

## Electronic Supplementary Information

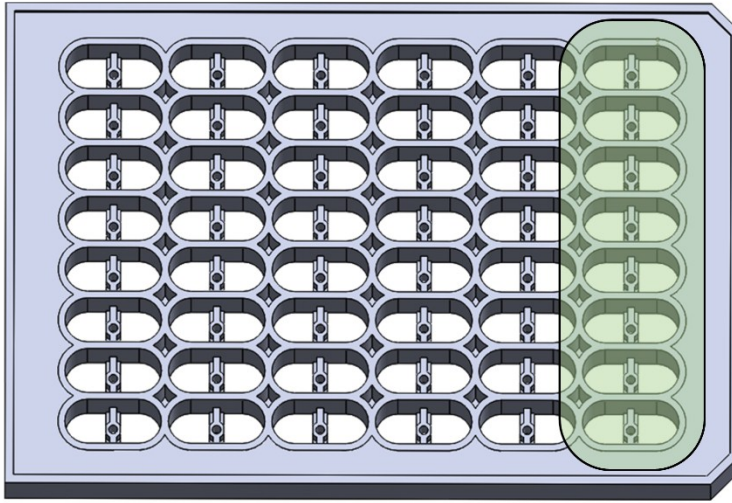
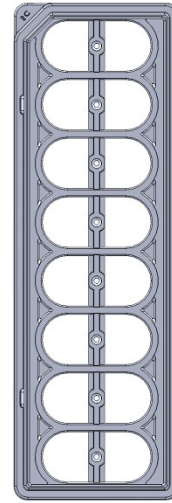
Tumor Spheroid-on-a-Chip:

A Standardized Microfluidics Platform for Investigating Tumor Angiogenesis

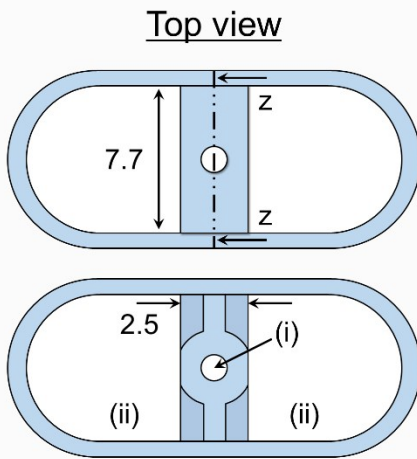
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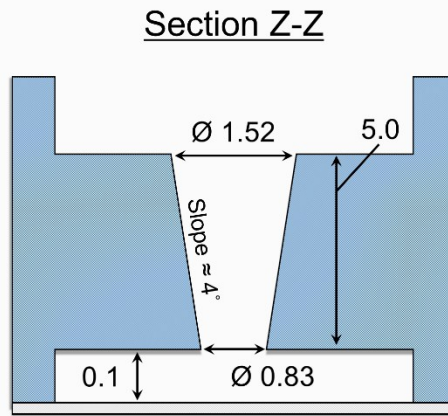
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**A****B**

96-well plate SBS format-based microplate

**C**

- (i) Patterning region
- (ii) Medium reservoir

**D**

Unit: mm

**E**

Figure S1. Overall schematics of the structural components of tumor spheroid-on-a-chip. (a) Illustration of 96-well plate SBS format-based microplate. (b) A single array of tumor spheroid-on-a-chip as a prototype model. (c-d) Schematic view detailing the dimensions of the device. (e) Photograph of the dye being patterned into the rail of the device.

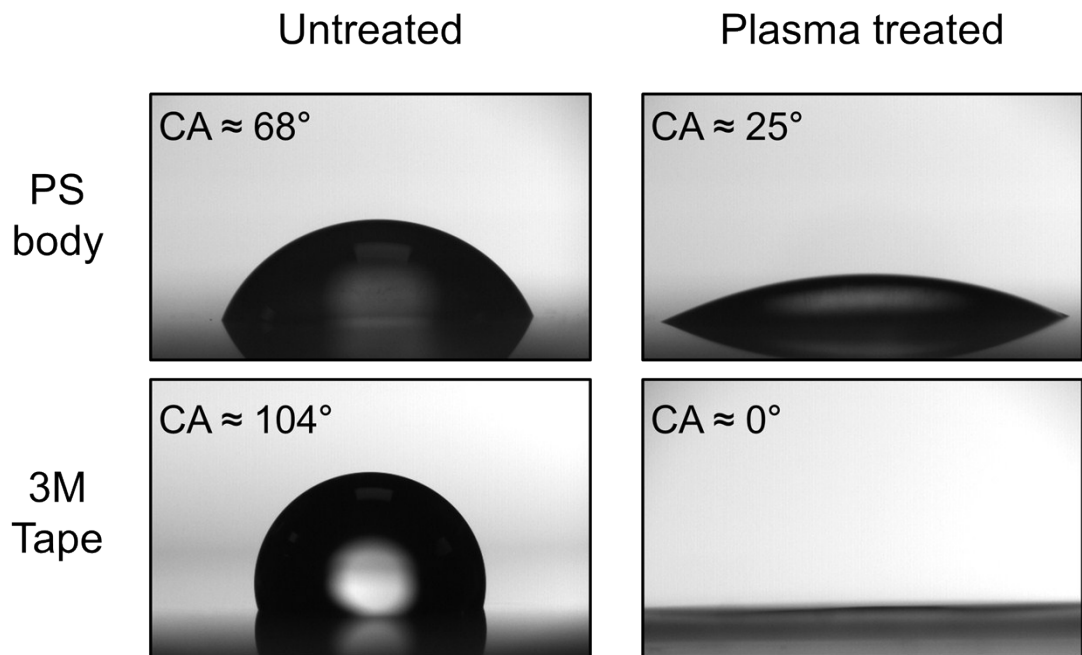
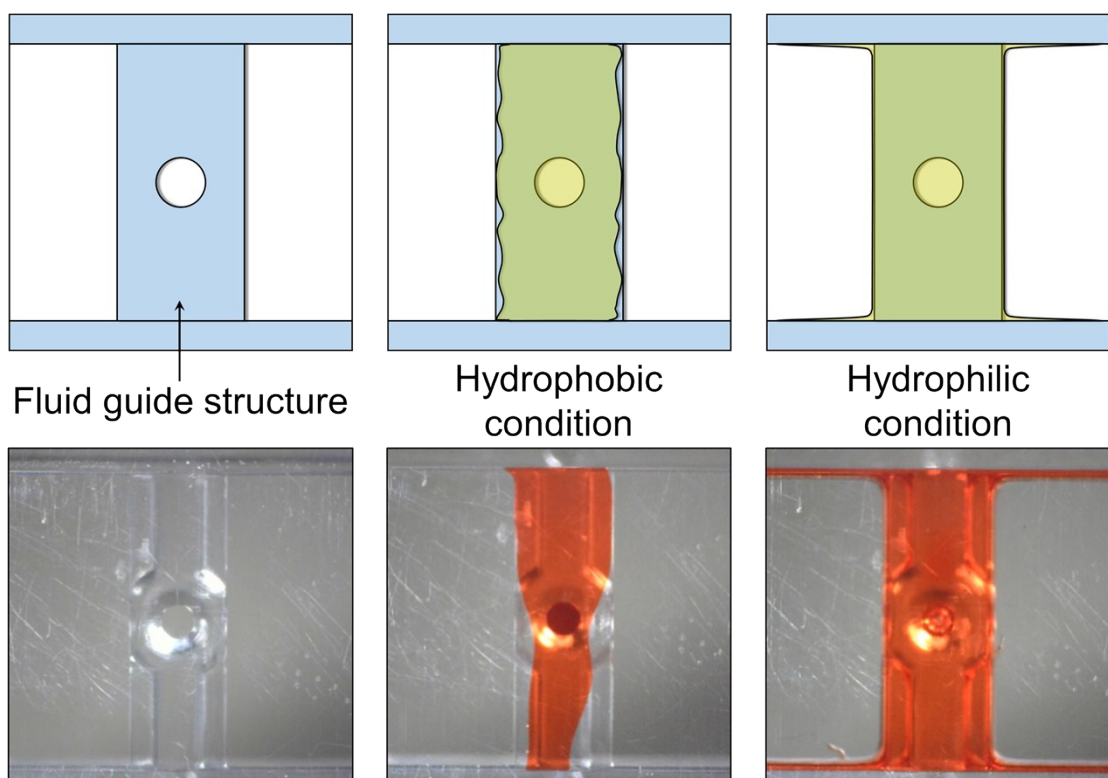

**A****B**

Figure S2. Fluid patterning under the hydrophilic and hydrophobic environment. (a) Investigation of the contact angle of the material used in the device under hydrophilic

conditions to establish the fluid patterning technique. (b) The fluid interface showed surface imbalance under hydrophobic environment. After the plasma treatment, the fluid was well patterned under hydrophilic environment.

	Production Volume					
Production Volume (ea)	Mold Fabrication	100	1k	10k	100k	1m
Soft Lithography (day)	1	7	70	700	7k	70k
Injection Molding (day)	10	1	1	10	100	1k

\* Injection molding conducted by Quick Delivery Mold (QDM) process

 Production time difference between soft lithography and Injection-molding

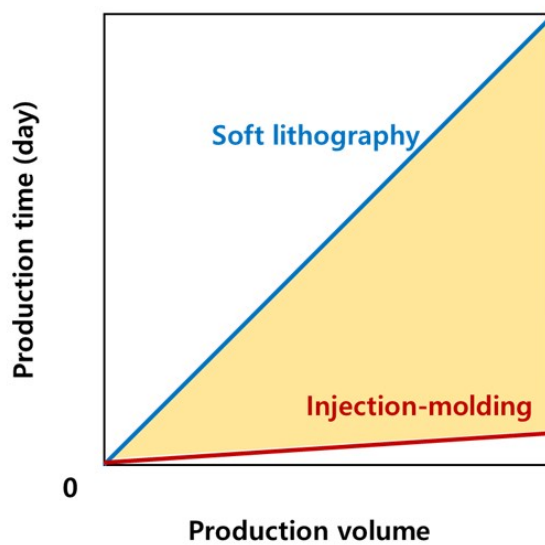


Figure S3. A diagram comparing the time required for production of the device between soft lithography and injection molding process.

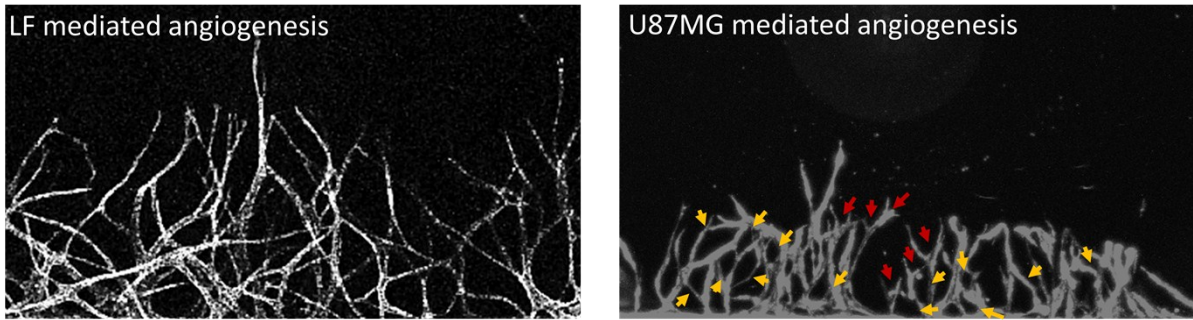
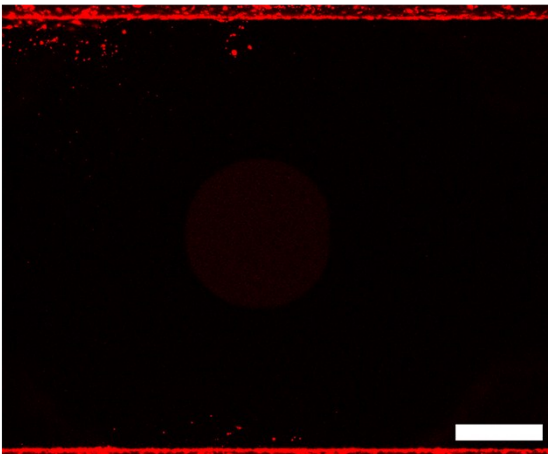


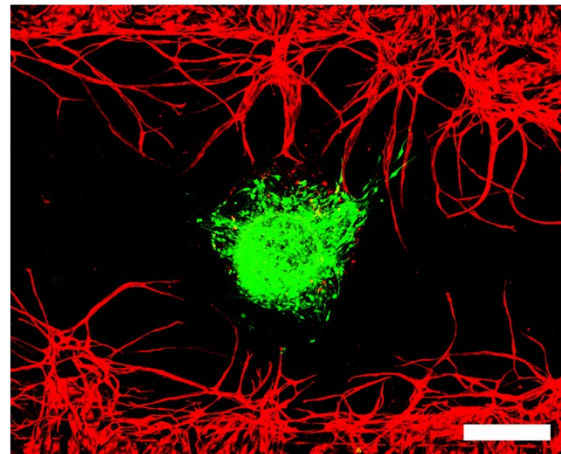
Figure S4. Comparison of angiogenic sprouts under different conditions. Under co-culture with U87MG cancer cells, angiogenic sprouts are characterized by the sprouts with branching tip cells (red arrow) and convoluted and aberrantly converged blood vessels (yellow arrow).

**A**

**(a) Negative control**



**(b) Positive control**



**B**

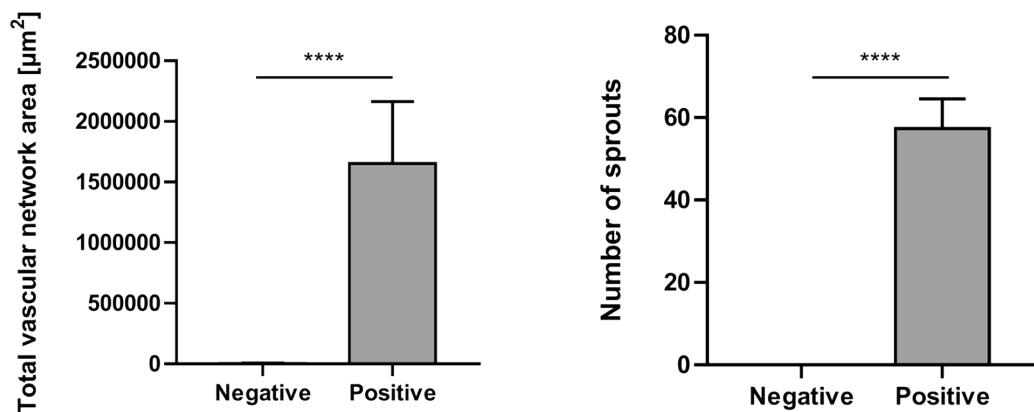


Figure S5. Identification of control models to establish the tumor angiogenesis model. (a)

Projection view of the fluorescence images to compare the control result for tumor angiogenesis. Scale bar = 500  $\mu\text{m}$ . (b) Quantitative analysis of control model based on total vascular network area and number of vessel sprouts. Bars present mean  $\pm$  SEM from at least 8 devices per condition. \*\*\*\* $p < 0.0001$  in unpaired two-tailed Student's  $t$ -test.