Fabrication of Biomimetic µfluidic Networks Using Image-Guided Photocavitation-Mediated Hydrogel Degradation

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Statement of Purpose: *In vivo*, tissue is highly vascularized to provide nutrients and oxygen necessary for cell survival. Furthermore, complex tissues, such as the liver, contain multiple fluidic systems (arterial, venous, biliary, and lymphatic) that do not directly connect yet interact via biomolecular mass transport. The ability to recapitulate complex *in vivo* fluidic architecture in synthetic constructs could open new avenues for the implementation of biomimetic µtissues for high-throughput drug screening applications. Toward this goal, we previously demonstrated the ability to induce endothelial cells (ECs) to adopt a 3D architecture derived from native tissue vasculature[1]. While this approach allowed for direct control over 3D EC organization, it did not result in perfusable fluidic networks. To overcome this limitation, we have developed a laser-induced, photocavitation-mediated fabrication technique, driven by optical breakdown and plasma formation[2], to locally degrade poly(ethylene glycol) diacrylate (PEGDA) hydrogels in desired 3D configurations for the fabrication of µfluidic systems. We demonstrate the ability to create complex, interconnected, 3D µfluidic networks derived from native tissue vasculature (Fig. 1) and independent intertwining µfluidic architectures that do not connect yet communicate via biomolecular diffusion (Fig. 2); demonstrating the potential to recapitulate *in vivo* fluidic architecture and transport in synthetic constructs.

Methods: Replica molded 5% weight/volume PEGDA hydrogels were fabricated via photopolymerization of 3.4 kDa PEGDA in HEPES-buffered saline (pH 8.3, 10 mM HEPES, 100 mM NaCl, 1.5% triethanolamine, 10 µL/mL Eosin Y, and 3.5 µL/mL N-vinylpyrrolidone) using white light (3.1 mW/cm^2) for 3 min between a poly(dimethylsiloxane) mold and a 3-(trimethoxysilyl)propyl methacrylate functionalized glass coverslip to create wells in the base hydrogel. Image planes of 3D models, *in vivo* cerebral cortex vasculature (Fig. 1A), 3D intertwining channels designed in SolidWorks (Fig. 2A), or rectangular channels (10x10x50 µm), were processed using MATLAB scripts to approximate the image features as a mosaic of quadrilaterals.[1] The quadrilateral coordinates were exported in a format recognized by Zeiss ZEN software to define regions of interest (ROIs) for each feature in each plane.[1] The ROIs guided a 790 nm 140 fs pulsed Ti:S laser focused through a 20X (NA1.0) water immersion objective operating at 21.9 - 37.7 nJ/µm^2 to define regions where the PEGDA gel was degraded via photocavitation. The extent of degradation was quantified by (1) intensity measurements of Eosin Y, (2) diffusion of 2000 kDa fluorescein-labeled (FITC) and 10 kDa tetramethylrhodamine-labeled (TRITC) dextran, and (3) intensity measurements of fluorescently-labeled acryl-PEG-RGDS coupled after degradation.

Results: We demonstrate the ability to fabricate complex, interconnected, 3D µfluidic networks derived from the architecture of native vasculature (Fig. 1B) as well as non-connected, intertwining µchannels developed in SolidWorks that interact via biomolecular transport (Fig. 2B) in PEGDA hydrogels. We also demonstrate that the degree of degradation, local pore size of the PEGDA, can be controlled by tuning the laser fluence or scan speed. A fluence of 37.7 nJ/µm^2 resulted in total degradation of the PEGDA and open µchannels completely free of polymer as verified by post photocoupling of fluorescently-labeled acryl-PEG-RGDS. Decreasing the laser fluence to 21.8 nJ/µm^2 and/or increasing the scan speed allowed for the fabrication of degradation gradients resulting in µfluidic channels with well-defined pore sizes ranging from ~2.36 nm to completely degraded channels as determined by quantifying diffusion of varying molecular weight fluorescently-labeled dextran, 10 to 2000 kDa, with corresponding Stokes radii of 2.36 to 27 nm respectively.

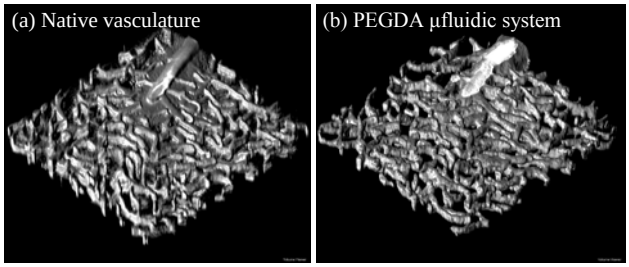

Fig. 1: Cerebral Cortex Vasculature-Derived µfluidic Network. 3D renderings of (a) native vasculature *in vivo* (450x450x30 µm) and (b) resulting vasculature-derived µfluidic system in PEGDA perfused with 2000 kDa FITC-dextran.

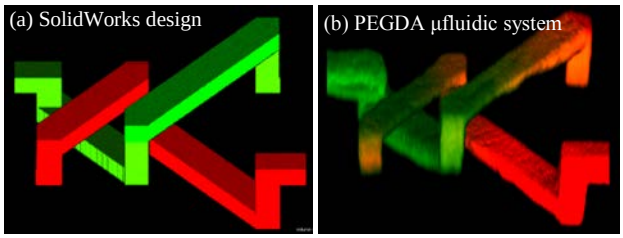

Fig. 2: Diffusion Between 3D Non-connected, Intertwining Channels. 3D renderings of (a) intertwining channels created in SolidWorks and (b) resulting µfluidic system in PEGDA; (*green*) 10 kDa FITC-dextran and (*red*) 10kDa TRITC-dextran. (b) Notice diffusion-mediated crosstalk between the two channels.

Conclusions: Image-guided, laser-based, photocavitation-mediated degradation allows for the fabrication of complex, biomimetic µfluidic networks difficult to achieve with current fabrication methods. This technique can alter transport properties through µfluidic systems by modulating local pore size and by bringing opposing fluidic networks into communicable range, thus opening new avenues for recapitulating *in vivo* transport *in vitro*.

References

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