

Top-down Fabrication of Endothelialized Capillary-like 3D Channel Networks throughout Thick Hydrogels

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In the cardiovascular system, blood is pumped from the heart to arteries, then to arterioles, to capillaries, to venules, and lastly to veins. Arterioles, which are less than 60-100 μm in diameter branch into capillary networks which are 5-10 μm in diameter. Capillaries are responsible for the exchange of gases, nutrients, and wastes between tissues and blood. While many researchers have demonstrated top-down fabrication approaches to show the ability to produce channels containing a layer of endothelial cells surrounded by a basement membrane, they have yet to show the scaling of these channels past the size of an arteriole. Top-down fabrication approaches start by patterning the micro-channels within hydrogels to create vascular networks, followed by the introduction of endothelial cells to line the channel walls. It is important to note, however, that capillaries are the vessels where critical exchange of soluble compounds occurs, leveraging their single-cell thick walls and high surface area. Tissue engineers, however, have yet to demonstrate top-down fabrication of endothelialized channels less than 60 μm , a threshold far larger than the ~ 10 μm diameter of a capillary; thus current approaches fail to replicate natural capillary bed architecture. The significance of this work lies in replicating the capillary architecture, complete with endothelialized vasculature in large volumes of engineered tissue. Our specific aim was to produce a network of interconnected capillary-sized channels lined with endothelial cells which can be perfused to produce an in vitro model of capillary networks within thick hydrogels. Hydrogel scaffold fabrication process involved using sacrificial fibers formed by solvent-spinning Soluplus®, a thermoresponsive polymer, embedded in gelatin, to make networks of channels with architectures that mimic the capillary bed. We performed initial control experiments to show diffusion of dextran from these capillary channels into the gel. We embedded GFP-HUVECs into our capillary network channels and were able to get them to successfully line the channel walls. Green Fluorescent Protein (GFP) expressing Human Umbilical Vein Endothelial Cells (HUVEC) were introduced into the channels and imaged with confocal microscopy at several timepoints. Future experiments will include replicating these experiments with endothelialized channels to demonstrate barrier properties and viability of these endothelialized cells in the capillary channels over time. In addition, future work will involve optimizing cell growth and proliferation, and incorporate cells from the parenchymal space like fibroblasts. Recent advances in vascularized microfluidic hydrogels have led to 50-100 μm endothelialized channels, however, this technique allows for patterning of the smallest to date (5-50 μm) channels that can be lined with endothelial cells and perfused to produce an in vitro model of capillary networks within a gelatin scaffold. This work is a major stepping stone towards engineering a complete microcirculatory system.

