Recapitulation of Endogenous Microvascular Structures Using Two Photon Absorption Laser Scanning Lithography

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Statement of Purpose: The integration of growth factors and adhesive ligands into a biocompatible support scaffold is widely acknowledged to be a key component of a successful tissue engineering strategy. Furthermore, the patterning of bioactive factors into physiologically relevant microstructures within a scaffold may provide a means to more precisely guide cellular migration and organization towards desired tissue development. In this work, we present the application of two-photon absorption laser scanning lithography (TPA-LSL) to create bioactive micropatterns that precisely mimic the architecture of endogenous microvascular networks. Specifically, vessels in the mouse cerebral cortex were imaged and converted to regions of interest files via custom written software. Regions of interest files were then used to control a tightly focused laser beam that initiated precise crosslinking of fluorescently labeled monoacrylate-PEG-RGDS within poly (ethylene glycol) diacrylate (PEG-DA) hydrogels.

Methods: *Imaging Mouse Microvasculature*

Functional vessels in the mouse cerebral cortex were imaged via confocal microscopy in fixed tissue after cardiac injection of fixable, fluorescently-labeled dextran.

Conversion of Images to Regions of Interest Files

Custom image processing software was utilized to convert tissue images to binary regions of interest files. Generated region of interest files were then loaded onto the computer controlling the LSM 510 META NLO confocal microscope to control scanning of a 720 nm laser.

PEG-DA Hydrogel fabrication

A glass coverslip was piranha etched and incubated with 85 mM 3-(Trimethoxysilyl)propyl methacrylate (ph 4.5) in ethanol to introduce surface acrylate groups. A prepolymer solution of 10% (w/v) PEGDA in HBS with 10 µL mL^{-1} of 300 mg mL^{-1} 2, 2-dimethoxy-2-phenylacetophenone (DMAP) in *N*-vinyl pyrrolidone (NVP) was then prepared and injected between an acrylated coverslip and a glass slide separated by a .5 mm spacer. The hydrogel was then pre-crosslinked and immobilized to an acrylated coverslip through a 45 second exposure to UV light (365 nm).

Fluorescent monoacrylate PEG-RGDS synthesis

Acrylate-PEG-SCM (Laysan) was reacted with RGDS at a ratio of 1.2:1 in DMSO. The resulting monoacrylate PEG-RGDS was then further reacted with an Alexafluor488 TFP ester (Invitrogen) in 0.1 M sodium bicarbonate buffer (ph 9.0) to form a fluorescent monoacrylate PEG-RGDS product that was further purified via dialysis.

Microvascular Patterning Strategy

The pre-crosslinked PEG-DA hydrogel was incubated in a solution of fluorescently labeled monoacrylate PEG-RGDS (50-100 nmol) in HBS with 10 µL mL^{-1} of 300 mg mL^{-1} DMAP in NVP. The hydrogel was then placed on the stage of a LSM 510 META NLO confocal microscope. The prepared region of interest file was then selected in the LSM software, and the desired Z plane was specified via focus adjustment. A two-photon titanium/sapphire laser tuned to 720 nm was then scanned across the designated regions of interest to crosslink free acrylate groups in the PEG-DA hydrogel with the fluorescent acrylate-PEG-RGDS. The microscope stage was then adjusted axially before again scanning across the regions of interest. This process was serially repeated to create patterns of desired axial dimensions. The hydrogel was then washed with HBS to remove unbound fluorescent acrylate-PEG-RGDS and the resulting pattern was imaged on the confocal microscope.

Results: *Micropatterning of Cortex Microvasculature*

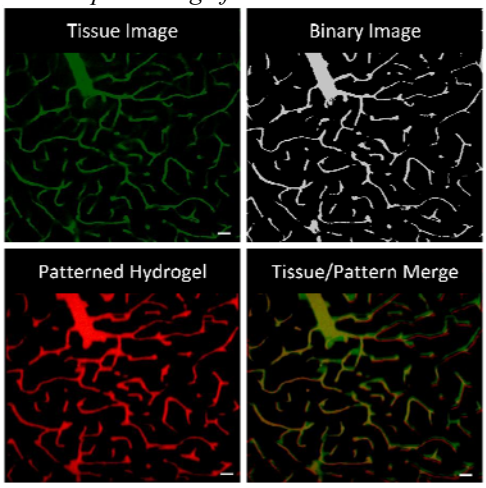

Figure 1. (Top left) Confocal image of vessels in mouse brain cortex. (Top right)Binary region of interest file (Bottom Left) Pattern of fluorescent acrylate-PEG-RGDS (Bottom right) Merge of original vessels image and acrylate-PEG-RGDS pattern. All scale bars 25 µm.

In figure 1, we show the ability to produce micropatterns that mimic the microvasculature of the mouse cerebral cortex with high fidelity. An image of vessels within the mouse brain was converted to a region of interest file that then directed a 720 nm laser to initiate designed crosslinking of fluorescent acrylate-PEG-RGDS within the PEG-DA hydrogel. A merge of the original image of endogenous vessels with that of a pattern in a PEG-DA hydrogel shows the ability of this technique to mimic complex structures with a high degree of precision.

Conclusions: In this work we have shown the ability to recapitulate the mouse cerebral cortex microvasculature with a pattern of fluorescently labeled monoacrylate-PEG-RGDS within a PEG-DA hydrogel. Specifically, we utilize TPA-LSL to crosslink fluorescent acrylate-PEG-RGDS moieties in minute three dimensional focal volumes specified by region of interest files that mimic the cortex microvasculature. Future work will focus on the mimicry of microvasculature networks in collagenase degradable PEG based hydrogels in vitro and in vivo.