FABRICATION OF 3D BIOMIMETIC MICROFLUIDIC NETWORKS

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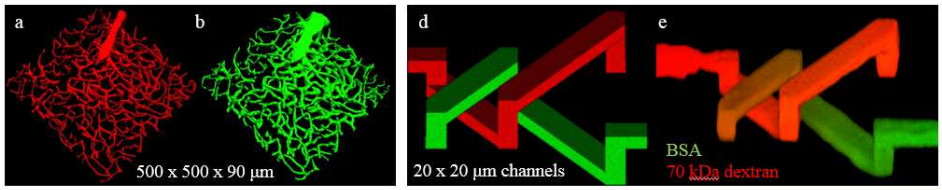
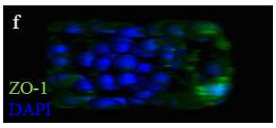
Introduction: Recapitulation of *in vivo* transport *in vitro* is essential in creating truly biomimetic microtissues for disease models and high-throughput drug screening. Toward this goal, we previously demonstrated the ability to recapitulate *in vivo* 3D endothelial cell organization in a synthetic hydrogel using image-guided, laser-based patterning of an adhesive peptide.[1] We further extended this laser-based strategy to fabricate perfusable, endothelialized, biomimetic µfluidic networks. We demonstrate the fabrication of 3D µfluidic systems derived from native vasculature (Fig. 1a,b), as well as multiple independent 3D µfluidic networks in close proximity that never directly connect, yet allow for transport similar to *in vivo* transport between vascular and lymphatic systems (Fig. 1d,e). These 3D µfluidic networks provide a means to recapitulate complex *in vivo* transport *in vitro*.

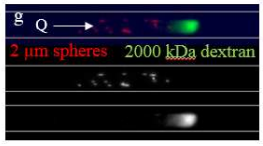
Materials & Methods: Micromolded, 5% w/v poly(ethylene glycol) diacrylate (PEGDA) hydrogels were photopolymerized under white light (3 min @ 3.1 mW/cm^2) via reaction of 3.4 kDa PEGDA in HEPES-buffered saline (pH 8.3, 10 mM HEPES, 100 mM NaCl, 1.5% triethanolamine, 3.5 µL/mL N-vinylpyrrolidone, and 10 µL/mL Eosin Y). 3D confocal stacks of *in vivo* vasculature and a 3D SolidWorks model of independent, intertwining µchannels were processed with a MATLAB script[1] to convert image features into spatially defined regions of interest (ROIs). ROIs guided the position of 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 induce hydrogel degradation via photocavitation. The µfluidic networks were characterized via diffusion of fluorescent bovine serum albumin (BSA) and varying molecular weight dextran. µchannels cultured with endothelial cells (ECs) were fluorescently immunolabeled for DAPI and ZO-1 and imaged with confocal microscopy to confirm lumen formation.

Results & Discussion: The ability to pattern perfusable µfluidic networks in a hydrogel with high resolution is demonstrated through recapitulation of cerebral cortex vascular structure (Fig. 1a,b); skeletonization analysis confirms the similarity of the networks (Fig. 1c). Fabrication of a SolidWorks model demonstrates the ability to construct multiple, independent, and intertwining µfluidic networks in close proximity (Fig. 1d,e). Quantification of diffusion of fluorescent BSA and dextran between µchannels (Fig. 1e) lends itself towards study of *in vivo* transport processes. These µchannels can be successfully endothelialized, as seen by the formation of an intact lumen (Fig. 1f), and fluidized via connection to a syringe pump. Furthermore, in lieu of creating a completely open channel system, the pore size of the hydrogel can be controlled by altering the laser energy. Pore size is assessed by measuring dextran diffusion into the degraded µchannels. By gradually decreasing the porosity within a channel, we show particle trapping via size-based exclusion (Fig. 1g).



c	In Vivo	µFluidic Network
Branch Points	418	418
Avg. Diameter	3.79 µm	3.78 µm
Total Length	23.36 mm	23.02 mm
Total Volume	3.28 x 10^5 µm^3	3.19 x 10^5 µm^3





(a) *In vivo* vasculature and (b) hydrogel µfluidic network demonstrate (c) high resolution and high fidelity network generation. (d) SolidWorks model used for transport studies in (e) µfluidic networks. (f) Endothelialized 50 µm channel with complete lumen. (g) Size-based species separation in porosity gradient channel, 50 µm channel, Q = 0.02 mL/min.

Conclusions: Photocavitation-mediated hydrogel degradation allows for superior architectural control over *in vitro* cell culture platforms by providing a means to generate 3D biomimetic µfluidic networks, multiple µfluidic systems in close proximity, and controlled hydrogel porosity.

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References: 1. Culver, JC. Adv. Mater. 2012:24:2344–2348.