Title: Creation of hierarchical microchannel architecture within tissue engineered hydrogels using melt spun sacrificial fibers

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Abstract:

Purpose: Creation of synthetic tissues with microvascular networks that mimic the architecture of capillaries remains one of the foremost challenges within tissue engineering. In previous work, we used a sacrificial microfiber technique whereby Pluronic F127 microfibers were embedded within a collagen matrix, leaving behind a patent channel, which was subsequently seeded with endothelial and smooth muscle cells, forming a neointima and neomedia. Here we describe two approaches to synthesize a biocompatible tissue-engineered construct, recapitulating the hierarchical organization of an arteriole, venule, and capillary bed.

Methods: 1.5 mm Pluronic F127 macrofibers were bridged by three-dimensional networks comprised of either 100-500 μm Pluronic F127 microfibers or 10-400 μm melt-spun Shellac microfibers. Networks were embedded in type I collagen, sacrificed and "intraluminally" seeded with $5x10^6$ cells/mL human aortic smooth muscle cells (HASMC) followed by $5x10^6$ cells/mL HUVEC. Constructs underwent 7 or 14 days of culture either static or dynamic. Both seeded and unseeded constructs were microsurgically anastomosed to rat femoral vessels and perfused *in vivo*. Architecture and cell viability were confirmed via hematoxylin and eosin (H&E) staining. Immunohistochemical (IHC) analysis was performed to determine the spatial relationship of seeded cells.

Results: Pluronic and Shellac/Pluronic three-dimensional constructs were successfully embedded and sacrificed in type I collagen, leaving patent microchannels ranging in size from 10 to 500 μm. The presence of a dense network of microchannels with adherent cells was confirmed via H &E after 7 and 14 days for both types of networks. Within microvessels 50 μm or less, CD31 expressing endothelial cells were identified, whereas larger microvessels demonstrated both CD31 and α-smooth muscle actin (SMA) expressing cells along luminal surfaces after 7 and 14 days. Additionally, following perfusion, CD31 expressing cells remained adherent along the walls of the micro- and macrochannels. Both types of constructs were successfully anastomosed to rat femoral vessels and perfused.

Conclusions: We have developed two techniques to create three-dimensional microvascular networks within tissue-engineered constructs using Pluronic F127 and Shellac sacrificial microfibers. Both techniques produce channels that support adhesion and growth of endothelial cells crucial to providing thrombosis-free flow. These results represent significant progress towards the fabrication of constructs with a hierarchical

vascular network analogous to that seen *in vivo*, necessary for the production of human-scale engineered tissues.