Supporting Information

A 3D printable perfused hydrogel vascular model to assay ultrasound-induced permeability

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Movie S1. Representative time-lapse of a perfusion-permeability study for an improved endothelialized gel. An endothelialized channel perfused with 70 kDa Rhodamine-Dextran at 100 μL min^{-1} and subjected to 5 minutes of ultrasound at 2.0 W cm^{-2}.

Movie S2. Demonstration of custom MATLAB permeability GUI. Video demonstrates the use of the permeability GUI to determine permeability coefficients, endothelialized channel behavior, and dye diffusion.

Movie S3. Time-lapse of VE-Cadherin-GFP HUVECs in response to ultrasound with no microbubbles. An endothelialized gel was subjected to ultrasound of power intensity 2.0 W cm^{-2} for 5 min with 5 min of perfusion prior to ultrasound and after ultrasound collected for a total acquisition time of 15 minutes.

Movie S4. Time-lapse of VE-Cadherin-GFP HUVECs in response to ultrasound with microbubbles. An endothelialized gel was subjected to ultrasound of power intensity 2.0 W cm^{-2} for 5 min with 5 min of perfusion prior to ultrasound and after ultrasound collected for a total acquisition time of 15 minutes.

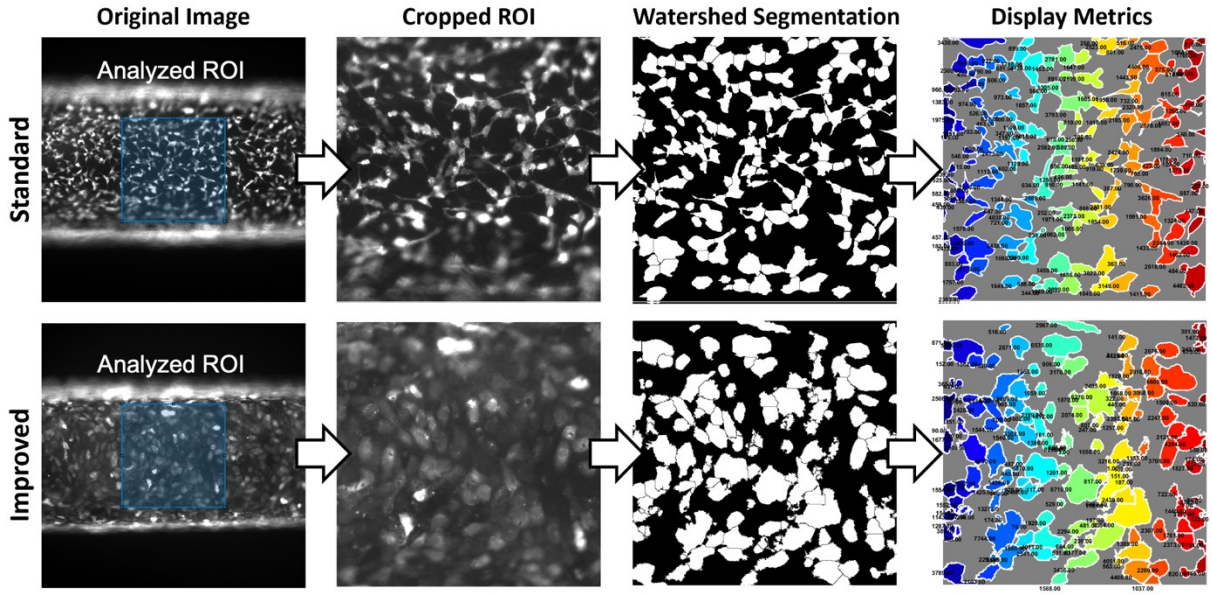

Figure S1. Workflow for quantification of cell morphology shown in Figure 1. Image quantification utilizes a custom MATLAB script and begins with importing the high-resolution TIFF, cropping to a desired ROI, performing a watershed segmentation, and then producing metrics such as cell coverage, cell area, and cell density for the ROI.

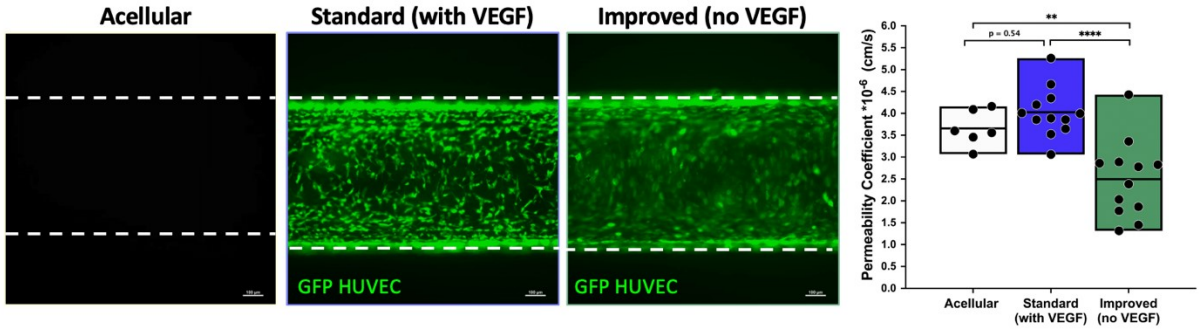

Figure S2. Permeability of acellular, standard, and improved gels before ultrasound application. Permeability was determined using 70 kDa Rhodamine-Dextran and the custom MATLAB GUI detailed in this manuscript. To utilize the GUI's edge tracking feature, 150 kDa FITC-Dextran was used to track the edges of the channels in the acellular trials when GFP HUVECs are not present. 150 kDa FITC-Dextran was able to be used as it will not diffuse through the gel in the timeframe of the acquisition. The resulting permeability coefficients showed a statistical significance (****) between the standard (n=12) and improved groups (n=12), as well as showing statistical significance (**) between the acellular (n=6) and the improved group. No statistical significance was seen between the acellular and standard groups. Significance was determined using a one-way ANOVA followed by Tukey's multiple comparisons test (Tukey-Kramer to adjust for unequal number of replicates), *$p < 0.05$, **$p<0.01$, ****$p <0.0001$.

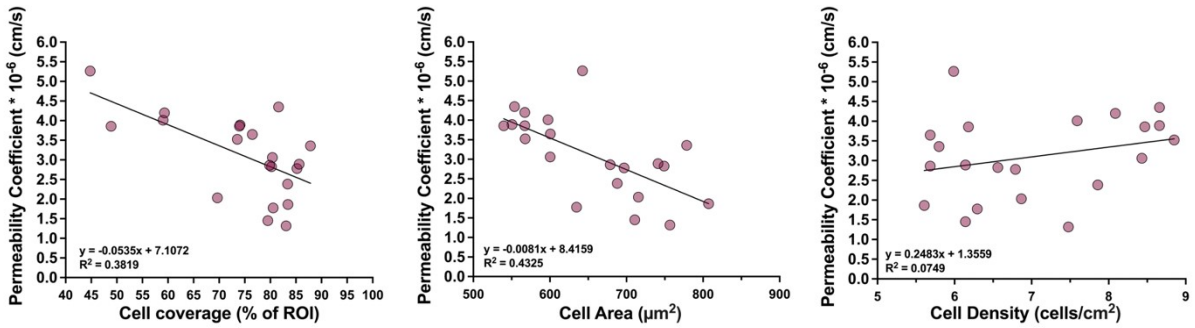

Figure S3. Linear regression of permeability coefficient correlations. A simple linear regression was performed on the data shown in Figures 3e-3g where the red circles are points from both the standard and improved groups.

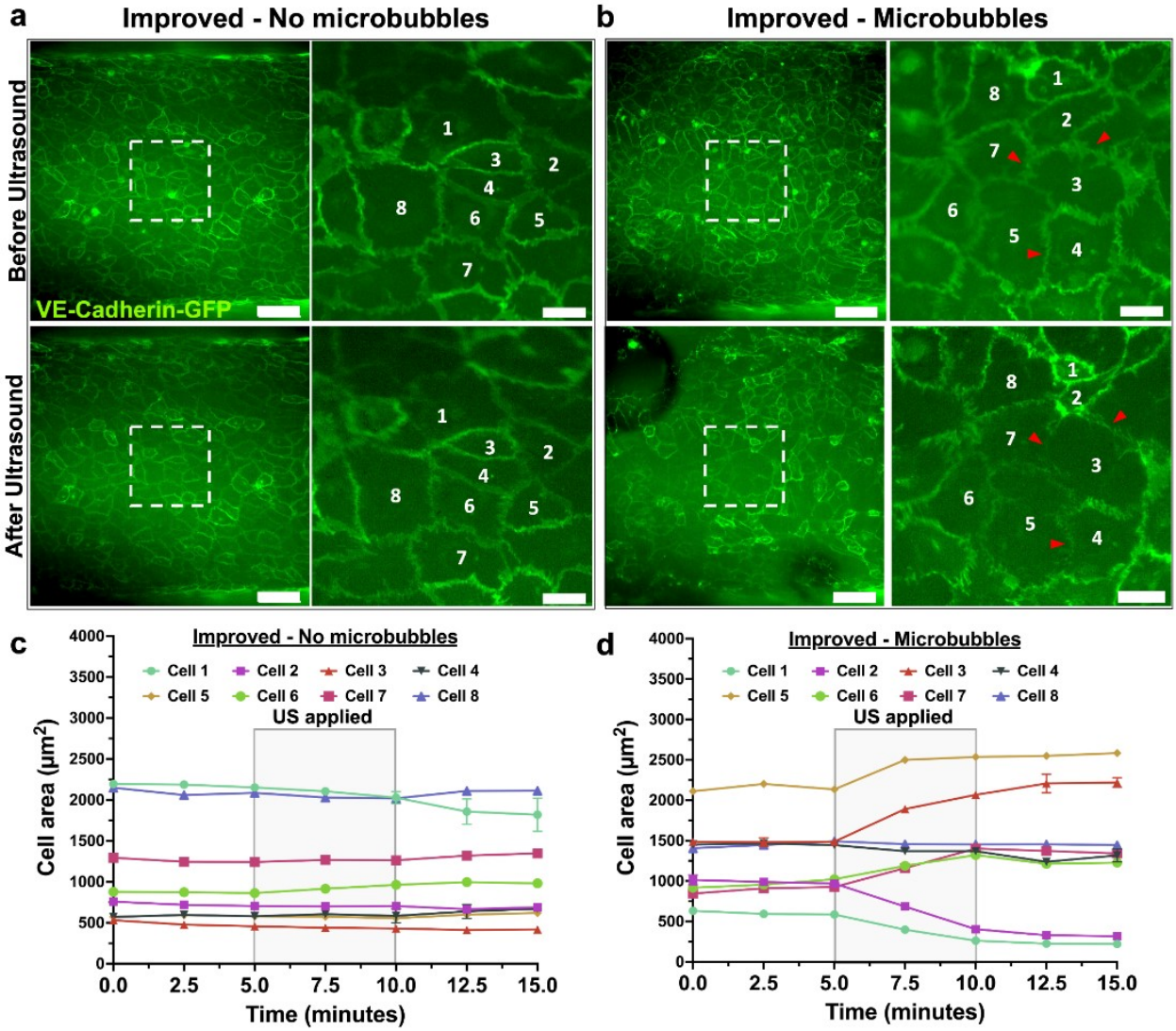

Figure S4. VE-Cadherin-GFP HUVECs enable the visualization of cell-cell junction (VE-Cadherin) dynamics in living cells under the application of ultrasound. VE-Cadherin-GFP HUVECs were seeded at 30M mL^{-1} within the vascular hydrogel model and underwent perfusion culture at 5 µL min^{-1} (improved condition, VEGF removed). During the study, perfusate (left (a, c): no microbubbles, right (b, d): with microbubbles) was perfused through the endothelialized channel at 100 µL min^{-1}. Panels a & b show representative images of the endothelial cell-cell junctions before and after applying ultrasound at 2.0 W cm^{-2} for 5 minutes (same conditions used with GFP HUVEC studies) with red arrowheads indicating disrupted junctions, scale bars, 100 µm and 25 µm (insets). Graphs c & d display cell area over time of eight individual cells (denoted in a & b insets before and after ultrasound) with a box indicating when ultrasound is applied.