Fabrication of Tissue Engineered Constructs with Stable Endothelial Lining After 28 Days

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Purpose: Synthesizing tissue-engineered constructs with an inherent vascular system remains one of the foremost challenges in regenerative medicine. *In vivo*, a healthy confluent endothelial surface is critical for thrombosis-free blood flow. Here we aim to recreate such blood vessels through the fabrication of tissue-engineered endothelialized microvessels within collagen hydrogels that are sustainable for up to 28 days in culture and suitable for *in vivo* perfusion.

Methods: U-shaped pluronic F127 fibers were sacrificed in type I collagen, creating a central microchannel, 1.5 mm in diameter. After fiber sacrifice, 5 $x10^6$ cells/mL human aortic smooth muscle cells (HASMC) and 5 $x10^6$ cells/mL human umbilical vein endothelial cells (HUVEC) were sequentially seeded into the microchannel 24 hours apart. Following 7,14 and 28 days of culture, constructs were fixed and processed. Histological and immnuohistochemical analysis was performed to determine density and spatial relationship of seeded cells.

Results: Microchannels were successfully seeded with HUVEC/HASMC. Hematoxylin and eosin staining demonstrated a confluent lining by 7 days of culture, which was thickened at 14 days and maintained at the 28-day time point. Immunohistochemical analysis revealed the formation of a neointimal and neomedial layers comprised of CD31 expressing endothelial cells along the luminal surface and α-SMA expressing smooth muscle cells in the subendothelial plane. In addition after 7 and 14 days we noted deposition of critical extracellular matrix proteins basal lamia collagen IV and fibronectin within the microchannel wall.

Conclusions: We have successfully fabricated tissue-engineered constructs with microchannels that support engraftment and proliferation of smooth muscle and endothelial cells forming a durable vascular network. Such constructs recapitulate *in vivo* organization of vascular cells and brings us a step closer to the fabrication of tissue-engineered flaps ready for immediate perfusion.