Encapsulation and Cardiac Differentiation of hiPSCs in 3D PEG-Fibrinogen Hydrogels

Petra Kerscher[1], Alexander J. Hodge[1], Joonyul Kim[1], Irene C. Turnbull[2], Blakely S. Bussie[1], Dror Seliktar[3], Christopher J. Easley[1], Kevin D. Costa[2], Elizabeth A. Lipke[1]

[1] Auburn University, Auburn, AL, USA, [2] Icahn School of Medicine at Mount Sinai, New York, NY, USA,
[3] Technion-Israel Institute of Technology, Technion City, Haifa, Israel

Statement of Purpose: The objective of this study was to create a 3D hydrogel system to culture and directly differentiate human induced pluripotent stem cells (hiPSCs) into mature, synchronously contracting engineered cardiac tissues. Current state-of-the-art hiPSC differentiation into contracting cardiomyocytes (CMs) follows either a 2D sheet approach or employs 3D cell aggregates (embryoid bodies). Both of these techniques require cells to be dissociated for downstream applications, including formation of engineered heart tissues and potential clinical applications, resulting in multiple cell handling steps and for electrophysiologically mature CMs, a high level of cell loss. In addition, the ability to manipulate microenvironmental cues provided to the differentiating cells is limited. PEG-fibrinogen (PEG-Fb), a hybrid biomaterial which provides structure, stiffness, cell adhesion sites, and degrades in response to cell-secreted factors, has previously been shown to support formation of engineered heart tissue using CMs derived from human embryonic stem cells[1]. In this study, clump and single hiPSCs were encapsulated into a PEG-Fb precursor solution and photocrosslinked to form a 200 µm thick tissue. HiPSCs remained viable within the PEG-Fb hydrogel and formed a contracting 3D cardiac tissue within seven days.

Methods: IMR90-1 hiPSCs were cultured on Matrigel in mTeSR-1 media. Clump and single hiPSCs were collected, centrifuged, and resuspended in PEG-Fb precursor solution, which consisted of PEG-Fb, triethanolamine (TEOA), N-vinylpyrrolidone (NVP), and the photoinitiator Eosin Y[2]. The cell-PEG-Fb suspension was transferred to a polydimethylsiloxane (PDMS) mold and photocrosslinked using visible light exposure to form the tissues. Tissues were cultured in mTeSR-1 media for three days followed by initiation of differentiation (day 0). Cell viability 24 hours post-encapsulation was assessed using LIVE/DEAD Assay. Proliferation was quantified following immunostaining for proliferating nuclear antigen (PCNA). Qualitative sarcomeric α-actinin, connexin 43 (Cx43), and Nkx2.5 expression of encapsulated CMs was obtained in the entire tissue by immunohistochemistry. Contracting cardiac tissues were dissociated to obtain electrophysiological characteristics. Recording of calcium transients in human CMs were obtained using an IonOptix Myocyte Calcium and Contractility Recording System. Samples were loaded with Fura-2AM dye and perfused with Tyrode's solution. CMs were paced from 0.5-2.5 Hz and stimulated at 30 V. Action potential durations (ADP) at 50% and 80% of repolarization were recorded.

Results: Encapsulated clump and single hiPSCs remained viable within PEG-Fb 24 hours post-encapsulation. Daily phase contrast microscopy and PCNA staining on tissues revealed hiPSC's ability to proliferate within PEG-Fb. Isolated contracting regions within tissues were visible starting on day 7 of differentiation. Contractile force and uniformity increased over time, resulting in a fully synchronous contracting cardiac tissue which remained its function for more than two months at a frequency of approximately 140 beats per minute. Positive sarcomeric α-actinin (Fig. 1), Cx43, and Nkx2.5 staining throughout the entire tissue thickness suggests uniform CM development independent of tissue location. In addition to spontaneous contraction, CMs cultured in PEG-Fb showed 1:1 correspondence to outside pacing signals up to 2.5 Hz. ADP50/ADP80 ratio at 1 Hz was 0.65 ± 0.04.

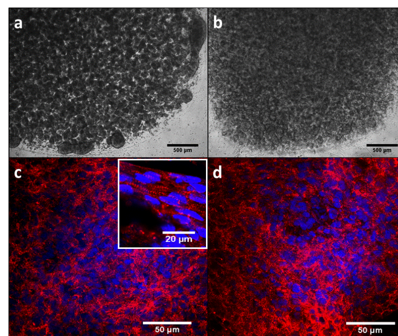

Figure 1. PEG-Fb hydrogels enable hiPSC culture and differentiation in 3D to produce mature cardiac tissues. 24 hrs post-encapsulation, PEG-Fb encapsulated clump (a) and single (b) hiPSCs start to proliferate and form networks. Clump (c) and single (d) hiPSCs derived CMs express sarcomeric α-actinin (red) throughout the entire tissue thickness. Cell nuclei are shown in blue. Insert shows sarcomeric pattern.

Conclusions: Here we describe the ability to culture and differentiate hiPSCs directly in 3D to create reproducible engineered cardiac tissues that mimic native myocardium. PEG-Fb is a suitable biomaterial that provides an ideal microenvironment for hiPSC culture and differentiation to obtain highly reproducible 3D cardiac tissues *in vitro*. HiPSCs can be successfully incorporated into PEG-Fb where they remain viable and proliferative. In addition to 3D hiPSC culture, clump and single stem cells can be differentiated into contracting CMs which retain their contractile function for several months. Tissues stained positive for cardiac-specific markers and responded to pacing frequencies up to 2.5 Hz. By providing a 3D tissue structure and microenvironment similar to the native heart, PEG-Fb is a favorable biomaterial to generate cardiac tissues *in vitro*. The instant tissue formation of hiPSCs in PEG-Fb provides the ability to guide cardiac differentiation, to produce mature CMs, and to obtain highly reproducible cardiac tissues.

References: 1. Shapira-Schweitzer, K. et. al. Journal of Molecular and Cellular Cardiology. 2008, 2,213-224.
2. Lipke, E.A., West J.L. Acta Biomaterialia. 2005, 1,597–606. **Acknowledgements:** Funding AHA, NSF

©2014 Society for Biomaterials