

Engineering Microvasculature for Mechanotransduction

Joanna McDonald, Crescentia Cho, William Polacheck

Joint Department of
**BIOMEDICAL
ENGINEERING**



ABSTRACT

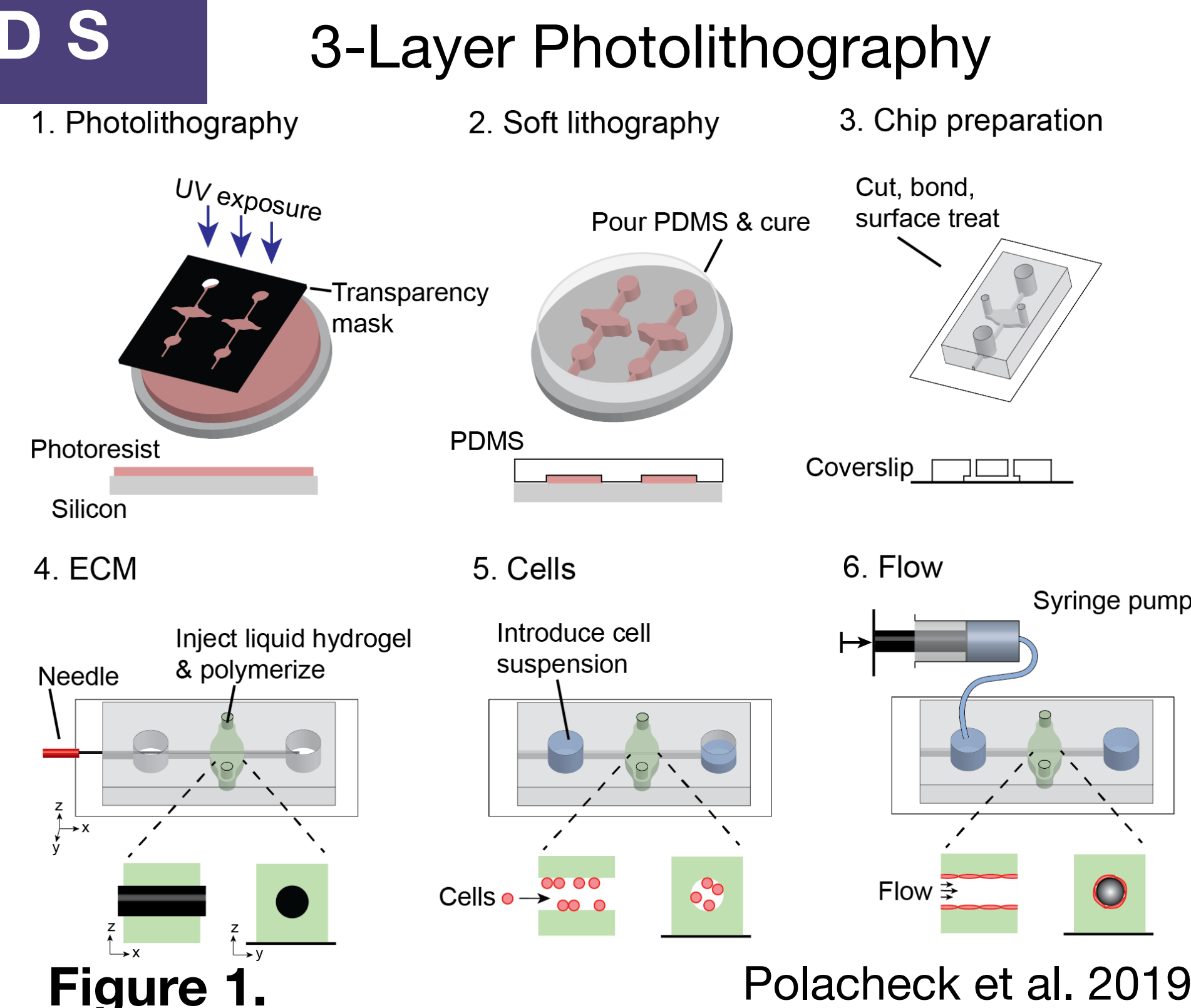
The Polacheck lab has engineered vascular networks in microfluidic devices by combining human umbilical vein endothelial cells (HUVEC) and human lung fibroblasts (HLF) in 3D fibrin hydrogels. However, microvasculature formed using this method produced vessels that were sparse, thin, and not perfusable due to a lack of patent lumens. A major contributor to this lack of function is thought to be fibrin hydrogel degradation due to HLF-mediated proteolysis contractile forces. Preliminary work suggests that limiting contact between HLFs and HUVECs results in better network morphology and hydrogel integrity. The aim of my project is to investigate why limited contact between the two cell types results in improved network topology and function and work towards creating more controlled microvascular networks that could eventually be implanted.

INTRODUCTION

Ischemic cardiovascular disease leads to stroke, heart failure, and impaired blood circulation and is one of the leading causes of death in the world. Current treatments include surgery and synthetic vascular grafting to replace upstream vessels, but these methods do not address downstream reperfusion problems located in the microvasculature. Therefore, there is a need to design and synthesize functional engineered vascular networks in vitro to study treatment methods.

METHODS

Fibrin gel was used to represent the ECM as it creates a soft, 3D ECM to enable cellular remodeling of the microvascular networks, allows access for biochemical assays, as well as high resolution imaging.



RESULTS

Figure 1 displays the double-channel microfluidic devices used in these experiments in which the microvascular network is patterned between the two channels. In terms of the microfluidic device fabrication process, I used larger biopsy punches for the gel ports to increase the surface area of interaction of the HUVECs with the HLFs. At the step of injecting the fibrin gel into the hydrogel ports, I first had to make a fibrin gel with the LifeAct GFP HUVECs, let that polymerize for 15 minutes, and then add a fibrin gel with the fibroblasts just on top of the gel ports and let that polymerize for another 15 minutes. Initially, I ran into several obstacles with getting this protocol to work, including the gels continuing to rip after removing the stainless steel needles. Increased thrombin concentration in the gel would increase its stiffness, so I conducted titration experiments to determine the ideal thrombin concentration. The initial protocol used 4 U/mL of thrombin, so I tried 6 U/mL and 8 U/mL and found that 6U/mL yielded the least amount of ripped gels. Using that thrombin concentration, microvascular networks formed and were imaged using an Olympus FV3000 confocal microscope to create z-stack images, as shown in Figure 3.

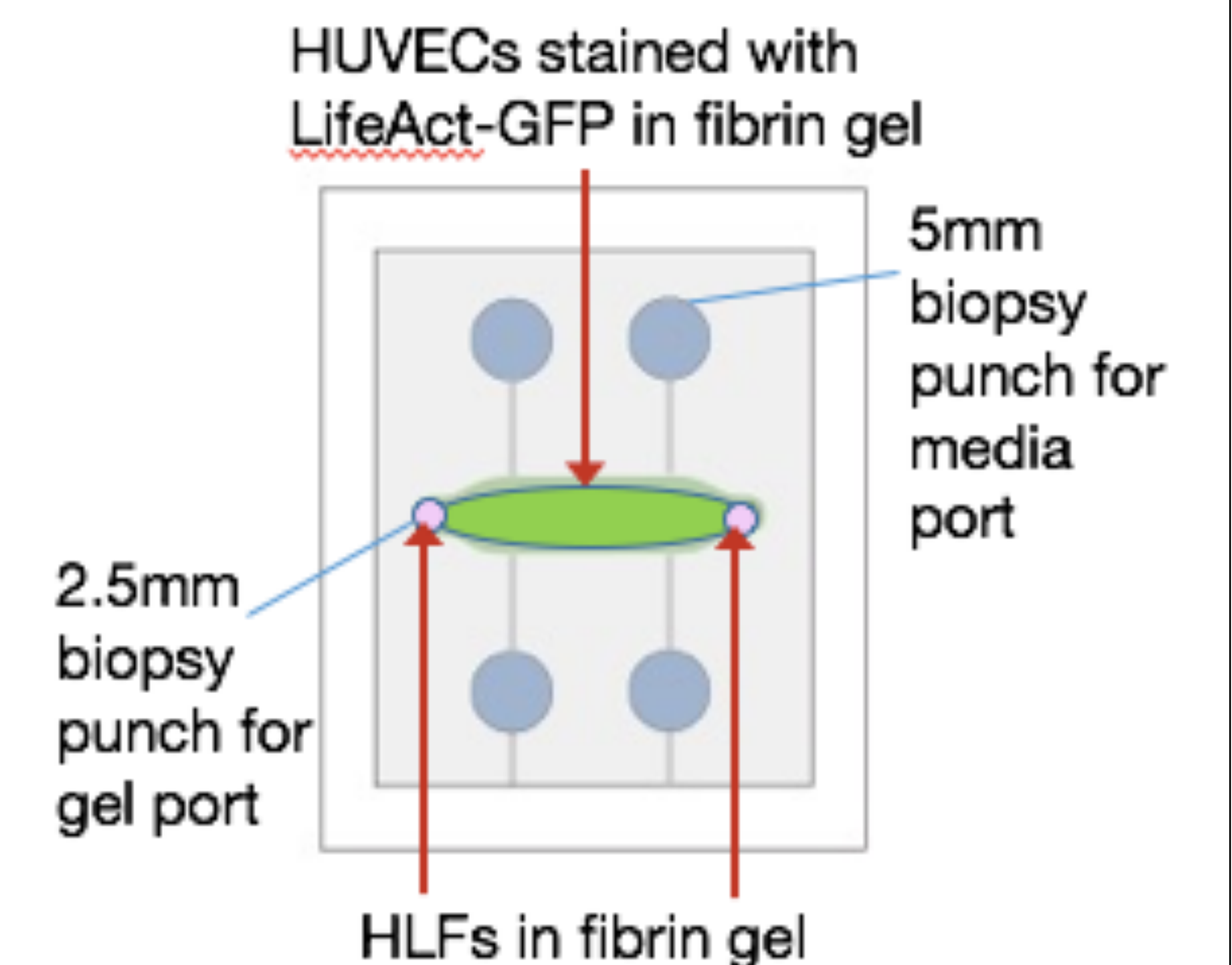


Figure 2.

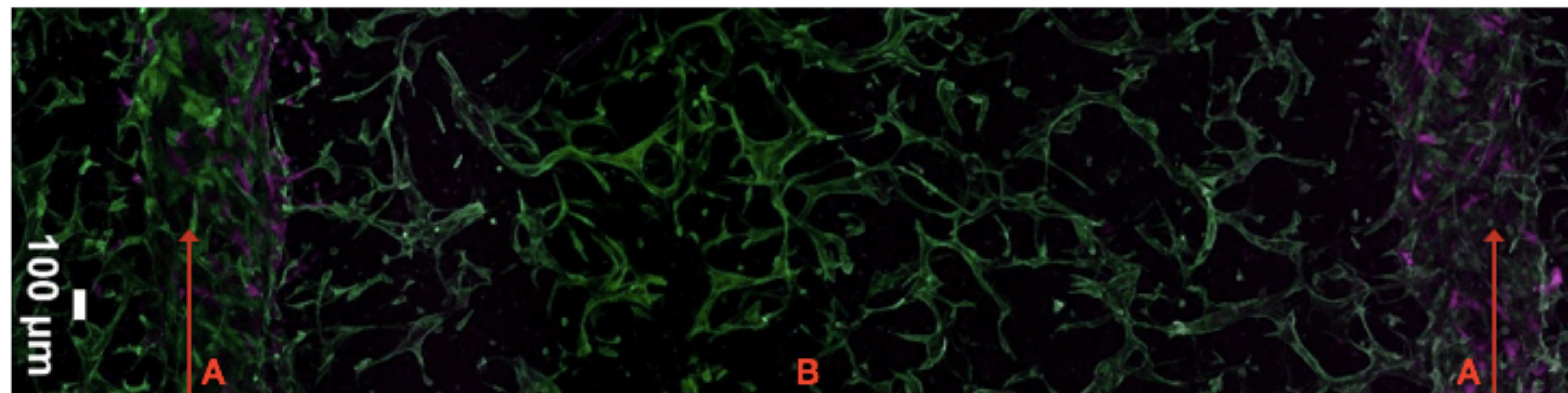


Figure 3.

- Imaged using a 10x objective lens on FV3000 confocal microscope
- Green:** HUVECs labelled with LifeAct GFP
- Magenta:** HUVECs labelled with LifeAct Ruby
- Imaged at 120 hours compared to previous 72 hour life span
- A** – Parent Channel
- B** – Network

CONCLUSION

Figure 3 above is a stitched image to show the entire network created, with the two parent channels seeded with HUVECs labelled with LifeAct Ruby in magenta and the network shown ingreen. With this modified protocol of separating out the HLFs from the HUVECs, these networks were able to grow for 120 hours without ripping compared to the previous 72 hour limitation. Figure 4 shows the network generated had patent lumens, which is important because microvascular networks are only useful if they can carry blood.

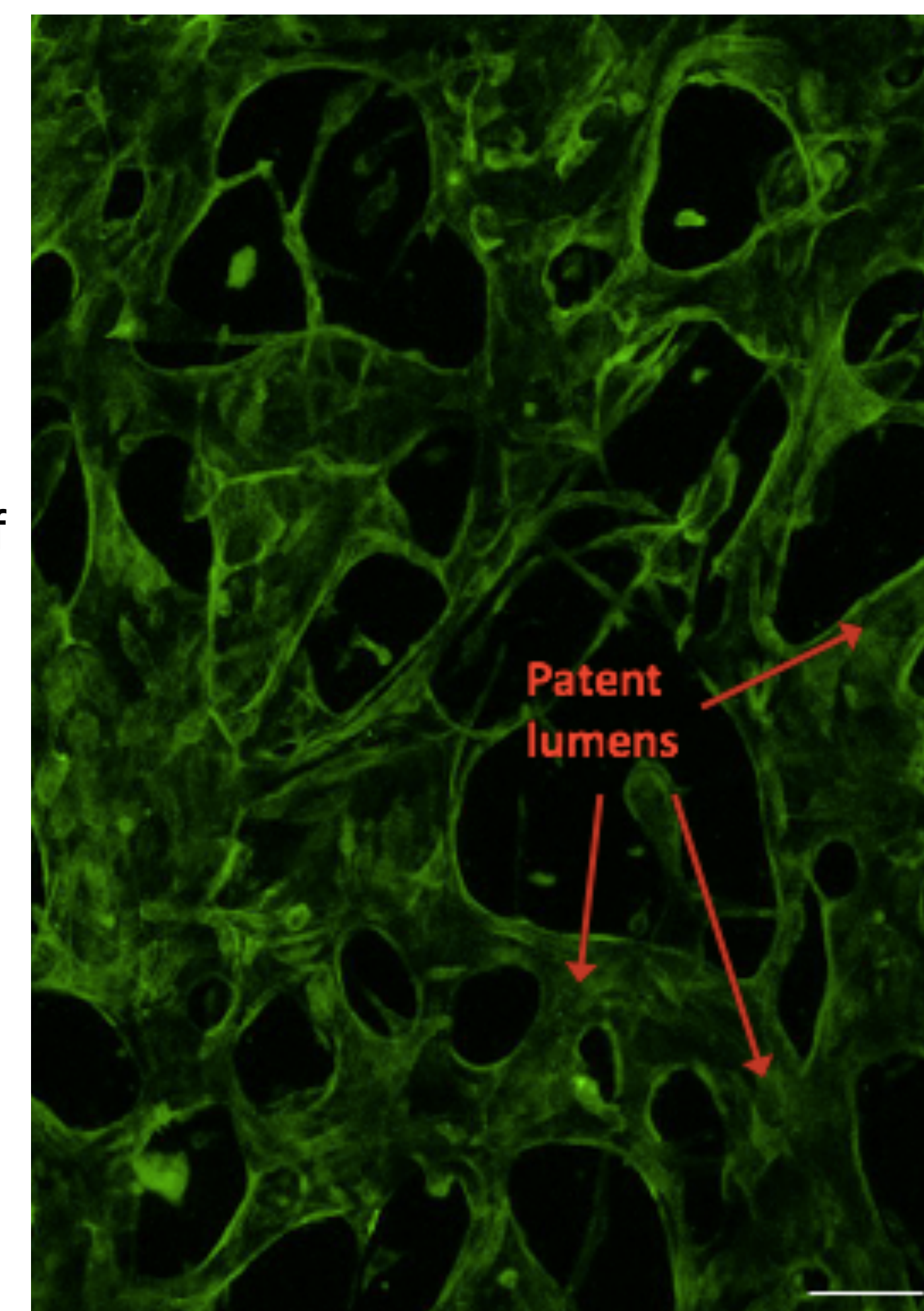


Figure 4.

FUTURE DEVELOPMENT

Future work will involve developing a protocol to increase the mechanical integrity of hydrogel by doping in collagen with the fibrin. Additionally, I will use far-red fluorescent fibrinogen (Alexa Fluor 647) to quantify the gel ripping through fluorescent imaging.

REFERENCES

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- Chen, Michelle B, et al. "On-Chip Human Microvasculature Assay for Visualization and Quantification of Tumor Cell Extravasation Dynamics." *Nature Protocols*, vol. 12, no. 5, 2017, pp. 865–880., doi:10.1038/nprot.2017.018.