Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information for

Facilitating Tumor Spheroid-Based Bioassays and *In Vitro* Blood Vessel Modeling *via* Bioinspired Self-Formation Microstructure Devices

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Supplementary information file includes 7 figures, 2 tables, 3 movies and 11 references

Supplementary Tables

Table S1. Parameters designed and evaluated for the circular or striped domain.

	Designs		*Experiments*[a]						*Variation of a_γ (% mean ± SD)*[b]	*Variation of V (% mean ± SD)*[c]
Domain	a_γ *(μm)*	V *(μl)*	h *(μm)*	a_γ *(μm)*	θ *(degree)*	R *(μm)*	Γ (h/a_y)	V *(μl)*		
Circle										
1	1500	1.5	918.9	1560.9	99.7	791.9	0.59	1.3		
2	1500	1	736.2	1583.1	87.7	792.2	0.48	1.0	1.5 ±	-13.5 ±
3	1500	0.5	490.7	1456.0	62.7	819.5	0.30	0.4	4.0[n.s.]	8.2[*]
4	1500	1	760.0	1488.0	86.4	745.5	0.47	0.8		
Stripe[d]										
1	600	1.5	125.3	588.8	50.8	380.2	0.24	1.1		
2	600	1.5	139.2	549.9	57.3	326.8	0.27	1.2	-3.1 ±	-26.2 ±
3	600	1	86.9	602.2	31.7	573.4	0.14	0.7	3.7[n.s.]	3.8[***]
4	600	0.5	37.0	583.9	17.8	956.7	0.08	0.4		

[a] The experimental data was measured from the fabricated PDMS structures either in circular or in striped domain.

[b] The variation of a_γ is defined as (a_γ_experiment – a_γ_design)/a_γ_design.

[c] The variation of V is defined as (V_experiment – V_design/V_design.

[d] The length of the striped pattern is 20 mm.

*p < 0.05 and ***p < 0.001.

Table S2. Fabrication of non-planer microstructures.

Technique	Feature geometry	Level of geometrical flexibility	Feature size (μm)	Feature aspect ratio	Application	Feasibility for tissue-based bioassay
Photoresist reflow [1]	Rhombus-shaped round channel	High	30.4	0.5	Microvascular networks	Yes
Grayscale lithography [2]	Semi-circular channel	Basic	200	0.2	Microvalve	Nil
Negative pressure [3]	Coaxial channel	High	200	0.38	Fabrication of microfibers and particles	Nil
Micro milling [4]	Round and multi-tiered channels	High	100	0.5	Microvascular networks and human liver sinusoid structure	Yes
Spin coating [5]	Round channel	Basic	100	0.5	Modeling of blood vessels	Yes
Non-printed/solid molding [6]	Round channel	Basic	150	0.5	Cancer invasion in artificial microvessels	Yes
3D Printed molding [7]	Serpentine channel	High	41.8	0.45	Nil	Nil
Liquid molding:						
Dipping (100% glycerol-hydrogel-based mold) [8]	Serpentine channel	High	50	0.097	Cell trapping	Nil
Drop on demand printing (50% glycerol-based mold) [9]	Semi-circular channel	Basic	100	0.08	Nil	Nil
Inkjet printing on liquid substrate (polymer ink-based mold) [10, 11]	Round channel and microwell	Moderate	100 for channel; 7 for well	0.5 for channel; 0.5 for well	Microfluidic reactor, single cell patterning and functional nanoparticle patterning	Nil
Our approach (bioinspired self-formation)	**Serpentine channel and microwell**	**High**	**200 for channel; 50 for well**	**0.27 for channel; 0.59 for well**	**Culture of tumor spheroid, high-throughput drug screening and modeling of blood vessels**	**Yes**

Supplementary Figures

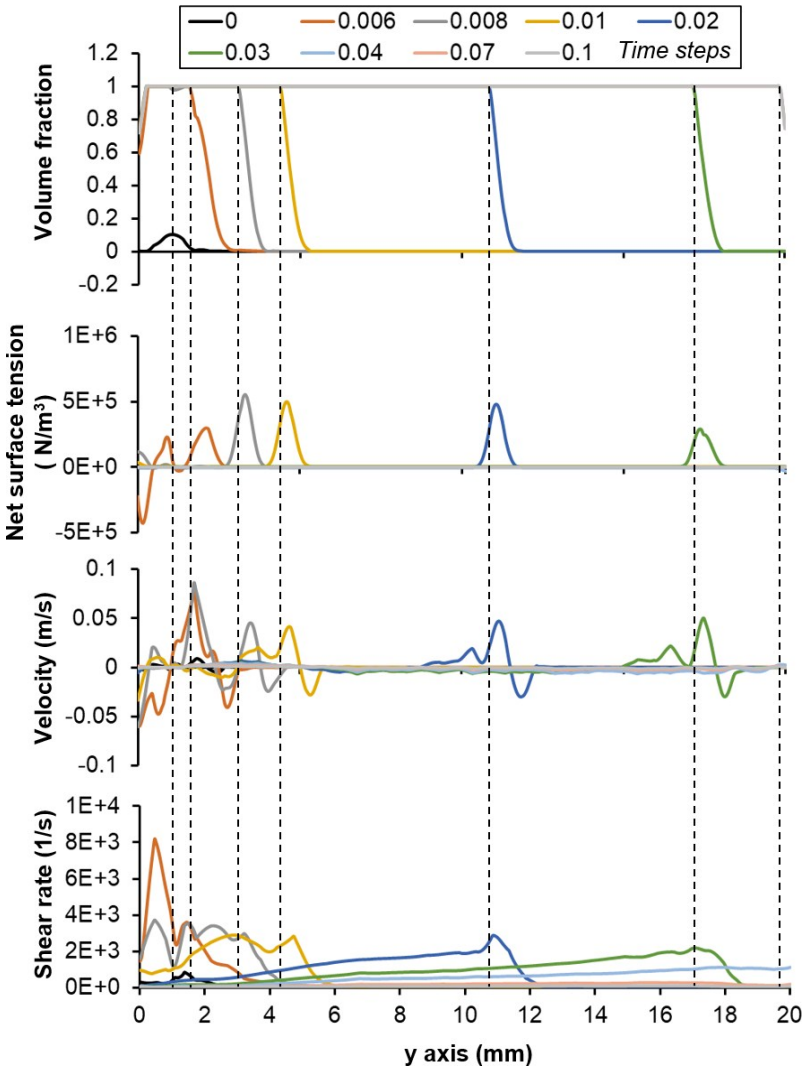

Figure S1. Time-lapse simulations of volume fraction, net surface tension, velocity and shear rate. The y-axis corresponds to the C-C' cross line in Fig. 2(d). Dash lines indicate the leading interfaces between air and dragged liquid by surface tension, during the calculated time steps.

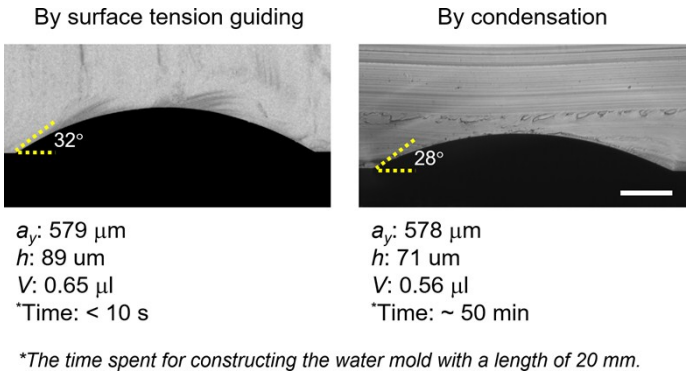

Figure S2. Comparison of similar sizes of microstructure made from surface tension-guided and condensation-induced water molding.

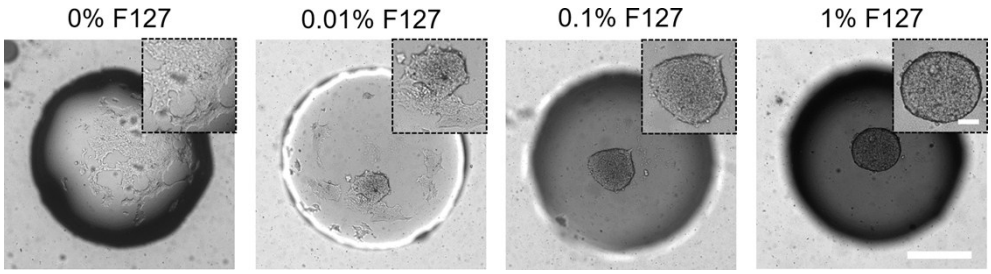

Figure S3. Comparison of MCF7 spheroids performed under different concentrations of F127 coating in the microwells. Initial 900 cells were loaded for each well and then cultured for 1 day. Scale bar, 500 μm; insert bar, 100 μm.

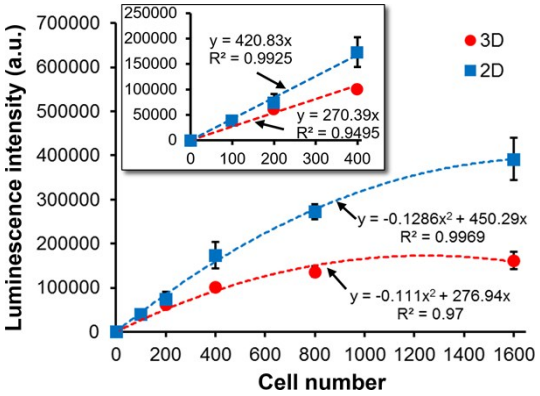

Figure S4. Detected luminescence from 3D or 2D cultured cells versus different cell numbers loaded. Cells were cultured for 1 day in 3D conditions. Note that there is a linear relationship between the detected luminescence and the loaded cells as the cell number is less 400. Each data represents the mean ± SD (n = 2 ~ 5).

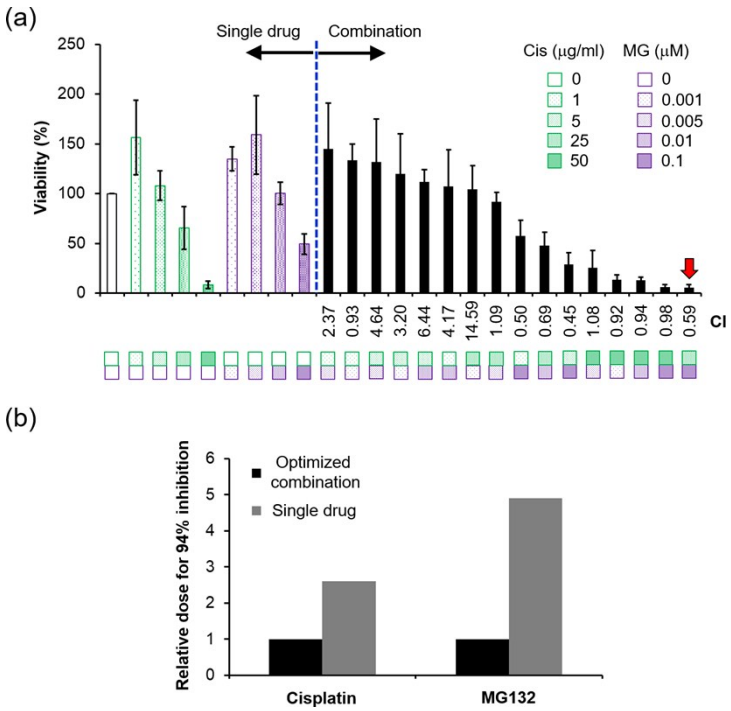

Figure S5. Synergistic effect of cisplatin and MG132 on SK-N-DZ tumor spheroid. (a) Cell viabilities of SK-N-DZ spheroids under the treatments of cisplatin and MG132 with different concentrations. The optimized drug combination is highlighted by a red arrow. Each data represents the mean ± SEM from 4 independent experiments. (b) Comparison of relative doses used for 94% cell inhibition by the optimized drug combination and by the single drug treatment (i.e., 25 μg/ml cisplatin or 0.1 μM MG132).

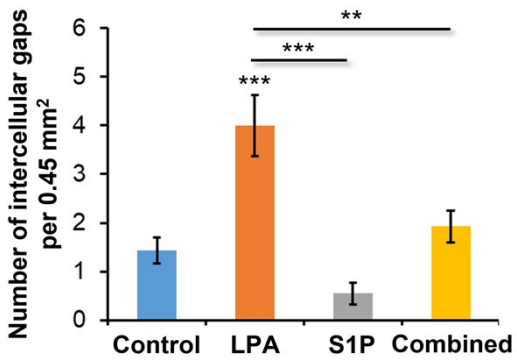

Figure S6. Number of intercellular gaps. The engineered blood vessels were treated with LPA (1 μM), S1P (0.5 μM) or combined treatment for 10 minutes. Each data represents the mean ± SEM from 2 independent experiments (n = 11 ~ 16 image fields per 0.45 mm^2). **p < 0.01 and ***p < 0.001 were compared to the control, except for the indicators.

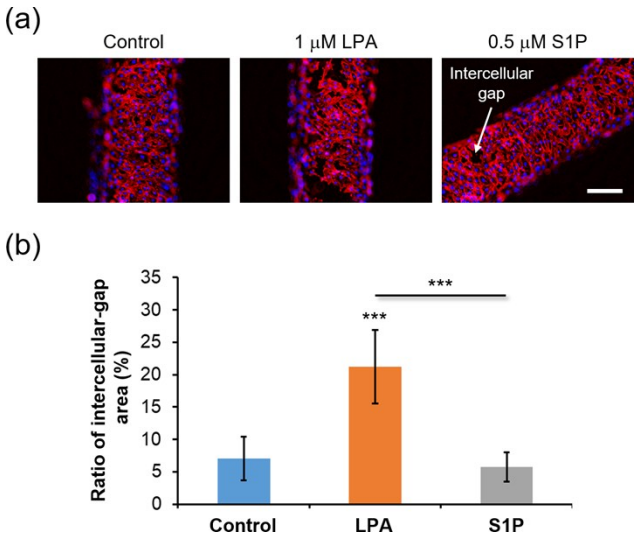

Figure S7. Effects of LPA and S1P on pre-impaired blood vessels. HUVECs were cultured within the blood channels for 1 day, resulting in a sub-confluent cell monolayer. The ratio of intercellular-gap area of control blood vessels is around 7%. The blood vessels were treated with LPA (1 μM) or S1P (0.5 μM) for 10 minutes. Each data represents the mean ± SD (n = 5 ~ 7 image fields per 0.78 mm^2). ***$p < 0.001$ was compared to the control, except for the indicators. Scale bar, 300 μm.

Supplementary Movies

Movie S1. Time-lapse volume fraction of a water droplet dispensed on a stripe-patternedly hydrophilic surface. The droplet volume is 0.7 μl. The width and length of the hydrophilic pattern are 600 μm and 20 mm, respectively.

Movie S2. Time-lapse surface shear rate of a water droplet dispensed on a stripe-patternedly hydrophilic surface. The droplet volume is 0.7 μl. The width and length of the hydrophilic pattern are 600 μm and 20 mm, respectively.

Movie S3. 3D view of the engineered blood vessel. Images were taken from a confocal microscopy and stacked by ImageJ software.

References

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