Controlled Electrophysiological Maturation of Stem Cell-Derived Cardiomyocytes

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Statement of Purpose: Creation of a viable cardiac tissue model would further the development of novel clinical treatments for patients with heart disease while providing researchers with an accurate platform for drug arrhythmogenicity screening. The effect of nitric oxide (NO) on cellular signaling pathways associated with cardiomyogenesis has been investigated by other groups (1, 2); however, the effect of NO addition on the electrophysiological properties of stem cell-derived cardiomyocytes has not been elucidated. In previous work, NO-generating ligands have been incorporated into the backbone of poly(ethylene glycol) hydrogels to provide localized NO delivery. In this study, a NO donor and nitric oxide synthase (NOS) inhibitor were periodically introduced into culture media supplied to maturing embryoid bodies (EBs). We examined the spontaneous contractile activity of EBs over a two week time frame to assess the effect of NO concentration on electrophysiological maturation.

Methods: Murine embryonic stem cells (mESCs) were expanded on murine embryonic fibroblast feeder cells in the presence of LIF; following a feeder layer subtraction, mESCs were formed into EBs using the hanging drop method. At day 2 of differentiation, EBs were transferred to culture in suspension. Culture dishes were coated with poly (2-hydroxyethyl methacrylate) (polyHEME) to prevent cell-surface interactions. On even numbered days starting on day 2, EB suspension cultures were supplemented with the NO donor S-nitrosocysteine (CysNO) (10, 50, or 100 μM) or NOS inhibitor N-nitro-L-argenine methyl ester (L-NAME) (1 mM); subsequently, spontaneous beating activity was assessed by determining the EB beating percentage and beat frequency for each culture condition. To determine the viability of differentiated cells exposed to each culture condition, EBs were isolated at day 18 and stained with ethidium homodimer-1 and calcein AM dyes (Invitrogen). Qualitative viability was determined by observation using fluorescence microscopy (Fig. 1).

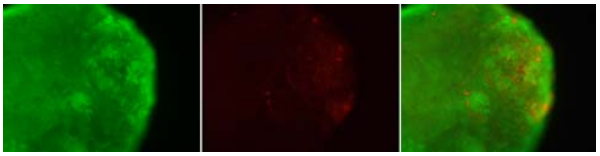

Figure 1. ***Viability analysis for day 18 EBs in suspension culture.*** Day 18 EB analyzed with ethidium homodimer-1 (left), calcein AM (middle), overlay (right); no significant regions of cell death were observed in EBs tested for each condition.

Results: Initial observations showed an increase in the percentage of spontaneously contracting EBs with NO treatment in 2 of 3 differentiation batches. However, pooled data for all 3 differentiations is inconclusive in statistically linking differences in spontaneous contractile behavior to NO exposure. To mitigate the effects of batch to batch variability, all data was normalized to the beating percentage values for the control on day 8, which was identified as the time point expressing the largest number of spontaneously contracting EBs (Fig. 2). Observations made at days 10, 12, and 14 do show a trend toward a higher percentage of beating EBs for populations treated with CysNO. Analysis of EB spontaneous beat frequency data for days 8-18 does not reflect any significant difference between conditions (Fig. 2).

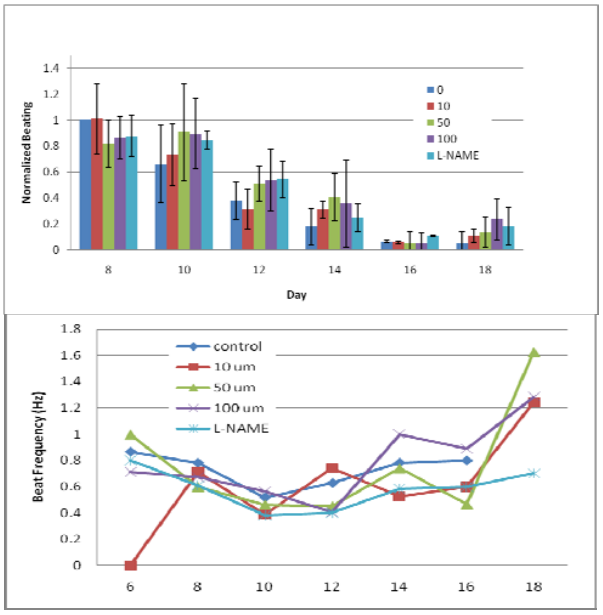

Figure 2. ***Analysis of spontaneous contractile activity of suspension-cultured EBs.*** Normalized beating values (top) and average beat frequency (bottom) for EBs cultured in different NO-conditioned media. (n=3 differentiations)

Conclusions: Incorporation of NO-generating ligands into biomaterial scaffolds may be useful in enhancing maturity of stem dell-derived cardiomyocytes. Although results do not indicate significant differences among the normalized percent beating and average beat frequency of EBs cultured under tested conditions, there is a trend toward higher spontaneous contractile activity for EBs treated with Cys-NO between days 10-14. Current experiments are further elucidating the developmental properties of EBs cultured in NO-rich environments. Spontaneous contraction is known to decrease as cardiomyocytes reach late-stage differentiation; therefore immunostaining, RT-PCR, and patch-clamp analysis will be used to more clearly elucidate NO-induced changes in electrophysiological maturity.

References:

1. Mujoo K. PNAS. 2008; 105:18924-18925
2. Kanno S. PNAS. 2004; 101:12277-12281