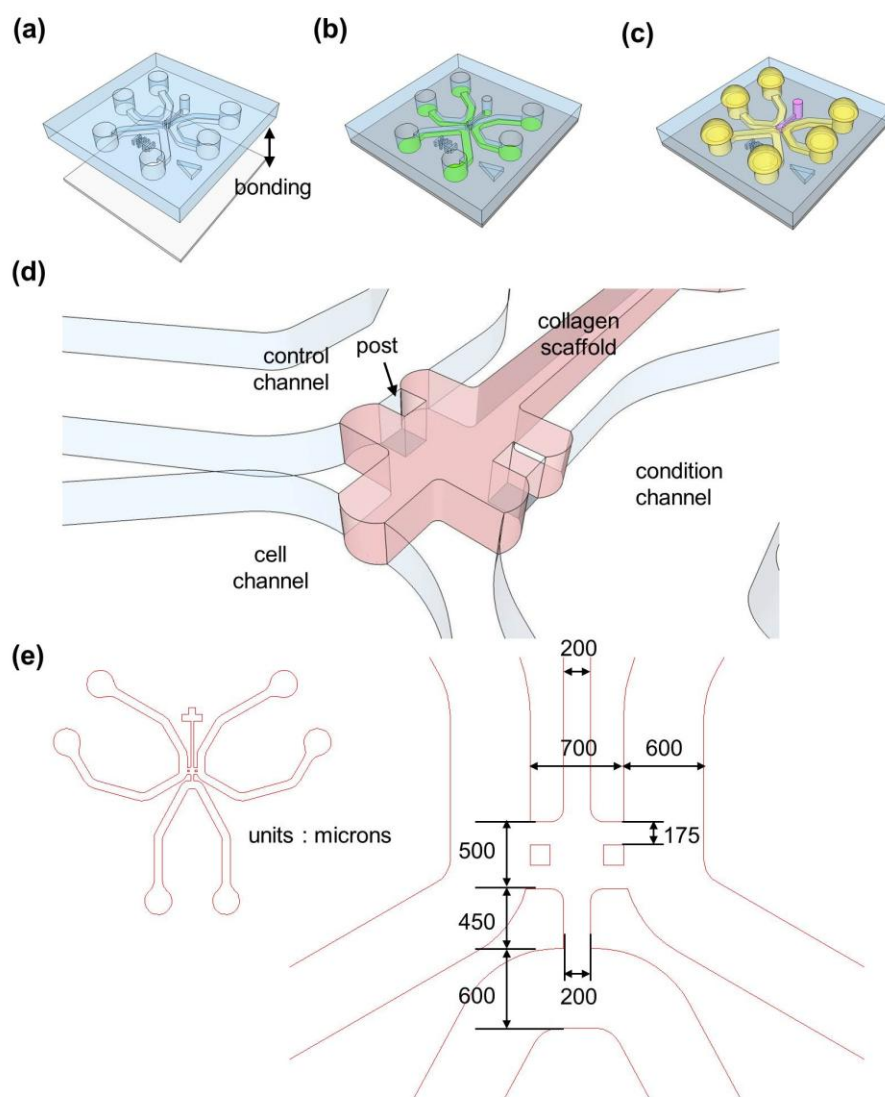


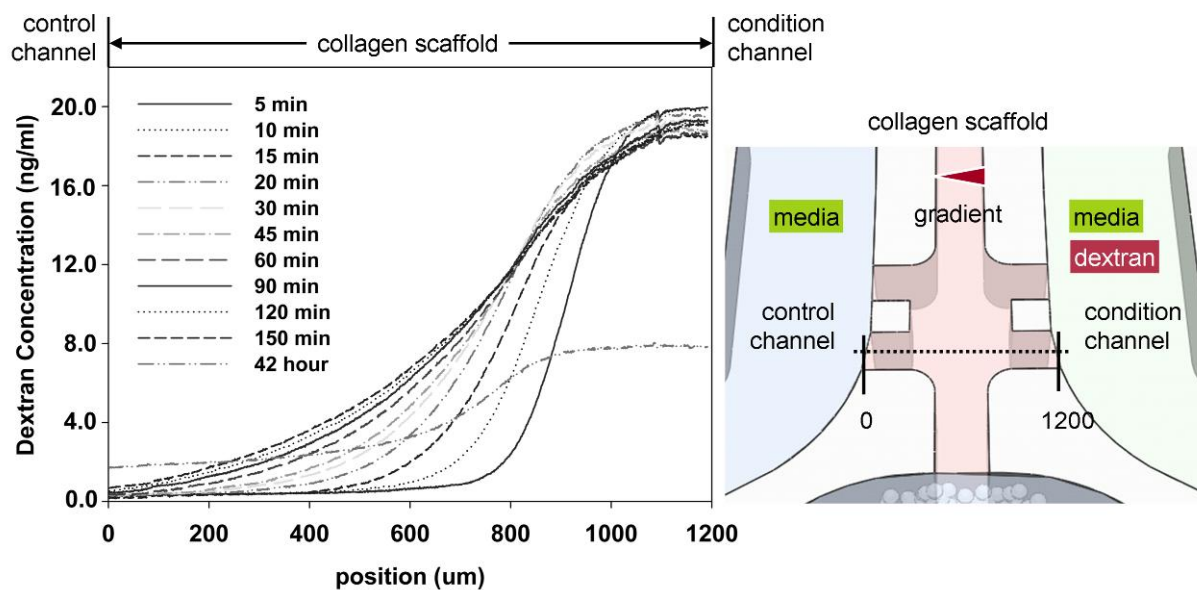
Supporting information

IN VITRO 3D COLLECTIVE SPROUTING ANGIOGENESIS UNDER ORCHESTRATED ANG-1 AND VEGF GRADIENTS

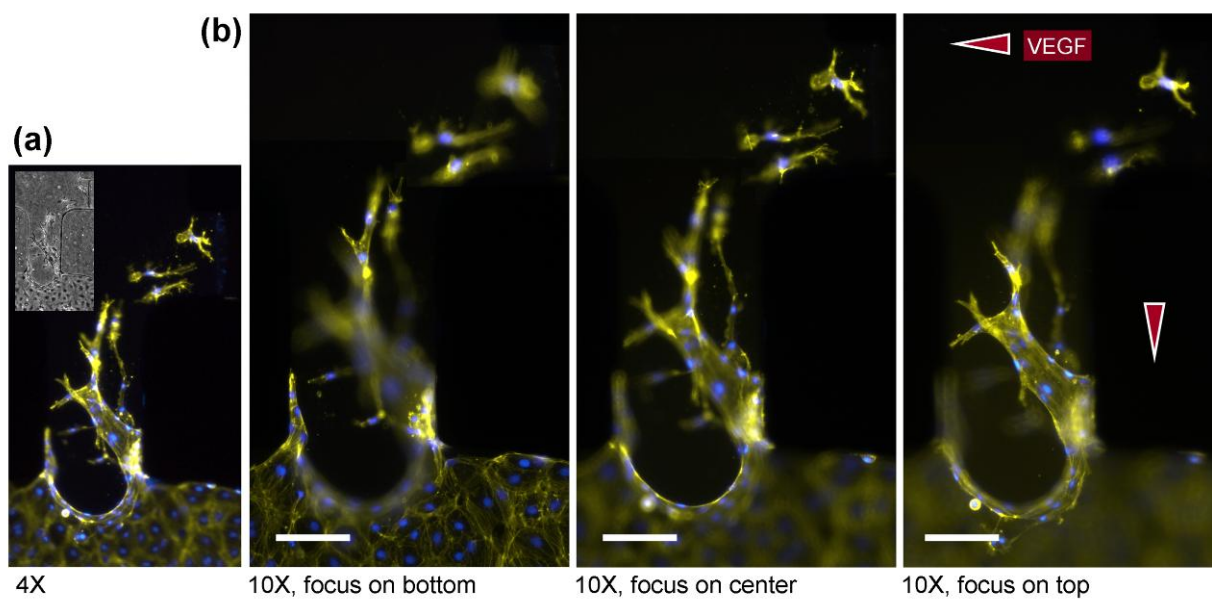
Y. Shin et al.



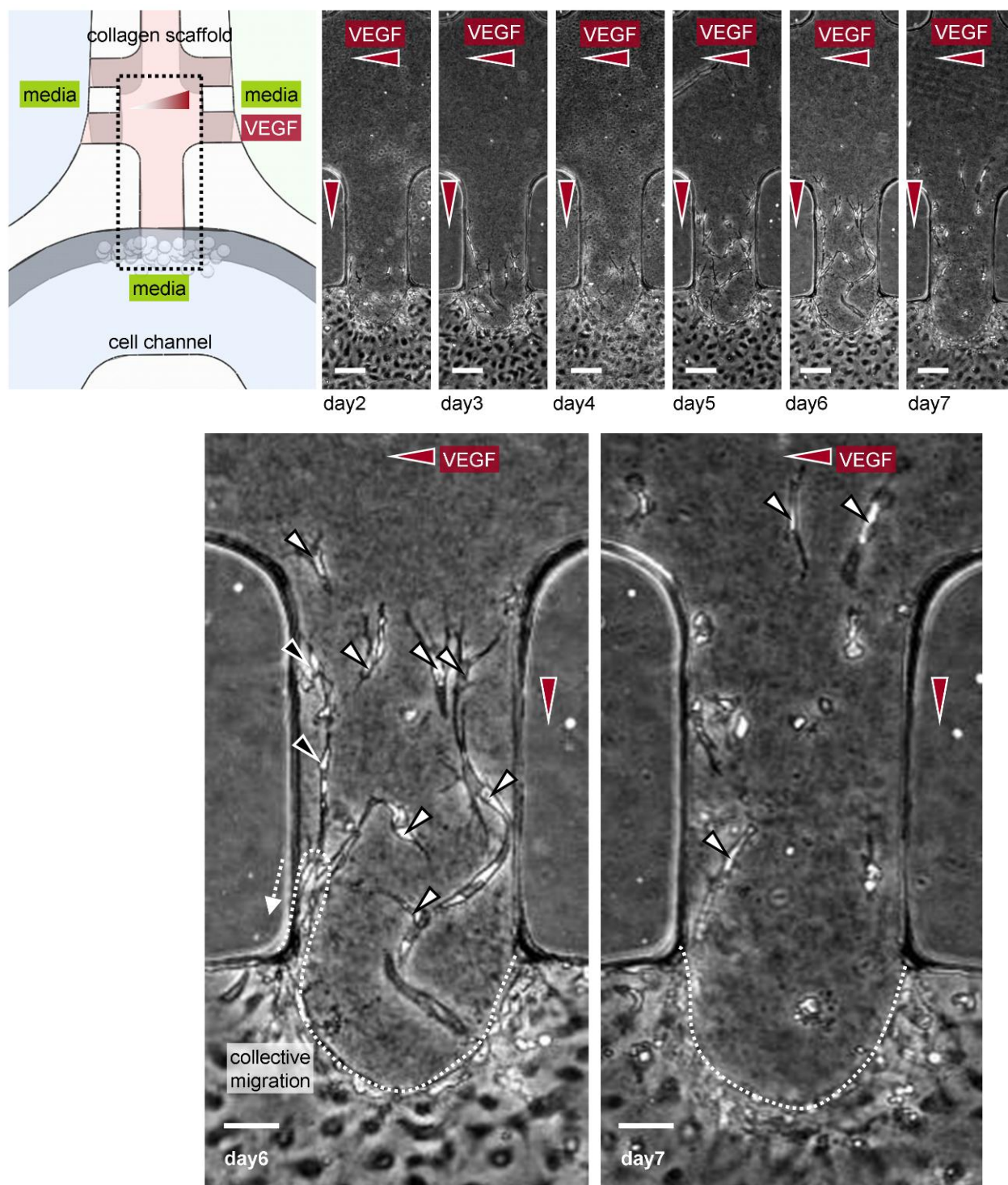
Supporting Figure 1. Fabrication process. (a) PDMS (poly-dimethyl siloxane) device prepared by a soft lithography process was bonded onto the glass coverslip by plasma treatment. (b) The closed channels were then coated with a PDL (poly-D-lysine hydrobromide; 1 mg/ml) solution (green color), injected just after surface plasma treatment and before introduction of the scaffold. (c) Type-I collagen, used as the scaffold material, was injected into the gel channel (pink color) and allowed to gel in a 37°C incubator for 30 minutes. (d) A schematic to show structure of 3 channels and collagen scaffolds (red color) and a picture of the fabricated device. (e) Dimension of the microfluidic channel in microns.



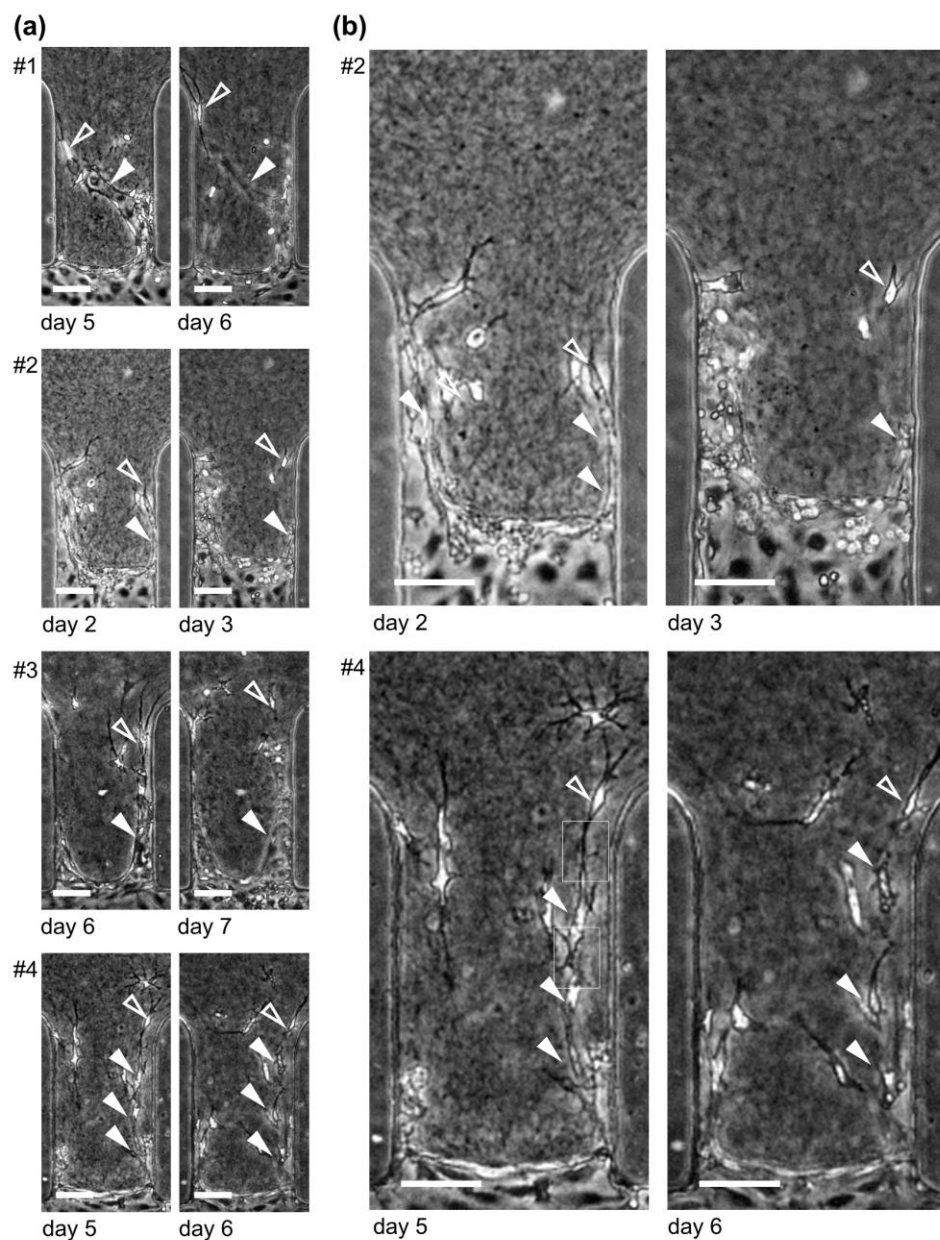
Supporting Figure 2. Characterization of the diffusion profiles (the details of Figure 1c). The normalized intensity plot of diffused 40kDa fluorescent dextran from the condition channel (right) to the control channel (left). Numbers in the plot indicate the elapsed time (minutes) after filling up with dextran solution. The intensity was normalized to unity at the center of the condition channel in each figure.



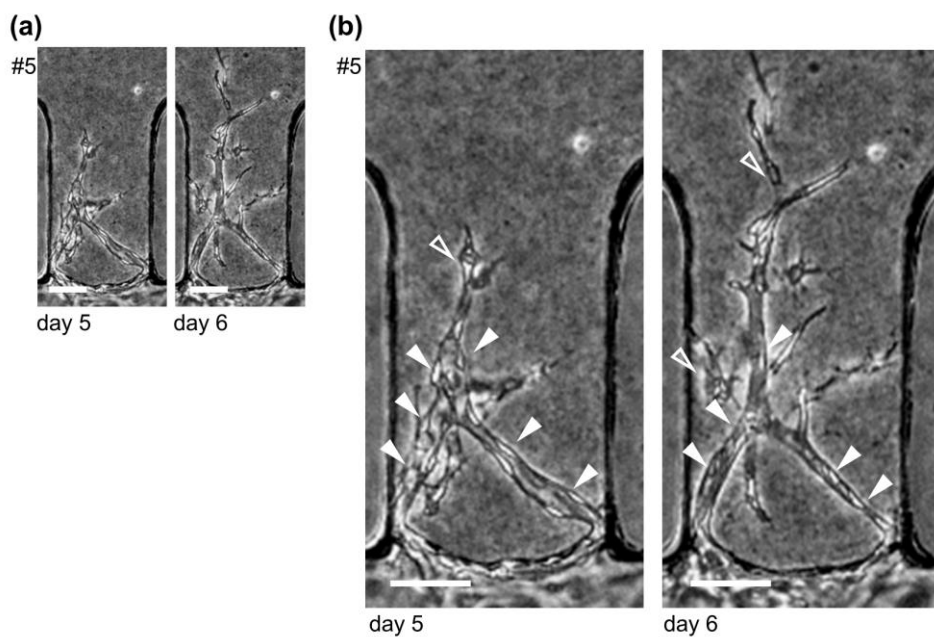
Supporting Figure 3. (a) HMVEC sprouting angiogenesis into the collagen scaffold, and (b) figures taken on different focal planes, viz., bottom, center, and top of the channel (the details of Figure 3b). Scale bars: 100 μm . Cells can be found in various focal plane, proving three-dimensional migrated structures and cells into type 1 collagen matrix.



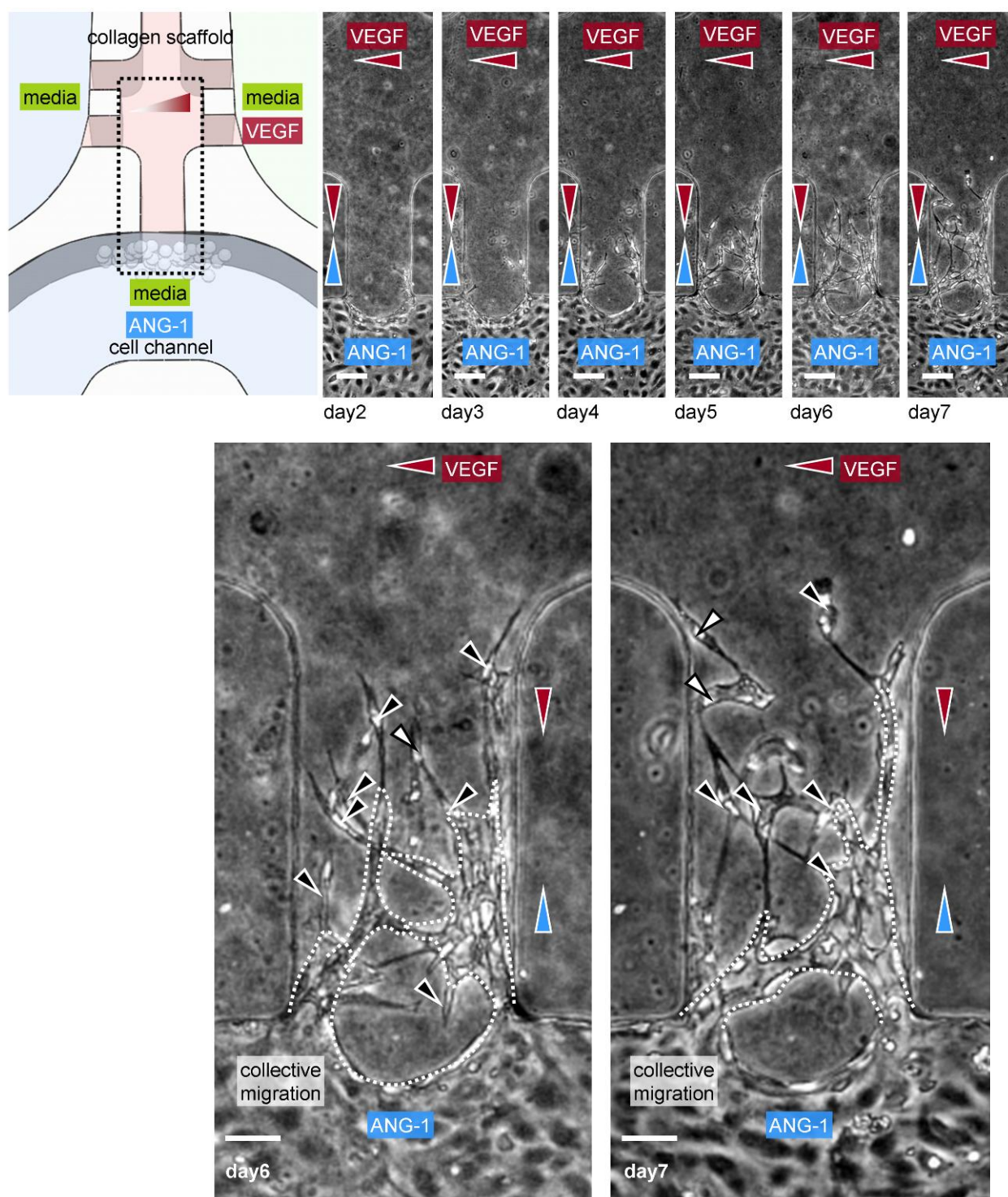
Supporting Figure 4. Sprouting angiogenesis under the VEGF gradient (the details of Figure 4a). White dotted lines indicate the perimeter of collective cell migration; black arrowheads, activated cells attached to the collective migration sites; and white arrowheads, activated cells separated from the collective migration sites. Scale bars: 100 μm .



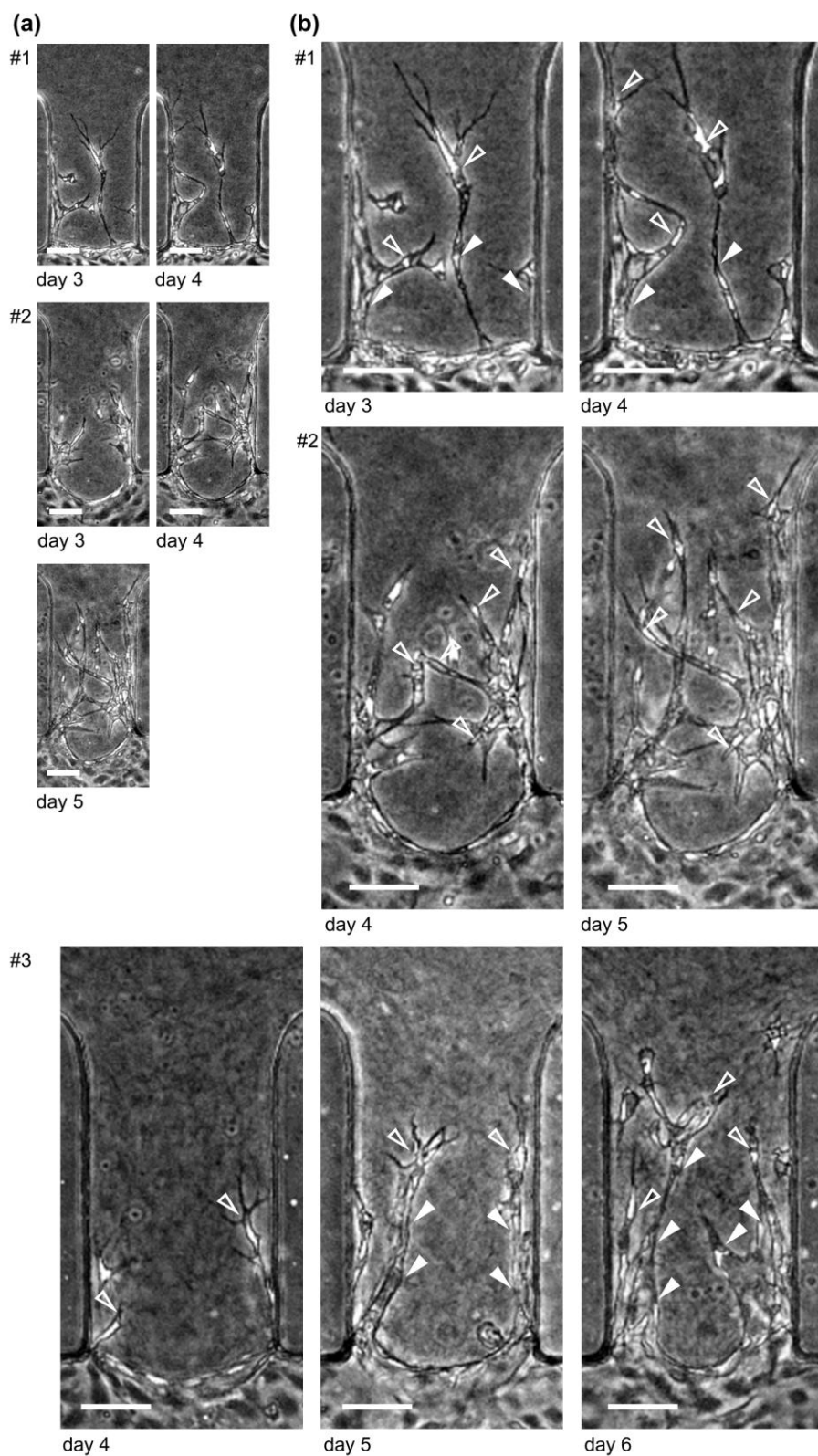
Supporting Figure 4-1. Examples of sprouting angiogenesis under the VEGF gradient (four devices #1~#4). In every device, stalk cells (white arrowheads) were regressed or disappeared in one day after they losing the connection with tip cells (empty arrowheads), while the detached tip cells migrated into the collagen scaffold, apparently responding to the horizontal and vertical gradient of VEGF. Scale bars: 100 μm .



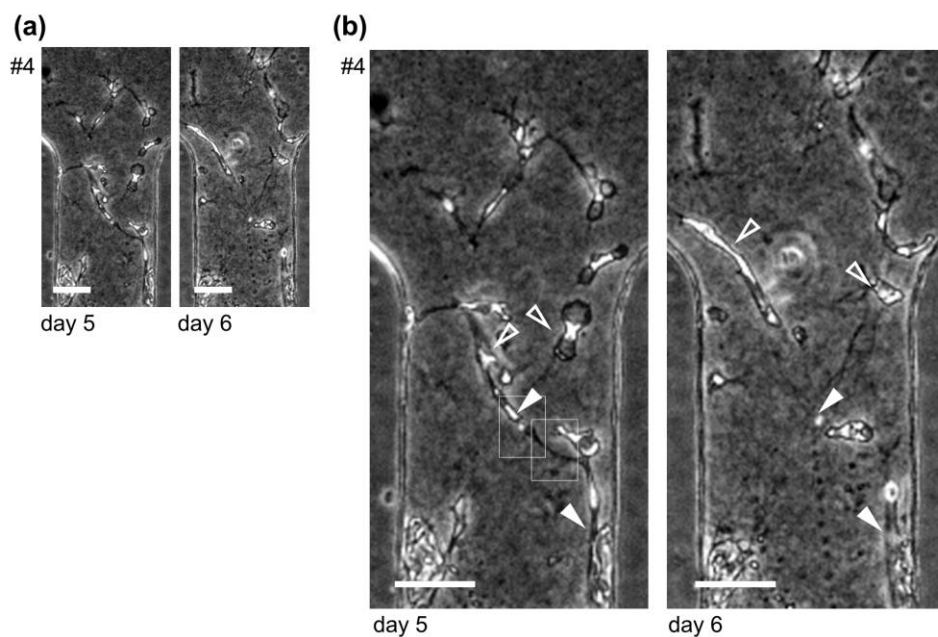
Supporting Figure 4-2. In a case ($n = 1$ device from total 17 devices), tip cells (empty arrowheads) and stalk cells (white arrowheads) kept the connection only under the VEGF gradient (#5). Stalk cells were not regressed only with VEGF gradient when they were connected with the tip cells. Scale bars: $100 \mu\text{m}$.



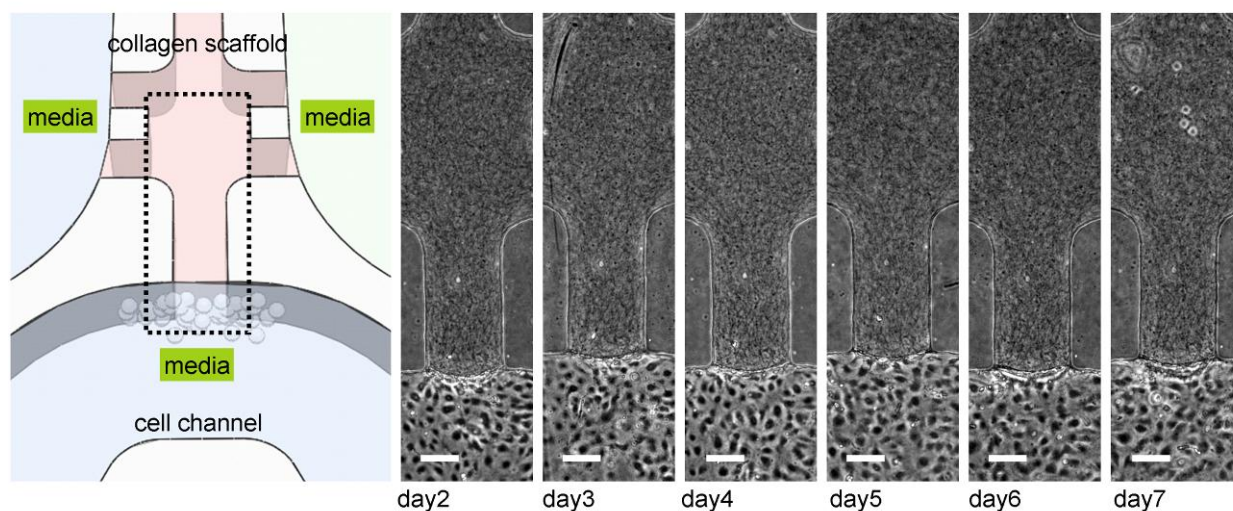
Supporting Figure 5. Sprouting angiogenesis under the VEGF+ANG-1 gradients (the details of Figure 4b). White dotted lines indicate the perimeter of collective cell migration; black arrowheads, activated cells attached to the collective migration sites; and white arrowheads, activated cells separated from the collective migration sites. Scale bars: 100 μm .



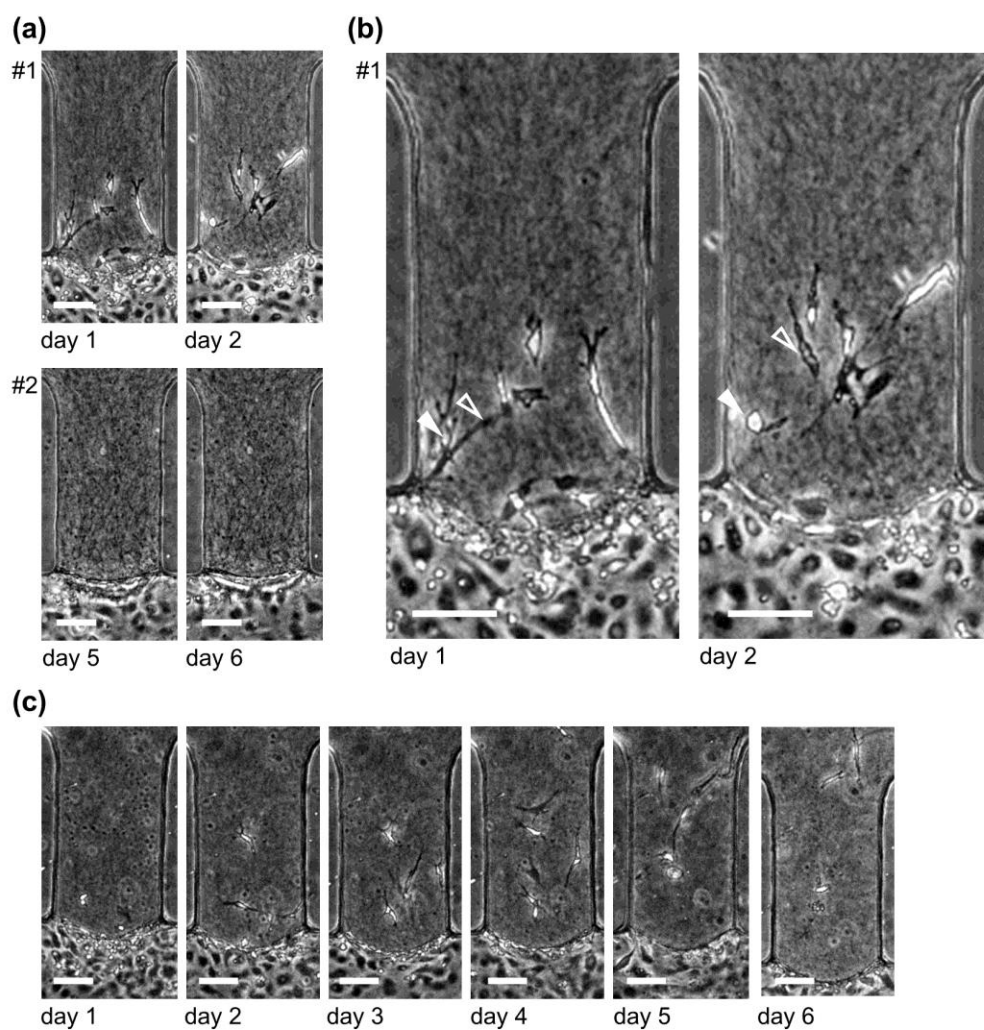
Supporting Figure 5-1. Examples of sprouting angiogenesis under the VEGF+ANG-1 gradients (three devices #1~#3). Note that the connection between stalk cells (white arrowheads) and tip cells (empty arrowheads) was maintained resulting connected and complex capillary structures. Scale bars: 100 μm .



Supporting Figure 5-2. In a case ($n = 1$ device from total 9 devices), almost all tip cells (empty arrowheads) were disconnected from the stalk cells (white arrowheads), and the stalk cells disconnected with tip cells were regressed and disappeared, even under VEGF+ANG-1 gradients (device #4). Scale bars: 100 μm .

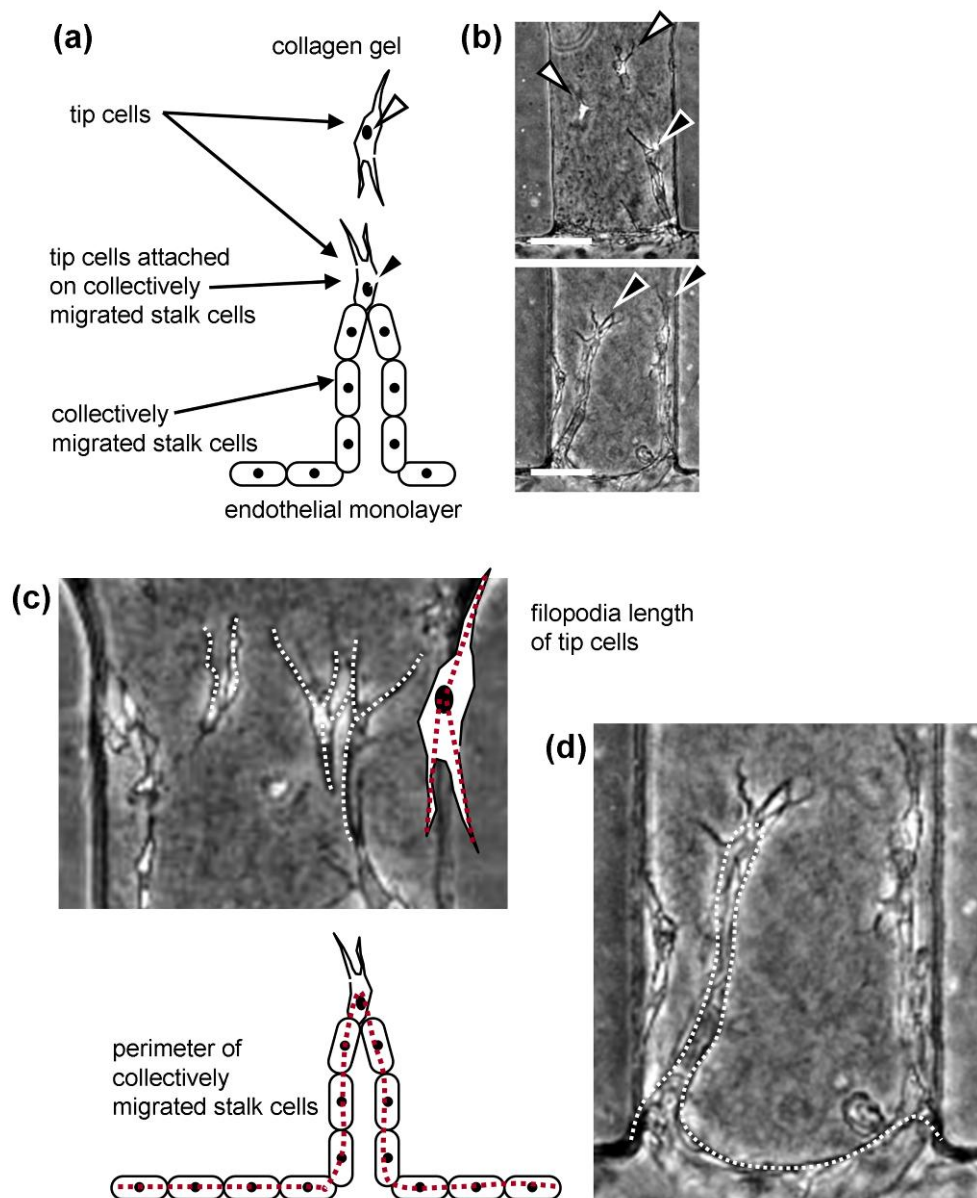


Supporting Figure 6. Sprouting angiogenesis in the control case supplemented by the control media. All control media initially contained 20 ng/ml of VEGF. Scale bars: 100 μm .

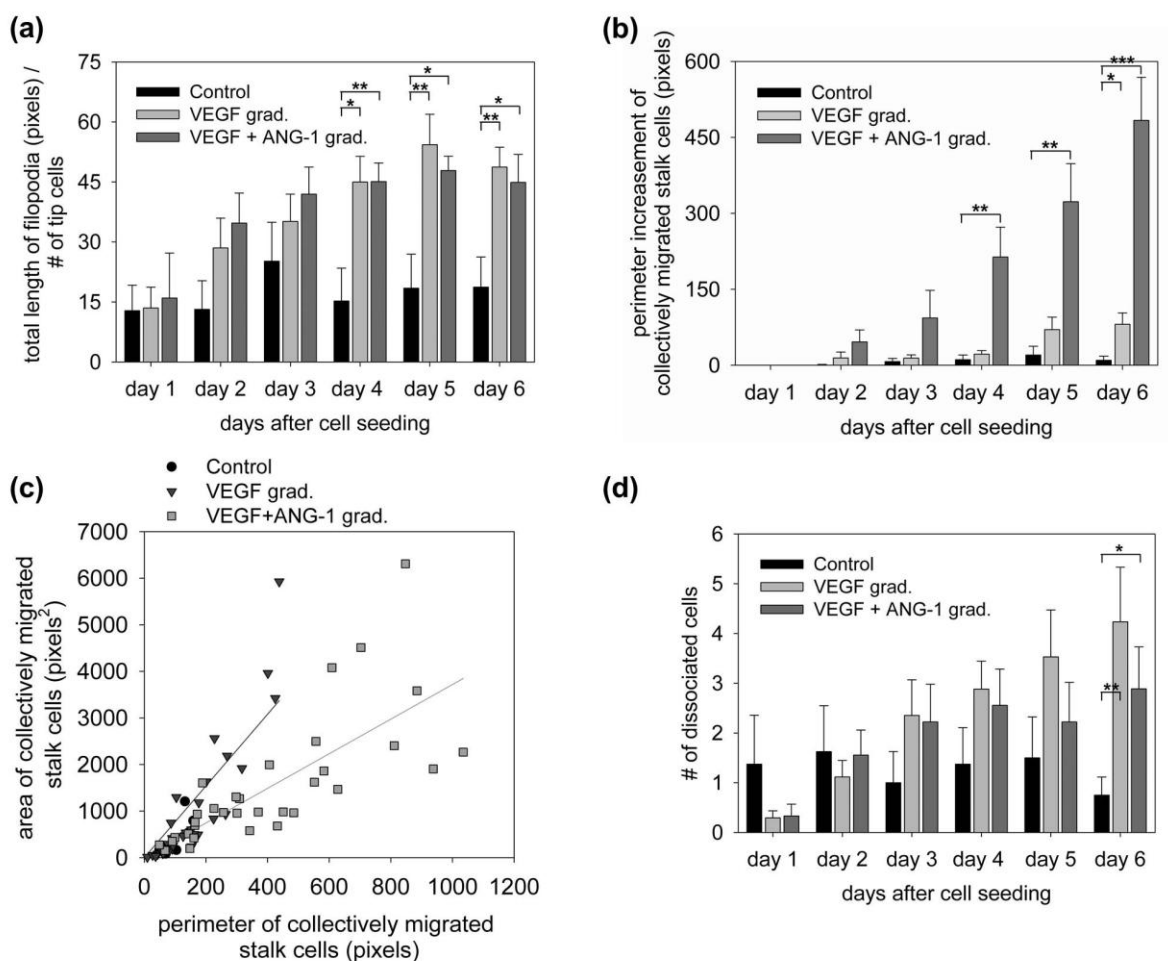


Supporting Figure 6-1. (a & b) Examples of sprouting angiogenesis in the control case supplemented by the control media (initially containing 20 ng/ml VEGF; two devices #1 and #2). Device #1 \rightarrow

Some cells are individually migrated into the collagen scaffold, just responding to the weak gradient of VEGF made by consumption of initially mixed 20 ng/ml concentration in the control media. Note that the connected cell (white arrowhead) was disappeared in day2, after losing the connection with the tip cell (empty arrowhead). Device #2: Even under the weak gradient, some devices do not show any sprouting angiogenesis response. (c) Only one device from total 6 devices only under ANG-1 supplements with control media (initially containing 20 ng/ml VEGF) showed individual migration into the collagen scaffold. Scale bars: 100 μm .



Supporting Figure 7. Method of quantification. Cell migration was monitored by phase-contrast microscopy by keeping the device in an incubator containing 5% CO_2 at 37 $^\circ\text{C}$. (a) Definitions of the terms used in graphs and (b) real images. Black arrowheads denote activated cells attached to the collective migration sites and white arrowheads, activated cells separated from the collective migration sites. Scale bars: 100 μm . White dotted lines indicate (c) the length of the filopodia of tip cells and (d) the perimeter of the collectively migrated stalk cells. All measurements were done by ImageJ (<http://rsbweb.nih.gov/ij/>). The cell boundary was tracked manually.



Supporting Figure 8. Quantification of (a) the average length of filopodia per unit tip cell, (b) the increase in the perimeter of collectively migrated stalk cells, (c) the relationship between area and perimeter of collectively migrated stalk cells, and (d) the number of dissociated cells from collectively migrated stalk cells. The experimental conditions were the same as those in Figure 5. Error bars indicate \pm the standard error. (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$.)