene expression of α- and β-MHC, ANP and SERCA2a in cardiomyocytes was compared to the stable housekeeping gene 18S, and analyzed using real-time RT-PCR after 1 and 7 days of culture. A sample size of 3 was used. A one-way ANOVA was performed ($p<0.05$ considered significant).

Results: Poly(HEMA) hydrogels of four crosslinking densities yielded average modulus values of 350 ± 25, 425 ± 54, 2000 ± 105, and 4760 ± 695 kPa (referred to as 350, 425, 2000, and 4800 kPa, respectively).

In vivo, the ratio of α-/β-MHC isoforms increases as the rat heart develops. In response to ultra stiff hydrogel substrates immobilized with collagen I, the ratio of α-/β-MHC expression did not change within cardiomyocytes cultured on the softer substrates, 350 and 425 kPa, but the mean values for the ratio decreased for the stiffer substrates, 2000 and 4800 kPa, from day 1 to day 7, although this decrease was not statistically significant (Fig. 1a). The expression of ANP, a signaling protein which falls in the developing heart, was found to increase from day 1 to day 7 for the softest substrate, although not significantly. There was a significant drop in ANP expression on the stiffest substrate (4800 kPa) from day 1 to day 7 (Fig. 1b). Additionally, ANP expression on the 4800 kPa hydrogels was significantly lower than the 425 kPa hydrogels by day 7. The mean expression levels for SERCA2a, a protein important for calcium transport and which increases in the developing heart, increased with culture time, but was not dependent on stiffness (Fig. 1c).

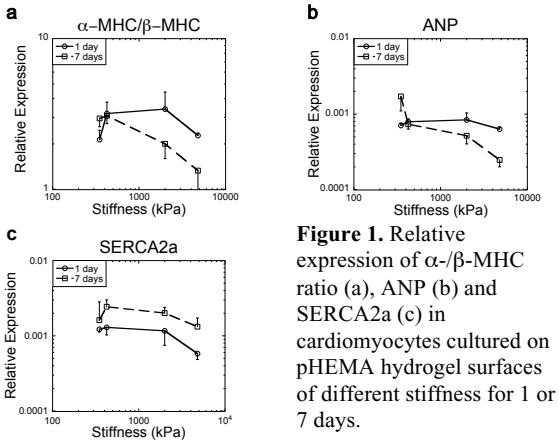

Figure 1. Relative expression of α-/β-MHC ratio (a), ANP (b) and SERCA2a (c) in cardiomyocytes cultured on pHEMA hydrogel surfaces of different stiffness for 1 or 7 days.

Conclusions: Maturing neonatal cardiomyocytes *in vivo* demonstrate an increase in α-/β-MHC and SERCA2a expression with a simultaneous decrease in ANP expression. Over the course of 7 days in an *ex vivo* environment, the gene expression response of cardiomyocytes cultured on stiff hydrogel substrates did not indicate a distinct maturation to what is observed *in vivo*. Nonetheless, there are interesting trends worth noting. Although the ratio of α-/β-MHC expression did not increase over time on the different substrates, the ratio remained higher for softer substrates after 7 days in culture. Additionally, the expression of SERCA2a showed an increasing trend on more compliant hydrogels and for longer culture times. However, ANP expression was the highest on more compliant hydrogels by the end of the study, which has been observed in cardiomyocytes cultured on PLGA scaffolds.[9] These findings suggest cardiomyocytes, when cultured on collagen immobilized ultra stiff pHEMA hydrogels, exhibit some signs of maturation, particularly on softer substrates. However, additional studies are necessary to elucidate the long-term effects of cardiomyocytes cultured on stiff substrates.

References: [1]Engler AJ. J Cell Sci. 2008;121:3794-3802. [2]Jacot JG. Biophys J. 2008;95:3479-3487. [3]Bhana B. Biotechnol Bioeng. 2010;105:1148-1160. [4]Berry MF. Am J Physiol Heart Circ Physiol. 2005; 290:H2196-H2203. [5]Miyake K. App Phys Lett. 2006;89. [6]Sucharov CC. Am J Physiol Heart Circ Physiol. 2006;291:H1299-H1308. [7]Fujimoto KL. J Am Coll Cardiol. 2007;49:2292-2300. [6]Martin SM. J Biomed Mater Res A. 2003;67A:334-343. [9]Brown DA. J Biomed Mater Res A. 2005;74A:419-429.