Image Guided 3D Patterning of Hydrogels to Recapitulate Microvascular Structures

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Statement of Purpose: The formation of a functional vasculature within a biomaterial scaffold will be necessary to engineer metabolically complex tissues. Physiologically, the structure of the microvasculature is tissue specific and directly related to tissue function. Therefore, when promoting the vascularization of scaffolds, it will be important to guide vessel formation to mimic the structure of the endogenous microvasculature. Here, we present the application of two-photon absorption laser scanning lithography (TPA-LSL to fabricate bioactive micropatterns that mimic the architecture of endogenous microvascular networks. Specifically, images of murine vessel networks were converted to regions of interest(ROI) files that were then utilized to control a two-photon laser beam to initiate crosslinking of monoacrylate-PEG-RGDS within an acrylate based poly(ethylene glycol) hydrogel. RGDS biomolecular patterns mimicked three-dimensional (3D) vascular structures derived from multiple distinct tissues, and hold the potential to guide the formation of a complex, tissue specific vasculature for use in regenerative medicine applications.

Methods: *Imaging Mouse Microvasculature*
Vessels in the murine coronary, cerebral cortex, and retina were fixed and imaged after cardiac injection of lysine fixable, fluorescently-labeled 70k dextran.
Conversion of Images to Regions of Interest Files
Vessel images were converted via thresholding to binary files. A segmenting algorithm was applied to recreate the image from quadrilateral regions of interest. ROIs were then combined into a single overlay file for each cross-section. Each file was loaded onto a computer controlling a Zeiss LSM 710 multiphoton microscope.
PEG hydrogel fabrication
Coverglass was piranha cleaned, and reacted with 85 mM 3-(Trimethoxysilyl)propyl methacrylate to introduce acrylate groups to the glass. A solution of 10% enzymatically degradable PEG-DA in HBS with 10 µL/mL of 300 mg/mL 2,2-dimethoxy-2-phenyl-acetophenone (DMAP) in *N*-vinyl pyrrolidone (NVP) was then injected between an acrylated coverslip and a glass slide separated by a 125 µm spacer. The hydrogel was then crosslinked and immobilized through a 45 second exposure to UV light (365 nm).
Microvascular Patterning Strategy
A hydrogel was first incubated in 50-100 nmol/mL of fluorescently labeled acrylate-PEG-RGDS in HBS with 10 µL/mL of 300 mg/mL DMAP in NVP for 30 min. The hydrogel was then placed on the multiphoton microscope stage with the first overlay file selected. A laser tuned to 720 nm with a laser scan speed of 25 µsec/pixel and a laser intensity of 60 mW/µm^2 was then used to excite photoinitiator molecules in precise locations designated by the overlay file, resulting in the crosslinking of free acrylate groups on acrylate-PEG-RGDS to free acrylate groups in the PEG based hydrogel. In order to pattern a 3D vessel bed, a new overlay file was selected for each successive scan as the focus was adjusted axially through the Z plane. In all cases, the distance of the focal adjustment corresponded exactly with the distance between the vessel image slices from which the overlay file was derived. Patterns were visualized after washing using traditional confocal microscopy.

Results: *Micropatterning Endogenous Microvasculature*

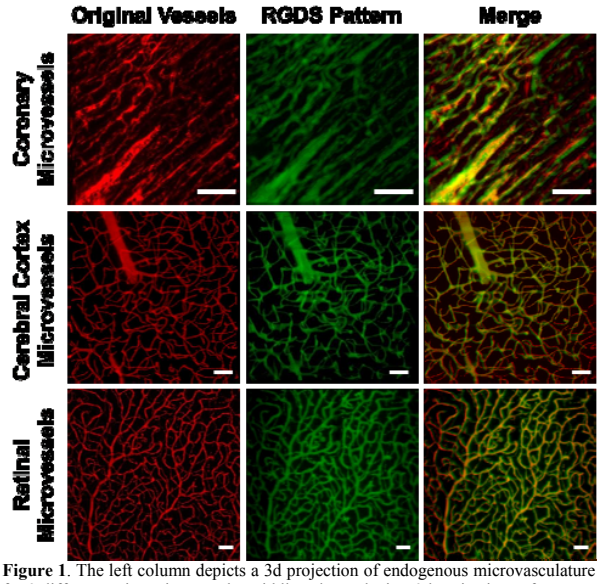

Figure 1. The left column depicts a 3d projection of endogenous microvasculature for 3 different unique tissues. The middle column depicts 3d projections of patterns of fluorescent acrylate-PEG-RGDS within PEG-PQ hydrogels. The right column shows a merge of the original vessels and the hydrogel pattern, demonstrating that the patterns mimic endogenous vessels with high fidelity. All scale bars = 50 µm.

In figure 1, we have shown the ability to fabricate micropatterns that mimic the 3d microvasculature of the murine coronary, cerebral cortex, and retina. After merging the original vessel images with the RGDS pattern hydrogel images, we have observed a very precise overlap (right column), indicating that our patterns mimic endogenous vessel structures with high fidelity.

Conclusions: In this work we have developed a novel two-photon patterning technique that utilizes a direct biomimetic approach to design an enzymatically degradable poly(ethylene glycol) scaffold with bioactive molecules patterned into tissue specific 3d vascular structures. This method serves as an important step toward the spatial control of vessel growth within an engineered tissue scaffold, and by extension, toward the regeneration of a variety of metabolically complex tissues.