Development of Human Induced Pluripotent Stem Cell Derived Co-Cultures of Cardiomyocytes and Endothelial Cells

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Cardiovascular disease is associated with myocardial infarction, arteriosclerosis, and cardiac arrhythmias, which lead to the segmental loss of functional beating cardiomyocytes. Numerous studies have investigated regenerative therapies involving stem cell transplantation using hydrogel scaffolds. However, these therapies have had limited success due to poor donor cell engraftment and low survival.[1] One major contributing factor to the lack of success is the limited vascularization within the ischemic cardiac tissue and the scaffold. In this study we started to address these limitations by creating a novel co-culture of cardiomyocytes and endothelial cells derived from human induced pluripotent stem (iPS) cells using TGFβ1 as a secondary differentiation factor. TGFβ1 was added to an existing protocol that differentiates human iPS cells into cardiomyocytes to increase the endothelial cell population. We targeted CD105 since it is a transmembrane receptor for TGFβ1, an important modulator of mesodermal differentiation, and is necessary for TGFβ1/ALK1 signaling which promotes the proliferation of endothelial cells.[3,4] We determined the distribution of differentiated mesodermal cells using cell markers specific to progenitor (CD105+) and mature (CD31+) endothelial cells along with cardiomyocytes (CTnT+) through fluorescence microscopy and flow cytometry analysis. We then assessed the differentiation of these iPSC-CPCs after seeding them into a hydrogel system of hyaluronic acid (HyA) to promote a 3D co-culture system

Methods: Wild type human iPS cells were obtained from the Gladstone Institute of Cardiovascular Disease and cultured on tissue culture polystyrene with mTesR medium prior to starting the Wnt-mediated differentiation protocol.[5] Briefly, cardiac progenitor differentiation was initiated with the addition of 12 uM CHIR to inhibit GSK3. Media was then exchanged with RPMI-B27 minus insulin to remove the CHIR. The Wnt pathway was then inhibited on day 3 with the addition of IWP4, and various concentrations (10 – 40 ng/mL) of TGFβ1 were added once the cells reached the cardiac mesoderm stage. On day 7, the media was exchanged to RPMI-B27 complete (with insulin) and prepared for analysis by immunostaining for flow cytometry. Differentiated human iPS cells were harvested from their culture plates with collagenase type 2 (1 mg/mL), 40 U/mL DNase and, 0.25% trypsin. They were then washed with PBS and fixed in 4% paraformaldehyde. The cells were then permeabilized with 0.2% Triton-X100. Non-specific binding was blocked with a Fc blocking solution and 2% bovine serum albumin. The cells were then immuno-stained with primary antibodies for CD105 and CD31, which are surface receptors for endothelial progenitor and endothelial cells, respectively. The cardiomyocyte populations was tested using a primary antibody for cardiac troponin T (CTnT). Cells treated with Wnt/TGFβ1 protocol were then encapsulated into a hydrogel scaffold composed of acrylated hyaluronic acid (AcHyA) (4mg), AcHyA-RGD (6mg), and heparin-SH (0.03 wt %) with TGFβ1 (40 ng/ml) dissolved in 0.3 mL of triethanolamine-buffer (0.3 M, pH 8). A thiol-terminated MMP-13 degradable crosslinker was then added to polymerize the scaffold. The cell viability after 24 hours in the hydrogel was assessed by a live/dead assay with propidium iodide and calcein staining.

Results: Flow cytometry and fluorescence microscopy demonstrated the presence of endothelial progenitor cells (CD105+) and endothelial cells (CD31+) mixed with cardiomyocytes (CTnT+) in all differentiation culture conditions. The addition of TGFβ1 increased the CD105 population significantly. Fluorescence microcopy indicated the cells grow in layers where cardiomyocytes grew over the CD105+ and CD31+ cells. The live/dead assay demonstrated the cells were viable in the HyA scaffold.

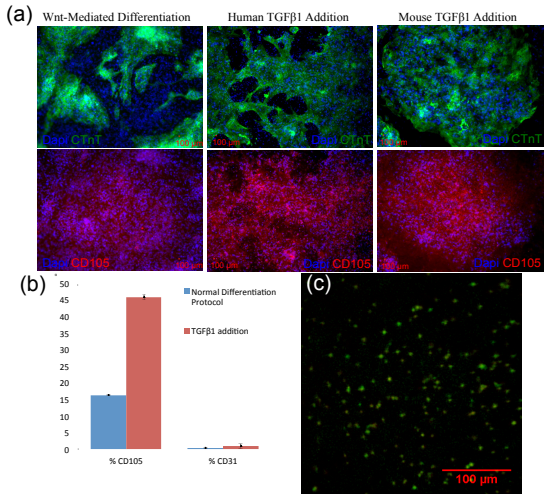

Figure 1. (a) Fluorescence microscopy (CTnT/CD105) (b) flow cytometry 7 days post TGFβ1 addition (c) confocal microscopy image of live/dead assay of co-culture with TGFβ1 *in HyA hydrogel (live cells: green, dead cells: red)*

Fluorescence microscopy and flow cytometry analysis demonstrate the addition of TGFβ1 to an existing Wnt-mediated differentiation protocol for human iPS cells promotes a co-culture of endothelial cells and cardiomyocytes. We are further exploring the development of these cells within HyA hydrogels.

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