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


**Supplementary Information**

# "Open-top" Microfluidic Device for in-vitro Three Dimensional Capillary Bed

Soojung Oh[‡a,b], Hyunryul Ryu[‡c], Dongha Tahk[a,d], Jihoon Ko[a,d], Yoojin Chung[e], Hae Kwang Lee[b], Tae Ryong Lee[b] and Noo Li Jeon*[a,d]

[a] School of Mechanical and Aerospace Engineering Seoul National University, Seoul, Korea
[b] Amorepacific Research and Development Center, Yongin, Korea. E-mail: soojung@amorepacific.com
[c] Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, USA
[d] Institute of Advanced Machines and Design, Seoul National University, Seoul, Korea
[e] Division of Computer & Electronic Systems Engineering, Hankuk University of Foreign Studies, Yongin, Korea

‡These authors contributed equally to this work.

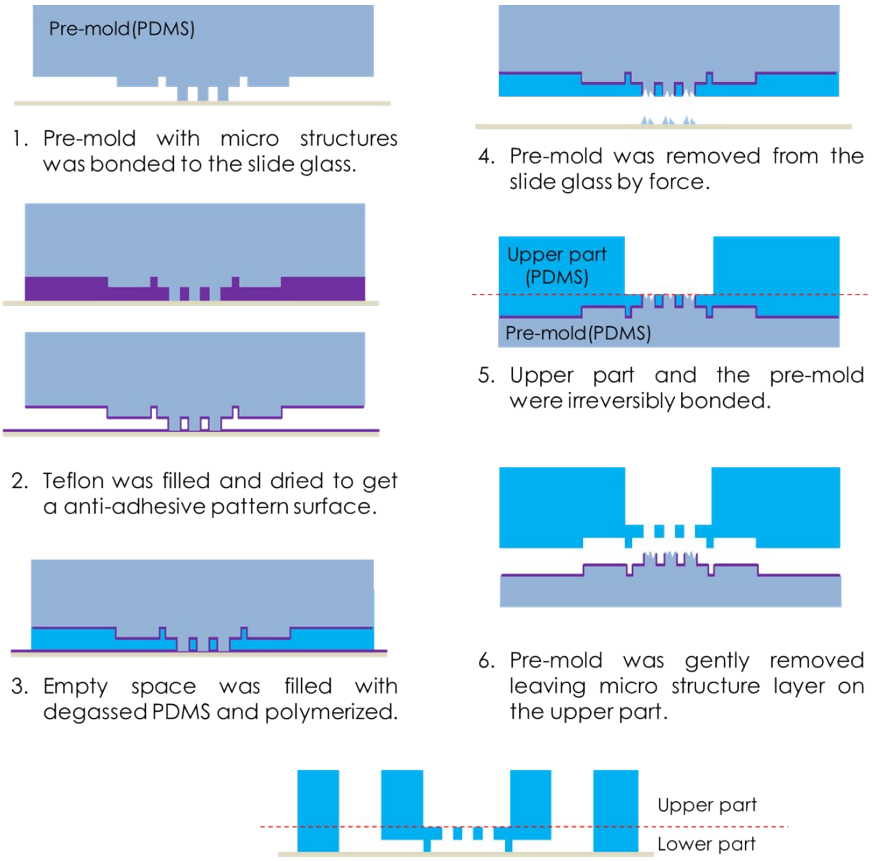


**Fig. S1.** Fabrication process of "open-top" microfluidic device.

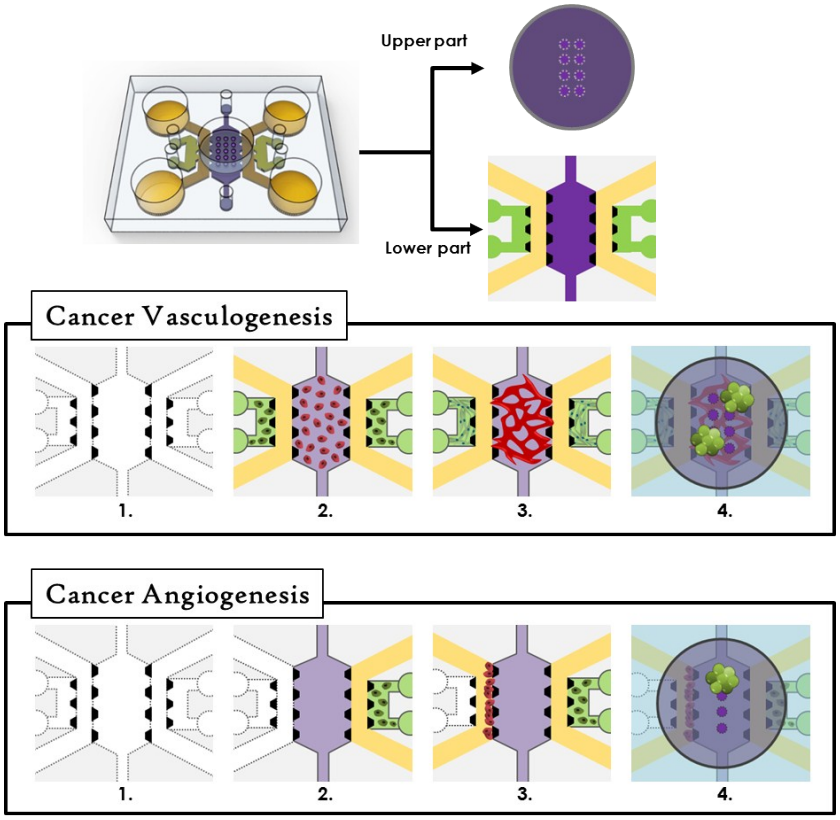


**Fig. S2.** Cancer spheroid placement for cancer spheroid induced vasculogenesis and angiogenesis. Right before transferring the spheroid into the device, mediums were removed from the reservoir except the reservoir in the middle. Then, the micropores began to slowly drain the medium in the reservoir due to the hydraulic pressure produced in the device. When the spheroid was gently transferred with 200µl pipette to prevent damage and placed near the micropores, it naturally plugged the nearest micropore within a few hours. After the spheroid anchor and settle down, all reservoirs were filled with fresh medium and maintained in humidified incubator with 37˚C and 5 % $CO_2$ exchanging medium every two days.

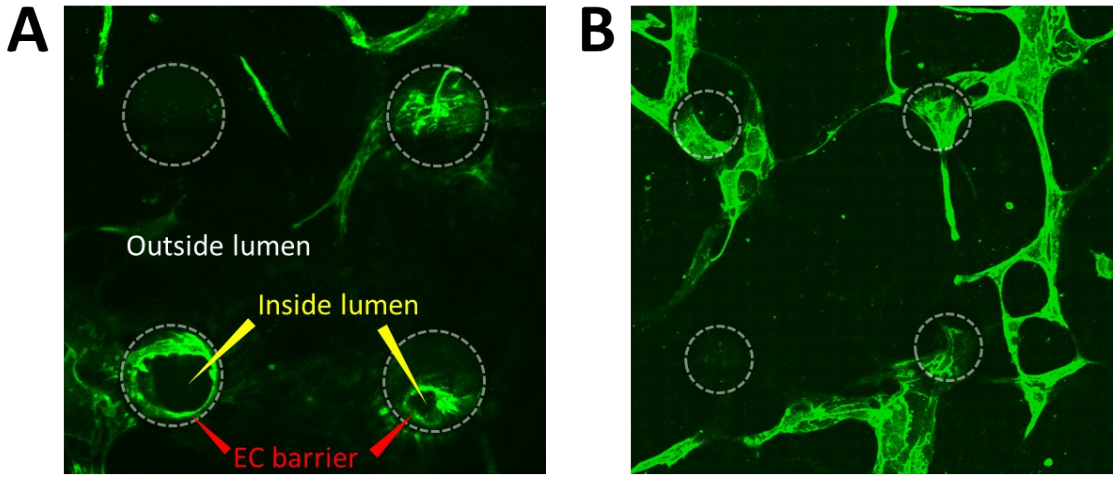


**Fig. S3.** Microvessel can sprout through 150µm sized micropore pathway. Doughnut shape in (Fig S3 A) indicate cross-section of microvessel sprouting toward vertical direction. Lumen can be observed (yellow arrow) within the micropore (white dotted circle). However, microvessel did not go through in the case of 100 µm pore (Fig S3 B). GFP=CD31

(This is the preliminary experiment data processed before the regular experiments. On the basis of this experiment, we have designed the “open-top” microfluidic device presented in this paper.)

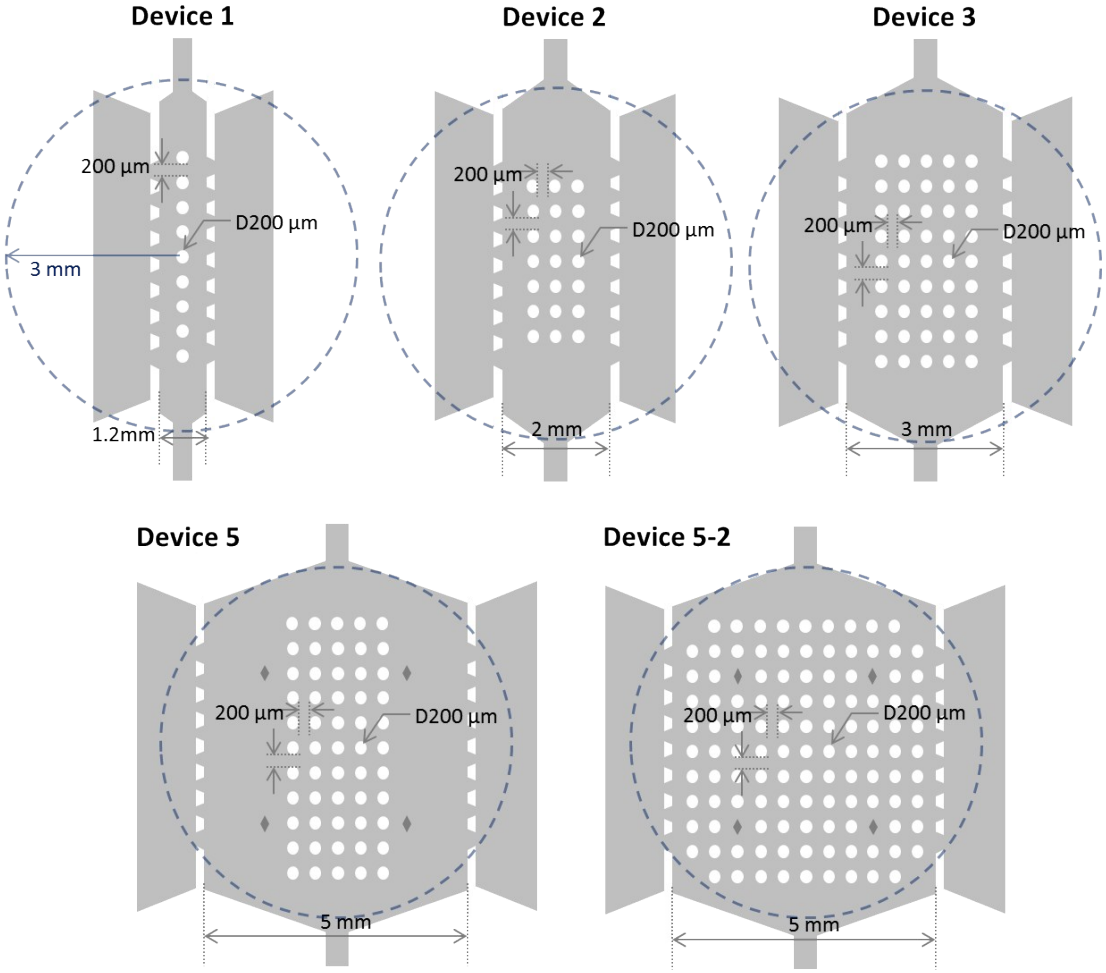


**Fig. S4.** Detail information of devices with different dimensions used in the paper.

Device 1 is used to observe angiogenesis induced with cancer cell spheroid and dispersed cancer cells.
Device 2, 3 and 5 is used in the experiment to compare microvessel formation with the device without micropores.
Device 5-2 is used in the experiment about microvessel recruitment co-cultured with cancer spheroid.

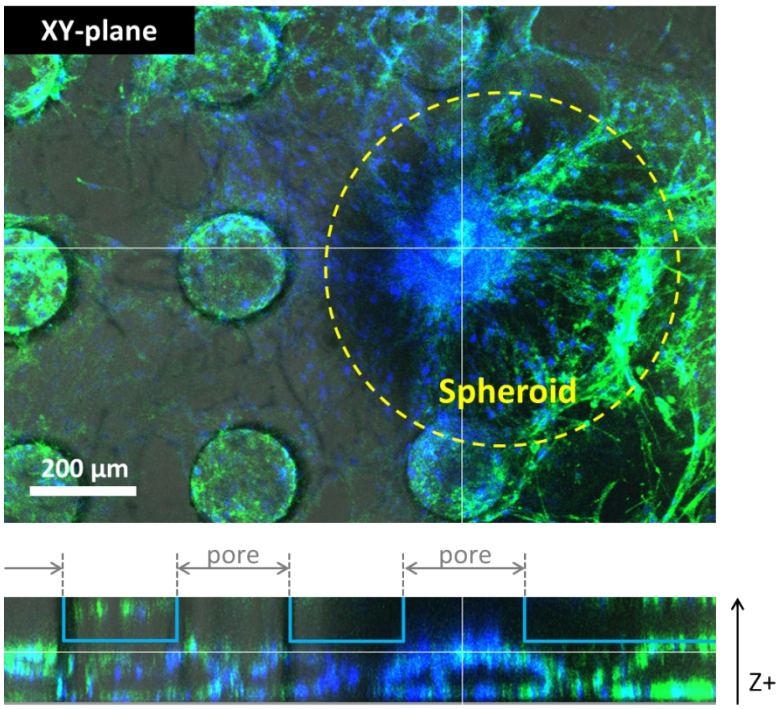


**Fig. S5.** Confocal microscopy **i**mage of microvessel and cancer spheroid. High concentrations of stained nuclei are observed where cancer spheroid is placed. As the cancer cells are agglomerated closely forming the spheroid, only outer few layers were stained with Hoechst (blue). We were able to detect intimate contact between vessel and the spheroid in the cross section image (z+ direction). GFP=CD31

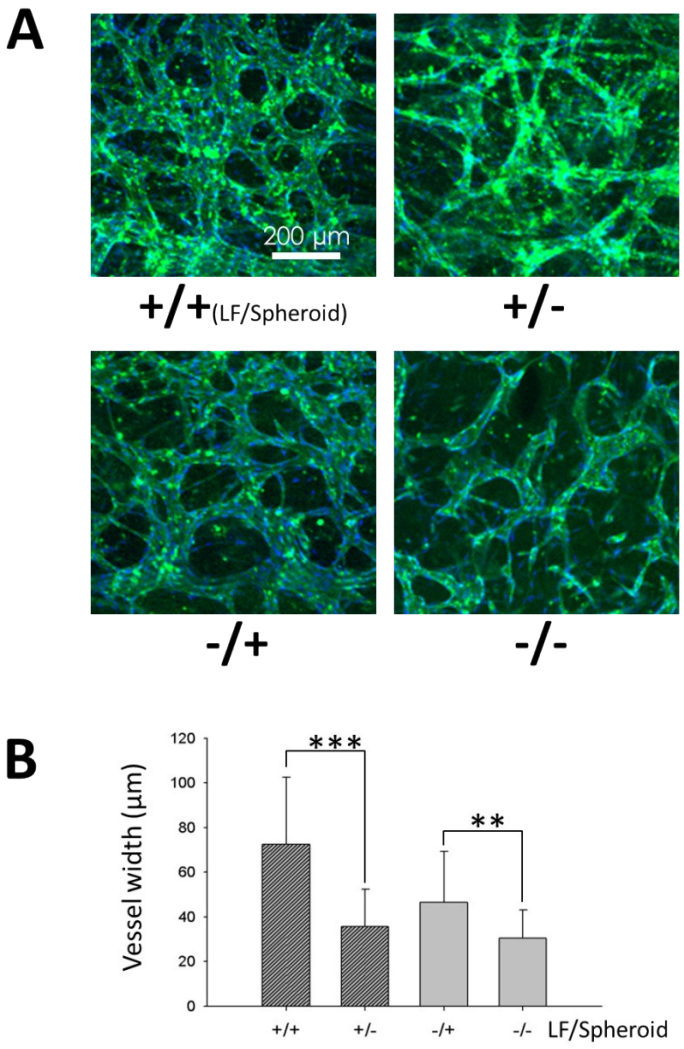


**Fig. S6.** In the experimental setup, fibroblasts were originally used to secrete growth factors to help the ECs to differentiate. Therefore, we co-cultured HDMECs with and without the spheroid or fibroblast during the culture. As the EC channel width must be wide enough to neglect the effect of the originally placed fibroblasts, we have selected a device with 5 mm EC channel to obtain any notable differences between the experimental sets. LFs were mixed with HDMECs in hydrogel at a 1:20 cell density ratio and a U87MG spheroid was placed on the top reservoir (+/+, LF/Spheroid) showed abundant vascular networks (Fig. S6. A). However, when there is no cancer spheroid (+/-), the microvessels seem thinner. However, the LFs help the ECs to elongate and connect with each other. If there are no LFs mixed with the ECs, the cells will generally fail to connect. The sample with only the cancer spheroid (-/+) has a better vascular formation compared to ECs with no VEGF source cells (-/-). From the results of this experiment, we understand the different outcomes from the sets and observed the effect of mixing LFs with ECs. When the LFs were spread uniformly mixed with the ECs, they can enhance the connectivity of the vessel. Similarly, the cancer spheroid can also enhance the growth of the endothelial cells by affecting the thickness of the microvessel. As the two types of cells are well known as VEGF-secreting cells, the use of both cells derives the best results. We have measured the thickness of the vessels and plotted in graph (Fig. S6. B). (n=90, **; $p < 0.01$, ***; $p < 0.001$)

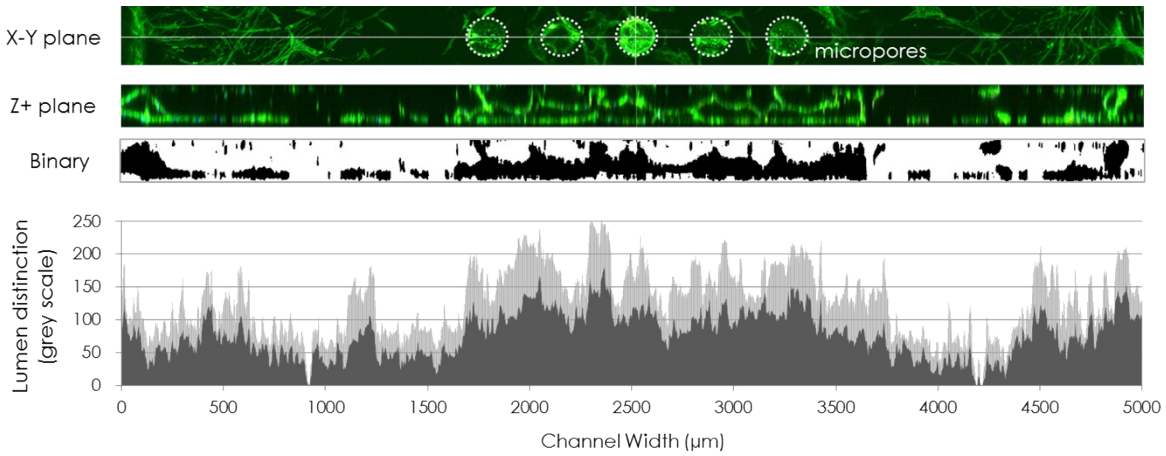


**Fig. S7.** The figure gives well information about formation of open lumen according to the distance from the micropores. The figure was selected from vasculature cultured in the device with 5 mm EC channel because it gave noticeable difference across the wide channel. In order to distinguish inner and outer lumen from the selected figure, we have switched 'Z+ plane' fluorescent image to binary image. And the portion of black area was calculated with ImageJ; dark grey area shows the average lumen distinction and light grey indicates the SD value (n=5). As you can see in the graph, lumens showed at the boundaries close to LF channel and seemed to decrease and appeared again at the center where the micropores are placed.

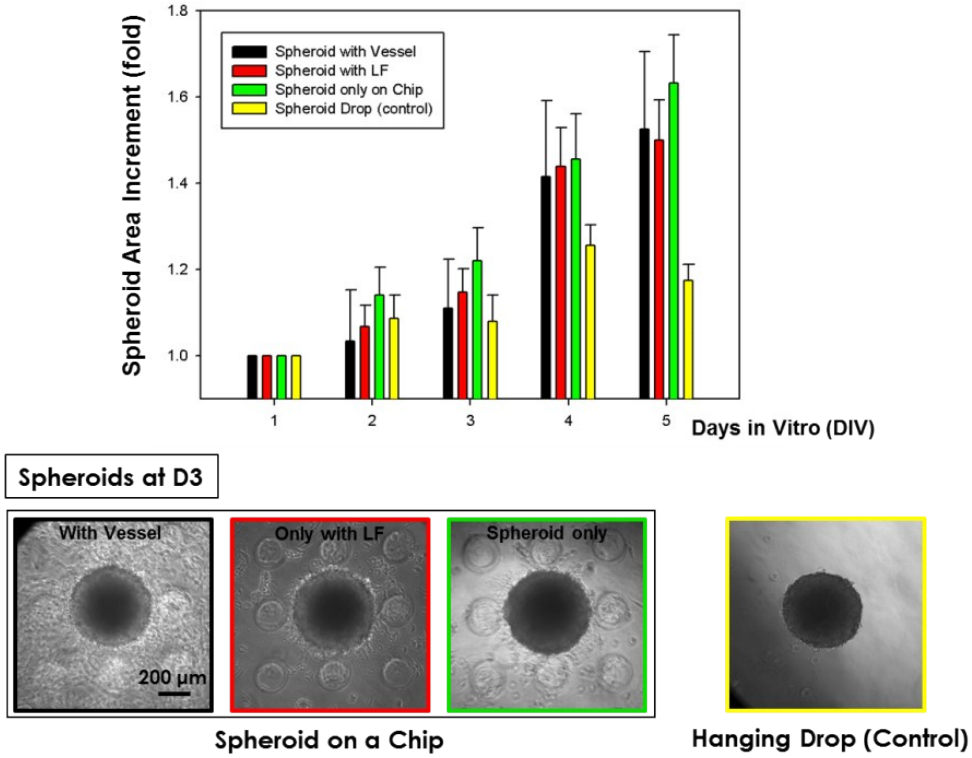


**Fig. S8.** The diameter of U87MG spheroid was measured. The spheroids were cultured in different conditions and compared their growth according to time. Spheroids were cultured (i) with vasculature, (ii) only with LF, (iii) only itself and left with (iv) hanging drop method. As you can see in the graph, all cases except (iv) showed proportional growth according to cultured time. It was because spheroids cultured in the device were well nourished with plenty of fresh media while very little amount of medium (only 20 µl) was supplied during hanging drop culture. Spheroid cultured in case (iii) was the largest because it was able to consume all the nutrients supplied from fresh media by itself, while it had to share media with other cells in the case of (i) and (ii). In spite of that, their differences are subtle enough to be considered as approximately equal. The spheroid was measured 450 µm at day 1 and 500 µm at day 3 in the case of (i).