Formation of Vascular Networks in a PEGylated Fibrin Hydrogel Increases Proliferation of Cardiomyocytes

Ethan J. Vanderslice[1], Staunton G. Golding[1], Jeffrey G. Jacot, PhD.[1,2]
[1]University of Colorado Denver | Anschutz Medical Campus, Aurora, CO 80045
[2]Childrens Hospital Colorado, Aurora, CO 80045

Statement of Purpose: Congenital heart defects are the most common congenital disorders, affecting approximately 1% of live births, with 25% requiring surgical correction within the first year of life [1]. Current patches become encased in scar tissue, which inhibits the electrical and mechanical coordination of the heart muscle leading to increased risk of arrythmias and sudden cardiac death [2, 3]. An engineered patch that causes regeneration of the native heart tissue would allow for better mechanical and electrical integration of the defect area, improving long-term function. Angiogenesis has been shown to precede cardiomyocyte proliferation and infiltration in regenerating rodent hearts [4]. Toward the goal of cardiac regeneration, this study develops vascular networks in a fibrin-based hydrogel and assesses how pre-formed vascular networks affect human cardiomyocytes compared to unorganized vascular cell and acellular hydrogels.

Methods: PEGylated fibrinogen was formed by crosslinking fibrinogen with bifunctionalized poly(ethylene glycol)-n-hydroxysuccinimide (PEG-NHS) (NOF America, White Plains, NY). PEGylated fibrinogen was combined with a cell solution comprised of human umbilical vein endothelial cells (HUVEC) and human dermal fibroblasts (hDF) at varying cell concentrations and cell ratios. Thrombin was added to the PEGylated fibrinogen + cell solution to create PEGylated fibrin hydrogels with encapsulated cells. These cellular hydrogels were cultured for 7 days to form vascular networks. Cardiomyocytes were differentiated from human induced pluripotent stem cells (hiPSC) following a modified GiWi protocol and seeded on top of the hydrogels. After 7 days, proliferation and gene expression were analyzed via EdU labeling and qPCR respectively. Cardiomyocytes seeded on hydrogels with pre-formed vascular networks were compared to those seeded on unorganized cellular hydrogels (hydrogels with encapsulated HUVEC and hDF which were not given the time to form networks) and acellular hydrogels (hydrogels without HUVEC and hDF encapsulated).

Results: Vascular Network Formation

To generate vascular networks in our PEGylated fibrin hydrogel, various cell concentrations and cell ratios were tested to determine the optimal cell loading that would assemble into networks over a 7-day culture period. Starting at a previously reported total cell concentration of 800,000 cells/mL, the ratio of HUVEC to hDF was varied from 3:1 to 6:1. Furthermore, addition of vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) were tested for impact on network formation. Average vessel length, total vessel length, and total number of junctions were quantified from fluorescent images using the ImageJ plug-in Angiotool. Results indicated that increasing the ratio of HUVEC to hDF improved network formation, however the addition of growth factors did not significantly improve network formation. Subsequently, the total cell concentration was varied from 500,000 cells/mL to 1,400,000 cells/mL with a constant ratio of 5:1 (HUVEC:hDF). Average vessel length increased at higher cell concentrations (1,100,000 and 1,400,000 cells/mL) compared to 800,000 cells/mL. Based on this data, hydrogels were made with a final cell concentration of 1,100,000 cells/mL and a cell ratio of 5:1 for the cardiomyocyte experiments.

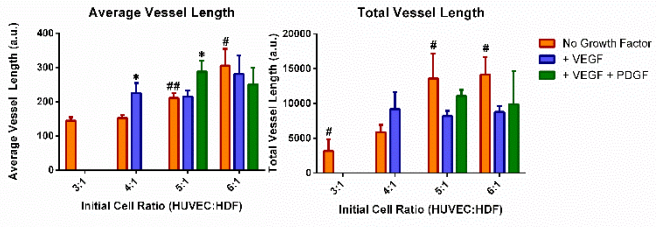

Fig. 1. Quantification of average vessel length and total vessel length of networks in hydrogels with varying cell ratios and growth factors. (# - significance from 4:1, no growth factor)

Cardiomyocyte Response to Vascularized Hydrogels: To assess how our hydrogel with vascular networks affects cardiomyocytes, human iPSC-derived cardiomyocytes were seeded onto the hydrogels for 7 days. EdU was added to the media for the final 24hrs to label proliferating cells. Both unorganized cellular hydrogels and vascular network hydrogels increased the proliferation of hiPSC cardiomyocytes compared to acellular hydrogels.

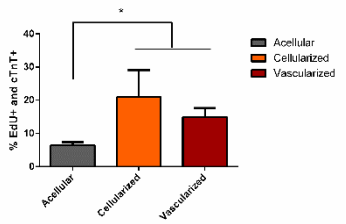

Fig. 2. Cardiomyocyte proliferation on acellular, unorganized cellular, and vascular network hydrogels.

Additionally, gene expression of cardiac maturity markers was assessed with qPCR. Vascular network hydrogels induced a less mature expression profile of myosin heavy chain and myosin light chain isoforms compared to unorganized cellular hydrogels. Altogether, this data shows that we can generate hydrogels with vascular networks and these hydrogels can induce and/or maintain cardiomyocytes in a more proliferative state. Thus, these hydrogels will be used for continued development of scaffolds for cardiac regeneration of structural defects.

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