Vascularization and Characterization of Perfused 3D Hydrogel Constructs

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Statement of Purpose: The objective of the study was to vascularize and characterize three-dimensional (3D) engineered tissue volumes for future use as breast cancer drug development and testing models. It is expected that this 3D system will act as a supplement to current drug testing platforms – *in vitro* cell culture and *in vivo* animal studies – to increase the prediction of cancer therapy safety and efficacy in human clinical trials. Increased accuracy will reduce costs associated with development of drug candidates that will ultimately fail in humans. Important aspects of this system are the presence of extracellular matrix (ECM)-cell interactions, large construct volume (2.4mm x 5mm x 10mm), and endothelial-lined microchannels, all of which increase the physiological relevance and complexity of the model. The engineered vasculature is necessary because current *in vitro* systems (e.g., rotating bioreactors, suspension of spheroids, and growth on a porous scaffold) are limited in size (< 1 mm) by their absence of micrometer-scale blood flow channels that allow for oxygen and nutrient diffusion into the tissue. The objective of this study is to characterize the hydrogel construct and engineer microvessels by endothelializing microchannels embedded in 3D hydrogel constructs, which is intended to support long-term endothelial and epithelial cell maintenance and growth and recapitulate physiological tissue function.

Methods: The hydrogel constructs were composed of bovine type I collagen (1.9 mg/ml) and growth factor reduced (GFR) Matrigel (10% v/v), which were supported by reticulated vitreous carbon (RVC), a continuous open-pore foam. Nanoindentation was used on the hydrogel constructs to determine the hydrogel elasticity, which was compared to breast tissue elasticity. Microchannels were created by inserting 400 µm diameter stainless steel rods through the RVC pores and polymerizing the collagen/Matrigel hydrogel around the rods and RVC struts. The constructs were placed in the flow-perfusion bioreactor to statically seed the microchannels with rat brain endothelial cells (6×10^6) followed by perfusion of the microchannels and 3D hydrogels via a peristaltic pump. Endothelialization of the microchannels was shown by staining histological sections with Hematoxylin and Eosin (H&E) and imaging using inverted (Figure 1A) microscopy as well as fluorescently staining with Cell Tracker Red and imaging with fluorescent microscopy (Figure 1B).

Results: Collagen/Matrigel hydrogels were successfully polymerized inside RVC pores with four internal parallel microchannels. Nanoindentation was used to measure the hydrogel elasticity at 0.65 ± 0.28 kPa, which is comparable to normal breast tissue elasticity. A flow-perfusion bioreactor was fabricated that has been shown to perfuse tissue volumes with media at specified flow rates and shear stresses along the microchannel walls. Incorporation of rat brain endothelial cells during static seeding followed by manipulation of fluid mechanics resulted in endothelial cell attachment to the microchannel walls and alignment along the direction of media flow (Figure 1). Additionally, fluorescent microscopy depicted endothelial cell localization to the microchannels.

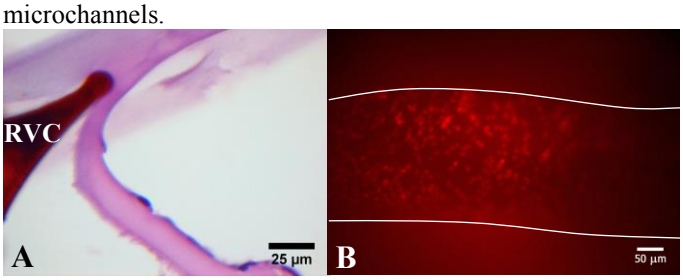

Figure 1. Endothelialization of hydrogel-embedded microchannels using a flow-perfusion bioreactor. A) H&E staining illustrates cell attachment and morphology, while B) fluorescent staining illustrates cell localization to the microchannel walls (white border).

Conclusions: There are two main conclusions that can be drawn from the results of this study. First, the 3D hydrogel constructs had an elastic shear modulus comparable to normal breast tissue. However, the hydrogel elasticity can be increased by enzymatic crosslinking (e.g., lysyl oxidase) of collagen to reach breast tumor elasticity (1.2-3.0 kPa). Second, this study has shown that the process of endothelialization is possible by statically seeding endothelial cells in the hydrogel embedded microchannels followed by manipulation of fluid mechanic parameters to meet physiologic conditions. Histology showed cell attachment to the microchannel walls and an elongated morphology along the direction of media flow (Figure 1A). Fluorescent microscopy illustrated the localization of endothelial cells to the microchannel spaces (Figure 1B). Protocol modifications will include coating microchannel walls with fibronectin for increased cell attachment and increasing the static seeding time to increase cell confluence. Future work will include further characterization of the endothelial cells (e.g., confluence, intercelluar junctions, and permeability/barrier function). It is anticipated that a confluent monolayer of endothelial cells will form along the microchannel walls once all parameters have been optimized. Therefore, this proof-of-concept work has shown it is possible to use 3D hydrogel constructs in a flow-perfusion bioreactor to engineer endothelial-lined microchannels (i.e., engineered microvessels). The successfully vascularized engineered tissues have the potential to not only mimic multiple disease states for drug development but also to revolutionize tissue replacement for diseased or traumatized tissues.

References:

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