Versatile Fabrication of Perfusable Human Microvessels with a Commercially Available 3D Printer

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Introduction: The inner lining of blood vessels is formed by the vascular endothelium, which integrates physical and chemical stimuli to regulate vessel barrier function.[1] Defects in vascular barrier function contribute to a host of health complications, including cardiovascular disease, which remains the leading cause of death for Americans.[2] Due to the link between vascular permeability and the progression of cardiovascular disease, investigating the cellular and molecular regulators of vascular barrier function is critical.[3] Microfluidic human engineered microvessel (hEMV) platforms offer three-dimensional in vitro methods to model microvasculature.[4] Microfluidic devices are traditionally fabricated by photolithography, but this method possess shortcomings in terms of the training, time, and overhead to produce a usable device. We present a novel method to develop perfusable human microvessels using a microfluidic device mold derived from a stereolithography 3D printer. The method presented here allows for the rapid prototyping of different designs as well as the creation of multiple blood vessels separated in 3D space.

Materials and Methods: Resin device molds were generated using a Form 2 Formlabs stereolithography 3D printer and Clear V4 resin. The molds were cured using UV light for 15 minutes at 60°C and surface treated with both a 2-hour ethanol wash, and a vapor disposition of trichloro(1H, 1H, 2H, 2H-perlfluorooctyl)silane for 1.5 hours at room temperature. Polydimethyl siloxane (PDMS) was mixed in a 1:10 crosslinker:base ratio, poured onto the molds, and left to cure overnight at 60°C. Devices were cut and bonded to a glass coverslip using oxygen plasma, with a 80 µm adhesive needle guide placed in each media port to raise the needles from the surface of the coverslip. The interior gel region of the device was then coated with 2 mg of dopamine hydrochloride in 2 mL of Tris-HCl buffer. Acupuncture needles were coated with 0.01% BSA for 30 minutes prior to insertion into the devices. A 2.5 mg/mL collagen gel was prepared and introduced into the device as previously described.[4] Following a 2-hour cross-linking period at 37°C, the needles were removed from the devices, and seeded with human umbilical vein endothelial cells (HUVECs) at 1.8 million cells/mL in growth media (EGM2, Lonza). The devices were flipped every minute to ensure thorough seeding and were rocked overnight. The devices were re-seeded the following day to reduce potential patchiness of the vessel. Devices were imaged using the Olympus FV3000 confocal microscope after staining with DAPI, AlexaFluor-488 Phalloidin, and in some cases DRAQ-5. Image J and CellProfiler were utilized to characterize the cell number, coverage, and dimensions of the acquired images.

Results and Discussion: The 3D printed microfluidic mold method was to fabricate an orthogonal microvasculature structure (Fig. 1). This structure demonstrated the merit of 3D printing as opposed to photolithography, as the mold's complexity along its height is difficult to achieve with photolithography. HUVECs adhered to collagen in devices from the 3D printed mold on all sides, creating an open, tube-like structure (Fig. 1g). The adhesive needle guide method effectively separated the two microvessels, ensuring a gap of approximately 160 µm between the two vessels (Fig. 1d).

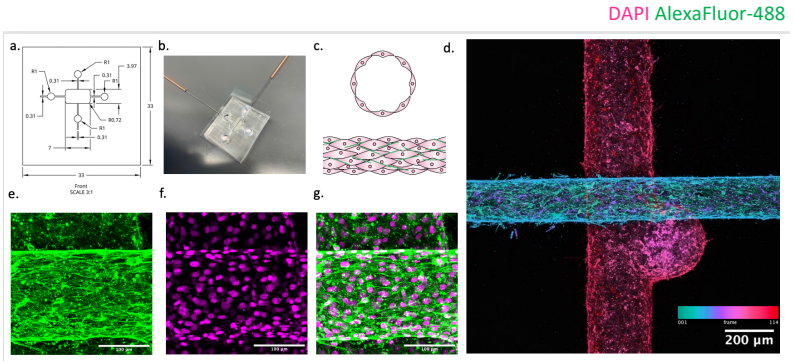

Figure 1. a) CAD drawings for orthogonal device. **b)** Fabricated microfluidic device from 3D printed mold with needles inserted. **c)**Schematic demonstrating cell orientation in device cross-section. **d)** Stained orthogonal microvessels (DAPI, AlexaFluor-488 Phalloidin).

Conclusions: We found that it is possible to create a functional microfluidic device using a mold made from a stereolithographic 3D printer and developed a working protocol for their continued fabrication. Additional experimentation seeks to stimulate angiogenesis between the two orthogonal microvessels. Overall, the ability to create a microvessel using a 3D printer introduces the possibility for rapid design prototyping and offers a dramatic decrease in production time as compared to previous methods.

References: [1]Kutys, M. Curr. Opin. Cell. Biol. 2016. 42: 73-79. [2]Rajendran, P. Int. J. Biol. Sci. 2013. 9:1057-1069. [3]Claesson-Welsh, L. Ups. J. Med. Sci. 2015. 120: 135-143. [4]Polacheck, W. Nat. Protoc. 2019. 14: 1425-1454