Direct Hydrogel Encapsulation of Pluripotent Stem Cells Enables Formation of Engineered Cardiac Tissues

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Statement of Purpose: The ability to manipulate microenvironmental cues provided to differentiating pluripotent stem cells is essential when mimicking the human myocardium. The objective of this study was to directly create a biomimetic model of the human developing heart using a simple and highly reproducible human induced pluripotent stem cell (hiPSC) encapsulation approach. Human cardiac tissues are currently created by a multi-cell handling approach, where hiPSCs are differentiated into contracting cardiomyocytes (CMs), dissociated, and finally resuspended in a biomaterial to drive CM maturation and improve CM function which is important for drug-testing or clinical applications. PEG-fibrinogen (PEG-Fb), a hybrid biomaterial which provides structure, stiffness, and cell adhesion sites, and which degrades in response to cell-secreted factors, has previously been shown to support formation of engineered heart tissues using CMs derived from human embryonic stem cells[1]. In this study, hiPSCs were encapsulated in PEG-Fb and photocrosslinked to form 200 µm thick tissues which formed contracting 3D cardiac tissues within seven days.

Methods: HiPSCs were cultured, collected, centrifuged, and resuspended in PEG-Fb precursor solution, which consisted of PEG-Fb, triethanolamine, N-vinylpyrrolidone, and photoinitiator Eosin Y. The cell-PEG-Fb suspension was transferred to a polydimethylsiloxane 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). Differentiation efficiency and CM proliferation was quantified by flow cytometry on day 20, using the cardiac marker cardiac troponin T (cTnT) and proliferation marker Ki67. Gene expression of pluripotency gene Oct4, cardiac genes myosin light chain 2v (MLC2v), α and β myosin heavy chain (αMHC, βMHC), and functional gene connexin 43 (Cx43) was quantified on days 10, 20, and 30 of differentiation. Calcium handling of 14 day old cardiac tissues was 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 0.5-2.0 Hz and time to baseline 50% and 80% of calcium transients were recorded. Qualitative α-sarcomeric actinin (αSA) and Cx43 expression of encapsulated CMs was obtained in the entire tissue by immunofluorescence. TEM images were acquired from cardiac tissues cultured in vitro for extended time frames (months). All quantitative studies of 3D cultured CMs were compared to the current state-of-the-art 2D sheet cardiac differentiation procedure[2].

Results: Encapsulated hiPSCs (Fig. 1a) remained viable and showed first isolated contracting regions within tissues on day 7 of differentiation, resulting in fully synchronous contracting tissues which retained their function for more than three months. On day 20, 3D tissues and 2D sheets contained 71.4±3.2% and 74.3±4.4% $cTnT^+$ cells (n=3), respectively (Fig. 1b). Throughout differentiation, Oct4 gene expression decreased significantly, and cardiac and functional genes MLC2v, αMHC, βMHC, and Cx43 show trends towards mature CMs (n=3, $p<0.05$). In addition to spontaneous contraction, 3D cultured CMs showed 1:1 correspondence to outside pacing signals up to 2.0 Hz. Long-term cultured CMs stained positive for αSA and Cx43 (Fig. 1d) throughout the entire tissue thickness visualizing aligned and well-defined sarcomeres with mature gap junctions. Ultrastructural features of 3D cultured CMs include Z-bands (Z), H-bands (H), intercalated discs (ID), gap junctions (GJ) (Fig. 1e) and basement membrane (bm), caveolae (c) and tranvserve tubule (T) (Fig. 1f).

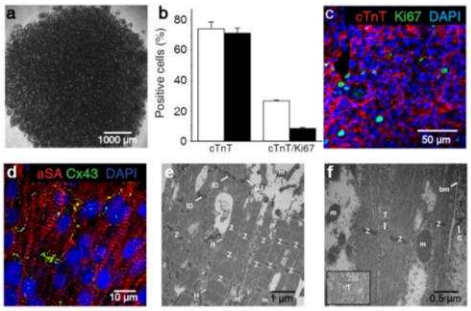

Figure 1. PEG-Fb hydrogels enable hiPSC culture and differentiation in 3D to produce mature cardiac tissues. (a) HiPSCs can be encapsulated into PEG-Fb hydrogels (b) 2D (white) and 3D (black) cultured cells showed similar differentiation efficiencies with >70% CMs and 10-20% of CMs proliferating, which was also observed using immunofluorescence (c). Long-term cultured CMs show (d) aligned sarcomeres and mature gap junctions, as wells as (e, f) ultrastructural features of mature CMs, important for excitation-contraction coupling.

Conclusions: PEG-Fb is a suitable biomaterial that provides an advantageous microenvironment for hiPSC culture and differentiation to obtain 3D cardiac tissues in vitro. PEG-Fb encapsulated hiPSCs survive, proliferate, and differentiate into contracting CMs which did not show any discrepancies in differentiation efficiency, gene expression, and calcium handling compared to the current state-of-the-art differentiation procedure. Furthermore, 3D tissues can be cultured for an extended culture period where CMs show mature ultrastructural features. 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. Lian, X. et. al. Nature Protocol. 2013, 8,162-75.

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