Supplementary Materials

Subtractive manufacturing with swelling induced stochastic folding of sacrificial materials for fabricating complex perfusable tissues in multi-well plates

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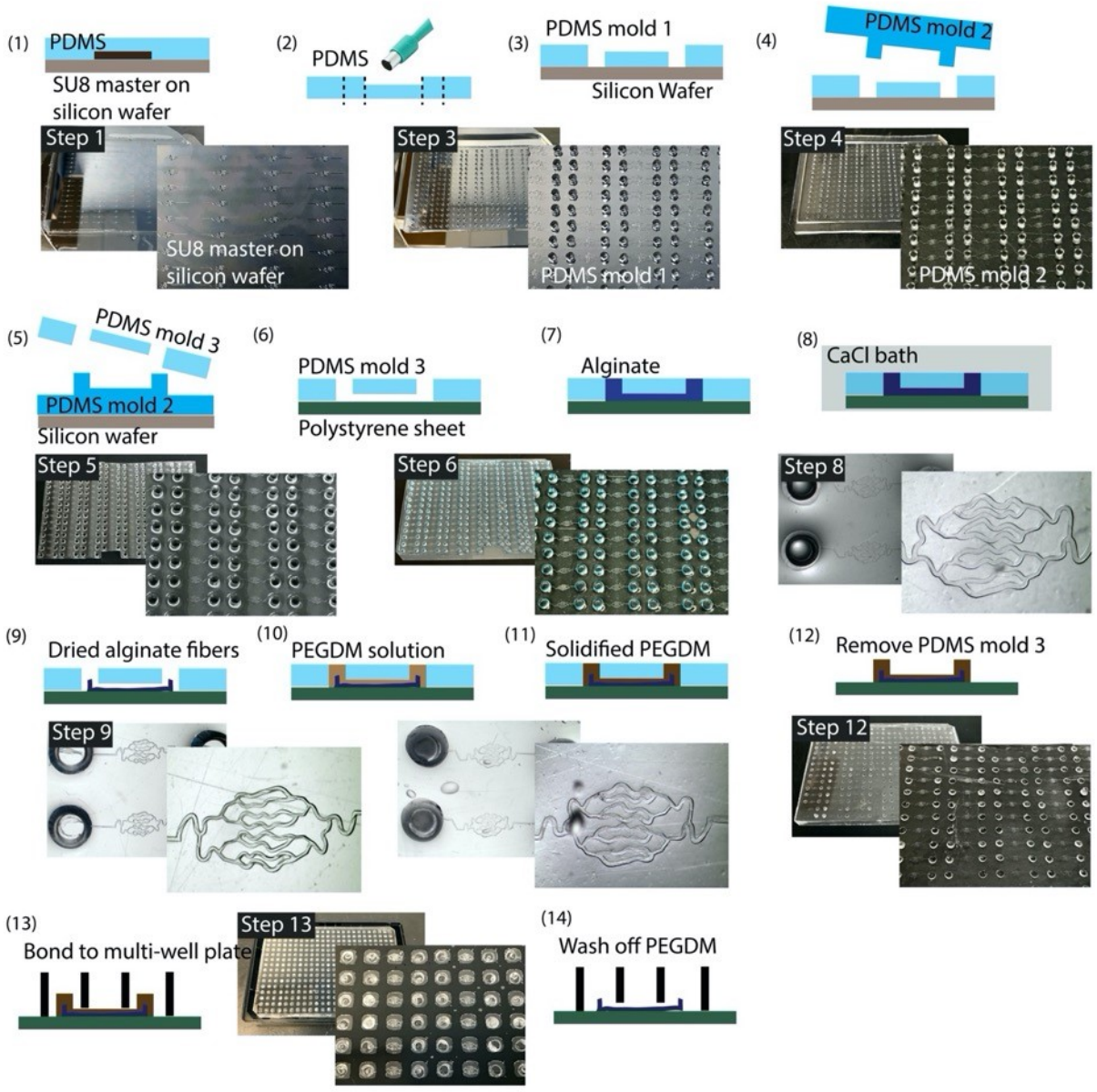
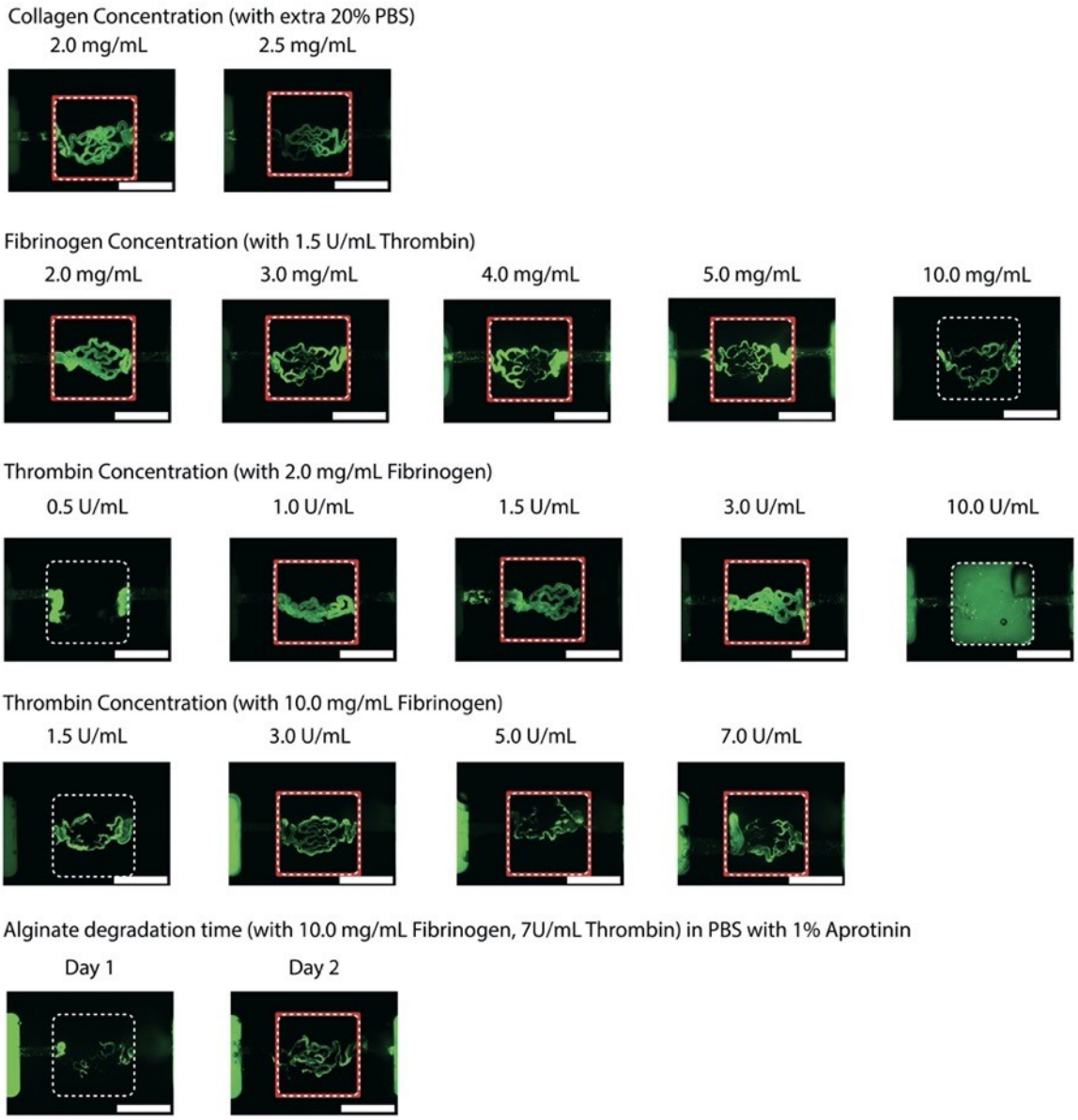
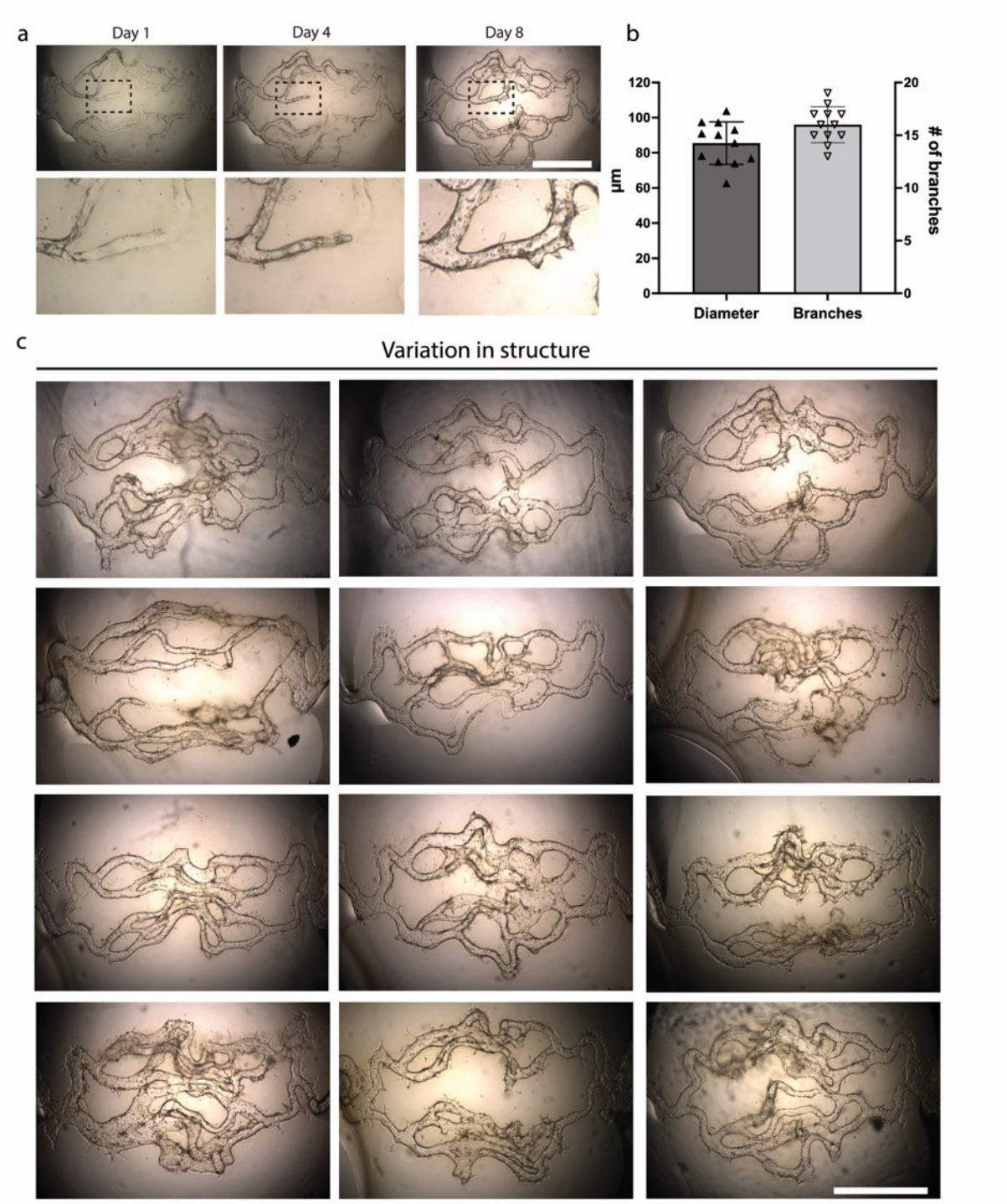


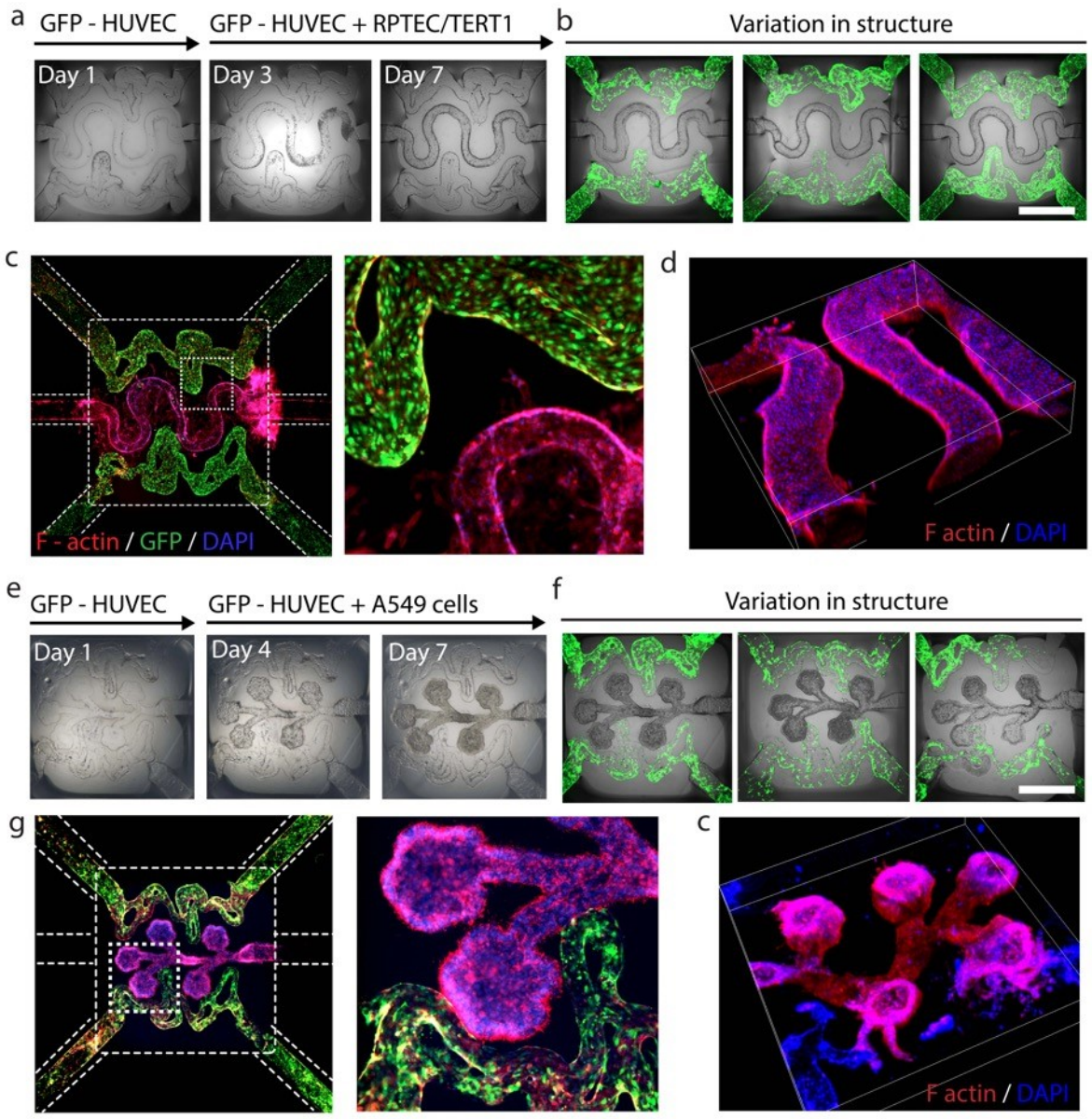
Supplementary Figure 1. Step-by-step fabrication of AngioPlate-384. First, using standard photolithography, we fabricated a PDMS mold with various patterns connected to an inlet and outlet well. The mold was then capped onto a polystyrene sheet to form an array of micro-channel networks. The networks were loaded with 3 wt.% alginate solution. Next, the entire mold was immersed in a calcium bath (1 mM), where calcium ions gradually diffused from the inlet and outlet wells into the alginate solution within the network, cross-linking the alginate overnight. With this approach, we were able to pattern 128 independent alginate fiber networks in the format of a 384-well plate. PEGDM solution was injected into the channels in the same way to encapsulate the alginate fiber to facilitate alginate release and to create the inlet/outlet channels. Finally, the polystyrene sheet patterned with alginate and PEGDM was assembled onto the base of a bottomless 384-well plate, encasing and sealing the alginate networks with a high viscosity PDMS glue.



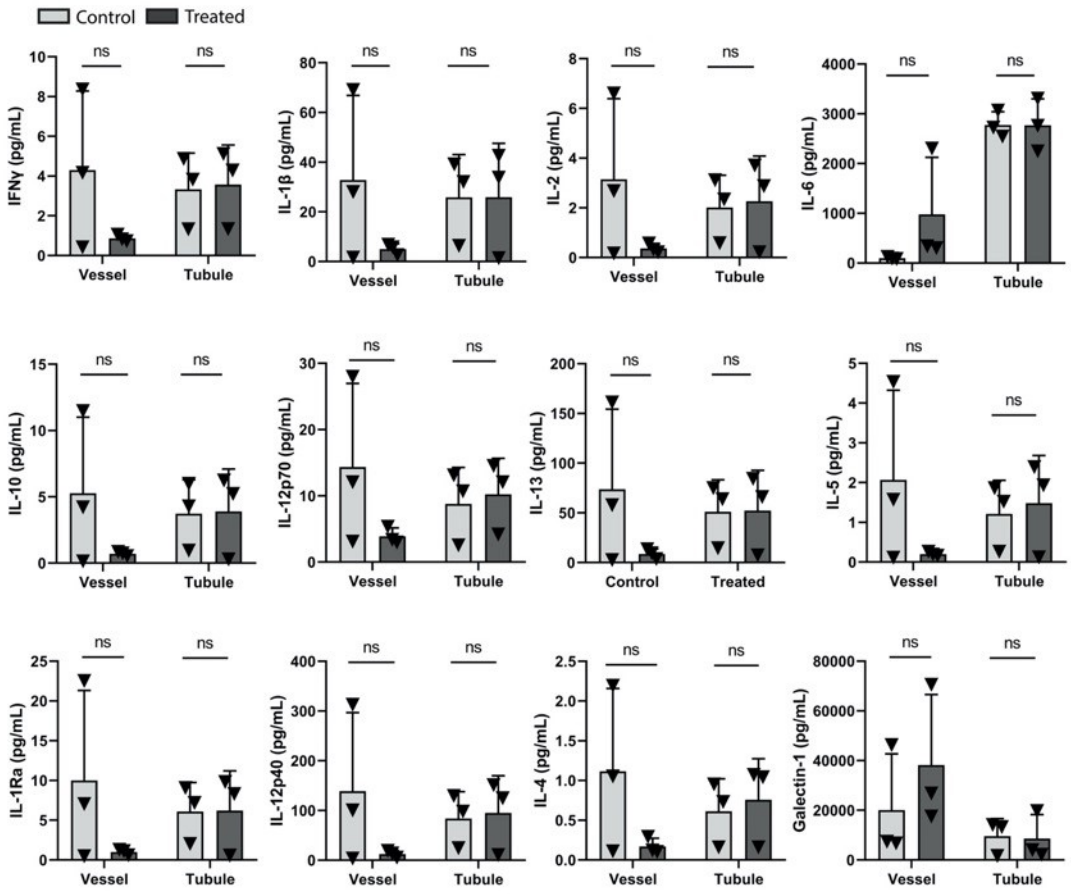
Supplementary Figure 2. Optimization of hydrogel matrix cross-linking condition for network formation. Fluorescent images of networks perfused with 1 μm fluorescent particles (green) under various gelling conditions in both collagen-based gel and fibrin-based gel. Red boxes label the good conditions that resulted in the formation of complete perfusable networks. Scale bar, 2mm.



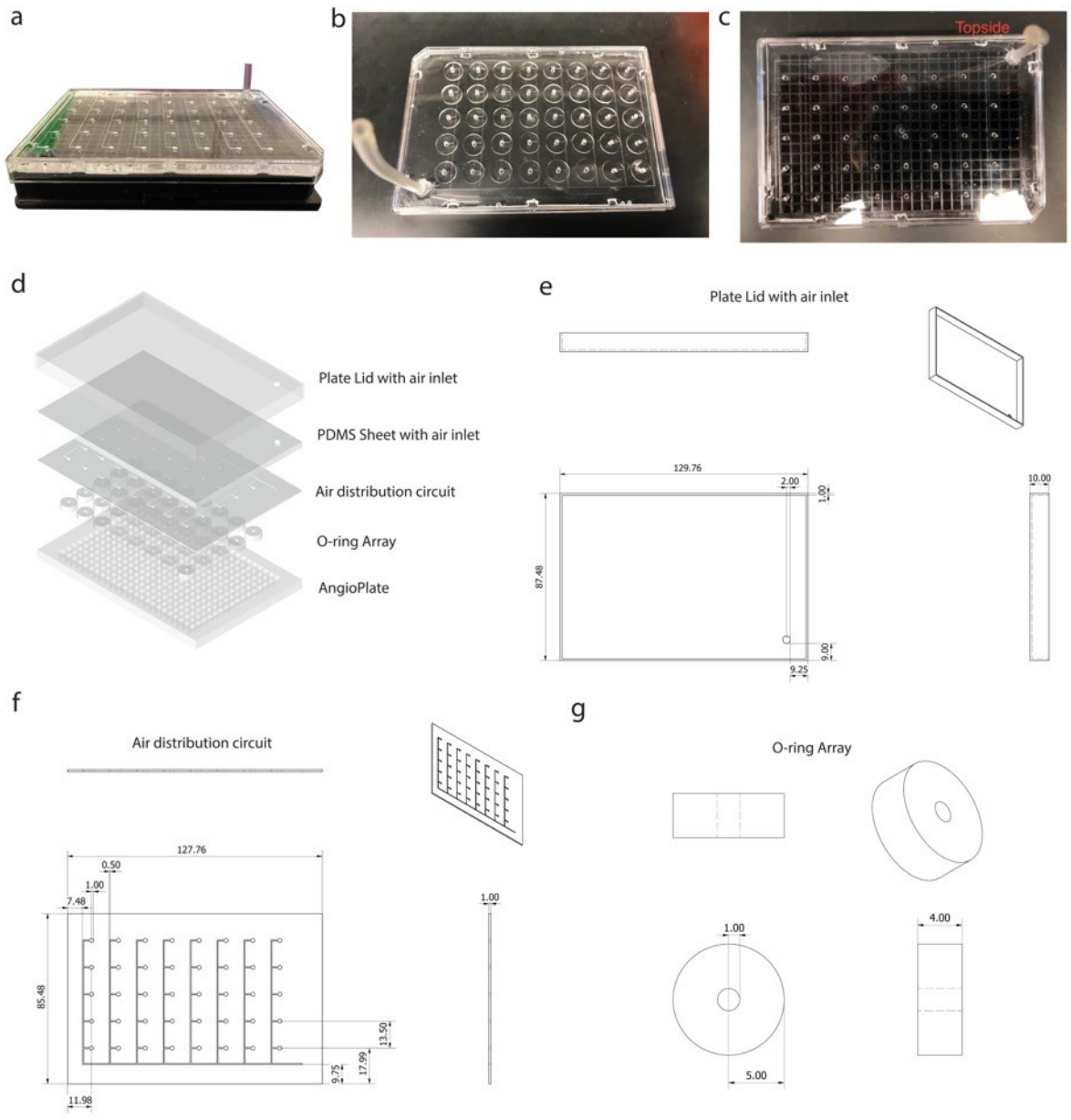
Supplementary Figure 3. Structural variation of vasculature in AngioPlate. a, Brightfield time-lapse images of networks seeded with human endothelial cells. Scale bar 1mm. **b**, Quantification of variation in vasculatures structure. n=12. **c**, Variation in the network structures from 12 different wells. Scale bar 1mm.



Supplementary Figure 4. AngioPlate-Lung and Kidney. a, Time-lapse brightfield images of a vascularized proximal tubule complex. Endothelial cells were seeded on day 0 and tubular cells were seeded on day 3. **b**, Variation in the structure of the vascular proximal tubule complex resulting from alginate folding. Scale bar 1 mm. **c-d**, Fluorescent images of vascular proximal tubule complex stained for F-actin (red) and DAPI (blue). **e**, Time-lapse brightfield images of a vascularized alveoli terminal. Endothelial cells were seeded on day 0 and Alveolar cells were seeded on day 3. **f**, Variation in the structure of vascularized alveoli terminal resulting from alginate folding. Scale bar 1 mm. **g-h**, Fluorescent images of a vascularized alveoli terminal stained for F-actin (red) and DAPI (blue).



Supplementary Figure 5. Cytokine assay of inflammatory kidney. Cytokine analysis of media perfusates from tissues treated with or without TNF-α, n=3. Statistical significance was determined using one-way ANOVA and one-way ANOVA on ranks with the Holm-Sidak method. ns indicates not significantly different. *P≤0.05 **P≤0.01 ***P≤0.001



Supplementary Figure 6. Mechanical actuation lid design, dimension and assembly. a, Image of the plate lid on an AngioPlate. **b**, Top-down view of the actuation lid. **c** Top-down view of the actuation lid on our device without the O-ring array to show alignment with the wells in an AngioPlate. **d**, Assembly of different parts in the actuation lid. **e-g,** Dimensions and design of parts of the plate actuation lid, including the air distribution circuit and the o-rings. All labels are shown in millimeters.

Supplementary Video 1. Swelling and shape changing alginate fibers on AngioPlate
Supplementary Video 2. Mechanical actuation of terminal alveoli on AngioPlate