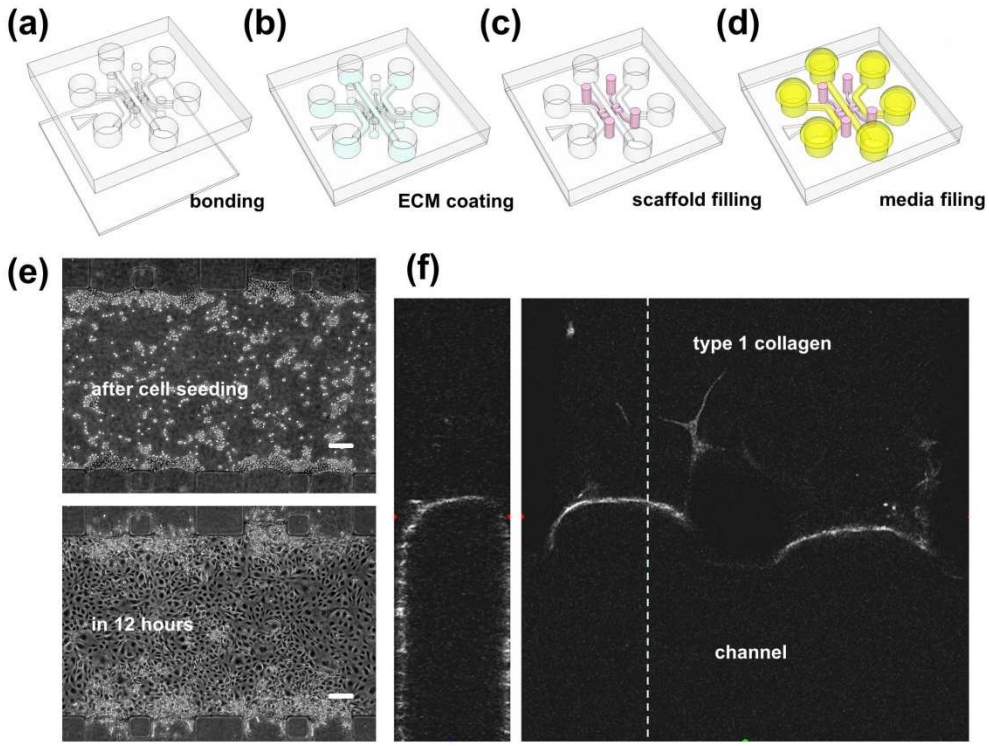
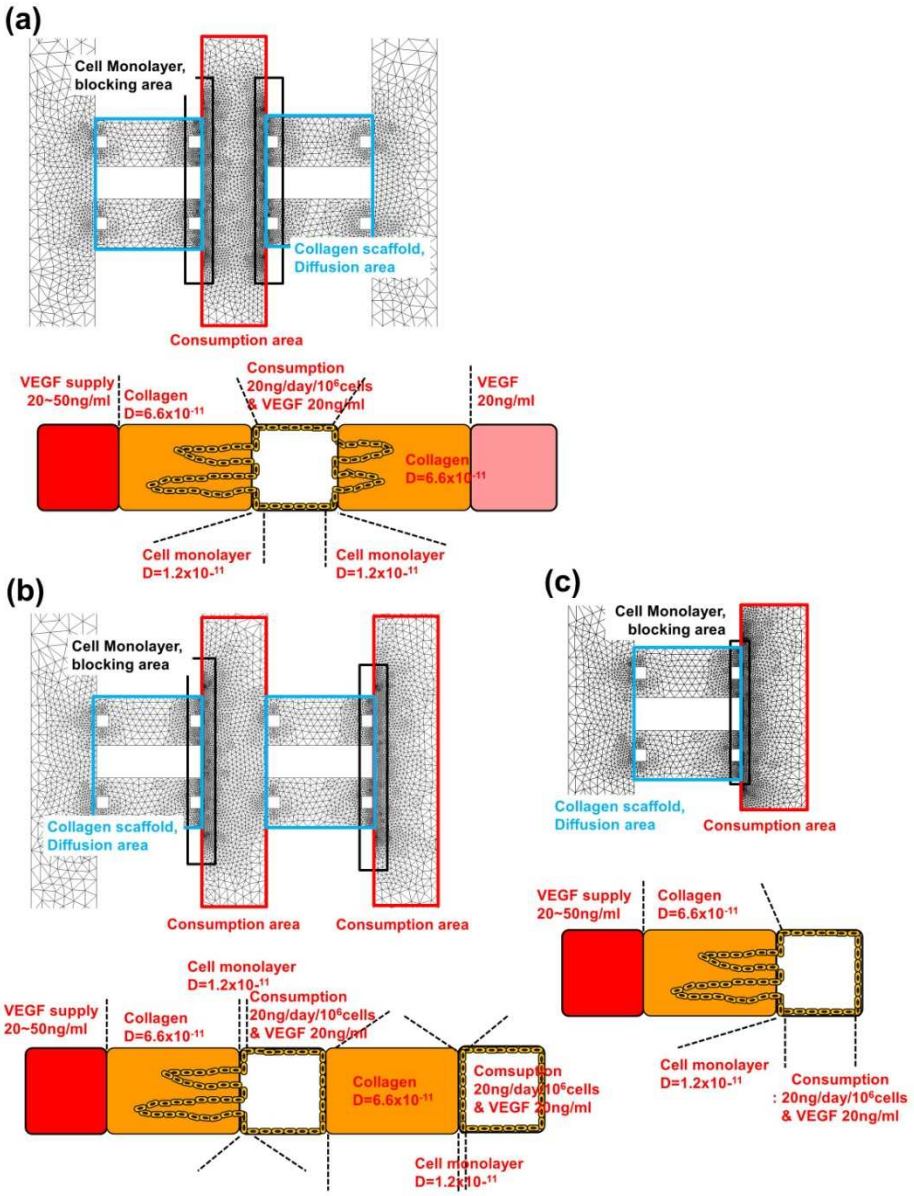
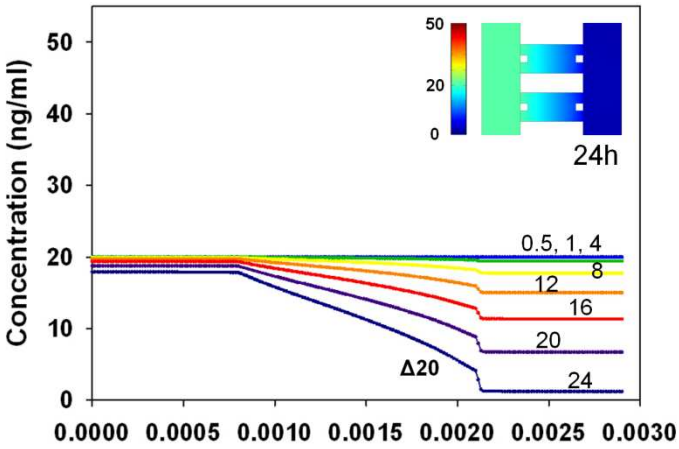
Supplementary Information



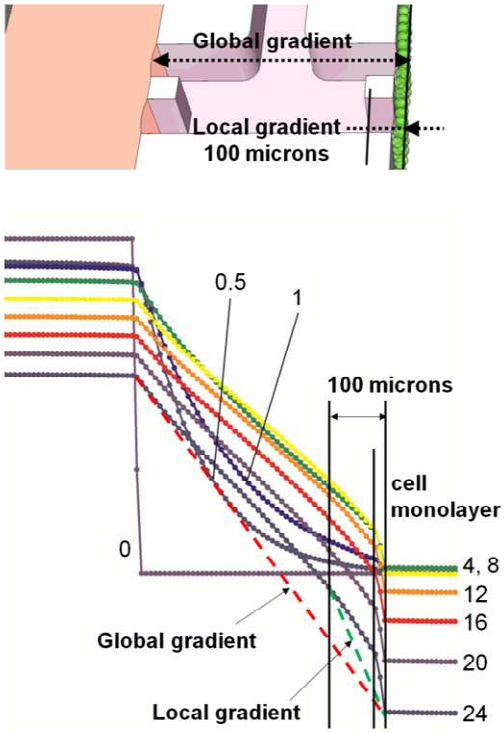
Supplementary Figure 1. Fabrication and preparation of the microfluidic cell culture devices incorporating collagen scaffolds. (a) Plasma treatment and bonding of the PDMS device to the glass coverslip. (b) ECM (PDL) coating in the channels. (c) Collagen scaffold filling into the gel region of the platform. (d) Media filling and preparation for cell seeding. (e) Immediately after cell seeding, the seeded cells attached to the side wall of the collagen scaffolds as well as on the channel surface, due to the applied flow (top). Within 12–24 hours, hUVECs formed a confluent monolayer on the collagen scaffold and channel surfaces. The scale bars indicate 150 microns. (f) A confluent monolayer was confirmed by confocal microscopy, showing a continuous monolayer of actin filaments (white; stained by Rhodamine phalloidin) in a cross-sectional view (left) and top view (right). The white dotted line indicates the position of the cross-section.



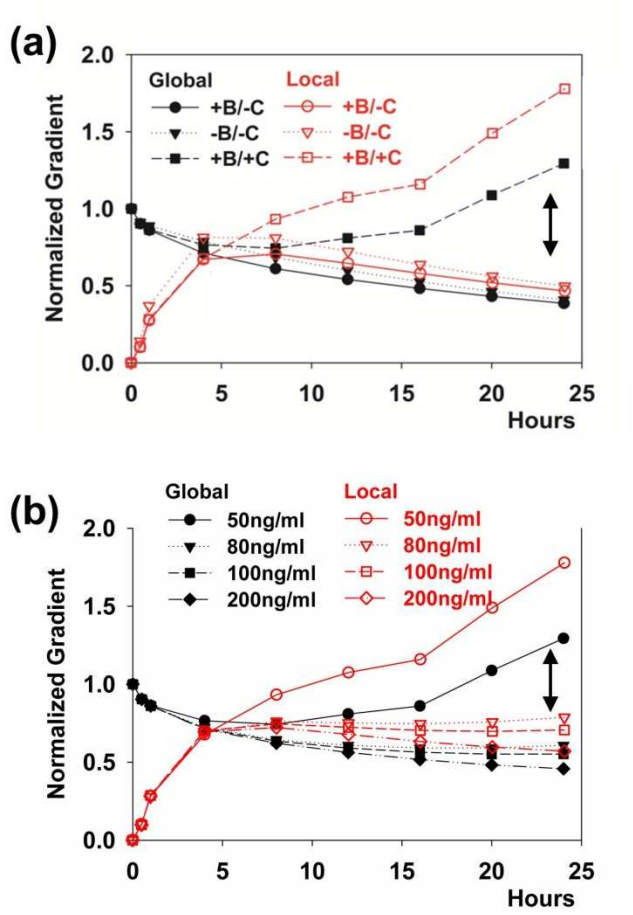
Supplementary Figure 2. Mesh structures for (a) the 3-Ch/1-Cell protocol, (b) the 3-Ch/2-Cell protocol, and (c) the 2-Ch protocol. The cell monolayer acted as a blocking region (black line box), a diffusion gradient was generated in the collagen scaffold (blue line box), and VEGF consumption was assumed across the whole region of the cell channel (red line box).



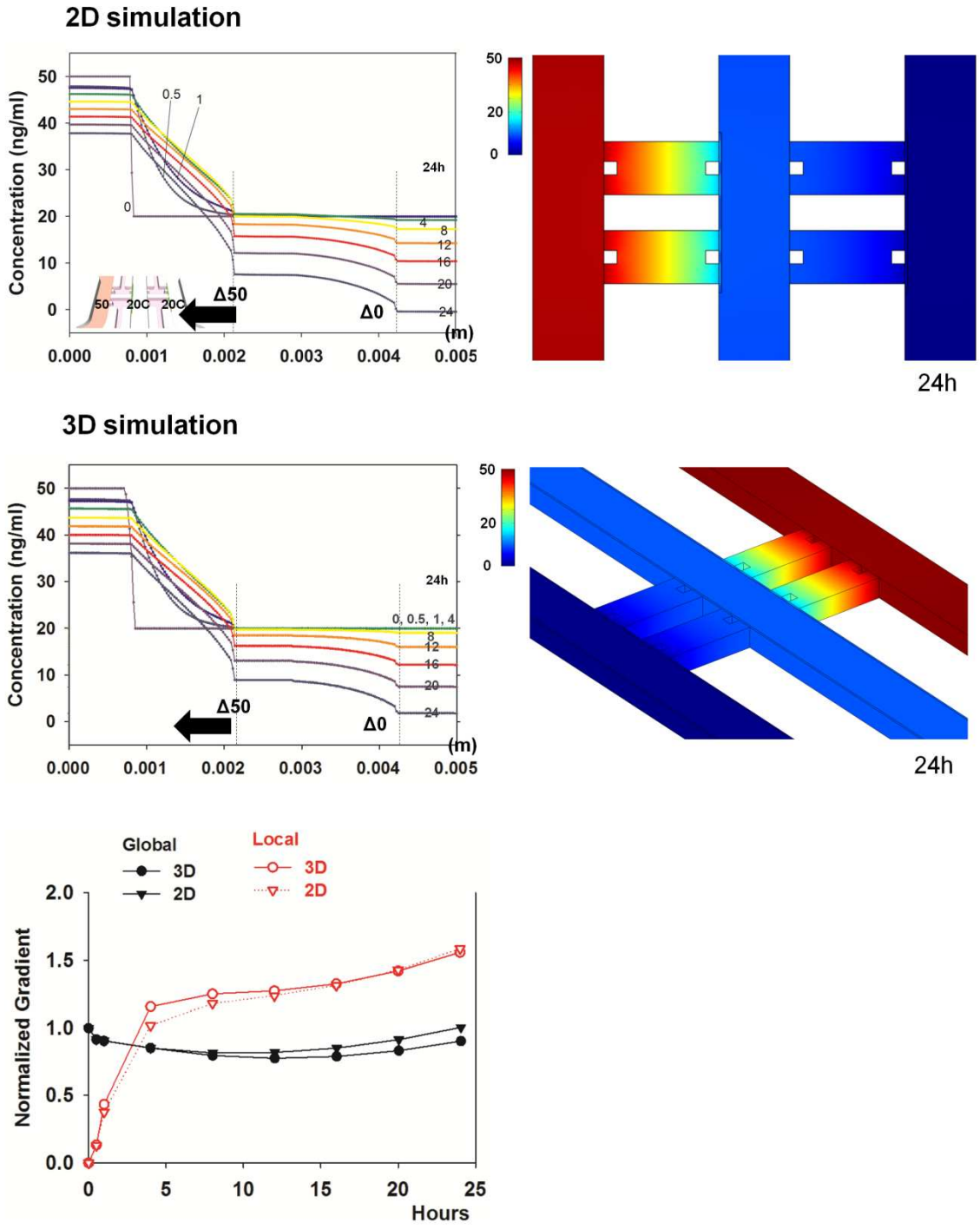
Supplementary Figure 3. Simulation of a VEGF gradient under passive VEGF supply (assuming that all channels were filled only with the control medium) in the 2-Ch protocol, with consideration for diffusion, inhibition, and consumption by the hUVEC monolayer cultured in the right-hand channel under 20–20C ng/ml initial conditions. The small figure (top right) shows the gradient within 24 hours.



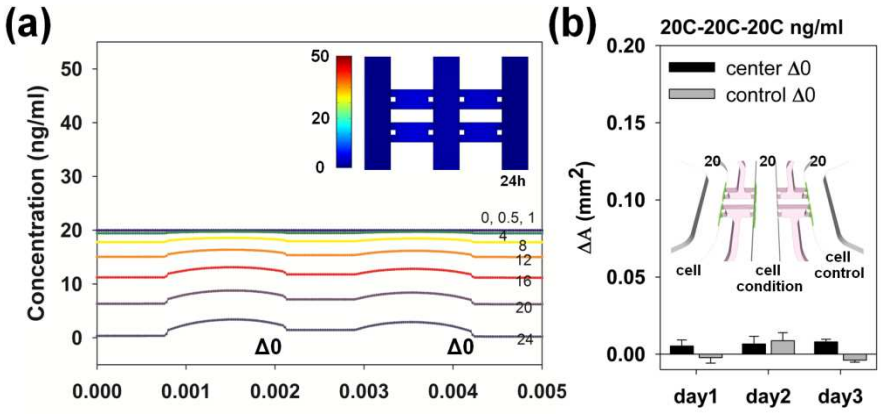
Supplementary Figure 4. Definition of the global and local gradients in a schematic drawing (top) and the concentration graph under molecular diffusion (bottom).



Supplementary Figure 5. Simulated normalized global gradient (across the entire length of the scaffold) and local gradient (across the scaffold to a depth of 100 μm from the cell monolayer, definitions are given in the Supplementary Figure 4). (a) The normalized global and local gradients in the 3-Ch/2-Cell protocol. The arrow at the right indicates the enormous difference between the +B/+C case and the others. (b) Normalized global and local gradients, under different initial concentrations of the applied VEGF, 50–20C–20C (50 ng/ml), 80–20C–20C (80 ng/ml), 100–20C–20C (100 ng/ml), and 200–20C–20C (200 ng/ml), in the +B/+C case. Under an active VEGF supply with a high concentration (80–20–20, 100–20–20, and 200–20–20), the normalized gradient decreased, as observed in the cases that did not consider the consumption of VEGF, because cell consumption was less dominant under a steep gradient.



Supplementary Figure 6. Comparison of the 2D and 3D molecular gradient simulations with consideration for VEGF consumption. (a) 2D simulated VEGF gradient in the 3-Ch/2-Cell protocol. The figure (right) shows a VEGF gradient over 24 hours (Fig 2e). (b) The time-varying VEGF gradient modeled in the 3D simulation was similar to that modeled by the 2D results. (c) The normalized global and local gradients, assuming 2D or 3D conditions, agreed well.



Supplementary Figure 7. Effects of consumption by the hUVECs on the gradient. hUVECs were cultured in all channels in a medium that was initially supplemented with 20 ng/ml VEGF. The simulations indicated that the concentration gradient remained flat over time, as evidenced by the insignificant increase in sprouting from the hUVEC monolayer on both the conditioned and control sides (n=8). The error bars indicate the mean ± standard error.