Pre-vascularized, 3D Printed Scaffolds for Optimization of Cell-based Therapies

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Statement of Purpose: Cell-based therapies are a promising treatment option for many diseases due to their ability to restore the functions of critical endogenous cells that may be lost to factors such as autoimmune attack. Historically, cell populations have been transplanted with supportive biomaterials and/or beneficial therapeutics to improve survival and engraftment; however, the insufficient diffusion of oxygen and other nutrients hinder the scaling these platforms to clinical applications. Our lab has previously engineered a porogen-leaching, highly porous polydimethylsiloxane (PDMS) scaffold for the housing of pancreatic islets. The scaffold further allows for the infiltration of host cells to support intra-device endogenous vasculature[1]. Implantation studies have shown long-term compatibility and support of both transplanted cells and endogenous vascularization. Unfortunately, the process of integration with host circulation occurs over a time scale of days to weeks, presenting a period of potential hypoxia. To overcome this limitation, we propose an alternative fabrication method using 3D printing to impart control over geometry and porosity to improve engraftment. In tandem, we implement an *in vitro* pre-vascularization protocol to allow for rapid anastomosis and more rapid graft perfusion post-transplantation[2].

Methods: To establish a more consistent fabrication process, address the variable pore-size of the previous platform, and permit further personalization of scaffold geometry, we employed a reverse-cast 3D printing technique to generate PDMS scaffolds with consistent shapes and pore sizes. By utilizing commercial 3D printing to quickly model and fabricate molds using polyvinyl alcohol (PVA) filament, a multitude of geometries and pore sizes could be generated consistently to observe the impact of geometry on vasculature both *in vitro* and *in vivo.* Sterilized scaffolds were then infused with human endothelial cells (HUVEC) and fibroblasts (NHLFs) at a 1:1 ratio suspended in fibrin hydrogel for culture over 7 days to form vascular structures *in vitro*. Resultant samples were stained for CD31 and Actin before imaging using confocal microscopy allowing for three-dimension visualization of vascular structures. For preliminary *in vivo* testing, scaffolds of varying pore size were infused with fibrin hydrogel alone and transplanted into the murine epididymal fat-pad (EFP) for 14-30 days before staining with tomato lectin to identify functional intra-scaffold vessels.

Results: Scaffolds with defined X, Y pore sizes of 150, 300, and 600 um and a thickness of 900 um were successfully fabricated. The loading of scaffolds of 150 and 300 um pore size with endothelial cells and fibroblasts resulted in the formation of construct-spanning, CD31+ structures, as determined by confocal microscopy. Compared to scaffold-free controls, pre-vascularized hydrogels cultured in both pore-size scaffolds retained their volume over the culture period without compressing. Quantification of TGF-β via enzyme-linked immunosorbent assay (ELISA) confirmed no significant change in secretion for both pore-size scaffolds over the culture period compared to scaffold-free controls. Lectin staining of transplanted hydrogel-scaffold constructs showed functional vessels within 300 and 600 um scaffolds after 30 days.

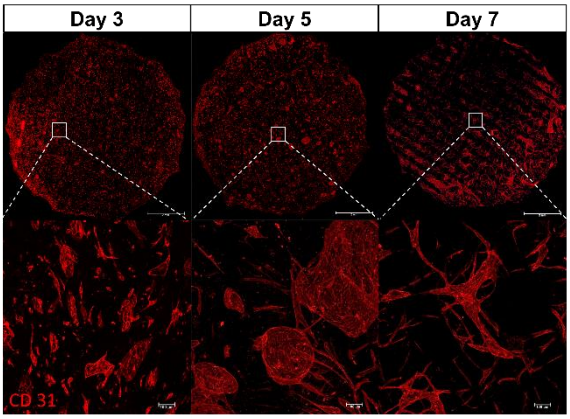

Figure 1. Time lapse of CD31+ structure formation within 300 um pore-size scaffold

Conclusions: Scaffolds with controlled pore-size were successfully fabricated using a 3D-print, reverse-cast technique. The open framework of the PMDS-based scaffold supported the formation of CD31+ structures that not only spanned the 9 mm diameter construct surface area, but also weaved within the scaffolds 0.9 mm thickness. Scaffold-hydrogels remained at similar volumes throughout the culture period, in contrast to the dramatic compaction observed for PMDS-free controls containing only fibrin and cells. Despite the lack of compression, quantification of secreted TGF- β indicated limited impact on overall cellular presence. Taking this same platform sans cells and transplanting within the murine EFP, a promising site for transplantation of cells, promoted the formation of functional intra-scaffold vessels after 30 days. The vessels appeared to follow the rungs of the scaffolds, suggesting potential control over vessel direction and structural conformation. Future work will focus on the further characterization of *in vitro* generated structures by quantifying other critical vascuologensis proteins including vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), and matrix metalloproteinase-9 (MMP9). Assessment of cellular gene expression will also be employed to further characterize the impacts of the 3D scaffold on cellular phenotype.

References: 1. Liang, et. al. ***Tissue Engineering Part A,*** 2021, 10.1089/ten.tea.2020.0287 2. Moya, et. al. ***Tissue Engineering Part C,*** 2013, 10.1089/ten.tec.2012.0430

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