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

Sara M. Meehan[1*], Mitesh Rathod[1], Stephanie A. Huang[1], Sarah E. Kubik[1], William J. Polacheck[1,2]

[1]Joint Dept. of Biomedical Engineering, The University of North Carolina at Chapel Hill and North Carolina State University
[2]Department of Cell Biology and Physiology, The University of North Carolina at Chapel Hill School of Medicine

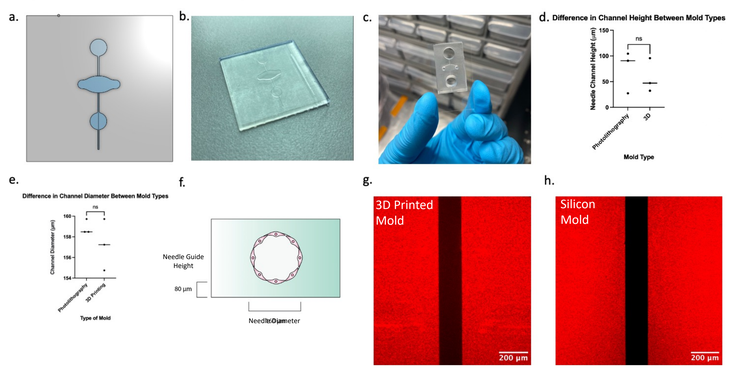
Introduction

- 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 the vascular transport barrier in vitro[4].
- Microfluidic devices are traditionally fabricated by photolithography, but this method presents challenges in terms of the training, time, and overhead to produce a usable design.

Goal: Investigate using stereolithography (SLA) 3D printing as a method for rapidly prototyping microfluidic device designs toward developing perfusable human microvessels.

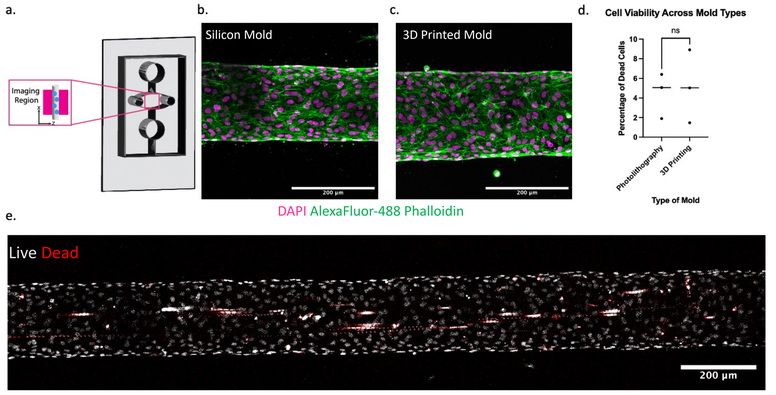
Device Fabrication

- Resin device molds were generated using a Form 2 Formlabs stereolithography 3D printer and Clear V4 resin.
- The molds were surface treated via an ethanol wash, oxygen plasma treatment, and a vapor disposition of trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane at room temperature.
- Following device fabrication, an 80-micron adhesive needle guide was cut using a Cricut die-cutting machine.



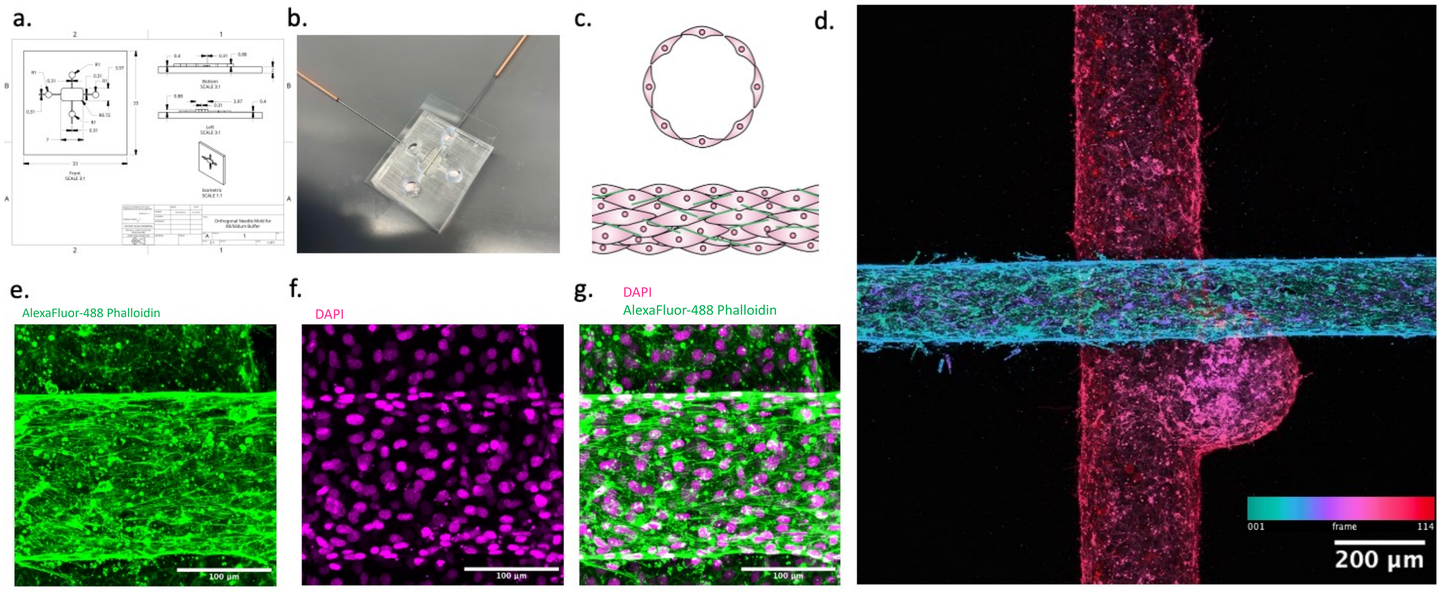
Cell Viability

- HUVECs adhered to type I collagen hydrogels in devices from both 3D printed (Fig. 2c) and silicon molds (Fig. 2b) on all sides, creating an open, tube-like structure.
- A live-dead assay consisting of DRAQ-5 (live) and ethidium bromide (dead) was done on the perfusable microvessels (Fig. 2e).
- Cell viability did not have a significant difference across mold types.



Results

- The cell viability demonstrated in earlier designs allowed for the creation of more complex mold designs, including multiple blood vessels located in different planes from the glass coverslip.
- The blood vessel height was determined using varying quantities of adhesive needle guides, specifically 2 and 5, to form hollow channels separated in space (Fig. 3b).
- The perfusable vessels were stained with DAPI (Fig. 3f, Fig. 3g) and Alexa-488 Phalloidin (Fig. 3e, Fig. 3g) for nuclei and actin, respectively.
- A spatial color code (Fig. 3d) was created using FIJI to demonstrate the differences in blood vessel heights.



Conclusions

- We developed a protocol to fabricate functional microfluidic devices using a mold made from a stereolithographic 3D printer, and we demonstrated that cells adhere to the vessel surface and form perfusable tubes.
- 3D printing also allows for orientation of multiple blood vessels in 3D space, specifically separated in the Z-plane, showing the merit of this method.
- Overall, the ability to create a microvessel using a readily available 3D printer introduces the possibility for rapid design prototyping and offers an increase in production throughput compared to previous methods.

References

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