Fabrication of a human cardiac tissue equivalent in vitro on synthetic 3D fiber scaffolds

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Statement of Purpose: Stem cell technology offers tremendous potential to recapitulate human myocardial tissue for understanding and screening new therapeutics. While several groups have successfully differentiated cardiac cells from human embryonic stem cells, there still remains a paucity of scaffolds that organize these cardiomyocytes and provide appropriate mechanical cues in a three dimensional structure. The goal of this project was to emulate the human myocardial ECM with an anisotropically aligned 3D surface with defined integrin engagement. To create 3D filamentous matrices of defined architecture and modulus, we used two-photon-initiated polymerization (**TPIP**) which has excellent 3D spatial resolution (~ 100 nm)[1] on the length scale of filamentous structures observed in natural tissue. Unlike other methods to create filamentous matrices like electrospinning, self-assembly of amphiphiles, 3D micropatterning of hydrogels, and protein gels, TPIP polymerized materials have unprecedented control over a wide range of matrix features such as architecture, porosity, and fiber dimensions, which are key regulators of cell function. For example, TPIP materials can be fabricated with controlled and variable porosity to allow cell infiltration to create a true 3D cellular structure, as compared to electrospun matrices with nanoscale pores that don't allow cell movement into the matrix. We envision that the appropriate 3D scaffold will be fabricated with an underlying filamentous structure generated by TPIP in combination with a biomimetic coating that controls integrin engagement. Here we sought to identify whether synthetic biomimetic scaffolds can promote EB differentiation into cardiomyocytes (CMs) and determine whether human CMs align onto TPIP synthesized 3D fiber scaffolds.

Methods: Human embryonic stem cell line H9 (α-MHC m-cherry positive, kind gift by Bruce Conklin, Gladstone Institue Cardiovascular Disease & UCSF) was used for all experiments. For cardiomyocyte differentiation, embryoid bodies (EBs) were formed by treating confluent cells with collaganese IV and suspending cells in low attachment plates in EB medium (Knockout-DMEM with 20% FBS, 0.1 mM non-essential amino acids, 2 mM L-glutamine and 0.1 mM 2-mercaptoethanol). At day 7, EBs were plated onto tissue culture polystyrene surfaces coated with either 0.2% gelatin or 0.1 wt% hyaluornic acid (HyA) conjugated with AG10 peptide (CGGNRWHSIYITRFG). In some cases, EBs were also plated directly onto fiber scaffolds. Fibers of dimensions 1.8 mm length, 10 μm diameter and 30 μm spacing in between fibers were fabricated as previously described (2). Briefly, glass bases, already coated with the UV curable organic–inorganic hybrid polymer (Ormocer®, US-S4, Micro resist technology), were assembled with ~ 1mm-thick spacers and filled with uncured monomer and crosslinker solution and the fibers were cured by high-repetition rate femtosecond laser irradiation.

Results: We have established that a thin coating of HyA conjugated with a peptide engaging the $\alpha_6\beta_1$ integrin (AG10) can support human embryonic stem cell derived CMs differentiated using the EB method (**Fig. 1**). CMs attached to the HyA-AG10 surface, began to beat spontaneously at Day 4 and continued to beat in culture for up to 30 days (experiment ongoing).

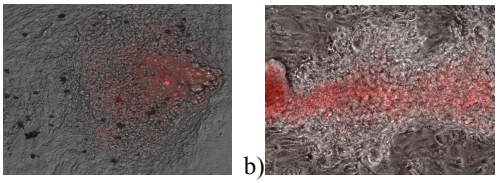

Figure 1. H9 αMHC-mCherry-CM (red staining) cultured on a synthetic HyA-Ag10 surface spontaneously started to beat at a) 4 days and continued to beat until b) 30 days. Fluorescent image is overlaid with corresponding phase image.

Also, we have shown that H9 derived EBs adhere on 3D TPIP generated fiber matrices, and over time proliferate and align along the fibers (**Fig. 2**). Interestingly, EBs attached onto all tested surfaces: fibers alone, fibers coated with gelatin, and fibers coated with synthetic AG10 peptide.

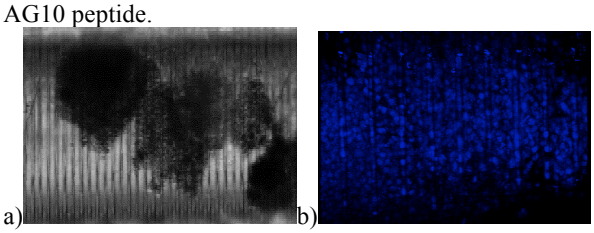

Figure 2: H9 Day 7 EBs plated on a 3D fibrous matrix for a) 2 and b) 13 days. b) Confocal microscopy 3D projection of a 40μm tissue stack shows nuclei (DAPI, blue) with alignment of cells along the parallel fibers.

Moreover, when human CMs (differentiated from H9 αMHC-mCherry-positive) are trypsnized and plated onto TPIP generated fiber matrices, the CMs attach onto the fibers and continue to beat (Fig. 3). We are currently evaluating mechanical and electrical coupling of these CMs on the fiber scaffolds.

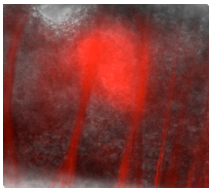

Figure 3: H9 αMHC-mCherry-CM (red staining) on a 3D fibrous matrix at 3 days. Fluorescent image is overlaid with corresponding phase image.

Conclusions: We have shown that synthetic surfaces with a $\alpha_6\beta_1$ integrin engaging peptide can support differentiation of hESC into cardiomyocytes. Moreover, we have shown that both human CMs attach, elongate, and beat on 3D fiber scaffolds.

References: 1) Hidai, H, Biomed Microdevices, 2009. 11(3): p. 643-52. 2) Jeon, H., Biomaterials, 2010. 31(15): p. 4286-4295