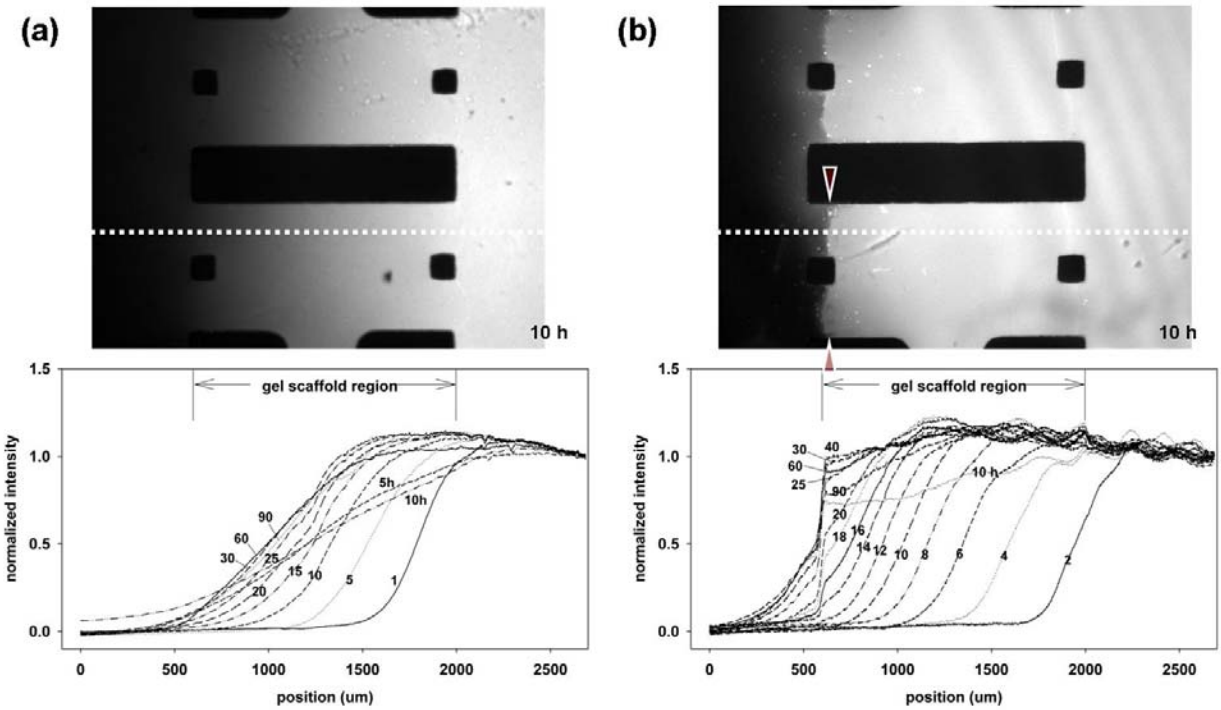
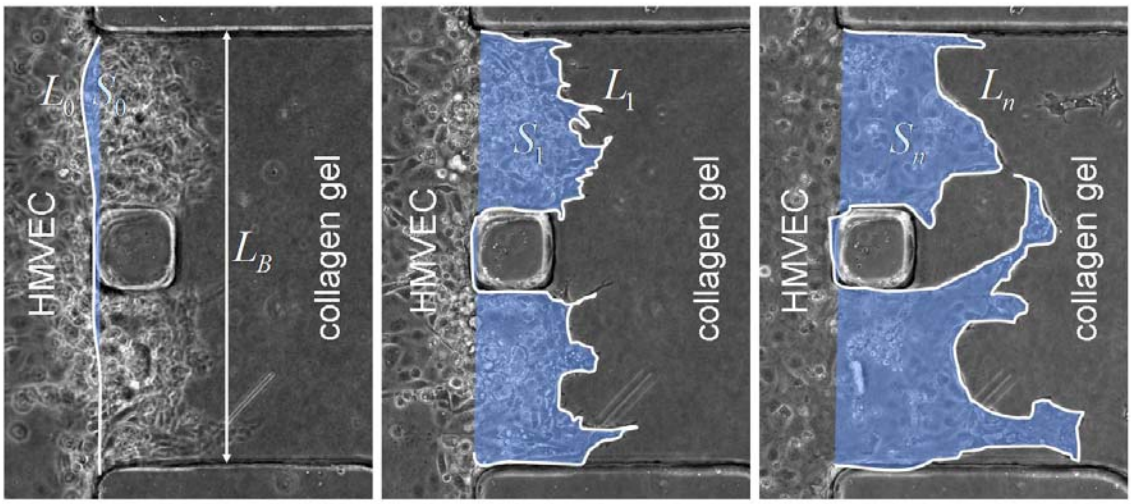
Supplementary information



Supplementary Fig. 1. Diffusion of fluorescent dextran into the collagen gel scaffold (region marked by a white rectangle in figure 1(g)). White lines indicate the measurement position in the pictures. (a) Gradient (up) and normalized intensity plot (bottom) of diffused 40 kDa dextran from condition channel (right) to cell channel (left). The numbers in the plot indicate the measuring time (minutes, 5 hours and 10 hours) after dextran solution filling. Fluctuations at earlier time points can be noticed to be stabilized within a few hours. (b) Gradient (up) and normalized intensity plot (bottom) of diffused dextran with cell monolayer. HMVEC were cultured 4 days to ensure monolayer in the cell channel (some sprouts can be noticed). The monolayer on the collagen scaffold is indicated by red triangles. Fluorescence intensity was normalized to equal 1 at the center of the condition channel (right channel). The outline of the cell monolayer is apparently functioning as a barrier to the diffusion of dextran into the cell channel. In these experiments, type I collagen (BD Biosciences, MA) at 2.0 mg/ml polymerized at pH 7.4 was used as a scaffold material.



L_B : basic perimeter = width of gel scaffold

S_B : basic area = area of gel scaffold (not indicated in the pictures)

To get normalized perimeter and normalized area. (eq. (1))

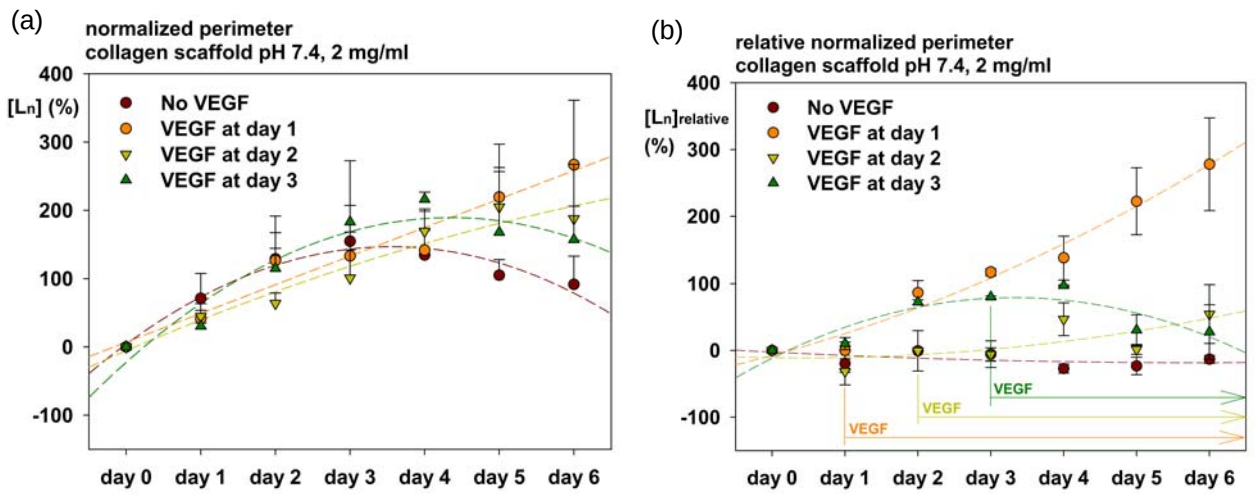
$$[F_n] = \frac{F_n - F_0}{F_B} : \qquad [L_n] = \frac{L_n - L_0}{L_B} \ \& \ [S_n] = \frac{S_n - S_0}{S_B}$$

To get relative normalized perimeter and relative normalized area (eq. (2))

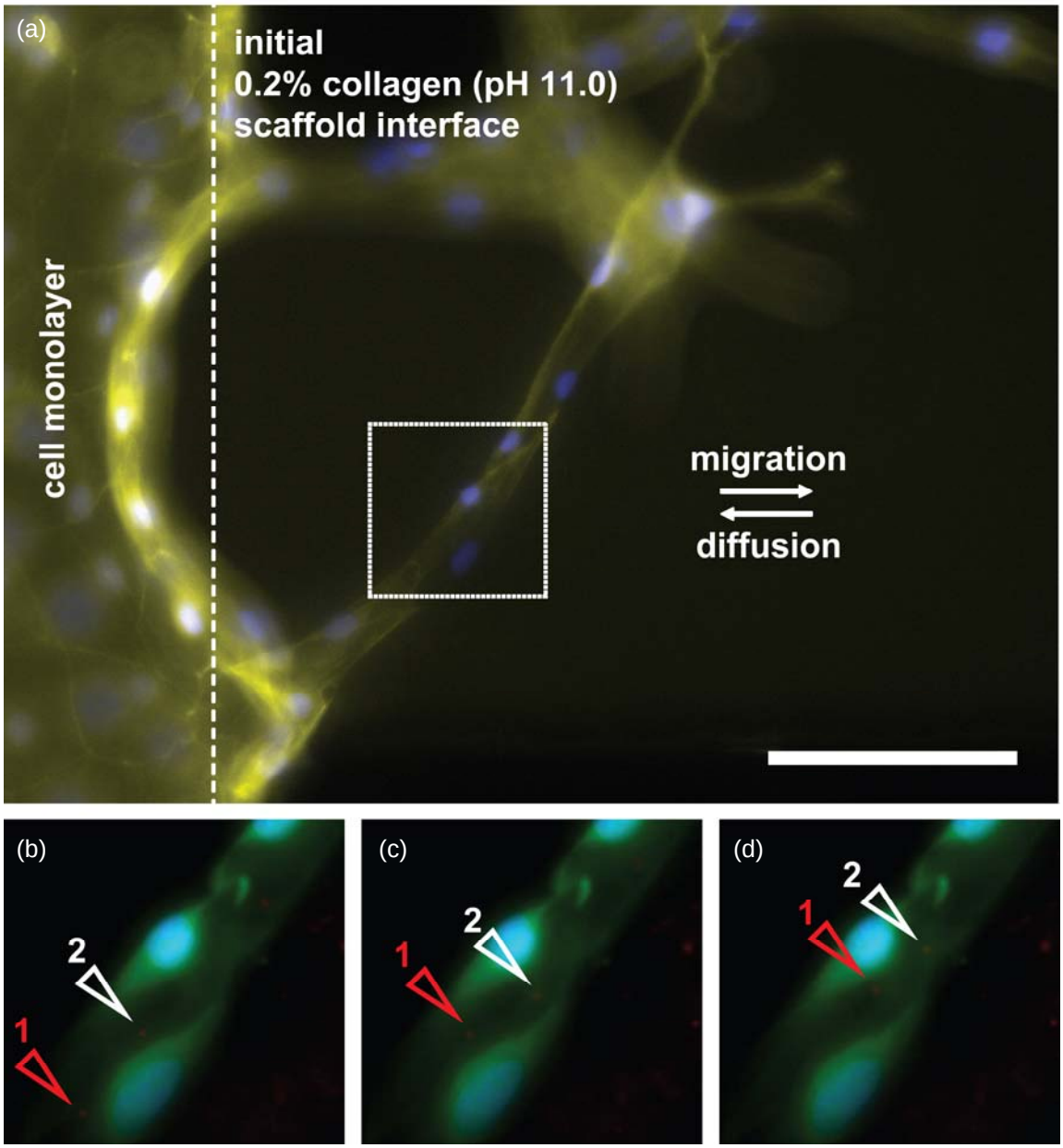
$$[F_n]_{relative} = [F_n]_{cond} - [F_n]_{contr} : \qquad [L_n]_{relative} = [L_n]_{cond} - [L_n]_{contr}$$

$$[S_n]_{relative} = [S_n]_{cond} - [S_n]_{contr}$$

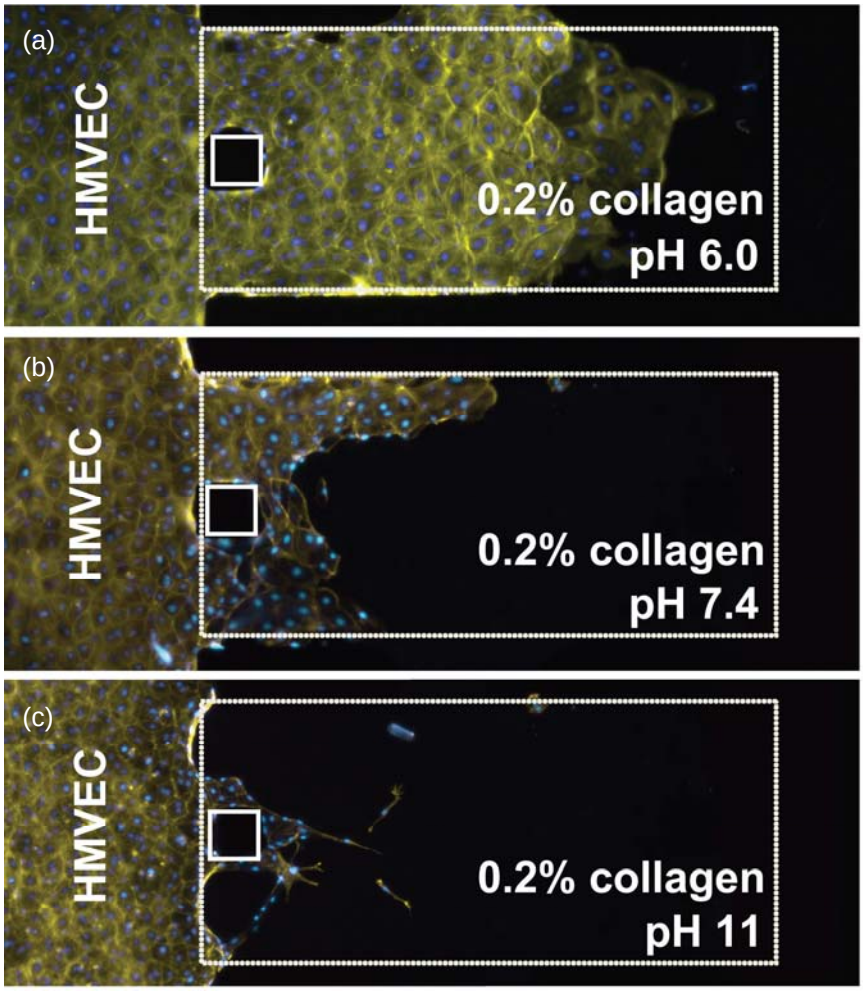
Supplementary Fig. 2. Area and perimeter of the migrated cell outline at day 0 and day n. L_0 and S_0 were measured in the picture taken in 6 hours after cell seeding (left). L_B is measured width of gel scaffold (indicated in the left figure) and S_B is measured total area of gel scaffold. With a same method, L_1 and S_1 were measured in the picture taken in 1 day after cell seeding (center) and L_n and S_n were measured in the picture taken in n day after cell seeding (right). The measured values are normalized by eq. (1). Then relative normalized data were acquired by eq. (2), with comparison of data at condition side from data at control side. Boundary perimeter of the monolayer and projected area of regions containing migrated cells were measured by ImageJ (http://rsbweb.nih.gov/ij/). The cell boundary was tracked manually. L_B and S_B are baseline, reference values for perimeter and projected area of the gel scaffold. Somewhat arbitrarily, we chose to use the gel scaffold area S_B and the gel scaffold width for L_B. The gel scaffold width is approximately equal to the initial perimeter of the cell monolayer.



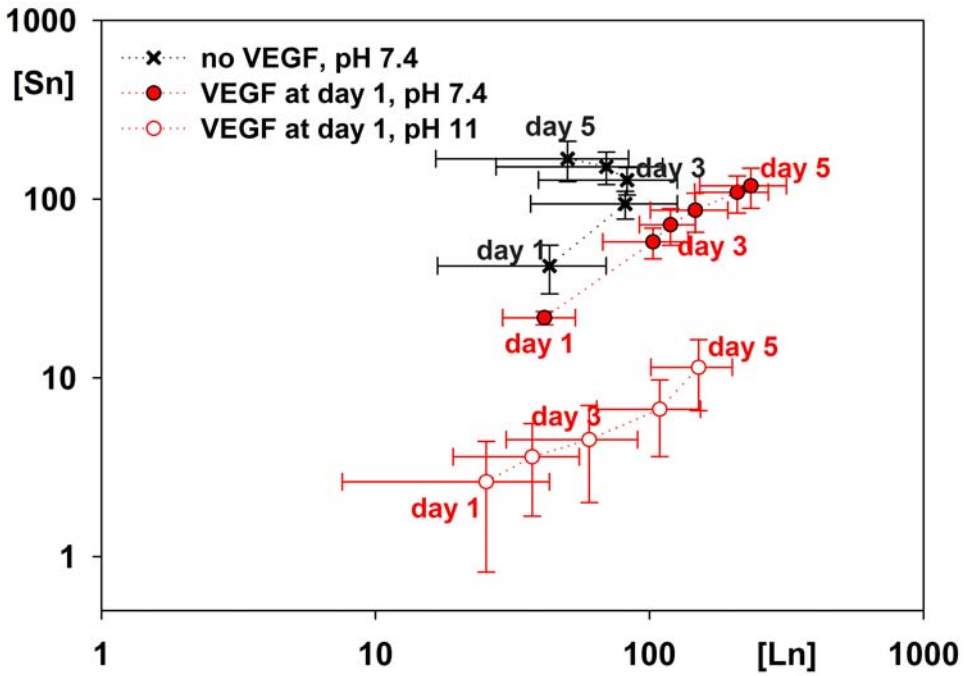
Supplementary Fig. 3. (a) Graph of normalized perimeter of migrated cells in the 2.0 mg/ml collagen gel scaffold polymerized at pH 7.4. 'No VEGF' serves as the negative control without VEGF gradient. 'VEGF at day n' means that VEGF was first applied n day after cell seeding to the end. Due to the chip-to-chip errors, trend and relation of graphs cannot easily be noticed. (b) Graph of normalized relative perimeter of migrated cells in the 2.0 mg/ml collagen gel scaffold.



Supplementary Fig. 4. (a) Tube-like structure formed in 2.0 mg/ml collagen scaffold polymerized at pH 11.0. Scale bar indicates 100 μm. The structure sprouted from the cell monolayer in the center cell channel into the collagen gel scaffold. The initial interface position of the monolayer is indicated by the white dotted line. (b~d) Microbeads flowing through hollow tube-like structures formed by microvascular cells. Two beads (marked with red (1) and white (2) triangles) can be identified flowing toward the end of the tube in the direction of the condition channel from the cell monolayer. Red dots outside of the tube structures were identified as background debris or beads that had become lodged in the matrix since they did not move under interstitial flow.



Supplementary Fig. 5. HMVEC migration behavior depends on gel stiffness. Stiffer gel (polymerized at a higher pH) generated shorter and narrower structures. (a) HMVEC migration in the 2.0 mg/ml (0.2%) collagen scaffold polymerized at pH 6.0, (b) pH 7.4 and (c) pH 11.0. Narrow tube-like structures were observed in the scaffold polymerized at pH 11.0 while wide sheet-like migration was observed in the scaffold polymerized at pH 7.4 and 6.0. All cells were fixed after 6 days of culture with 5 days of VEGF gradient application. Cells were stained for actin (yellow) and nuclei (blue). White dotted lines show the outlines of the gel scaffold and white rectangles indicate PDMS posts (150 μm ×150 μm).



Supplementary Fig. 6. Quantified structures of capillary morphogenesis. The graph shows the relation between the normalized area [Sn] (ordinate) and perimeter [Ln] (abscissa) of the migrated structures at day n. Each plot represents the values on each day from day 1 to day 5. All error bars represent standard deviation (n=8). 'VEGF at day 1' means VEGF was applied from 1 day after cell seeding to the end. 'pH 7.4 or pH 11' indicate pH when the scaffolds polymerized. The graph shows the different location of narrow tube-like structure formation (at pH 11.0, Supplementary Fig. 4(c)) from wide sheet-like structure formation (at pH 7.4, Supplementary Fig. 4(b)). Migration in the condition of 'no VEGF, pH 11.0' is omitted since all values were densely clustered in the lower left-hand corner of the graph.