Supplementary Information

# A microfluidic hanging drop-based spheroid co-culture platform for probing tumor-angiogenesis

Didem Rodoplu[1] , Jefunnie Sierra Matahum [1] and Chia Hsien Hsu [1,2,3*]

[1] Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes, Zhunan, Miaoli, 35053, Taiwan
[2] Institute of Nanoengineering and Microsystems, National Tsing Hua University, Hsinchu 30013, Taiwan
[3] Tissue Engineering and Regenerative Medicine, National Chung Hsing University, Taichung 40227, Taiwan

*Corresponding author

Chia-Hsien Hsu, Ph.D.
chsu@nhri.edu.tw

# Table of content

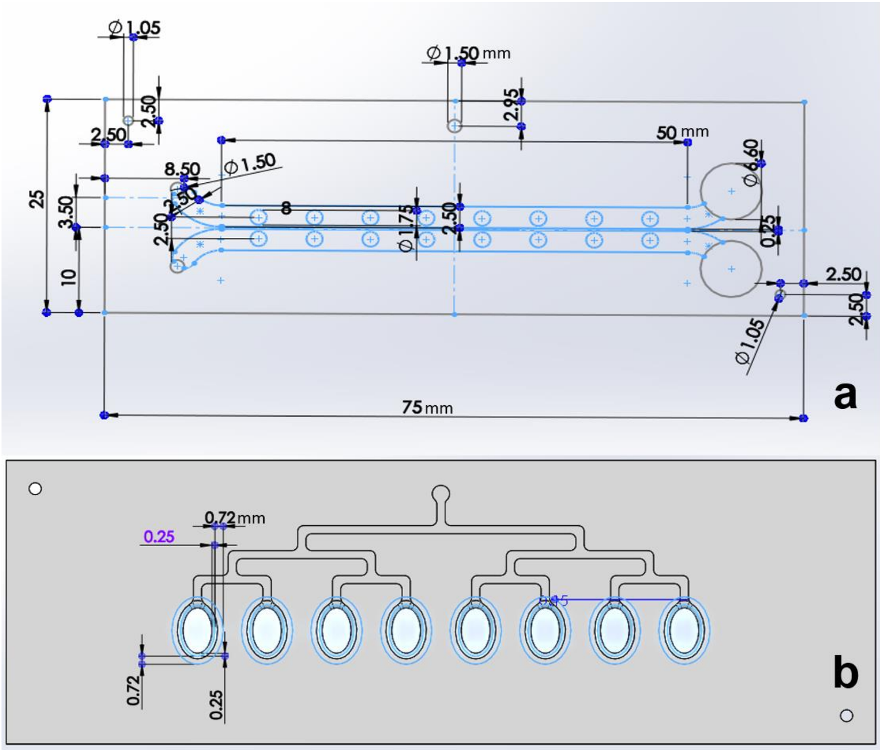


**Fig S1.** Schematic drawing of the μ-CCD device a) middle layer b) bottom layer

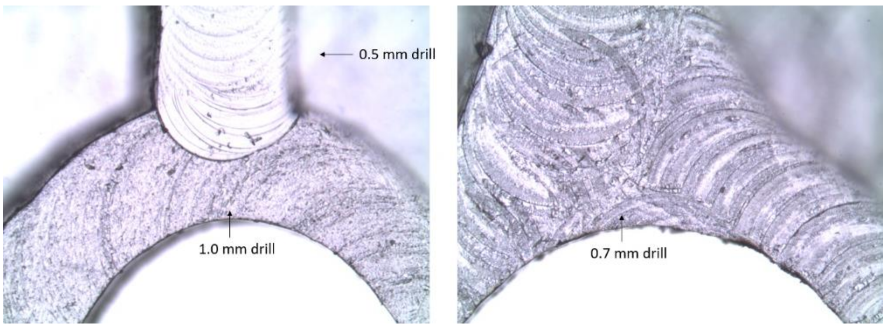


**Fig S2.** Images of confrontation channel surfaces that carved using various CNC drill bits

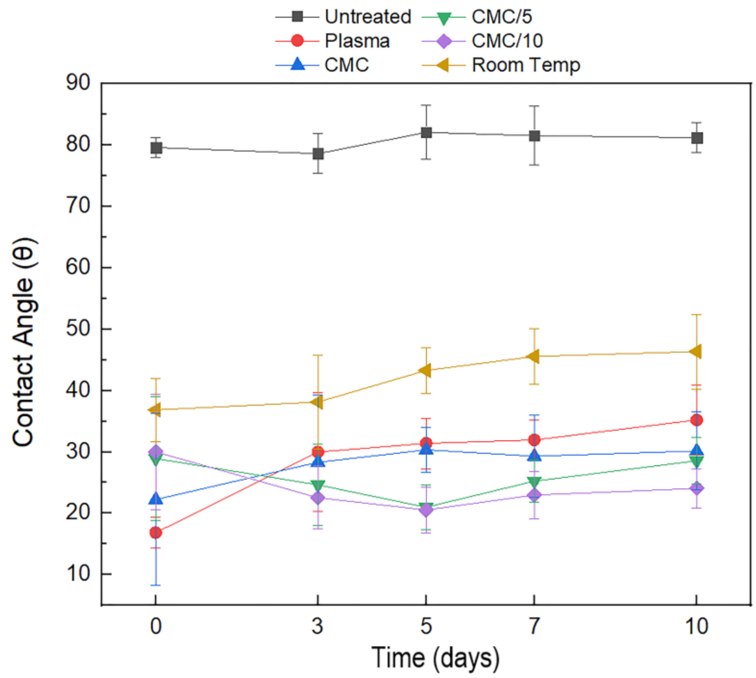


**Fig. S3.** Time-dependent wettability results of untreated, plasma treated and Pluronic™ F-127 treated surfaces, n=4 (error bars=±SD)

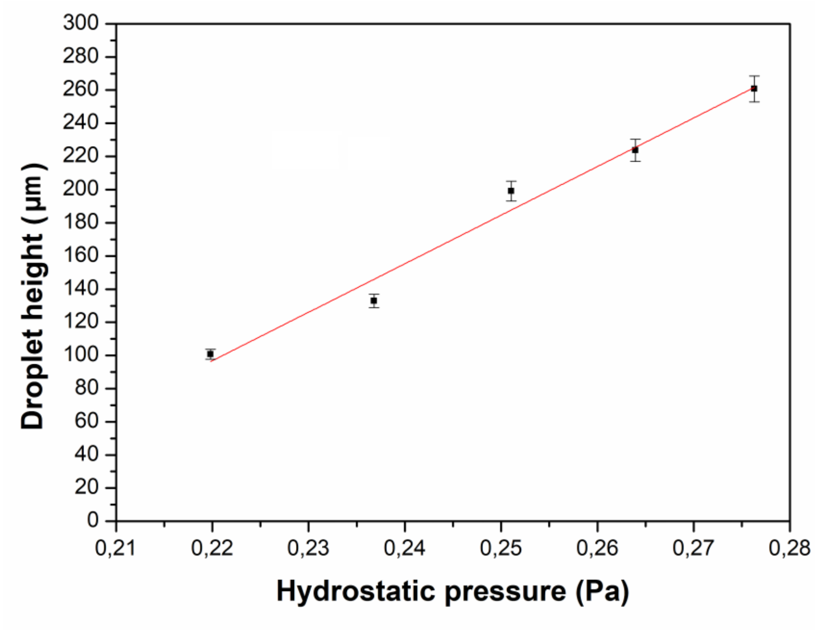


**Fig. S4.** The measured droplet heights under various hydrostatic pressure for the spheroid generation in the µ-CCD device

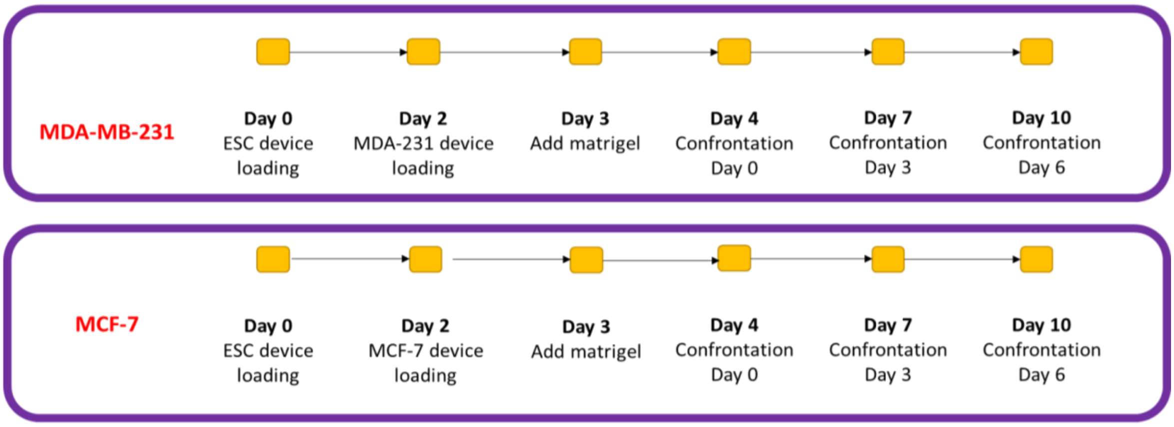


**Fig. S5.** Schematic timeline for tumor-angiogenesis

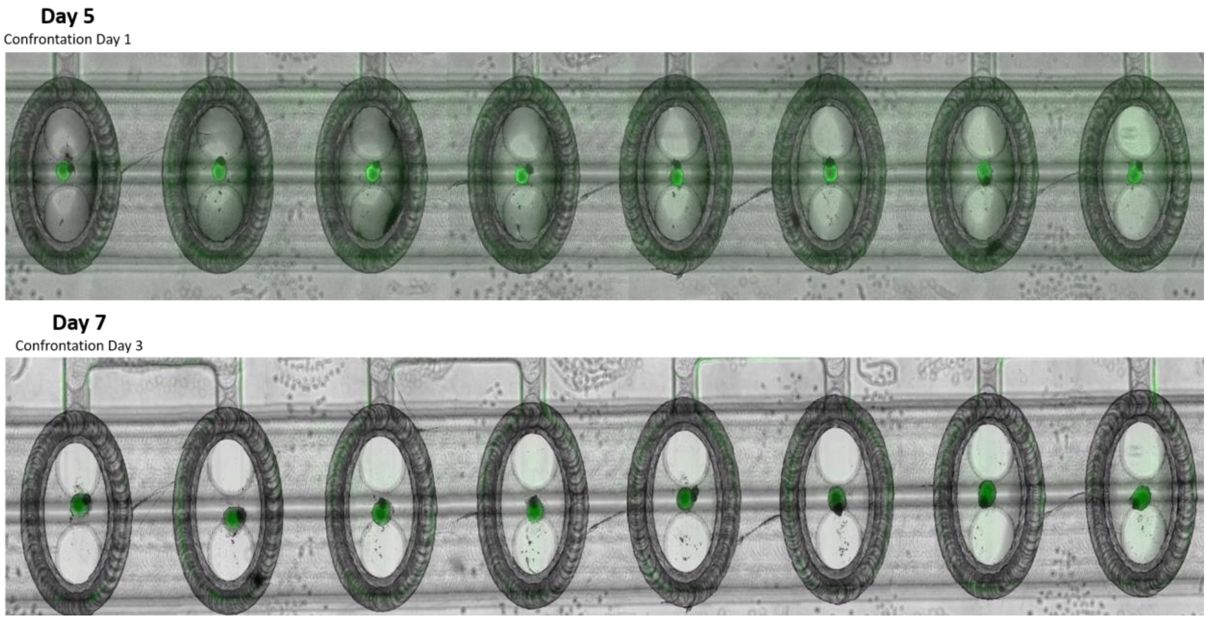


**Fig. S6.** Large-scanned Nikon inverted-fluorescence microscopy image of μ-CCD originated EB@MCF-7 with a CSR of 100%

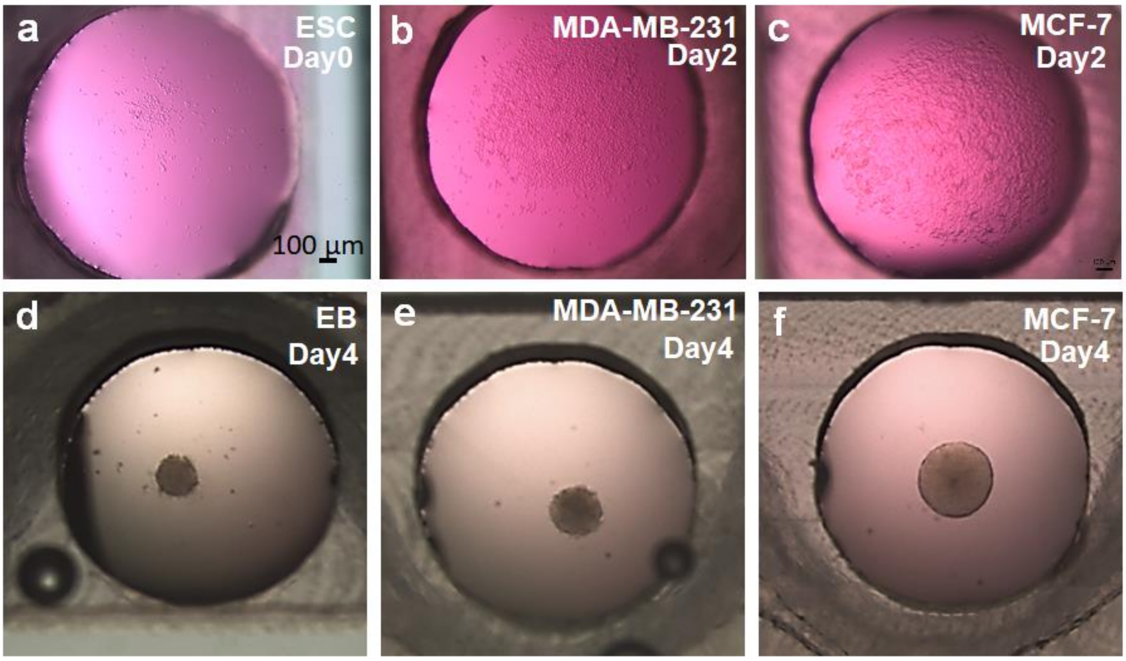


**Fig. S7.** The zoom-in images of EB and TS droplets. Shown are: a–c) individual cells after device loading, d–f) spheroid formation on day 4.

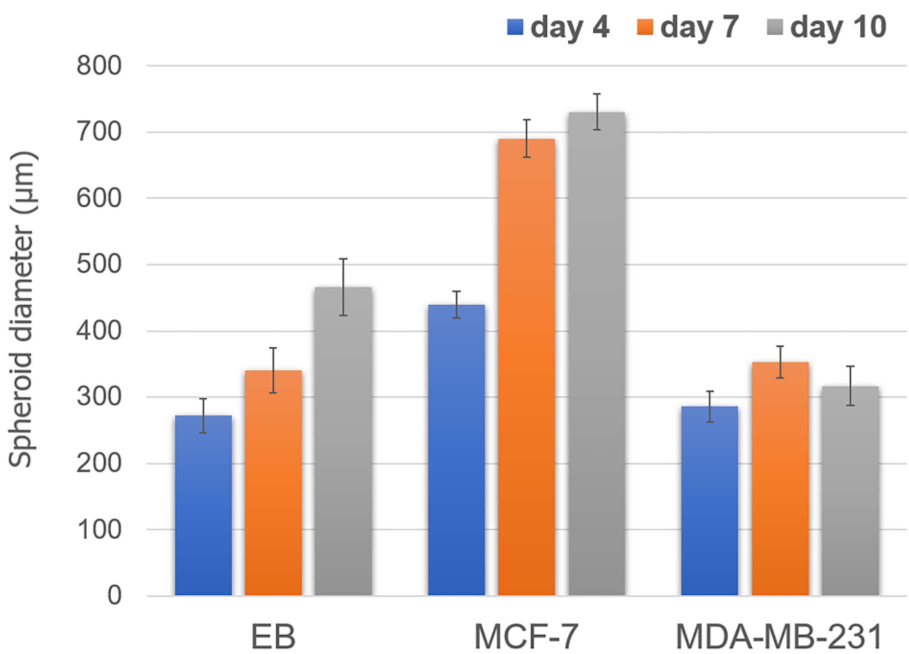


**Fig. S8.** Spheroid size variations of confrontation culture of EB@TS on experiment day 4, day 7 and day10 (n=4, error bars=±SD)

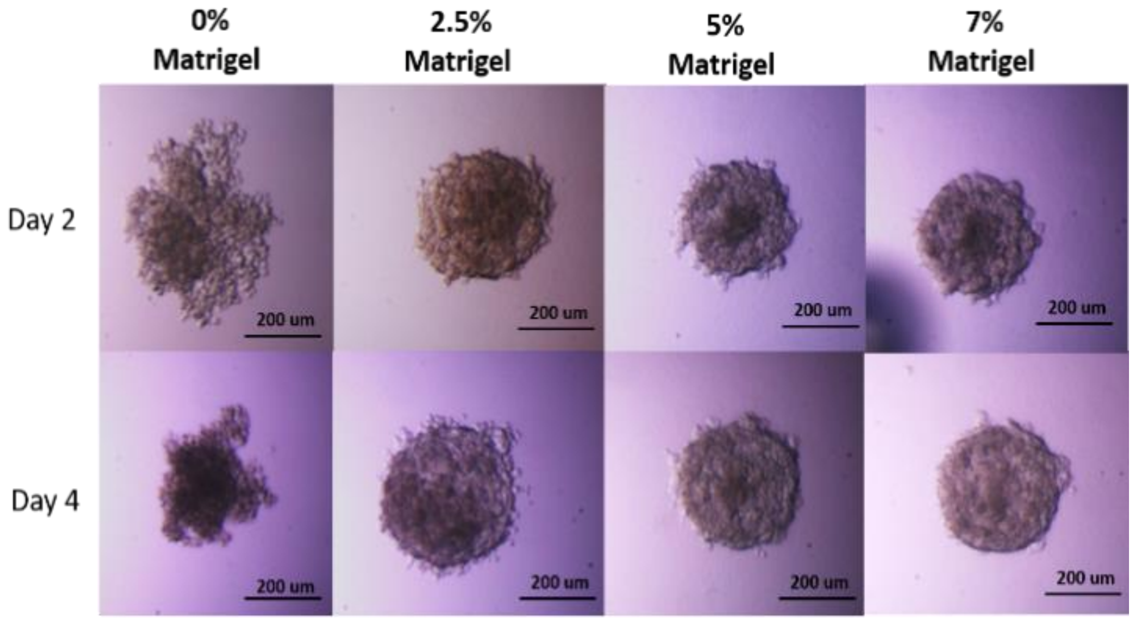


**Fig. S9.** The representative images for the influence of Matrigel concentration on MDA-MB-231 spheroid formation (initial seeding density of $2\times10^4$ cells/ml)

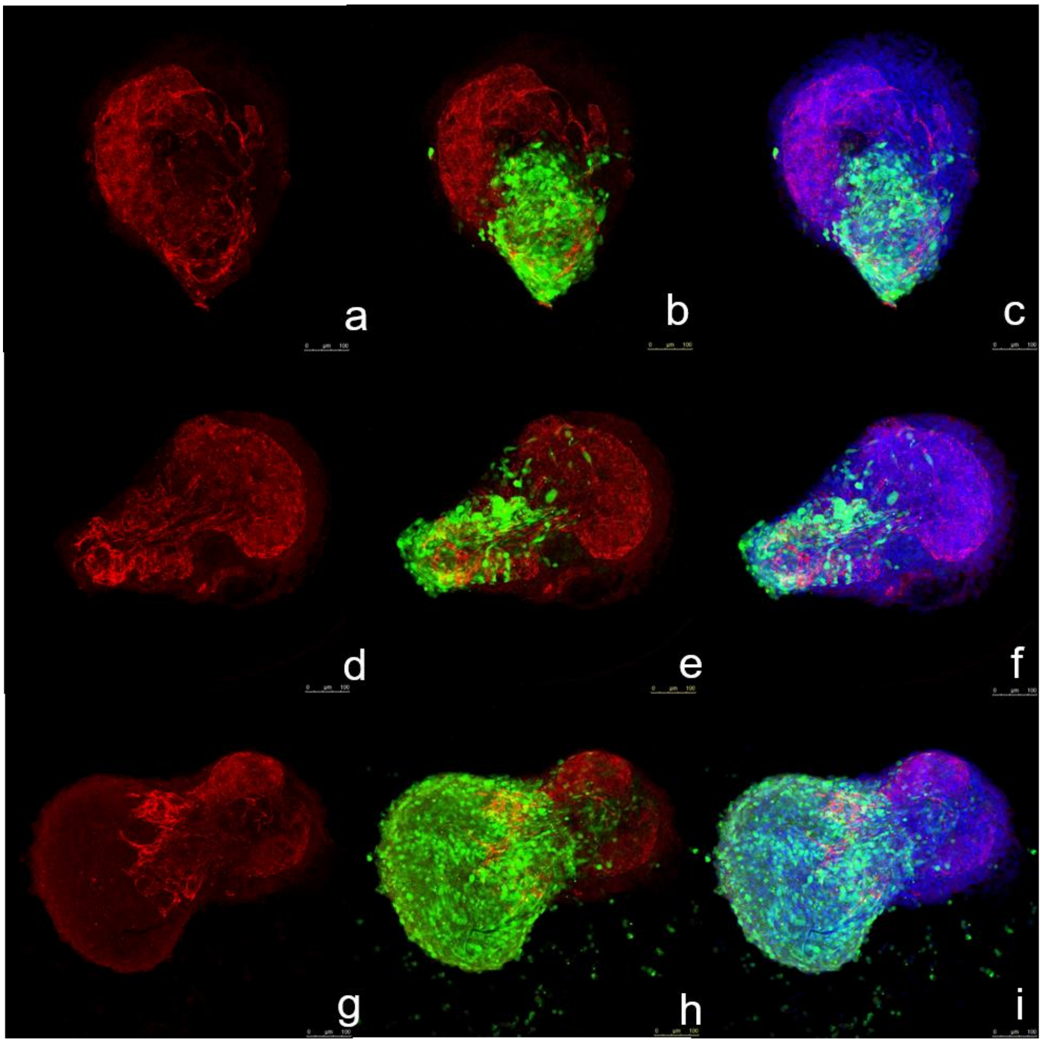


**Fig. S10:** Representative confocal images of tumor induced angiogenesis observed after 3 days of confrontation; a-b-c) without Matrigel, d-e-f) 1% Matrigel additive, g-h-i) 2.5% Matrigel additive, a-d-g) TRITC channel, b-e-h) merged TRTIC-FTIC channels, c-f-i) merged TRTIC-FTIC-DAPI channels

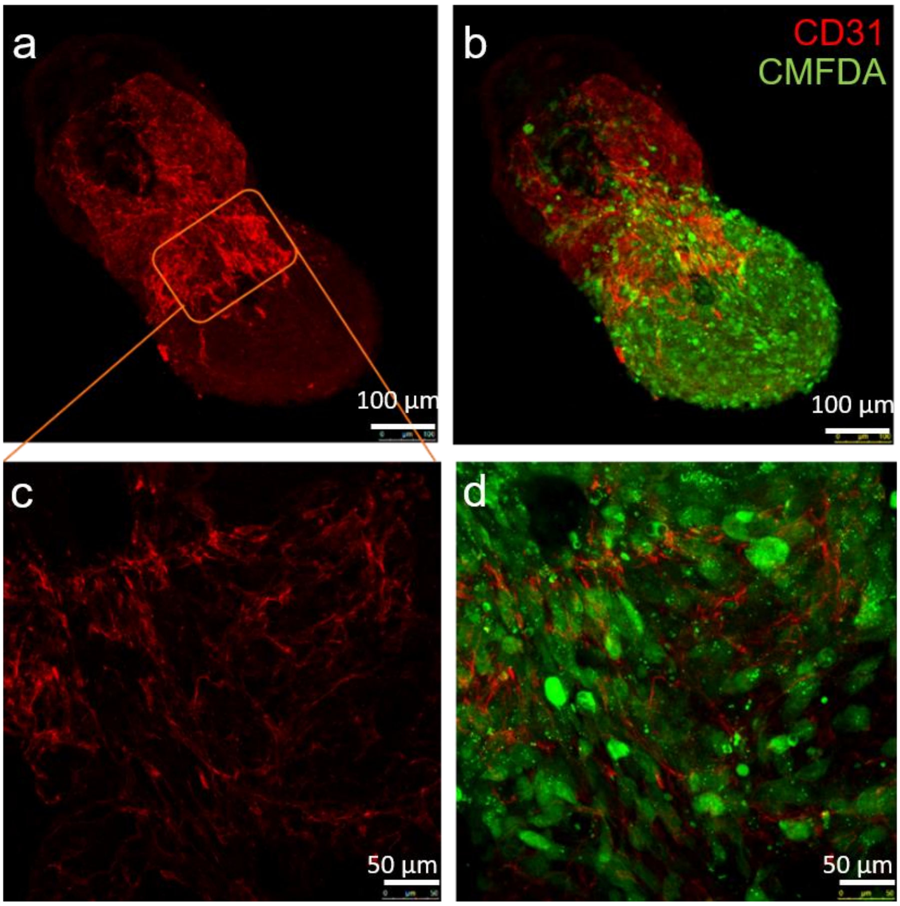


**Fig. S11.** The demonstration of a directed vascular network. Shown are: confocal images of EB@MDA-MB-231 in an ultra-low attachment 96-well plate a-c) TRITC channel, b-d) merged FTIC-TRITC channels, using a-b) 20X objective and a c-d) 40X objective.

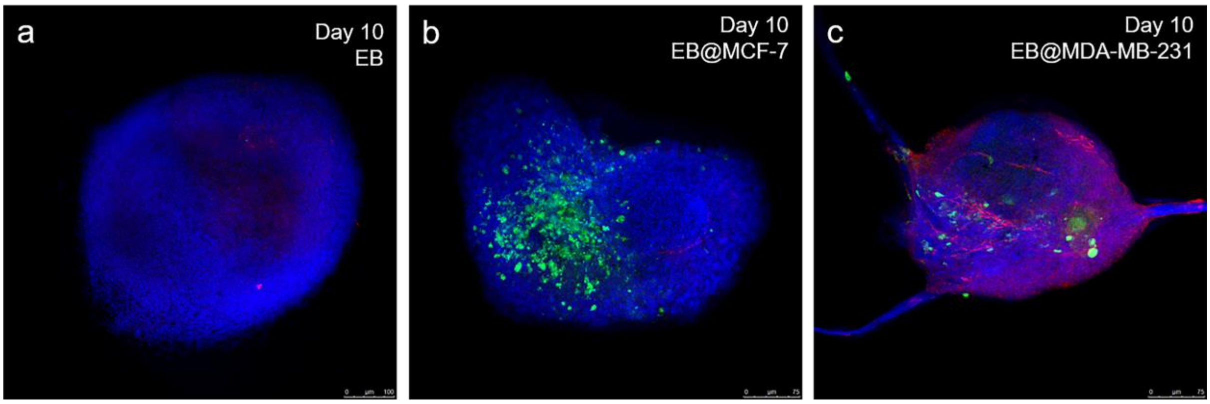


**Fig. S12.** The representative immunostaining images for probing tumor-induced angiogenesis (without Matrigel) a) EB control, b) EB@MCF-7, c) EB@MDA-MB-231 (CMFDA: green, CD31: red)

## Supplementary Note 1

### Principle of SCF criteria and Bond number

The shape of the hanging drops generated on the surfaces [1] and apertures of the suspended microfluidic channels' [2] have been widely studied. Previous studies on the axis-symmetric meniscus of the pendant drop suggested that relative importance of gravitational and surface tension force can be quantified by the bond number (Eq. 1)[1],

$$Bond\ number = \frac{gravitational\ force}{surface\ tension\ force} = \left(\frac{rd}{Ic}\right)^2 \tag{1}$$

where *rd* is the base radius of the pendant drop and *Ic* is the capillary length. In our model, we use ellipse confrontation aperture, which has two axes of symmetry, generates ellipsoid shaped wetted perimeter.

Casavant et al. described the spontaneous capillary flow (SCF) phenomenon for different geometries [2]. Using a quasi-steady-state surface analysis, SCF criteria was defined for most of the open and suspended microchannels as the following (Eq. 2)

$$\frac{Pf}{Pw} < \mathrm{Cos}(\theta) \tag{2}$$

where *Pf* is the free air-liquid interface and *Pw* is the wetted perimeter. Herein, to determine optimum minor axis diameter of the apertures, we have modified the SCF equation (Eq. 3) for suspended microfluidics by including minor axis diameter and the contact angle measured from the minor axis images, while major axis of the aperture was kept constant. The $\frac{Pf}{Pw}$ ratio can be calculated as the following (Eq. 3),

$$\frac{Pf}{Pw} = \frac{w+\delta}{2h+w-\delta} \tag{3}$$

where *h* is the total liquid height, *w* is the width of reservoirs, and $\delta$ is the minor axis of the droplets that w+ δ corresponds to the total free air-liquid interface.

[1] A Hollow Sphere Soft Lithography Approach for Long-Term Hanging Drop Methods, Tissue Engineering Part C: Methods 16(2) (2010) 249-259.
[2] B.P. Casavant, E. Berthier, A.B. Theberge, J. Berthier, S.I. Montanez-Sauri, L.L. Bischel, K. Brakke, C.J. Hedman, W. Bushman, N.P. Keller, D.J. Beebe, Suspended microfluidics, Proceedings of the National Academy of Sciences 110(25) (2013) 10111-10116.

# Supplementary Note 2

## The confrontation success rate

After confrontation, large, scanned images of μ-CCD devices were taken using inverted microscopy and the confrontation success rate (CSR) of the developed platform was calculated. We defined the CSR of the device as the percentage of converged spheroids, and calculated CSR using the following Eq. 4;

$$CSR\ (\%) = \frac{Nm}{Nt} x100 \quad (4)$$

Here, $N_m$ is the number of wells containing merged spheroids and $N_t$ is the total number of confrontation wells.