

Supporting information:

Integrating Perfusable Vascular Networks with A Three-Dimensional Tissue in A Microfluidic Device

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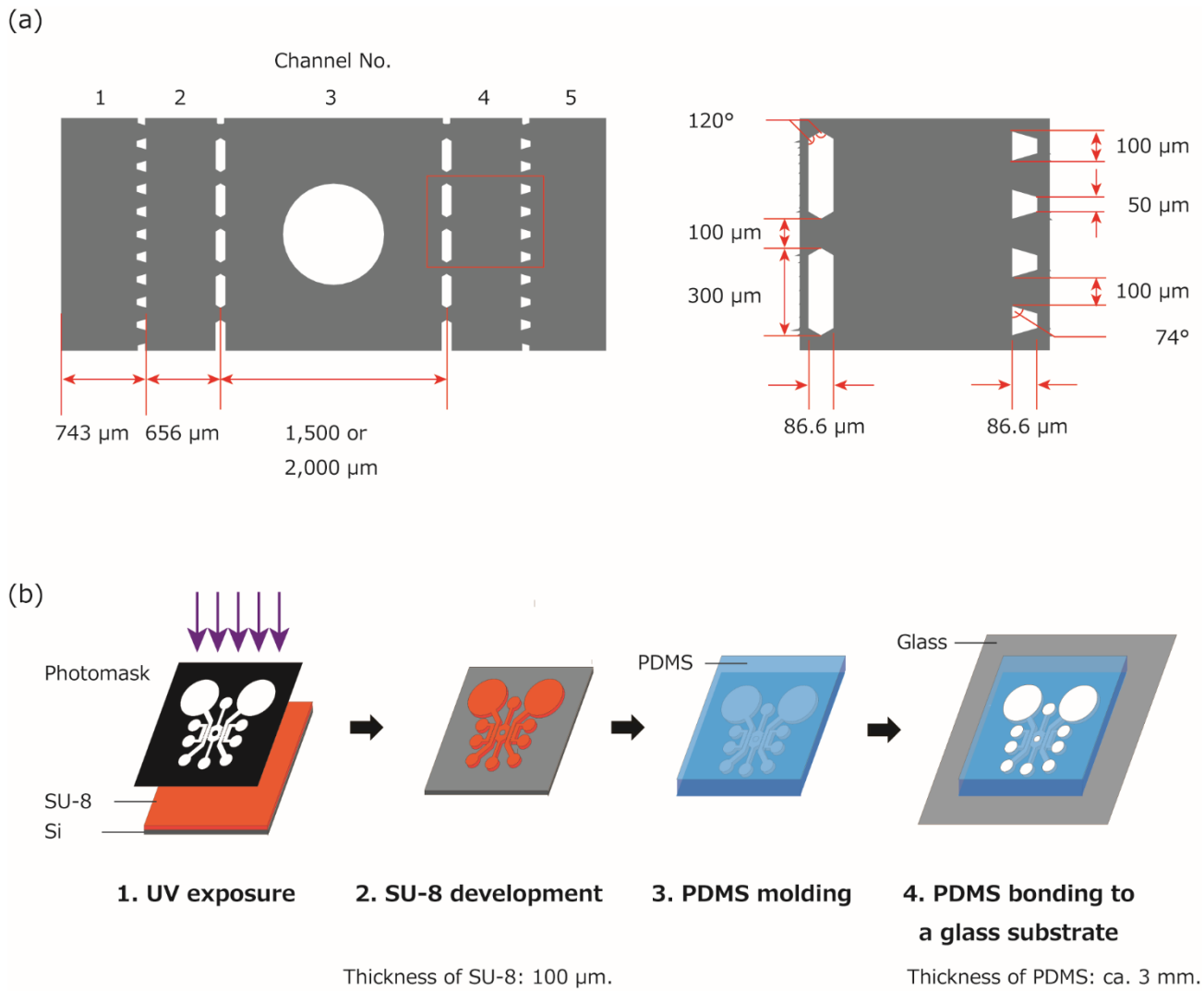


Figure S1 (a) Channel configuration and (b) fabrication process flow of the microfluidic device.

(a) Enlarged view at the center of the device. Post dimensions are indicated in the enlarged illustration on the right. (b) Fabrication process for the microfluidic device.

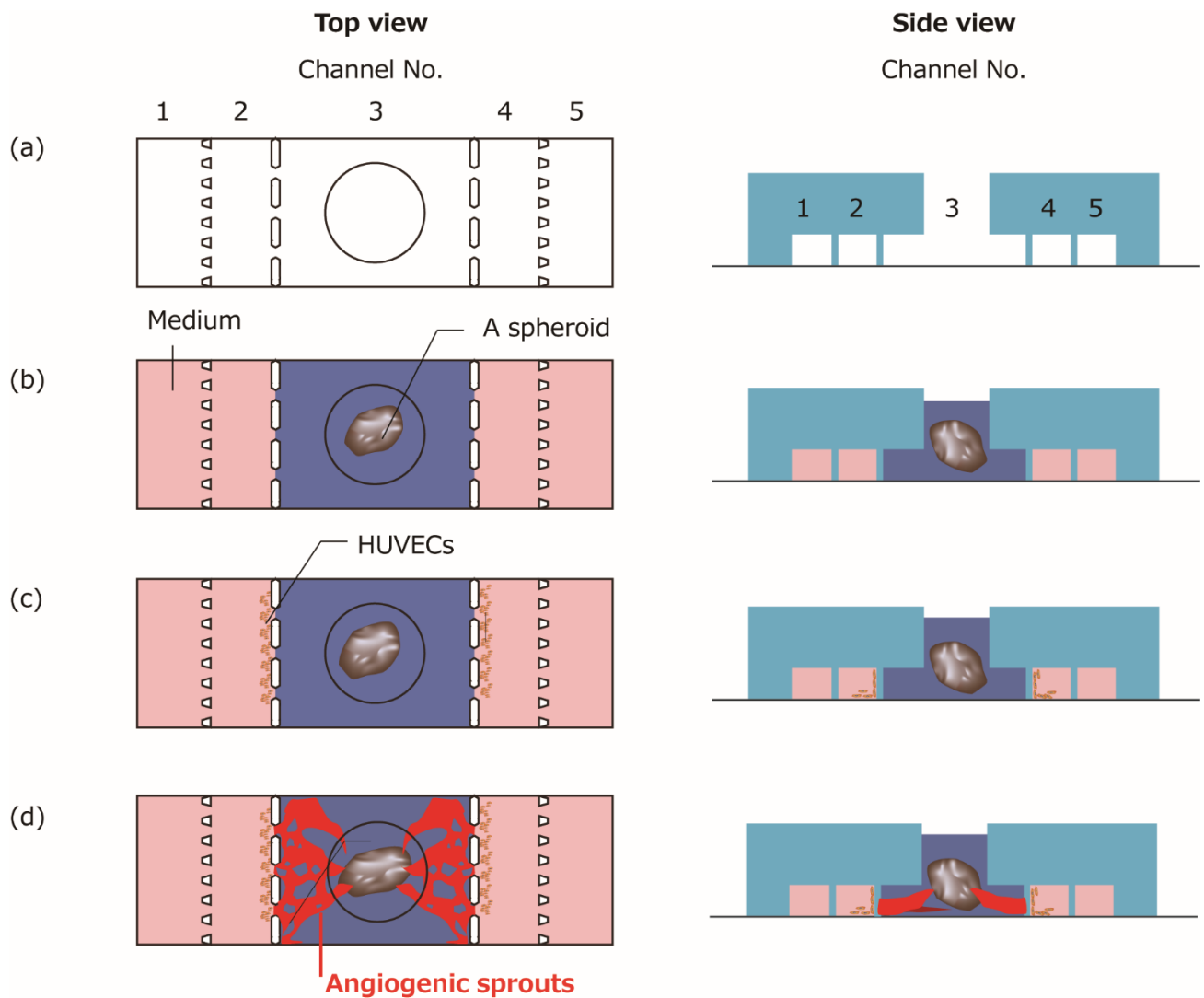


Figure S2 Schematic view of the cell seeding procedure. (a) A device before seeding cells. (b) A spheroid suspended in a fibrin–collagen gel was introduced into a spheroid well centered at channel 3. After the gelation, EGM-2 medium was added to channels 1, 2, 4 and 5, and the device was incubated for 24 h to remove air bubbles. (c) HUVECs (5×10^6 cells/mL) were introduced into channels 2 and 4. The device was tilted by 90 degrees and incubated for 30 min, which made HUVECs adhere to the side of the gel in channel 3. (d) The spontaneous vascular formation was induced by the secretion from hLFs in the spheroid.

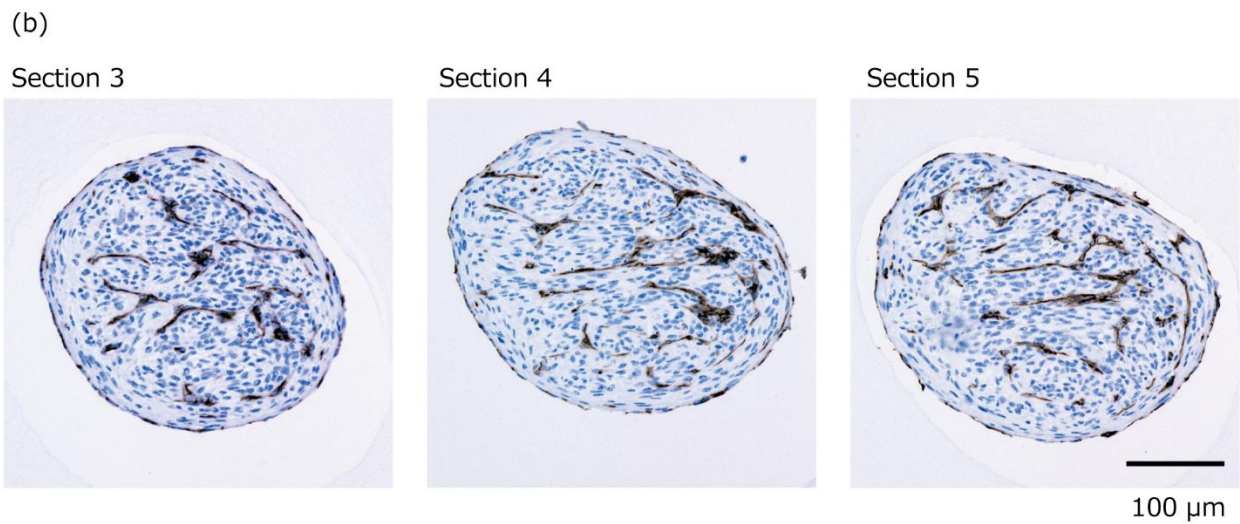
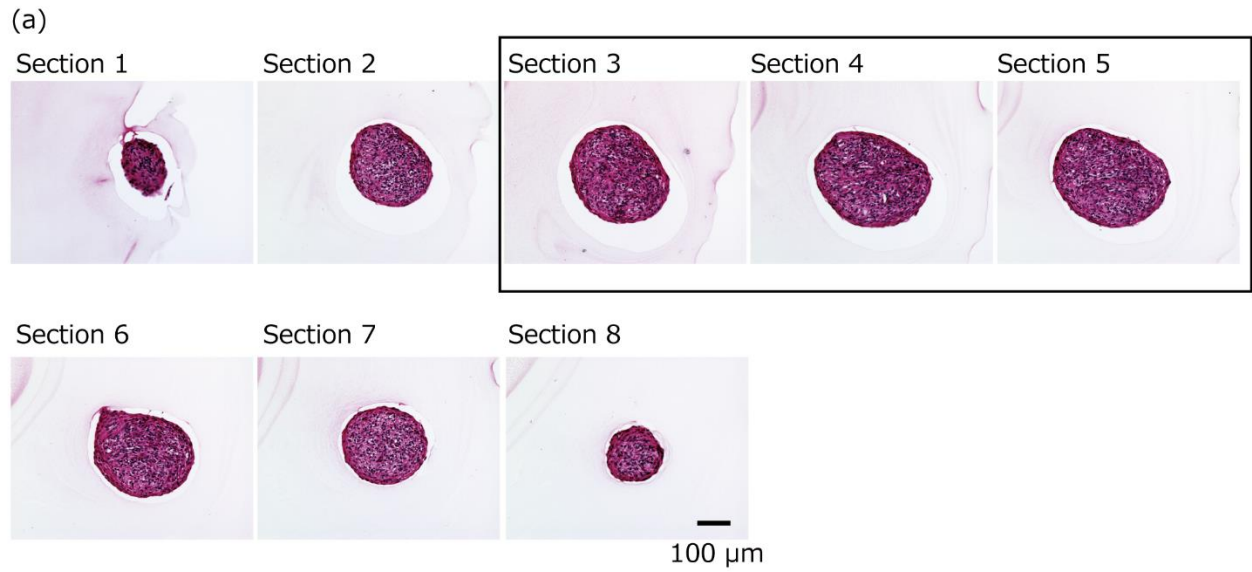


Figure S3 Organisation of vessel-like structures in a coculture spheroid after 4 days in culture in a 96-well. (a) Series of 8 consecutive, H&E stained sections of the paraffin-embedded coculture spheroid. (b) Series of 3 consecutive immunohistochemically stained sections (brown; CD31, blue; nuclei). Three sections were taken at the same position as indicated by the rectangle in (a).

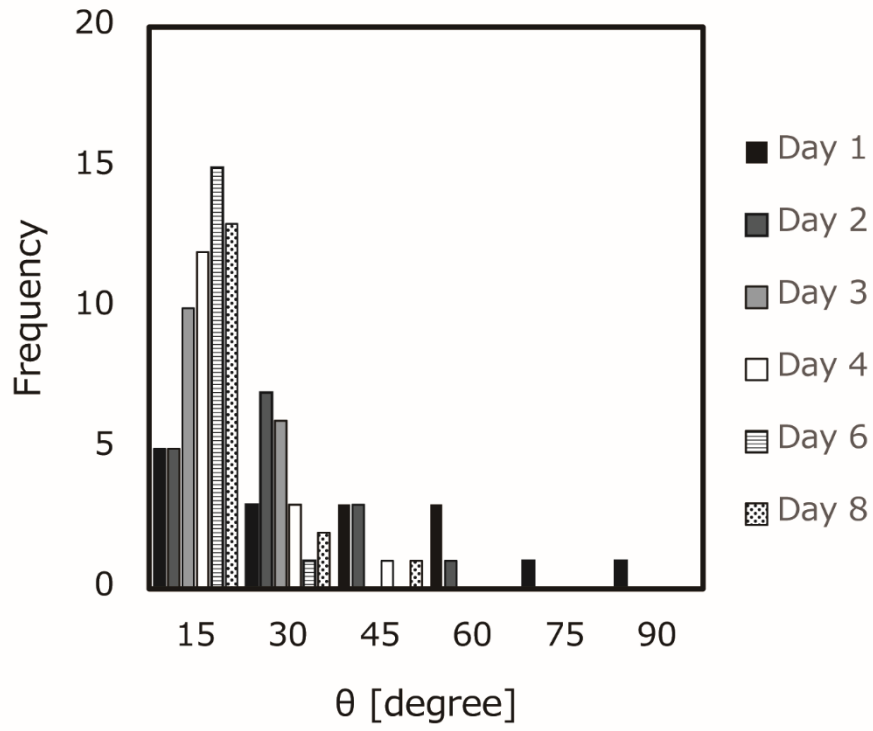


Figure S4 Histogram of angles of angiogenic sprouts. The histogram was created from the same data in Fig. 2b. $n \geq 16$.

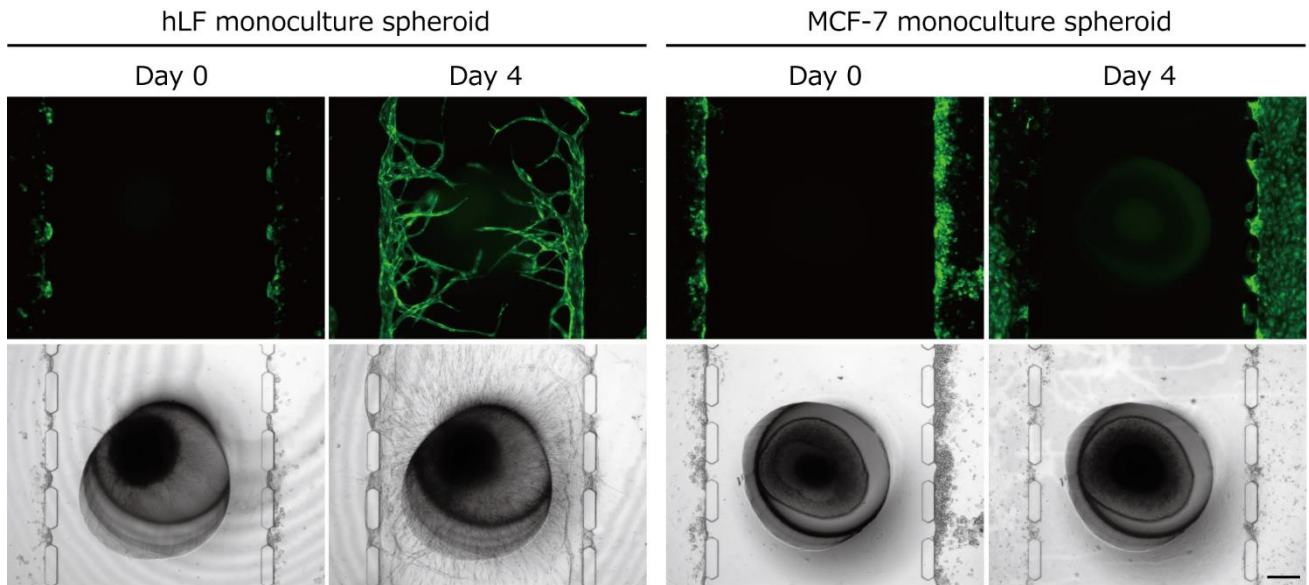


Figure S5 Comparison of sprouts induced by a monoculture spheroid comprised of hLFs and MCF-7. Each spheroid comprised of 2.0×10^4 cells was formed in a 96-well plate. After 2 days in suspension culture, each monoculture spheroid and GFP-HUVECs were introduced into the microfluidic device as described in the method section. MCF-7 monoculture spheroid did not induce any significant sprouts from microchannels. Scale bar = 200 μm .

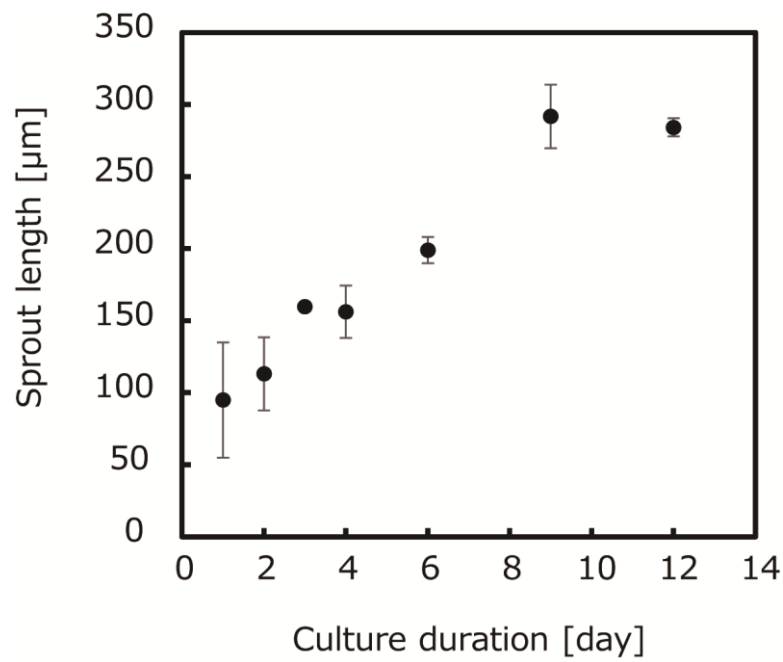


Figure S6 Time course of sprout length from coculture spheroids in a microfluidic device. Sprout lengths was defined by the distance between the spheroid surface and the vascular tip and was measured using imageJ software. Mean \pm SEM., n = 3.

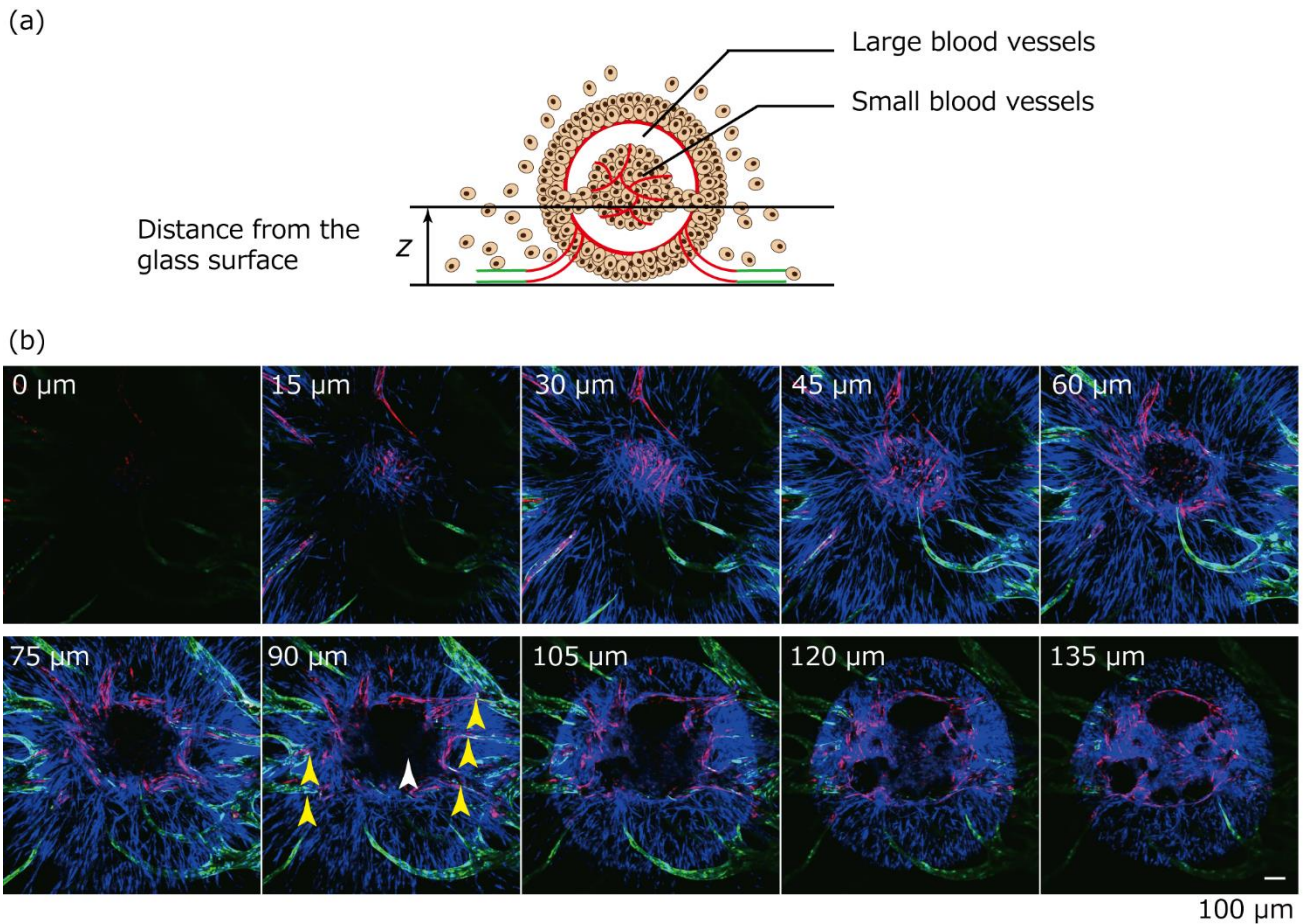
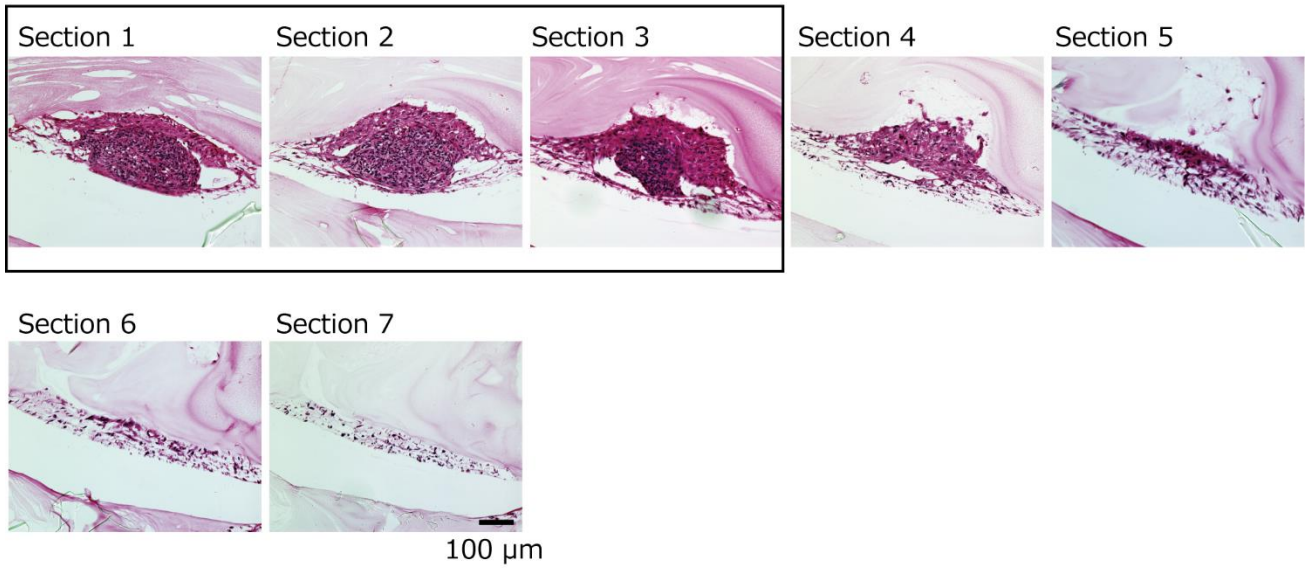


Figure S7 Coculture spheroid after 14 days in device culture. (a) Schematic illustration of the side view of the coculture spheroid in a device. Red lines indicate the vascular network formed by pre-existing endothelial cells (RFP-HUVECs) in a coculture spheroid and green lines indicate the angiogenic sprouts from microchannels (GFP-HUVECs). (b) Sectional images of confocal microscopy for the spheroid that is identical to one shown in Figures 6a and 6b. The distance from the glass surface corresponding to z in Figure S6a is indicated in the micrographs. White and yellow arrow heads indicate a large blood vessel and an angiogenic sprout connected with the large vessel.

(a)



(b)

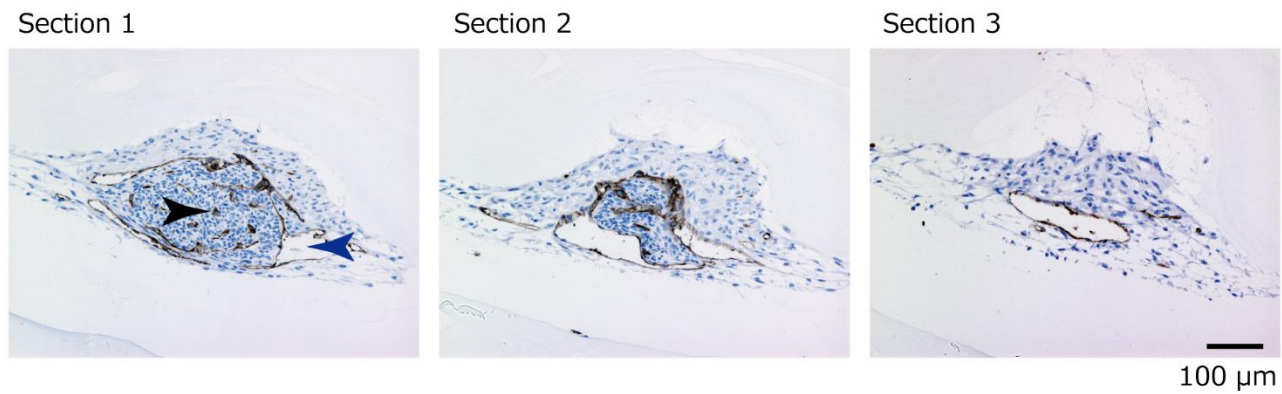


Figure S8 Vertical sections of a spheroid cultured in a microfluidic device for 14 days. The direction of vertical sectioning is illustrated in Figure 6c. (a) Series of 7 consecutive, H&E stained sections of the paraffin-embedded coculture spheroid. Separation distance between sections is 30 μm . (b) Series of 3 consecutive, immunohistochemically stained sections (brown; CD31, blue; nuclei). Three sectional positions correspond to the black rectangle in (a). Black and blue arrow heads indicate small and large vessels constructed in the spheroid.

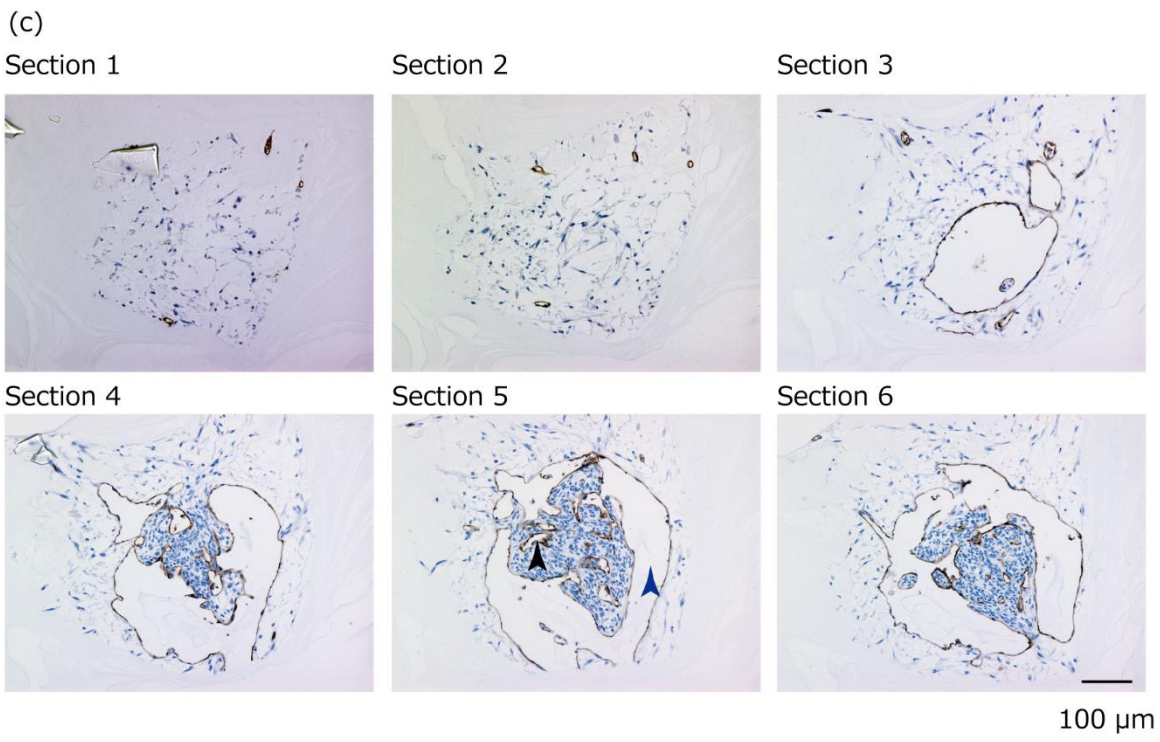
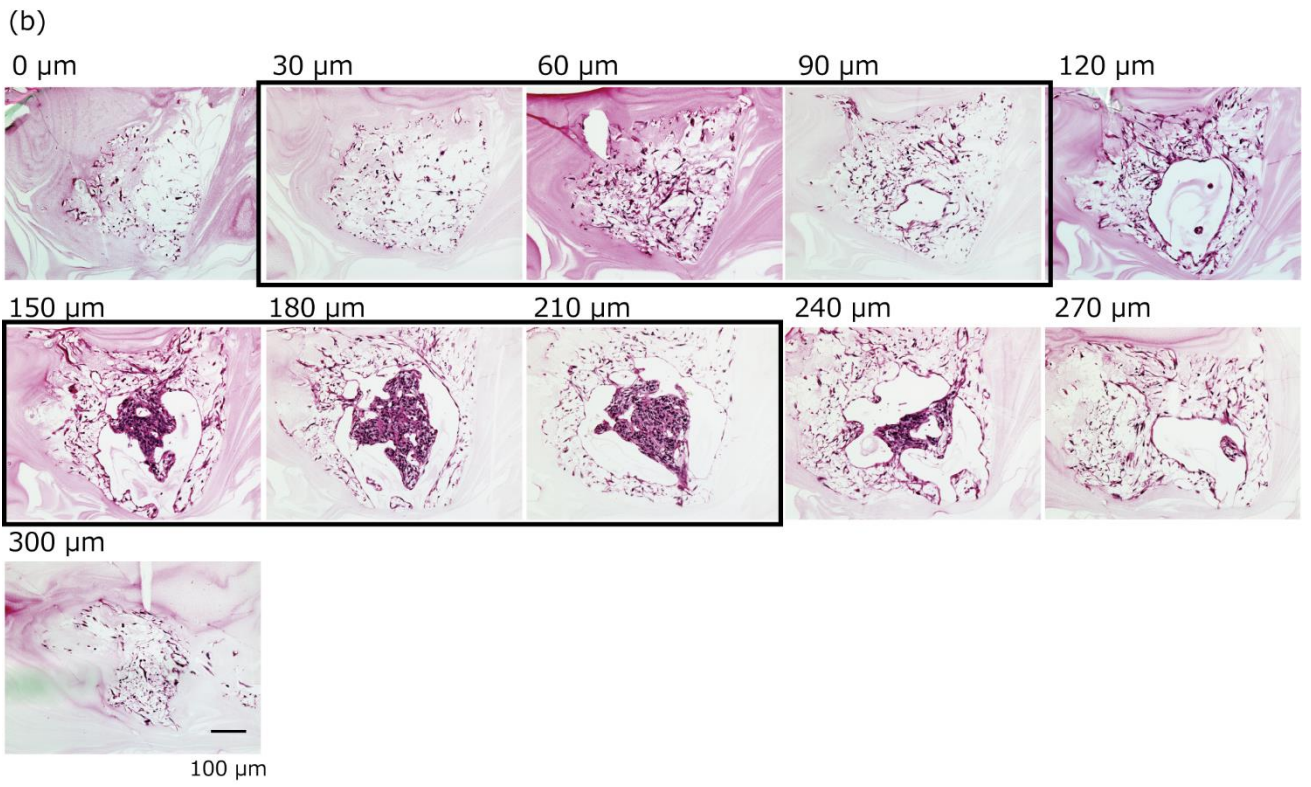
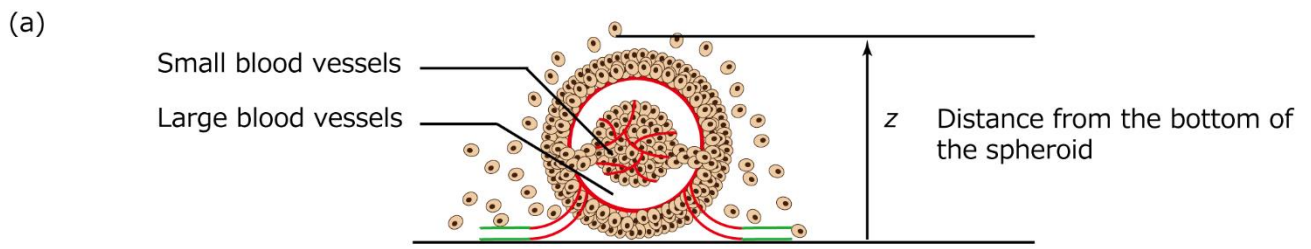


Figure S9 Horizontal sections of a spheroid cultured in a microfluidic device for 14 days. The direction of the horizontal sectioning is illustrated in Figure 6c. (a) Schematic illustration of the side view of the coculture spheroid in a device. Red lines indicate the vascular network formed by pre-existing endothelial cells (RFP-HUVECs) in a coculture spheroid and green lines indicate the angiogenic sprouts from microchannels (GFP-HUVECs). (b) Series of 11 consecutive, H&E stained sections of a paraffin-embedded coculture spheroid. Separation distance between sections is 30 μm . The distance from the bottom of the spheroid corresponding to z in Figure S8a is indicated in the micrographs. (c) Immunohistochemically stained sections (brown; CD31, blue; nuclei) corresponding to black rectangle sections in (b). Black and blue arrow heads indicate small and large vessels constructed in the spheroid.

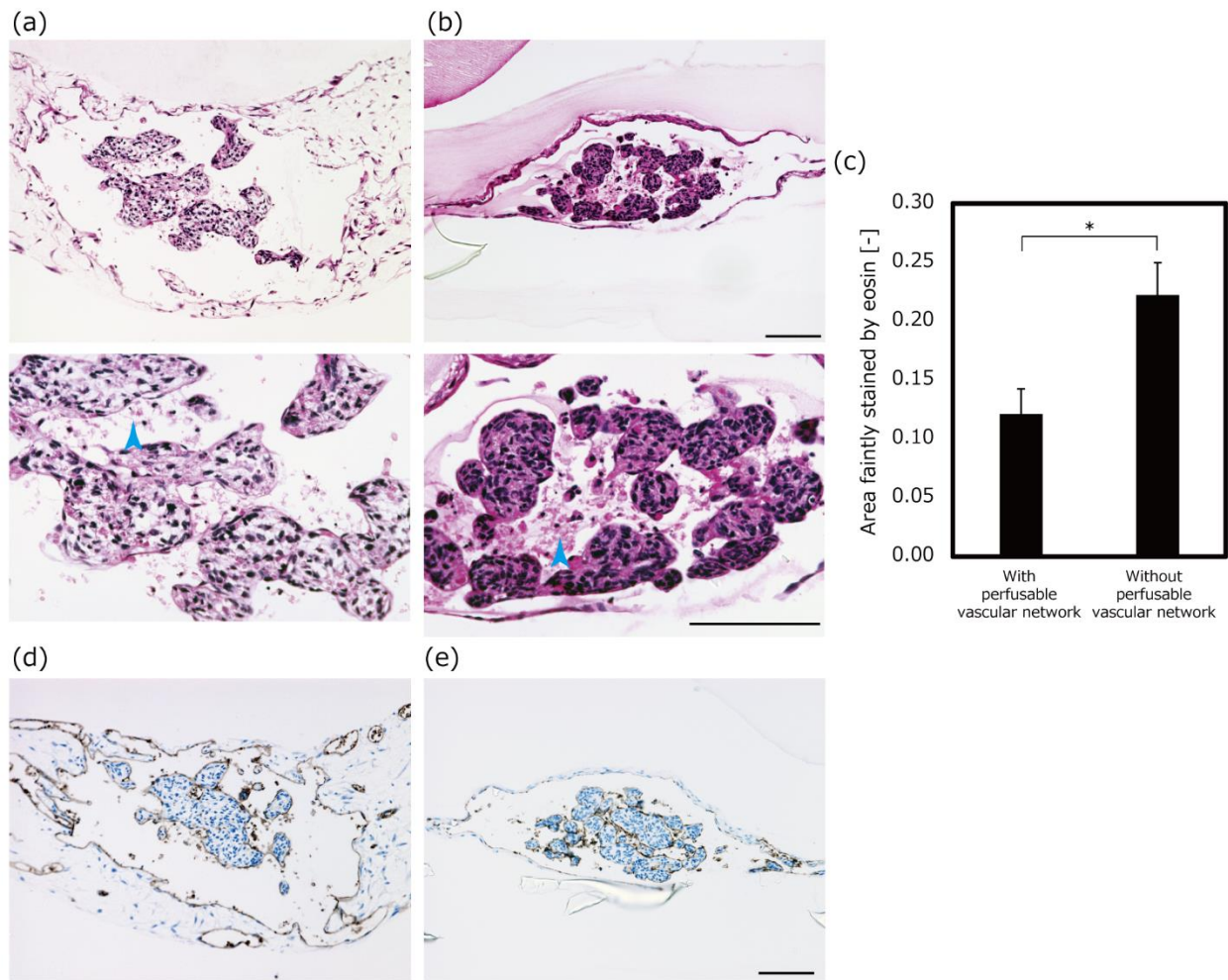


Figure S10 Preventing the necrosis at the center of the spheroid by perfusable vascular networks.

(a, b, d, e) Histological and immunohistochemical image of the spheroid with a perfusable vascular network (a, d) and without a perfusable vascular network (no HUVECs was cultured in channels 2 and 4, b, e). The sectional direction was approximately same as the vertical direction shown in Figure 6c. (a) (b) H&E staining, enlarged views are shown at the bottom of the corresponding images. Blue arrow heads indicated the areas faintly stained by eosin. (b) (e) CD31 staining. In immunohistological image, brown indicates HUVECs (CD31) and blue indicates nuclei (haematoxylin). (c) Quantitative analysis of the area faintly stained by eosin in the spheroids. Area was normalized by the hollow area in the section. *: $P < 0.05$ (t -test); $n = 3$. Scale bar = 100 μm .

Movie S1

Introduction of the fluorescent microbeads (red, dia. = 1 μm) into the vascular network through channel 2 (30 \times real time).

Movie S2

Time-lapse images of sprouting toward a spheroid in channel 3. Red: RFP-HUVECs in the spheroid, green: GFP-HUVECs cultured in the microchannels.

Movie S3

The reconstructed 3D image of a blood vessel comprised of RFP-HUVECs in the spheroid and GFP-HUVECs from microchannels. Image size = 320 \times 320 \times 91 μm .

Movie S4

Flow of FITC–dextran (green) from channel 2 to channel 4 through the vascular network and spheroid (4 \times real time). Blue indicates cellular nuclei (Hoechst 33342).

Movie S5

Flow of fluorescent microbeads (red, dia. = 1 μm) from channel 2 to channel 4 though the vascular

network and spheroid (3× real time).

Movie S6

Staining of the vascular network and spheroid using calcein red orange introduced from channel 2 (real time).

Movie S7

Staining of the vascular network and spheroid using calcein red orange introduced from channel 2 in the condition without HUVECs cultured in channels 2 and 4 (real time).