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

Supplementary Information (SI) to accompany

Three-dimensional tissue model in direct contact with an on-chip vascular bed enabled by removable membranes

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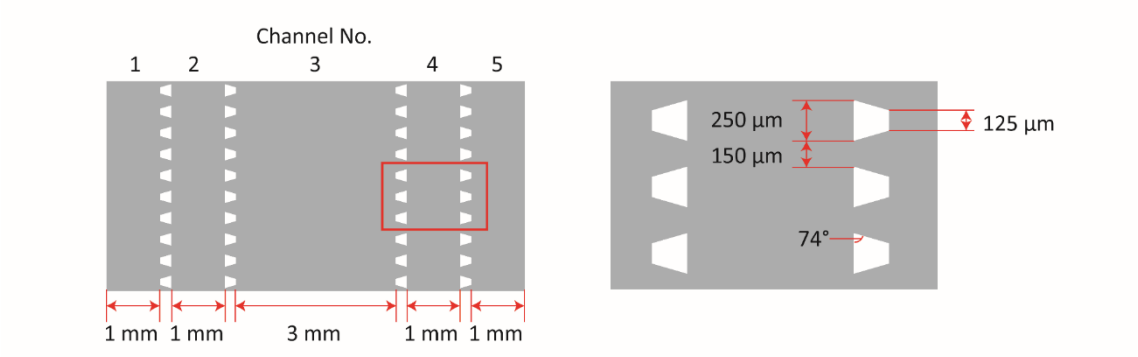

Fig. S1 Channel configuration. Dimensions for the micro-posts are shown in the enlarged illustration on the right. The grey area indicates fluid channels. The white trapeziums are micro-posts.

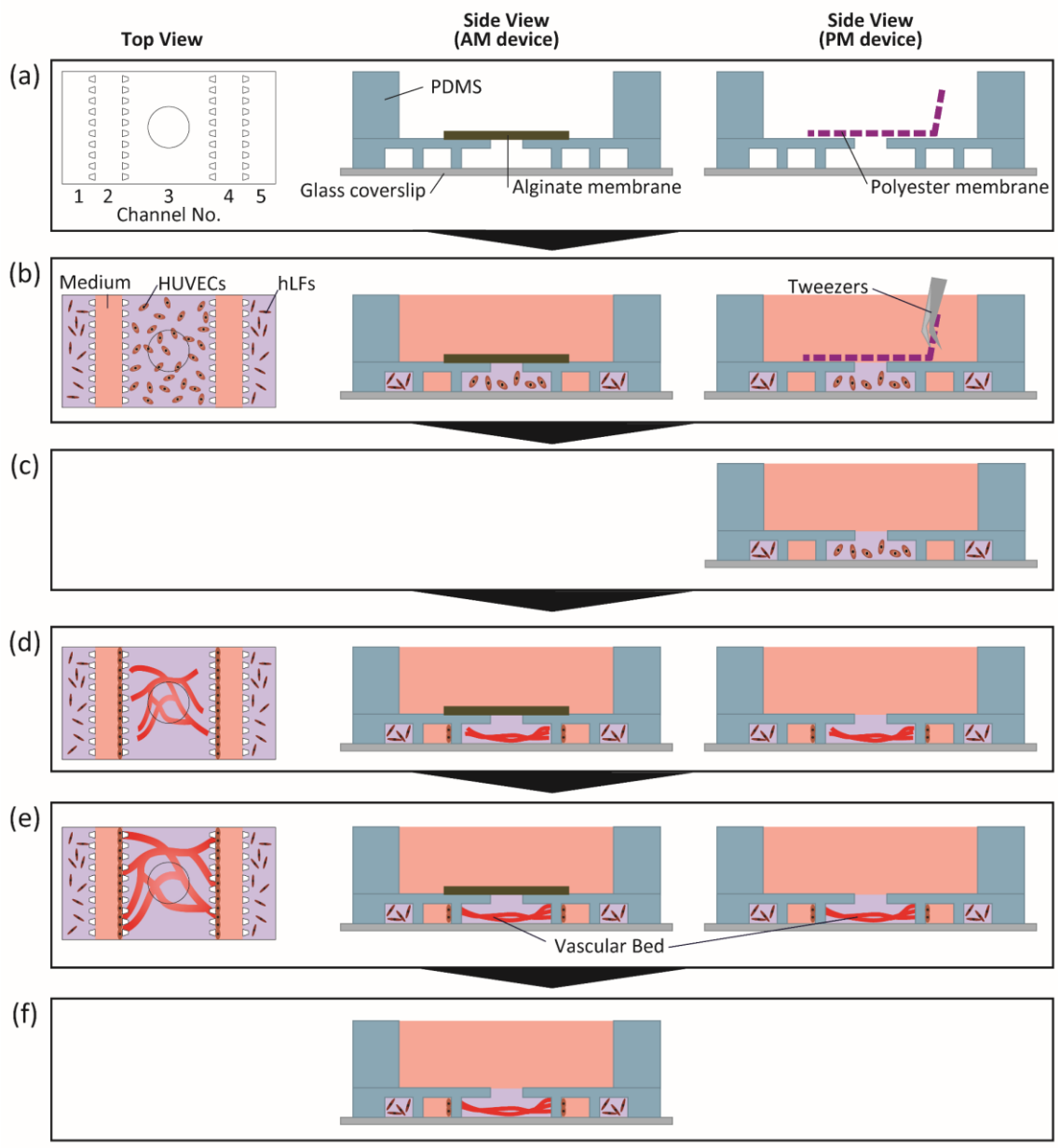

Fig. S2 Assay protocol for vascular bed formation. (a) The microfluidic device before seeding cells. (b) HUVECs were introduced into channel 3 and hLFs were introduced into channels 1 and 5. (c) In the PM device, the polyester membrane was removed using tweezers to open the open top after cell injection. (d) On day 2, HUVECs were introduced into channels 2 and 4. The device was tilted by 90° and incubated for 30 min, allowing HUVECs to adhere to the side of the gel in channel 3. (e) After one week in culture, the vascular bed was formed. (f) In the AM device, the alginate membrane was treated with EDTA to be dissolved before a spheroid was introduced.

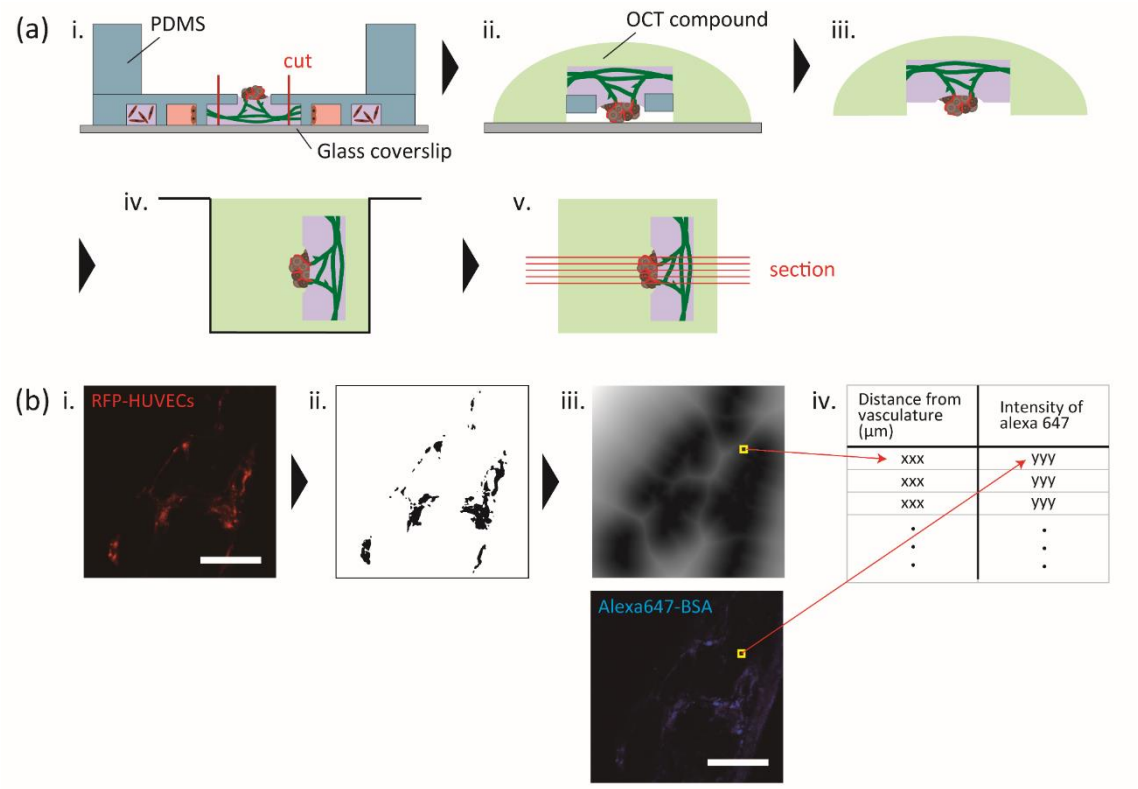

Fig. S3 Sample preparation and analysis procedure. (a) Preparation of the cryosectioned samples of the spheroid. i) PDMS around the spheroid was cut out with a biopsy punch (diameter: 2 mm). ii) The spheroid on the vascular bed with PDMS was removed using tweezers and placed upside down on the glass coverslip. Then, OCT compound was dropped onto the spheroid and frozen. iii) After removal from the glass coverslip, PDMS was peeled off from the spheroid. iv) The spheroid was embedded in OCT compound again and re-frozen to prepare a cryoblock. v) The block was sectioned using a cryostat. (b) Procedure to measure the fluorescent dye distribution. i) RFP fluorescence images were obtained. ii) The images were binarised. iii) The binarised images were converted to distance map images. iv) The distance x from the spheroid vasculature and the intensities of Alexa647-BSA in each pixel were measured using the distance map images and the fluorescence images, respectively. Scale bars: 50 μm.

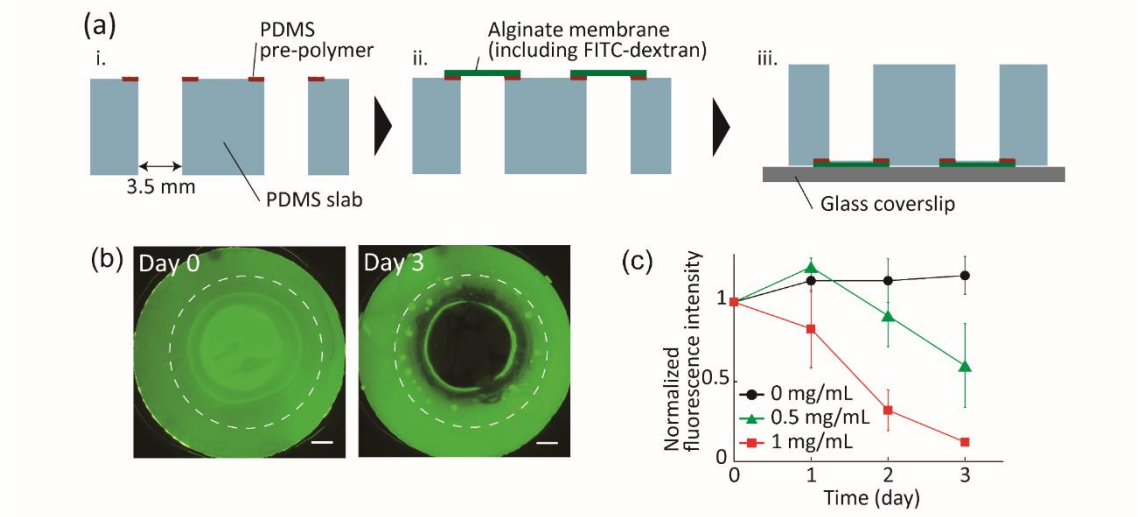

Fig. S4 Evaluation of alginate lyase for dissolving the alginate membrane. (a) Fabrication of wells for the alginate lyase evaluation. i) Wells (diameter: 3.5 mm) were punched out of a PDMS slab and PDMS pre-polymer was thinly coated around the wells. ii) Alginate membranes including FITC-dextran were bonded to the slab and cured at 70 °C for 30 min. iii) The PDMS slab was bonded to a glass coverslip using atmospheric plasma. The fabricated wells were filled with 30 µL of EGM-2 containing alginate lyase (Alginate lyase S, Nagase ChemteX, Kyoto, Japan) at 0, 0.5, and 1 mg mL^{-1}, and incubated at 37 °C. Membrane degradation was observed under a fluorescence microscope over three days. The average fluorescence intensity in the well was measured and normalised to the initial fluorescence intensity for each condition. (b) Images of the alginate membrane including FITC-dextran before and three days after 1 mg mL^{-1} alginate lyase was introduced. The white dashed circles indicate the area of the well. The fluorescence of FITC-dextran was no longer detected in the centre of the membrane within three days. Scare bars: 500 µm. (c) Time-course of fluorescence intensity of the alginate membrane in three different concentrations of alginate lyase. It took three days to completely dissolve the membrane by 1 mg mL^{-1} alginate lyase. Error bars represent the S.E.

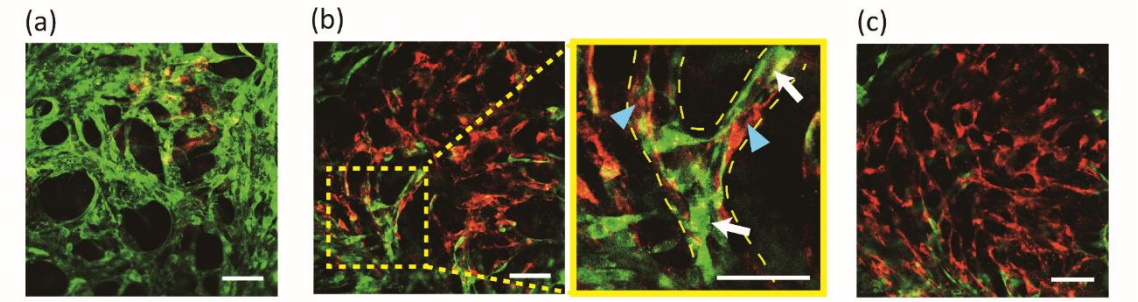

Fig. S5 Co-culture of an hLF and HUVEC spheroid with a vascular bed in the AM device. (a) Confocal images in the bottom (a), middle (b), and top (c) layers. In the middle layer, the vasculature, indicated by the yellow dashed line, was composed of GFP-HUVECs from the vascular bed (white arrows) and RFP-HUVECs from the spheroid (blue arrowheads). Green: GFP-HUVECs. Red: RFP-HUVECs. Scale bars: 100 μm.

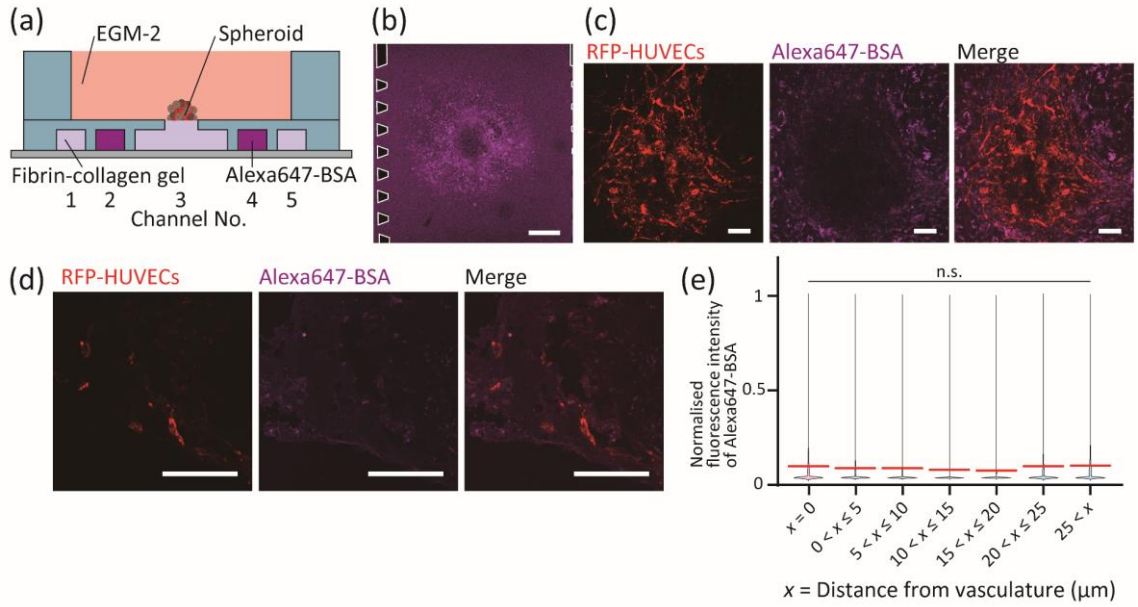

Fig. S6 Alexa647-BSA injection into the device where the spheroid was cultured on the gel. (a) Schematic diagram of the assay. (b) Confocal image in channel 3 one day after Alexa647-BSA injection. Alexa647-BSA fully diffused into the channel. (c) Confocal images of the spheroid. (d) Confocal images of the cryosectioned spheroid. (e) Distribution of the Alexa647-BSA fluorescence intensities categorised by distance from the vasculature. Red bars indicate the mean fluorescence intensities. The mean value and distribution of the fluorescence intensity were similar in the spheroid vasculature to the other areas. n = 25,000–120,000 pixels. *p < 0.05 vs. 0 μm group by Dunn's test. Scale bars: 500 μm (b), 100 μm (c, d).

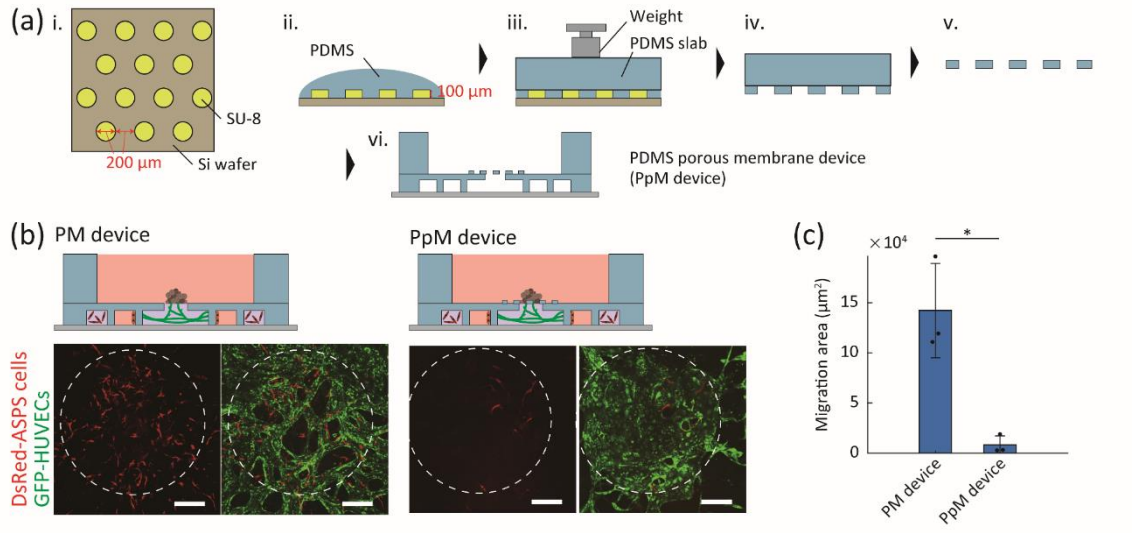

Fig. S7 Comparison of ASPS cell migration in the PM device and the device integrated with a PDMS porous membrane (PpM device). (a) Fabrication of the PpM device. i) The mould to fabricate the PDMS porous membrane. ii) Initially, PDMS pre-polymer was poured onto the mould. iii) The PDMS slab pre-treated with trichloro-silane was applied. Then, a 60 g weight was placed on the PDMS slab to allow SU-8 pillars to penetrate the uncured PDMS layer. iv) After curing at 70 °C overnight, the PDMS porous membrane was peeled off from the mould with the PDMS slab. v) The membrane was carefully peeled off from the PDMS slab. vi) Finally, the membrane was cut into a round shape using a biopsy punch with a 3 mm diameter and irreversibly bonded to the open top of the device using atmospheric plasma. (b) Confocal images taken after four days of co-culture with the ASPS spheroid and the vascular bed in the PM and PpM devices. The white dotted line indicates the edge of the open top. Scale bars: 200 µm. (c) Quantification of ASPS cell migration in the PM and PpM devices. After DsRed fluorescence images were binarised, DsRed positive areas were measured. Error bars represent the S.E. (n = 3 devices). *p < 0.05 by t-test.

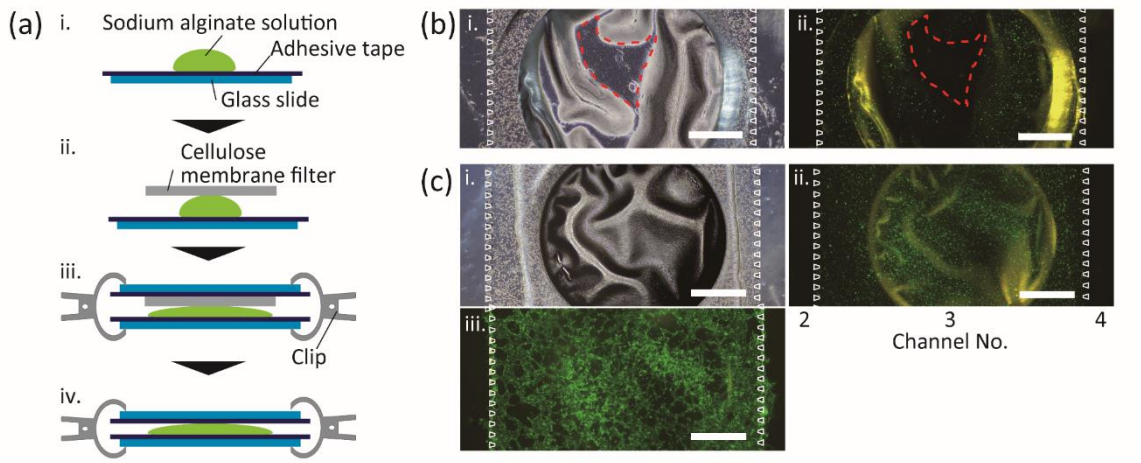

Fig. S8 Optimisation of the fabrication process of the alginate membrane. (a) Alginate membrane preparation. i) An alginate solution containing 1% or 4% (w/v) sodium alginate in deionised water was prepared. Then, 100 µL of the solution was placed on a glass slide covered with adhesive tape. ii) A cellulose membrane filter (ADVANTEC, Tokyo, Japan) was immersed in 100 mM calcium chloride in deionised water for more than 1 min. The sodium alginate was cross-linked with calcium ions by placing the membrane filter on the sodium alginate solution for 1 min. iii) The other glass slide covered with adhesive tape was placed on the filter and clamped with clips for 10 min. The alginate gel was then thinned and flattened. iv) The cellulose membrane filter was removed, and the membrane was again sandwiched between two glass slides and dried at 70 °C for more than 1 day. Finally, the membrane was cut into a rectangular shape (ca. 4.5 × 5 mm) to be bonded to the top PDMS layer. (b) Bright field (i) and fluorescence images (ii) after the injection of HUVECs into channel 3 with the 1% sodium alginate membrane. The membrane was attached to the bottom glass coverslip (the area indicated by the red dotted line) and HUVECs were removed from the area. (c) Bright field (i) and fluorescence images (ii) after the injection of HUVECs into channel 3 with a 4% sodium alginate membrane. (iii) A fluorescence image on day 6 after HUVEC injection. Green: GFP-HUVECs. Scale bars: 1 mm (b, c).

Movie S1

Flow of rhodamine-dextran (70 kDa) from channel 4 to channel 2 though the vascular bed in the PM device (x20 speed). Green: GFP-HUVECs, Red: Rhodamine-dextran.