Rapid Fabrication of Injectable Engineered Cardiac Tissue Spheroids Using a Novel Microfluidic Device

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Statement of Purpose: Cardiovascular disease is the leading cause of death in the world; this is in part due to the heart's inability to regenerate after damage is caused. Regenerative medicine, specifically, engineered cardiac tissue, offers hope for improvement over the current prevention and treatments for cardiovascular disease. Due to the inability to culture adult cardiomyocytes (CMs) *in vitro*, cardiac differentiation of human induced pluripotent stem cells (hiPSCs) is critical to realize this potential. In order for engineered cardiac tissue to become a viable treatment, the production of the tissue-engineered product must be scalable and able to be manufactured in a clinical grade setting. Currently, cardiomyocyte production is typically done using two-dimensional cell sheets. However, this method has limited scalability and fails to mimic the natural environment of the heart.

Building on prior work in the laboratory directly differentiating hiPSCs into cardiomyocytes in a 3D environment[1], here we present a rapid, scalable, and single-cell handling approach to manufacture three-dimensional functional cardiac tissue spheroids using a novel microfluidic technique. In our system, hiPSCs are combined with a biomaterial, PEG-fibrinogen (PEG-Fb), and spheroids are formed using a modified oil-and-water microfluidic emulsion technique. This custom system provides tight control over size and circularity of the spheroids, has also been used to encapsulate cells for injectable cell therapy, and has the potential to be leveraged for robust tissue production in a bioreactor.

Methods: To create the spheroids, a cell pellet of hiPSCs was combined with the PEG-Fb precursor solution[1] and transferred to a syringe to be used as the aqueous solution of the oil-and-water emulsion microfluidic technique. Using two separate syringe pumps, this PEG-Fb aqueous cell suspension and mineral oil were infused into opposite sides of a custom fabricated PDMS microfluidic device. Spheroids were formed at the junction and traveled along the outlet channel of the device where the PEG-Fb was photocrosslinked using high intensity visible light. The resulting spheroids were collected in a centrifuge tube, washed with cell culture media, and maintained three days prior to initiation of cardiac differentiation using the previously published method[1,2]. The size, uniformity, and normalized area growth of the spheroids was determined using Image-J software. The elastic modulus was determined using a Microsquisher parallel plate mechanical testing system. Dissociated cardiomyocytes were seeded onto a multielectrode array (MEA) for recordings of spontaneous contractions and drug response to isoproterenol and propranolol. To determine the percentage of positive cardiomyocytes, the spheroids were dissociated and labeled with cardiac troponin T (cardiac marker), Ki67 (proliferation marker) and P4HB (fibroblast marker) and analyzed using flow cytometry.

Results: Our novel microfluidic device enabled rapid fabrication of highly uniform spheroids with high cell density (25 million cells/mL) and a crosslinking time of less than 1 second. Following encapsulation, the cells remained viable and continued to grow within the PEG-Fb hydrogel matrix to produce denser and larger tissues. Compared to day 0, the normalized tissue cross-sectional area increased 1.27 and 1.7 times by day 3 and 7, respectively. The spheroids had an elastic modulus of approximately 40 Pa on day 5 of cardiac differentiation. Spontaneous contractions initiated on day 8 in suspension culture conditions. First, isolated areas of contraction were typically seen on day 8, with spheroids establishing synchronous tissue-level contraction by day 14 and retaining their function for several months. Spheroids supported high efficiency cardiac differentiation, containing 71.6±8.4% cTnT+, 7.1±1.7% cTnT+/Ki67+, and 8.4±6.5% P4HB+ cells (day 20, n=3 separate batches). Resulting CMs responded to both β-adrenergic agonist, isoproterenol, and β-adrenergic antagonist, propranolol. The spheroids exhibited a 1:1 capture up to 6.0 Hz when paced on the MEA on Day 50.

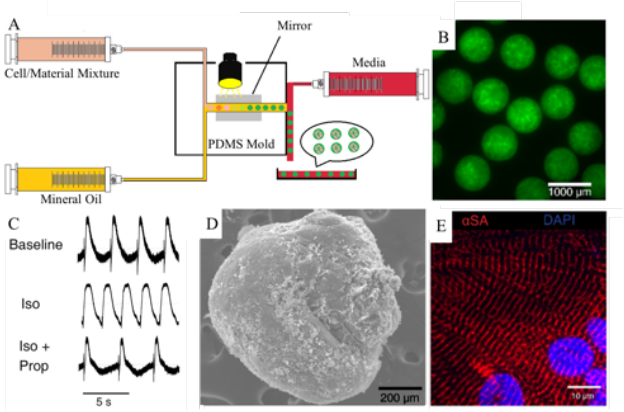

Figure 1. Production of Cardiac Spheroids. (A) HiPSCs were encapsulated within a PEG-fibrinogen hydrogel using a novel microfluidic system. (B) Highly uniform spheroids visualized by autofluorescence of the photoinitiator. (C) CMs responded to the β-adrenergic agonist, isoproterenol (Iso), increasing the contraction rate. (D) Cells remodeled their PEG-fibrinogen microenvironment to form dense cardiac microspheres. (E) Resulting cardiomyocytes with aligned sarcomeres.

Conclusions: We have developed a novel microfluidic technique for the fabrication of functional cardiac spheroids derived from hiPSCs. This system uses an oil-and-water microfluidic emulsion technique to produce uniform spheroids with tight control over size and shape. Spontaneous contractions begin consistently on day 8; resulting cardiomyocytes respond appropriately to β-adrenergic agonists and antagonists and can be paced up to 6.0 Hz. This technique has the potential for scale-up production and differentiation of engineered cardiac tissue in a bioreactor, which is necessary for regenerative medicine to replace the current treatments for cardiovascular disease.

References: 1) Kerscher P. Biomaterials. 2016;83:383-395. 2) Lian X. Nat Protoc. 2013;8:162-175.