MERCK
INVENTING FOR LIFE

Tech Session 4: Additive Manufacturing

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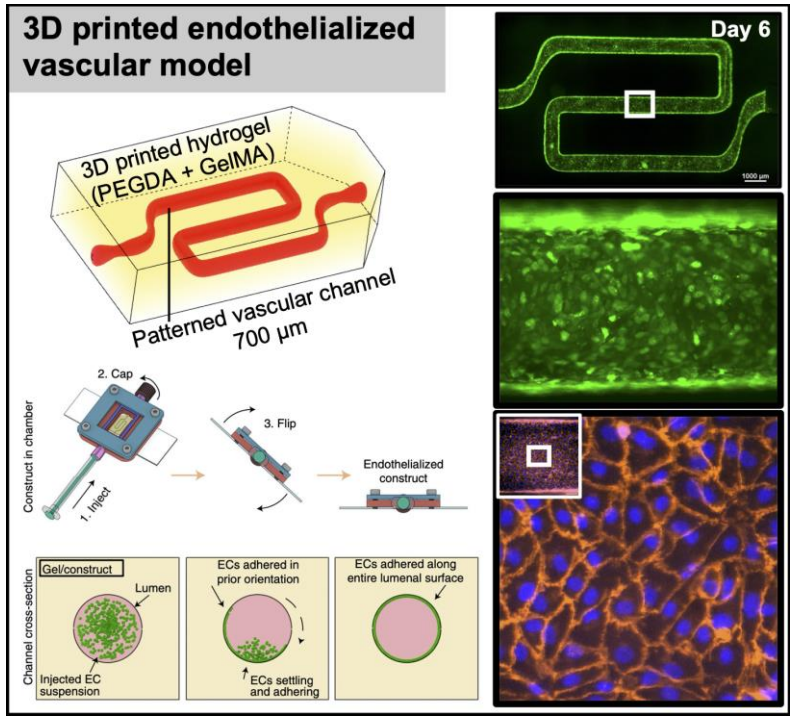
Advanced Delivery Science

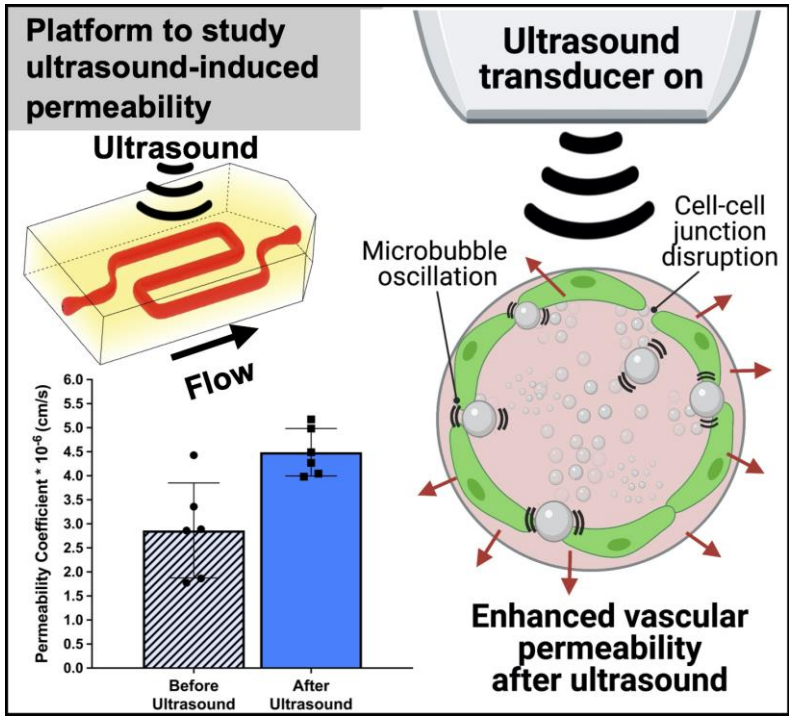
Leveraging Additive Manufacturing to Assay Ultrasound Enhanced Permeability

Goal: To develop a 3D printed *in vitro* vascular model that can be used to assess transport through vasculature for therapeutic applications

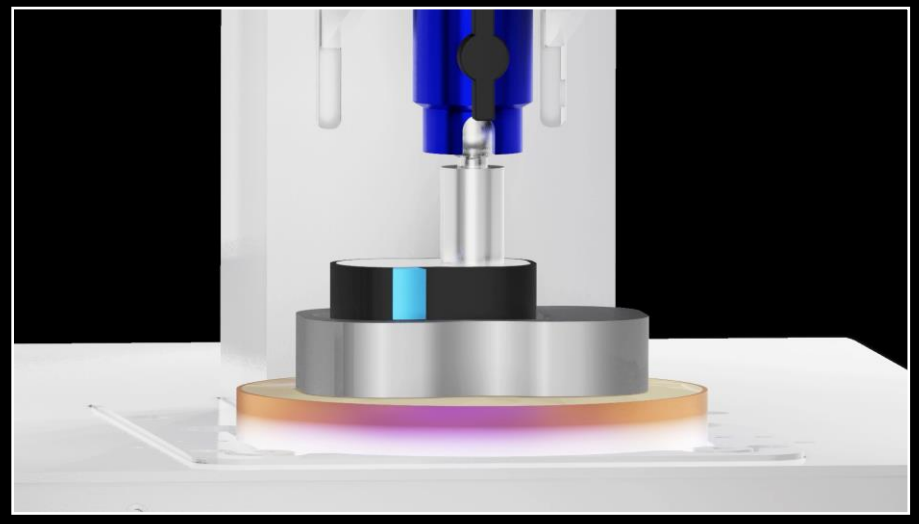
Specific Aim 1: To develop a perfused endothelialized vascular model within 3D-printed tissues

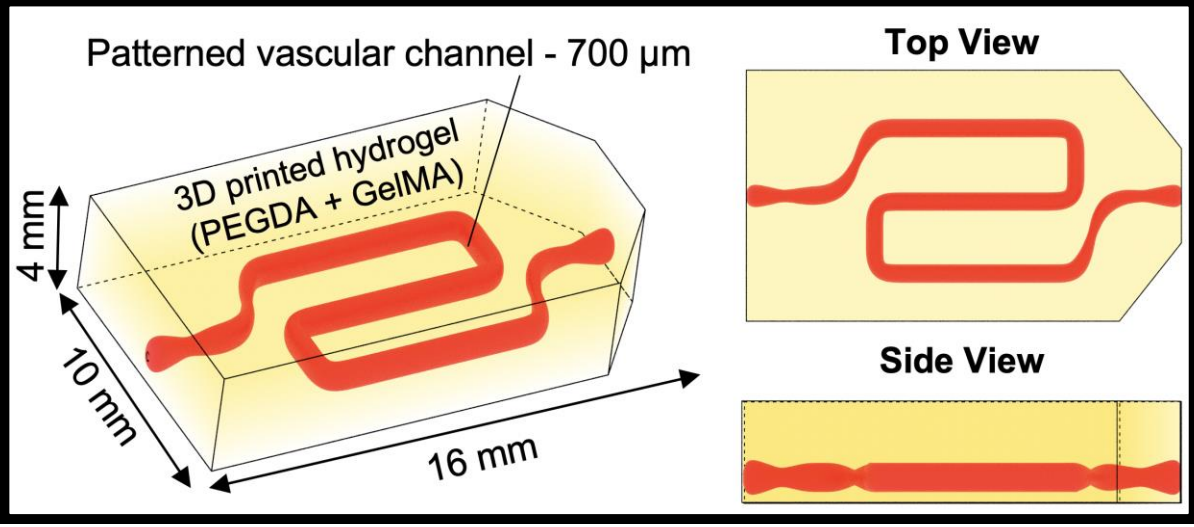


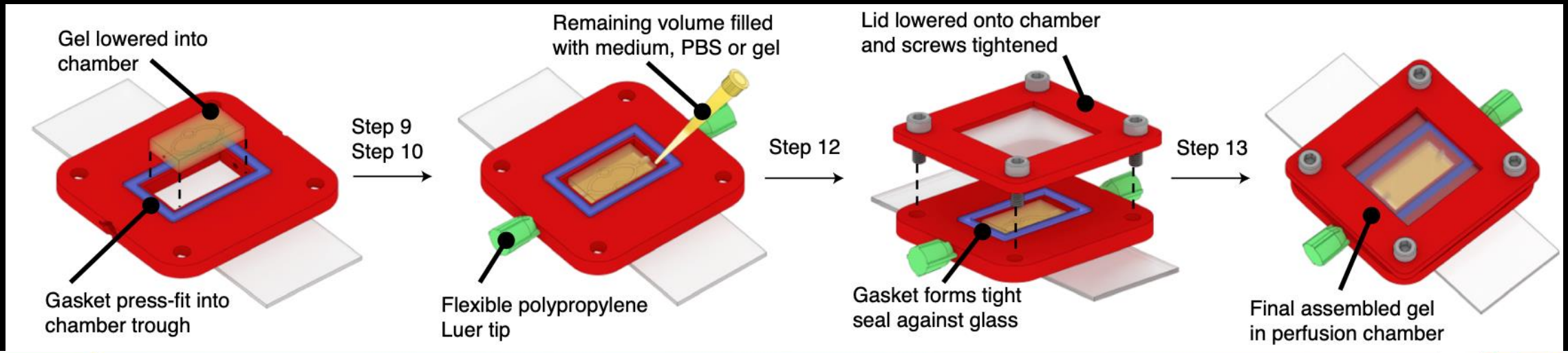
Specific Aim 2: To demonstrate this vascular model as an *in vitro* screening tool for assaying ultrasound-induced permeability



Demonstrated a method of consistent endothelialization within 3D printed hydrogels

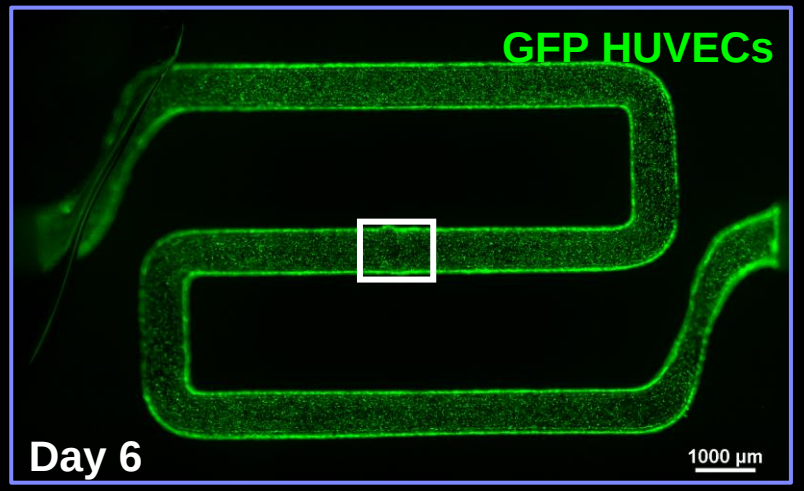


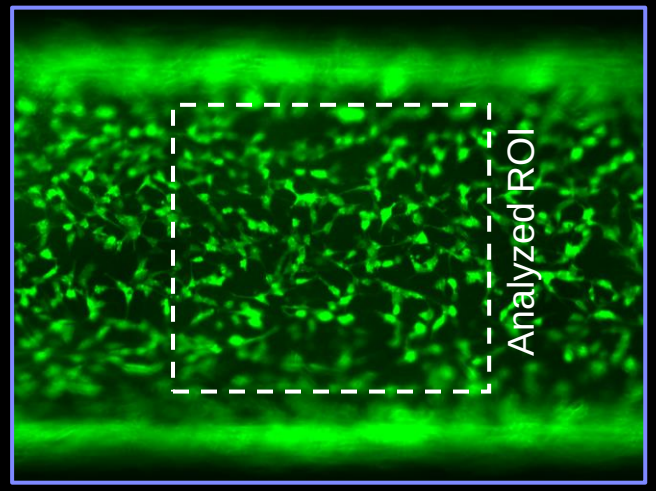


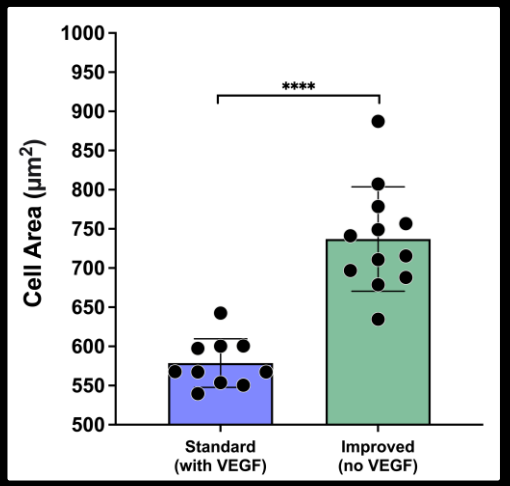


Removal of VEGF resulted in better coverage and more cobblestone morphology

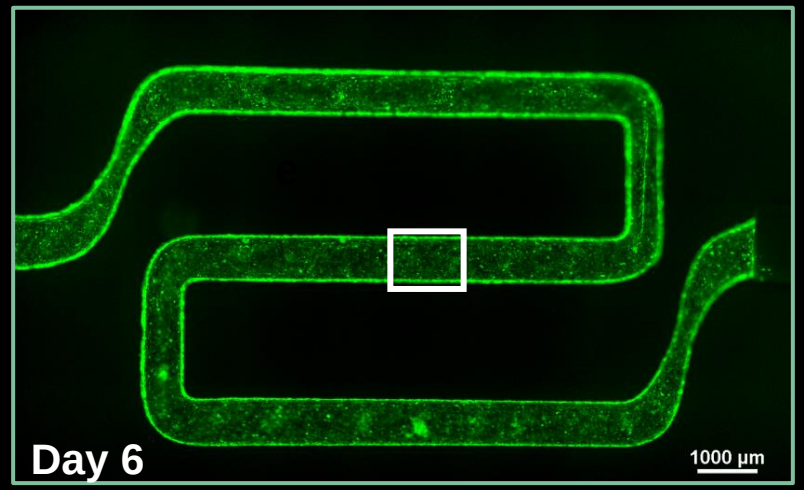
Standard: Complete Vasculife

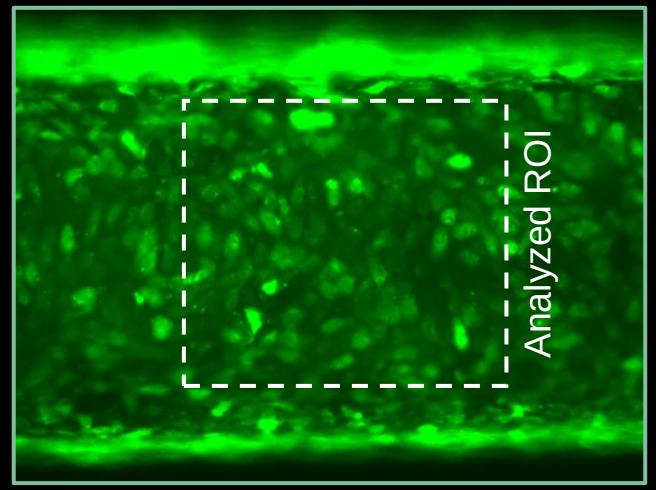


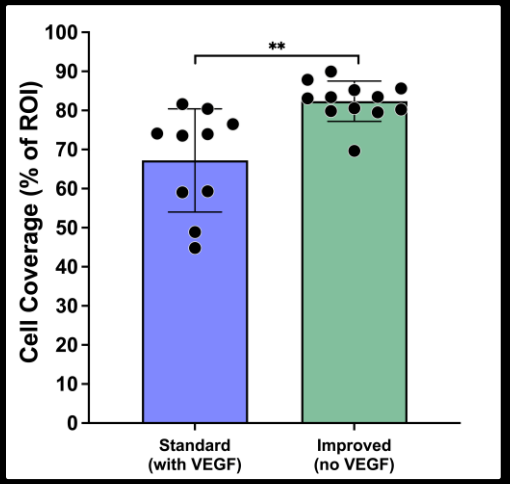




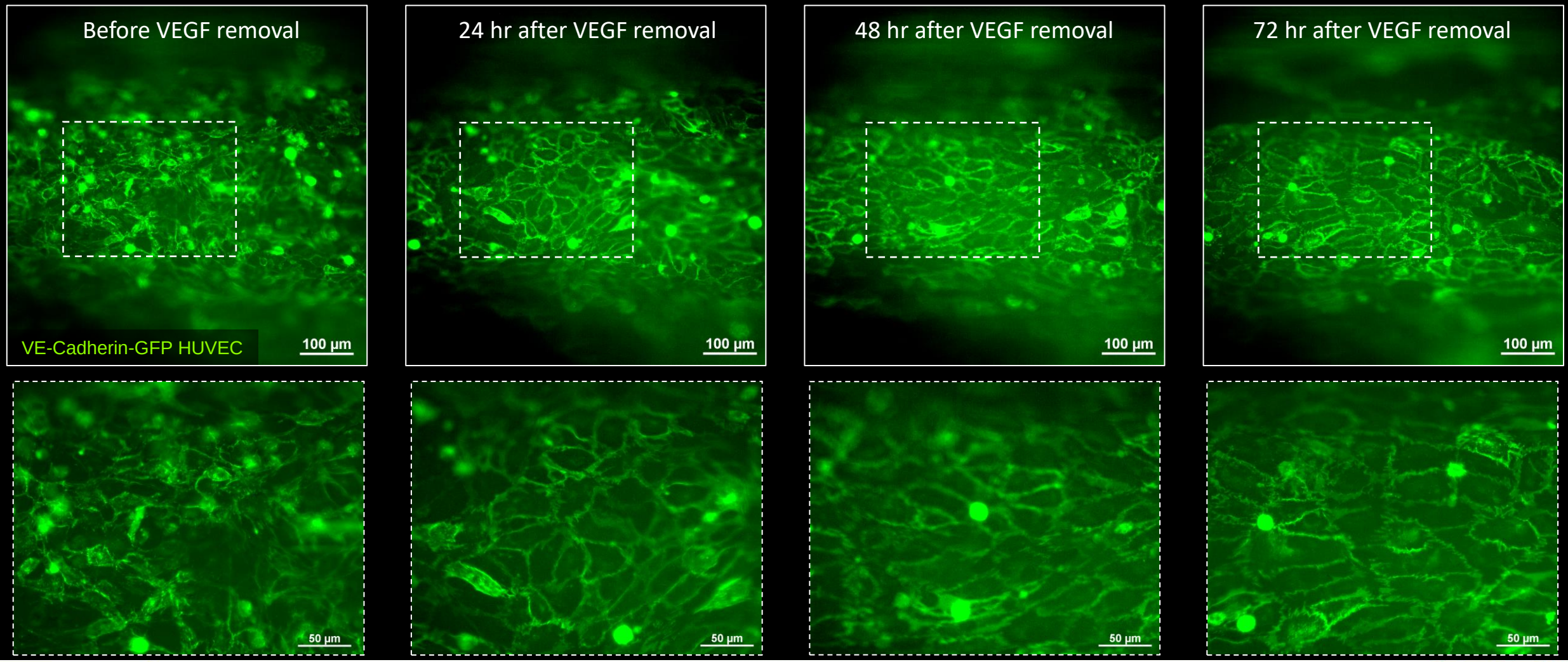
Improved: Vasculife with VEGF removed





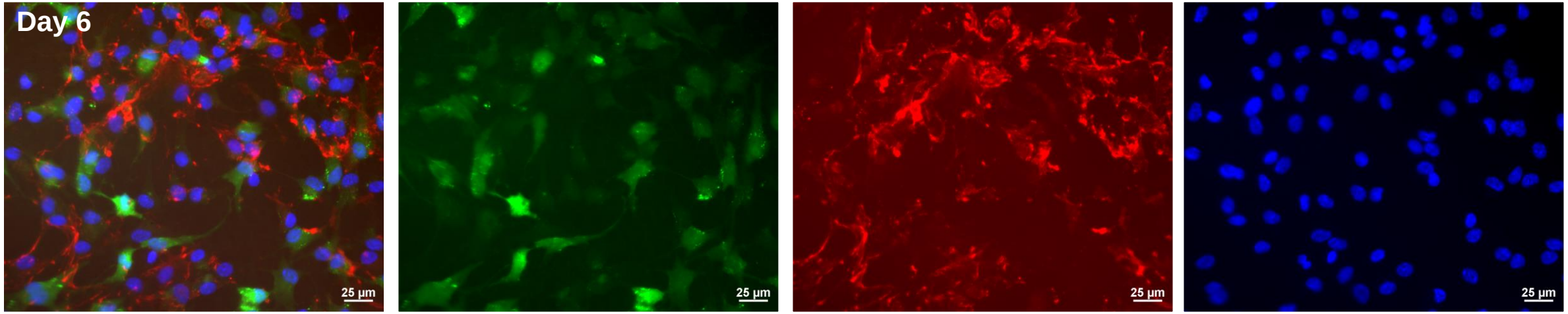


Cell-cell junction morphological changes observed within 24 hours of VEGF removal



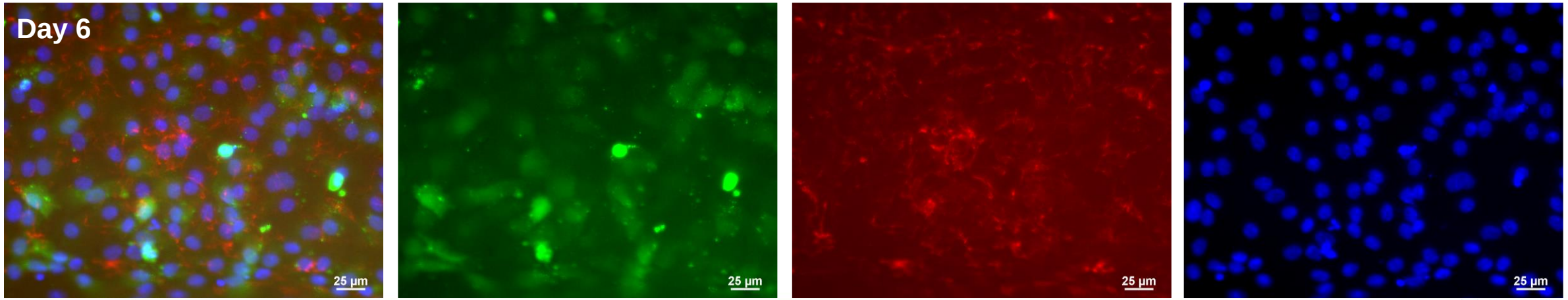
Confirmation of vascular maturity demonstrated by endothelial production of collagen IV

Standard: Complete Vasculife media



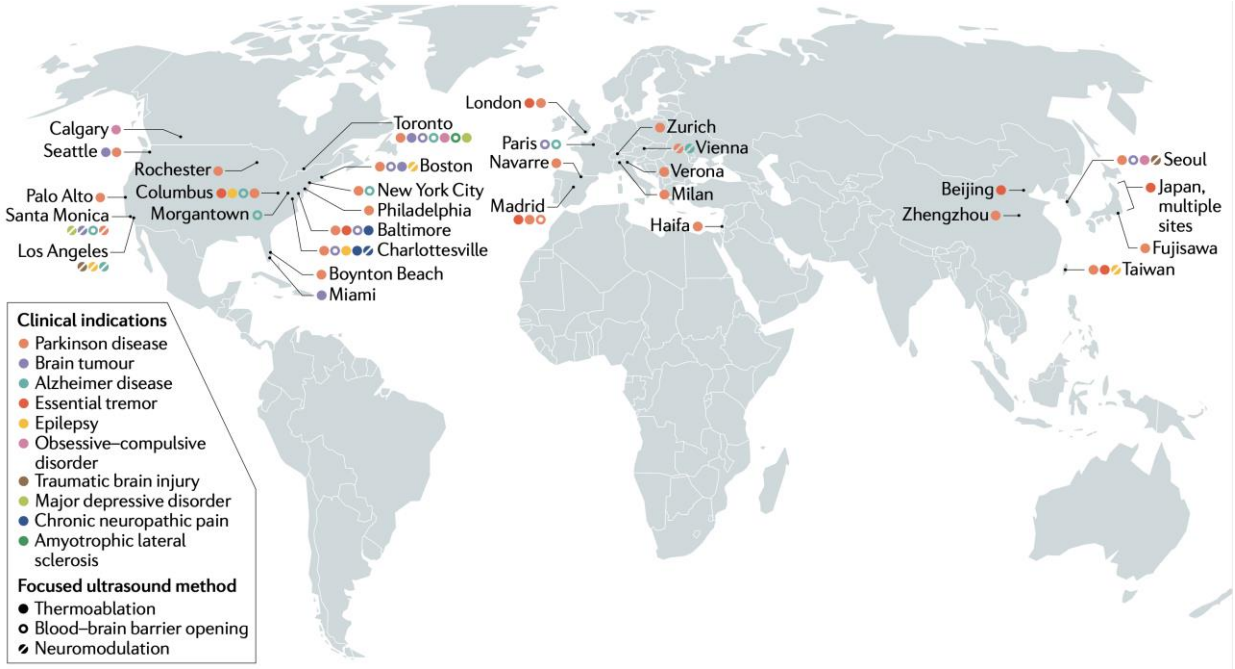
GFP HUVEC Collagen IV Hoechst

Improved: Vasculife with VEGF removed



Specific Aim 2: To demonstrate this vascular model as an *in vitro* screening tool for assaying ultrasound-induced permeability

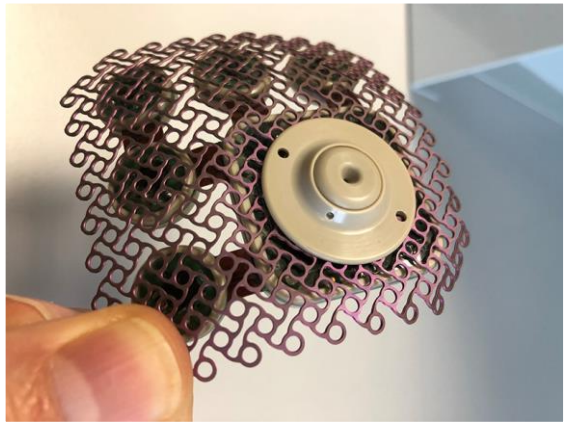
Active clinical trials using focused ultrasound



Ying, M. et. al. Nature Reviews Neurology. 2021.

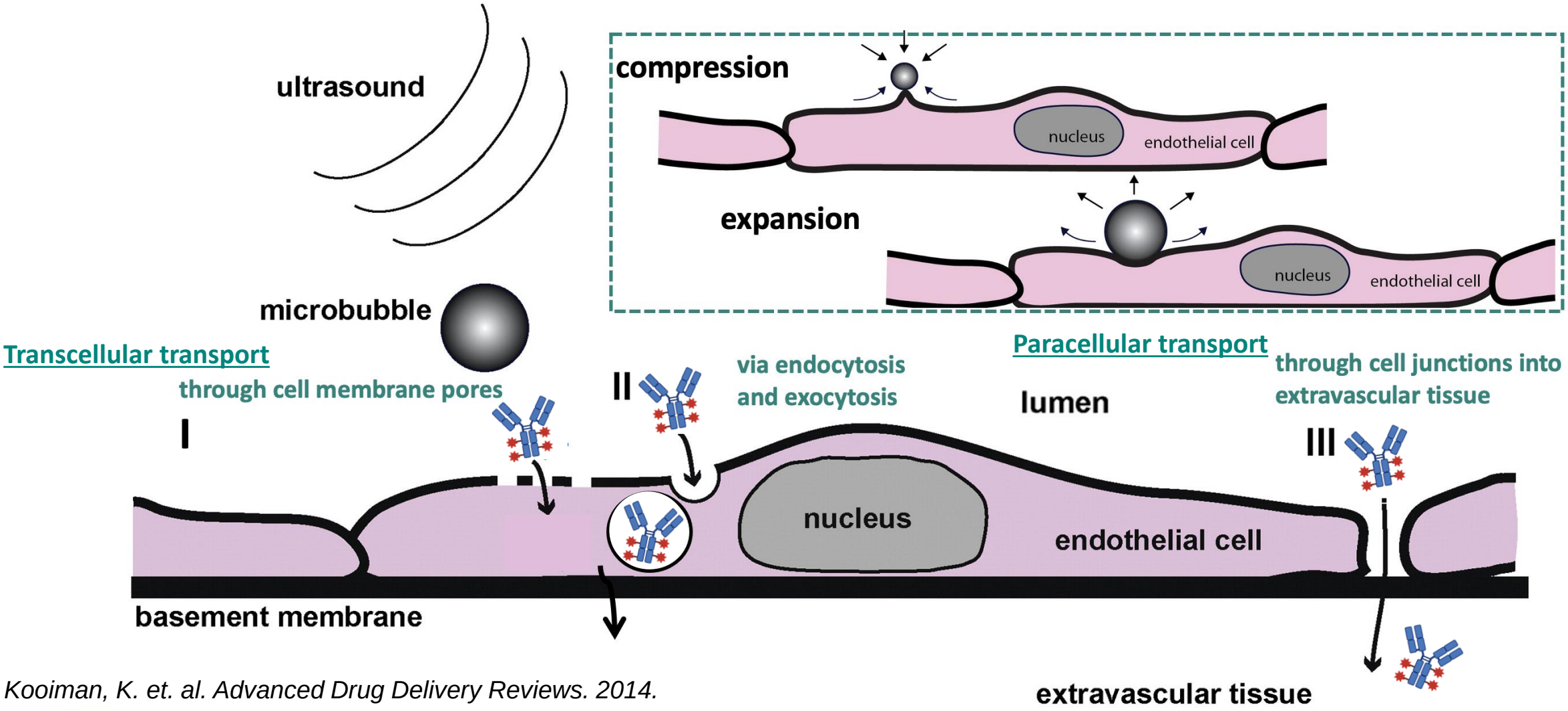
Diagnostic (unfocused) ultrasound

- Uses a flat transducer to supply sound wavs to a distributed area;
- Relies on low-intensity ultrasound/stable microbubble cavitation to trigger mechanical effects (sonoporation);
- Benefit: allows use of lower intensity ultrasound which helps minimize ultrasound bioeffects such as tissue heating;
- **Increasing interest in the use of diagnostic ultrasound for therapeutic applications**



SonoCloud - an implantable, unfocused ultrasound device that provides low intensity ultrasound for blood-brain barrier disruption

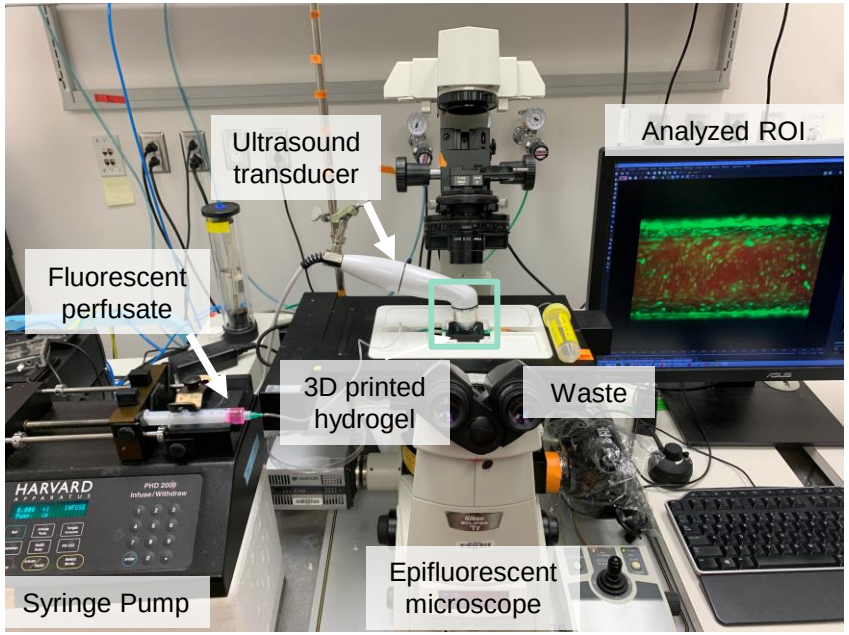
Sonoporation is utilized to enhance drug delivery through vasculature



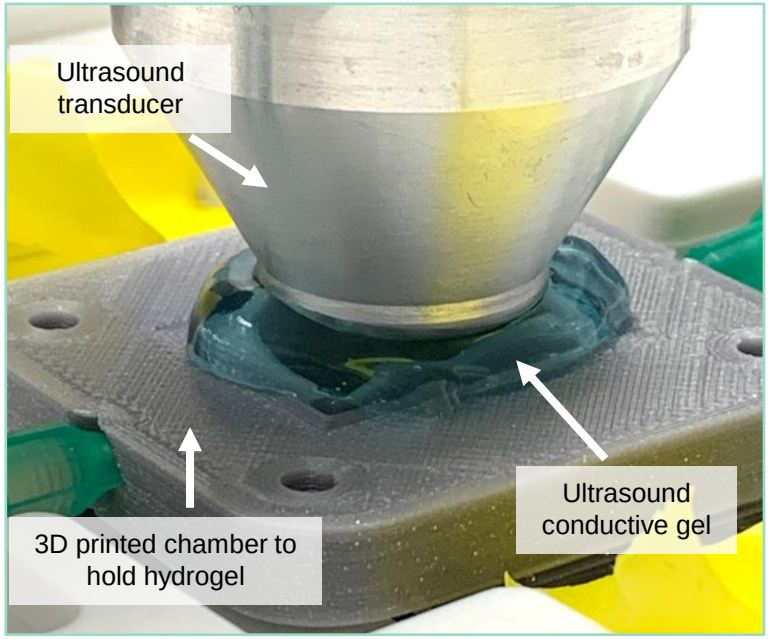
Kooiman, K. et. al. Advanced Drug Delivery Reviews. 2014.

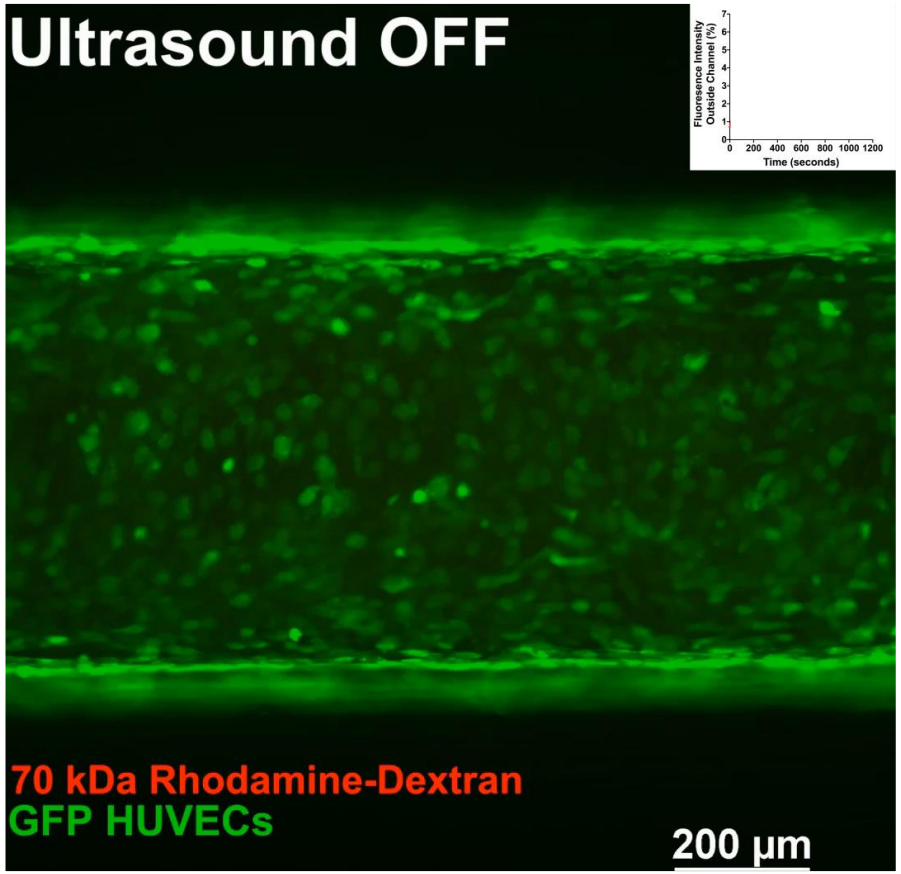
Epifluorescence imaging enabled study of ultrasound-enhanced transport

Experimental set-up

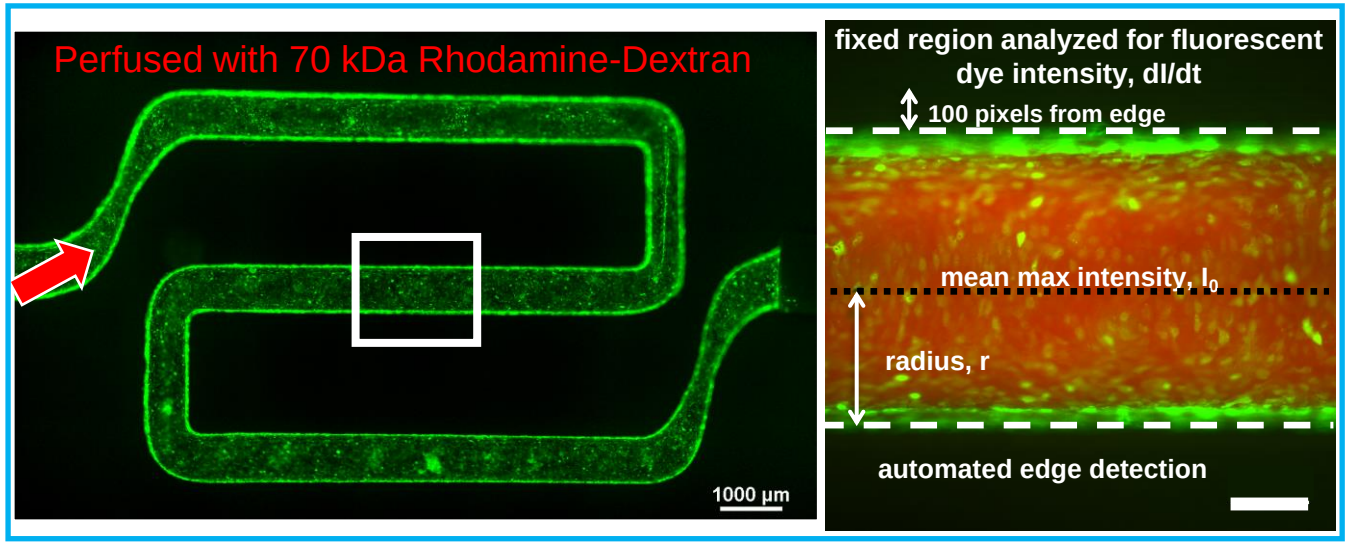


Ultrasound probe positioned on hydrogel





Development of a high-throughput method to quantify vascular permeability changes due to ultrasound



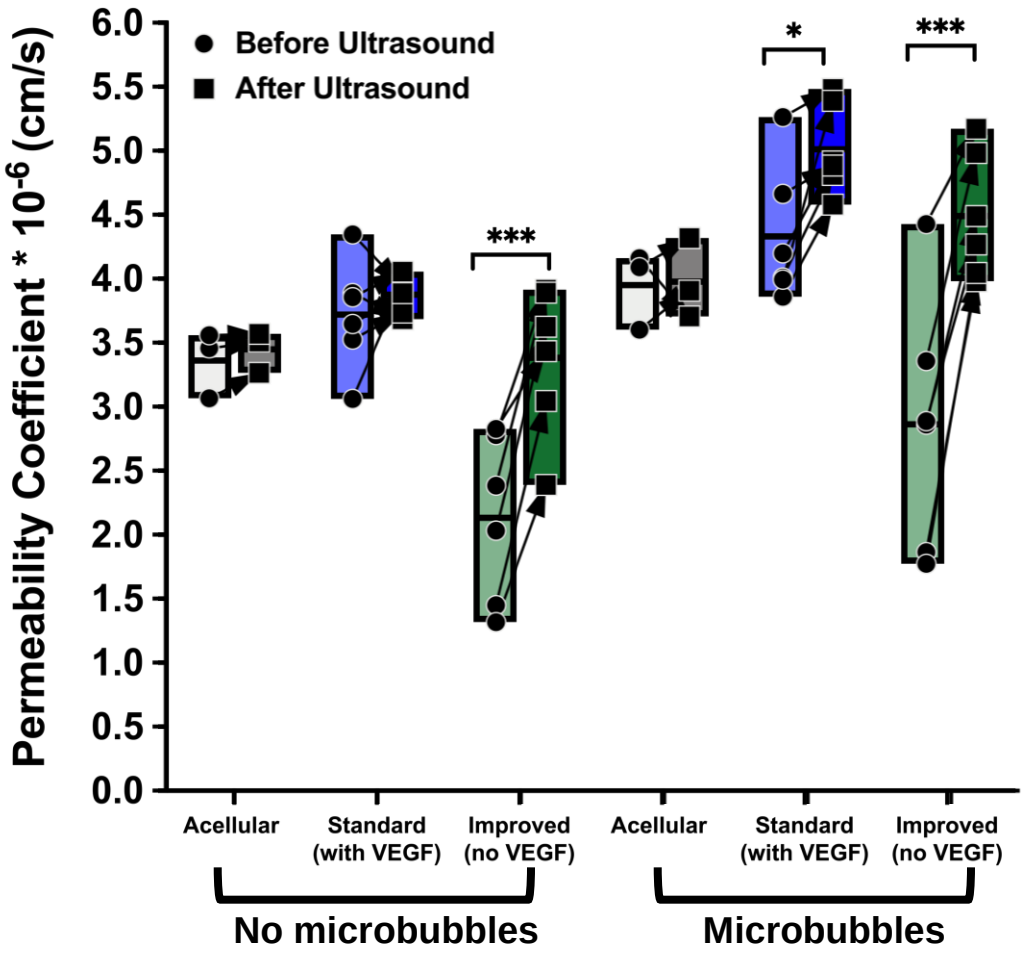
Apparent Permeability Coefficient Calculation

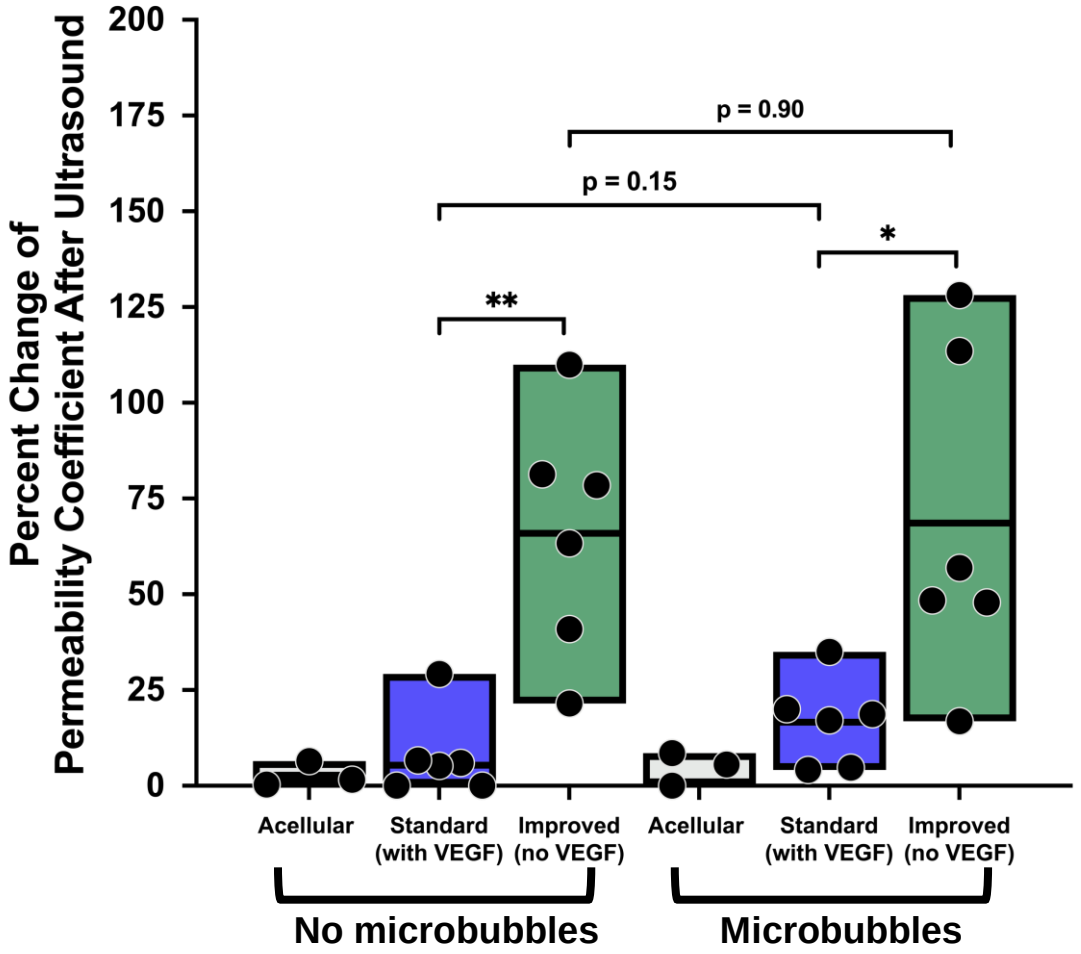
$$P = \frac{dI}{dt}\frac{r}{2I_0}$$

GUI output: permeability coefficient

	Before US	During US	After US
Whole gel	1.0967	4.5654	5.1727
User ROI	0.8592	2.6132	5.4743
Sliding ROI	1.0930	3.2585	5.7458

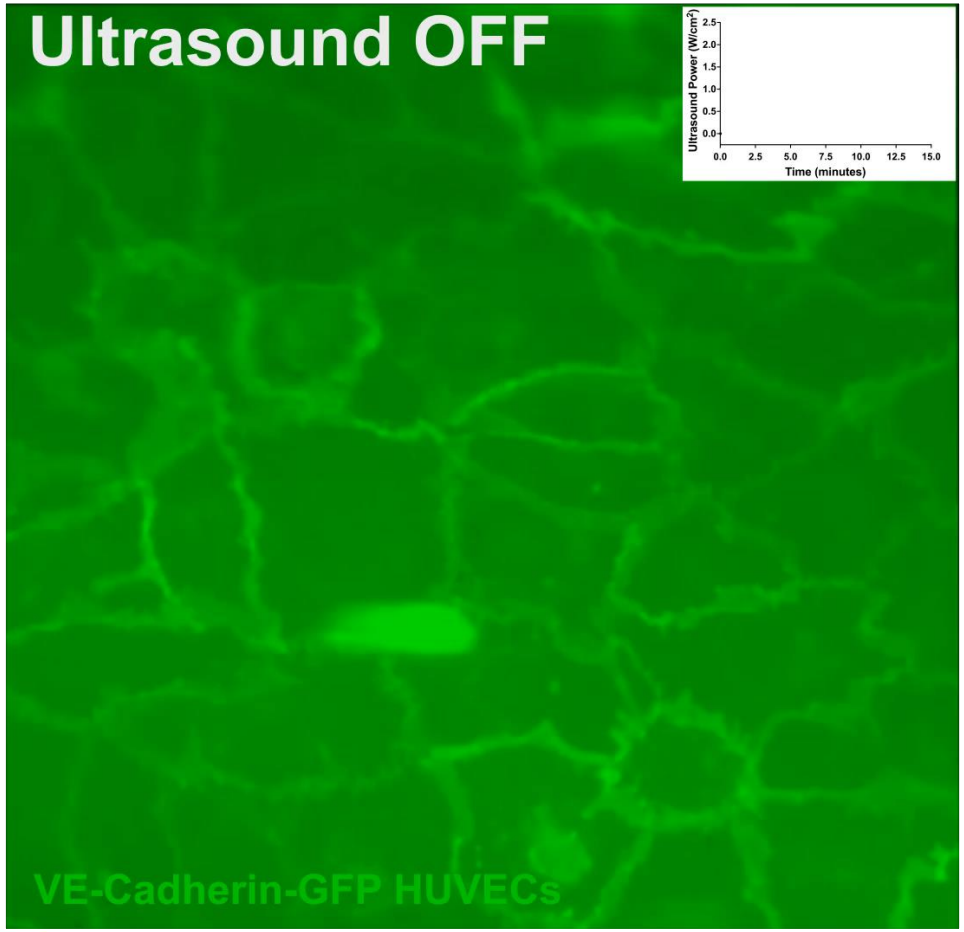
Ultrasound treatment increased apparent permeability coefficient in this hydrogel model



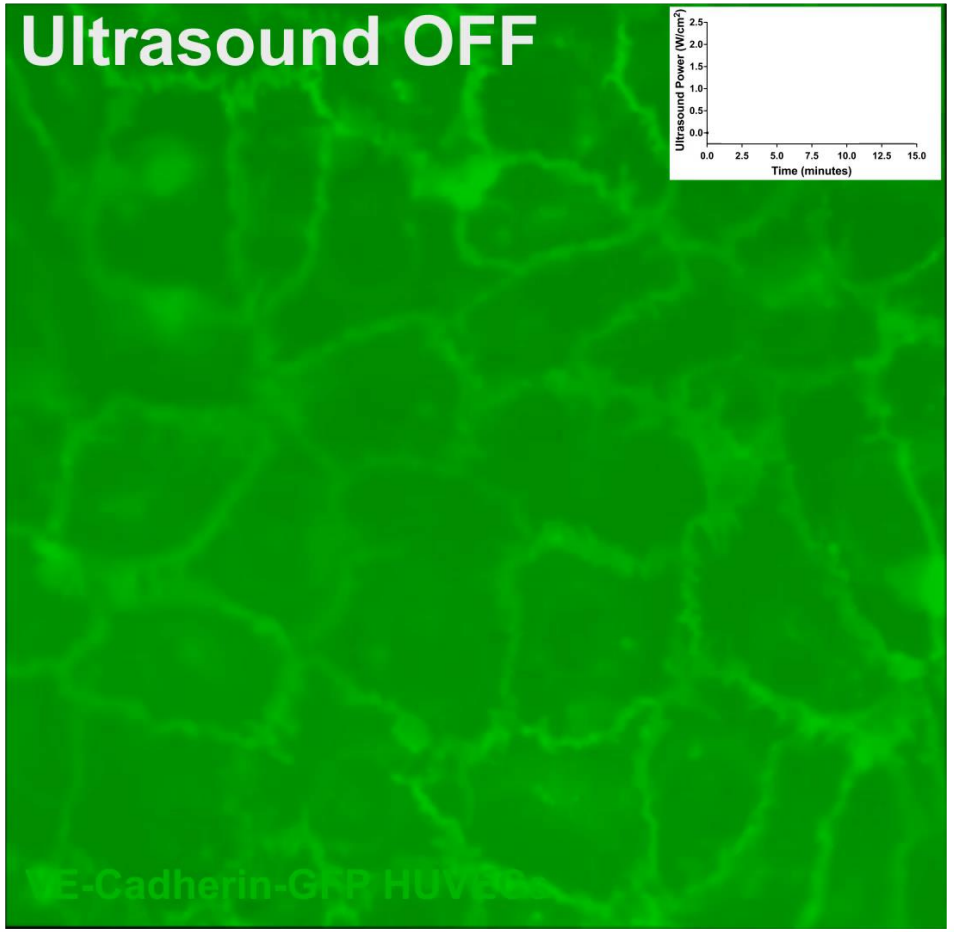


VE-Cadherin-GFP HUVECs provided first real-time acquisitions of endothelial cell-cell junction dynamics under ultrasound

No microbubbles



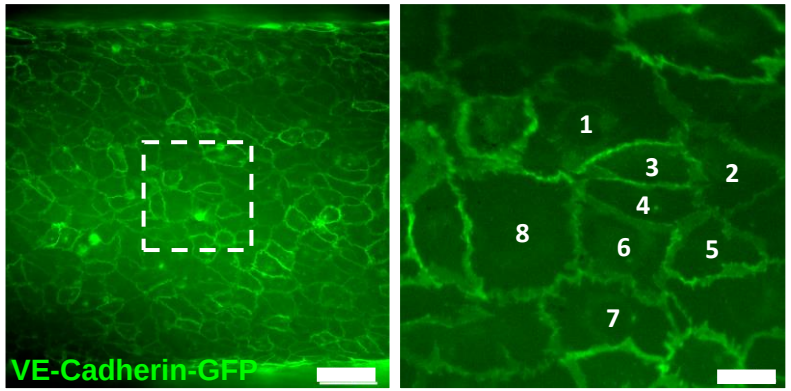
Microbubbles



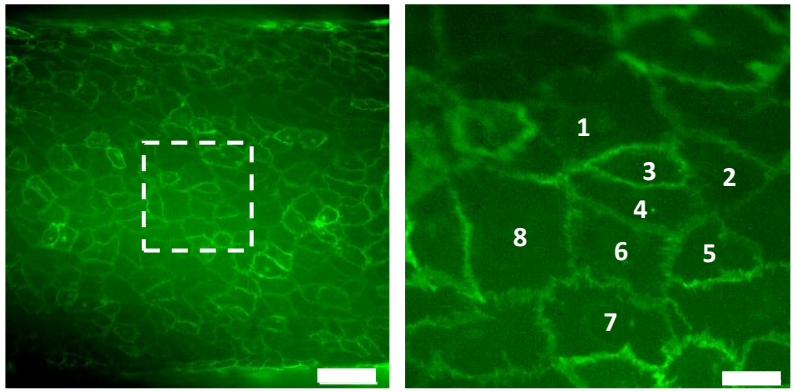
Cell area tracking demonstrated dynamic nature of endothelial cells in response to ultrasound and microbubbles

No microbubbles

Before Ultrasound

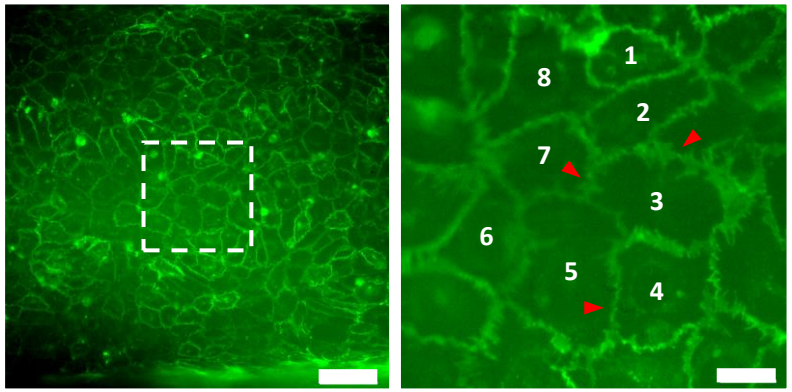


After Ultrasound

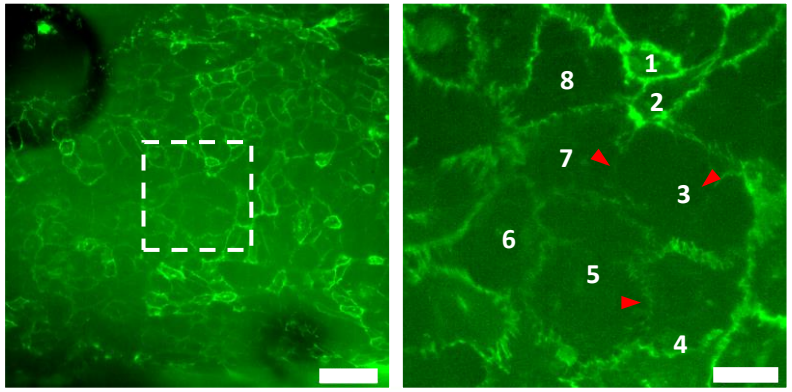


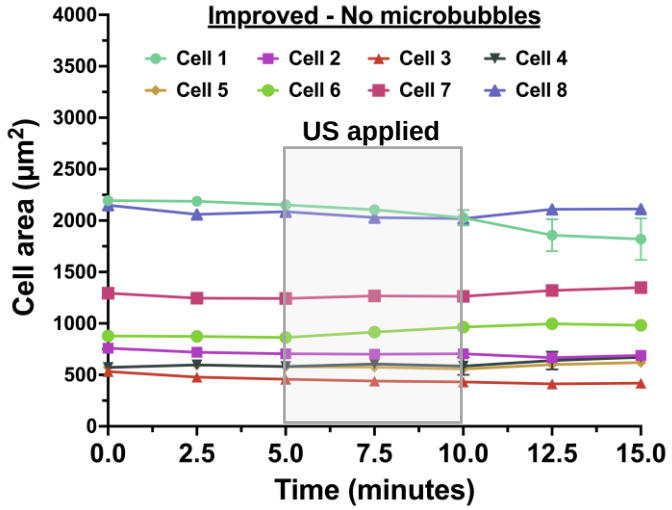
Microbubbles

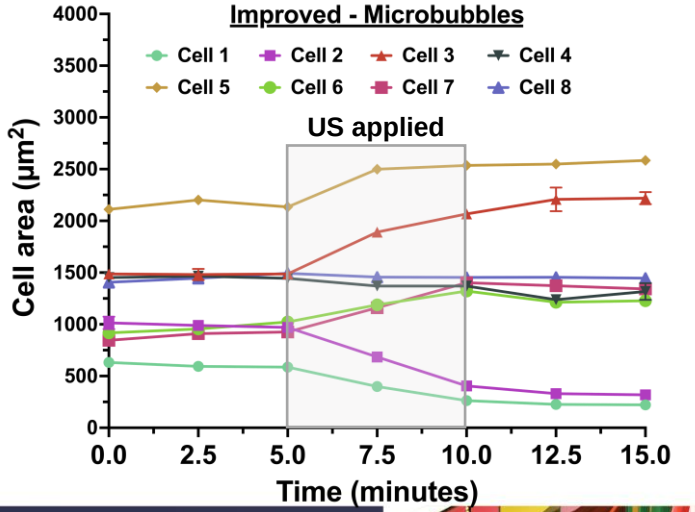
Before Ultrasound



After Ultrasound

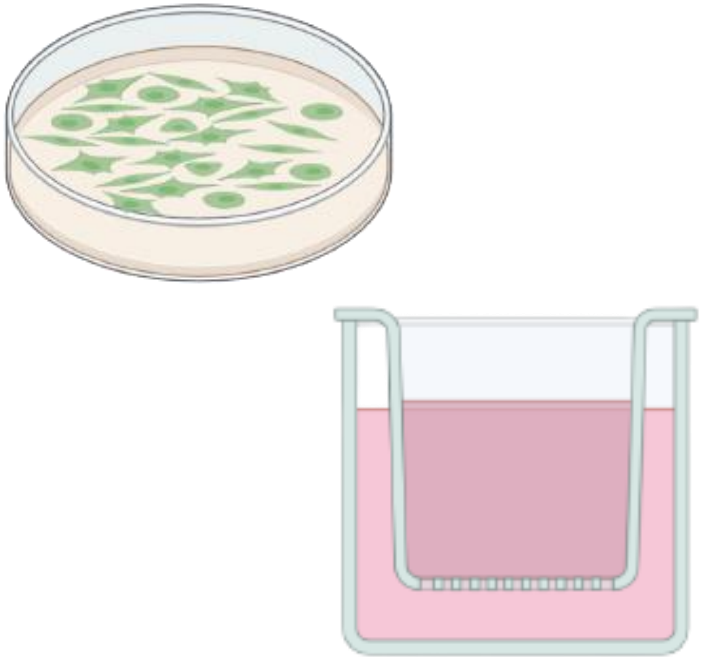




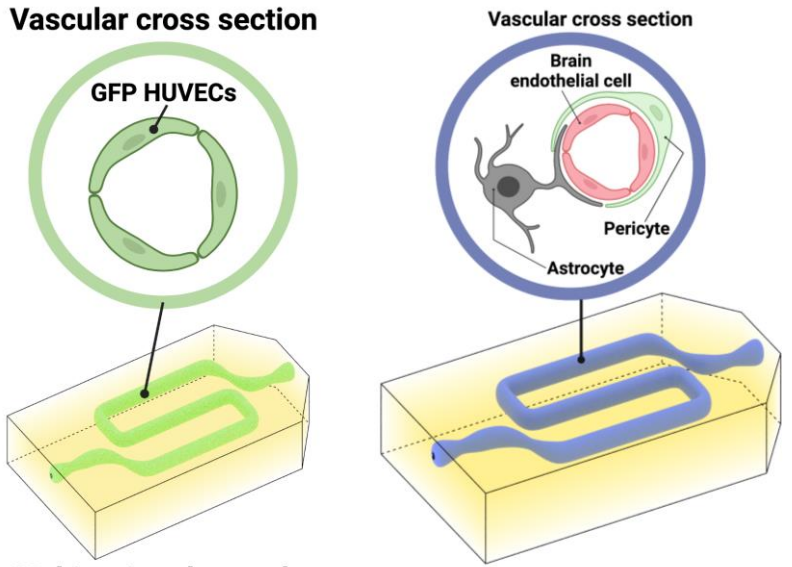


Conclusion: Developed a new screening platform to help predict therapeutic outcomes *in vitro* before preclinical translation

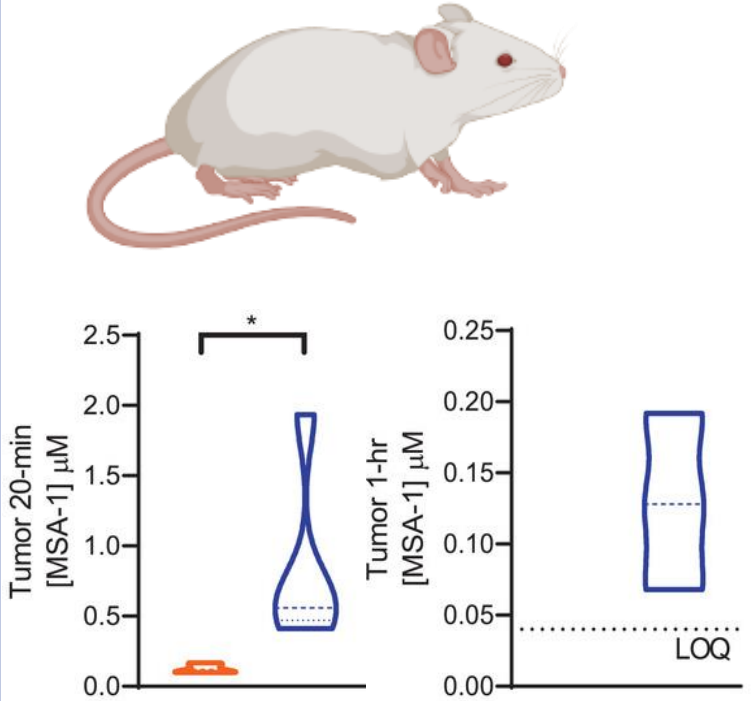
2D *in vitro* cell culture



3D-printed *in vitro* vascular model



In vivo animal studies



Adv. Sci. ***2020***, 7, 190-3394; *Adv. Therap.* **2021**, 4, 2100154; *Biomaterials Sci.* **2022**,10, 3158-3173