Microtopographical Effects on Differentiation of Embryonic Stem Cells into Cardiomyocytes

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Statement of Purpose: Cardiovascular disease is the leading cause of death in the United States and its prevalence is increasing with time (CDC, 2013). During a heart injury, such as myocardial infarction, cardiomyocytes are damaged and cannot be regenerated (Schneider et al. 2000). Left untreated, this damage can have fatal results. Due to organ shortage, lack of tissue grafts for transplantation, and lack of success from current therapies, cardiac tissue engineering is a potential approach to replace damaged muscle and treat heart injuries. For cardiac tissue engineering to be successful, elasticity and surface topography are important factors to consider. Elasticity of the scaffold is vital for proper expansion and contraction of the muscles during beating, while surface topography ensures a highly ordered alignment of cardiomyocytes for effective transduction of electrical signals. Our objective is to understand the effect of surface topography on differentiation of mouse embryonic stem cells (mESCs) into functional cardiomyocytes. To accomplish this objective, hydrogels will be employed as the supporting matrices due to their tailorability, viscoelastic nature, and tissue-like characteristics, while mESCs will be the cell source, as they have the ability to differentiate into cardiomyocytes. Validation of cell attachment and differentiation will be completed using surface morphology analysis, histochemical analysis, and PCR to detect expression of cardiac specific genes.

Methods: Hydrogel scaffolds are synthesized using free-radical polymerization of various blends of poly(ethylene glycol) dimethacrylate (PEGDMA) and methacrylic acid (MAA). The micropatterned glass slides are used during photopolymerization process to produce micropatterned hydrogel surfaces. The patterned hydrogels are rinsed for 10 days, sterilized and surface treated to promote cell attachment. Embryoid bodies (EBs) are generated from mESCs using the hanging drop technique and are seeded onto the three different surfaces: unpatterned surface (control), microchannels (width 700µm), and squares (700 x 700µm) and monitored for attachment, alignment, and differentiation into cardiomyocytes. EB seeded hydrogel scaffolds are evaluated using histochemical analysis to visualize the nuclei, actin filaments and myosin heavy chain within the cardiomyocytes on the micropatterned features.

Preliminary results:

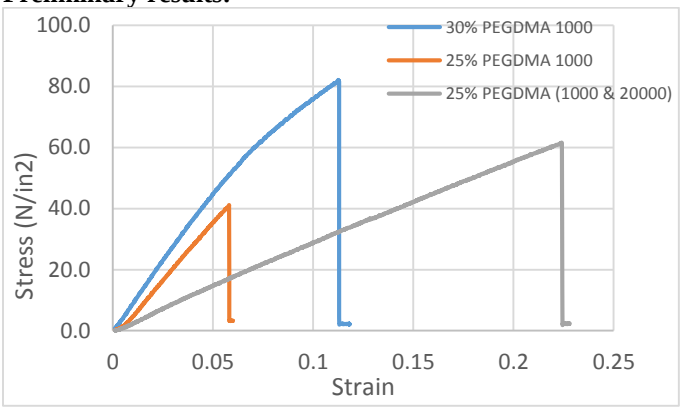

Figure 1: Stress vs. Strain plot of different PEGDMA polymer concentrations and molecular weights to determine the elastic modulus.

The stress-strain curves were plotted to obtain the elastic modulus. The 30% PEGDMA M_w 1000 displayed the highest elastic modulus (**1400 kPa**), 25% PEGDMA M_w 1000 (**1100 kPa**) while 25% blend PEGDMA with the lowest elastic modulus (**400 kPa**). The decrease in polymer concentration with higher molecular weights result in more elastic hydrogels.

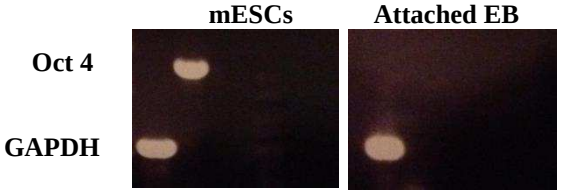

Figure 2: An ethidium bromide stained agarose gel showing cDNA fragments produced by PCR amplification.

The absence of Oct 4 expression, a marker of pluripotency, in differentiated EBs is an indicator of differentiation while GAPDH, a loading control was present both in ESCs and differentiated EBs.

Conclusion: To engineer cardiac tissue in vitro requires evaluating the influence of underlying substrate on differentiation of ESCs towards cardiomyocytes. The information gathered from this study can be used to improve generation of cardiac tissue constructs mimicking native tissue present in vivo. A combination of other biomimetic cues, such as electrical stimulation is a future approach to improve the platform used for engineering cardiac tissues in vitro.