Supplementary information

Transfer of Two-Dimensional Patterns of Human Umbilical Vein Endothelial Cells into Fibrin Gels to Facilitate Vessel Formation

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Materials and Methods

Materials

Bovine serum fibrinogen (Sigma-Aldrich, St. Louis, MO), bovine plasma thrombin (Wako Pure Chemical Industries Ltd., Osaka, Japan), bovine serum albumin(Wako Pure Chemical Industries Ltd., Osaka, Japan), polyethyleneimine (PEI, average Mw 600, Wako Pure Chemical Industries Ltd., Osaka, Japan), heparin (sodium salt, Wako Pure Chemical Industries Ltd., Osaka, Japan), CellTracker™ Green and Orange (Invitrogen Corp., Carlsbad, CA), CD31 immunostaining Kit including Mouse anti-human CD31, Goat anti-mouse IgG AlkP Conjugate and 5-bromo-4-chloro-3-indolyl-phosphate(BCIP) solution (Kurabo Inc, Osaka, Japan), fetal bovine serum (FBS, Lonza ltd., Basel, Switzerland), and all other chemicals were used without further purification or modification.

Microelectrode System

The fabrication of a Pt disk-type microelectrode and its control system has been previously described.[1,2] Briefly, a fine Pt wire was inserted into a glass capillary, and shielded by thermal fusing of the glass. The tip of the capillary was subsequently polished to give a disk-type microelectrode. The diameter of the Pt disk at the tip was typically 10 μm, while the tip diameter, including the insulating glass part, was ca. 30 μm. The oxidation of Br^- was carried out in a two-electrode configuration with a Ag/AgCl (saturated KCl) counter electrode in a phosphate-buffered saline (PBS) solution containing 25 mM KBr. Using a potentiostat and a motor-driven *xyz* stage connected to a PC, we could control both the potential and the position of the microelectrode.

Cell Culture

Cellular manipulations were performed within a sterile culture hood with the cells maintained at 37 °C under a 5% CO_2 atmosphere. Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza Ltd., and maintained in endothelial cell basal medium (EBM®-2, Lonza Ltd., Basel, Switzerland) supplemented with EGM™-2 SingleQuot® (Lonza Ltd., Basel, Switzerland). HUVECs from passages 4-6 were used in the experiments.

Cell Staining

For staining with CellTracker dye, cells were trypsinized; washed with medium; suspended in 10 μM CellTracker in phosphate-buffered saline (PBS); and incubated for 30 min at room temperature. Then the stained cells were washed with fresh medium and seeded onto a prepatterned substrate at a cell density of

$1x10^6$ cells mL^{-1}. For immunostaining with CD31, HUVECs culture was fixed with 70% ethanol for an hour and washed with PBS containing 1% BSA. Subsequently, the sample was immersed into primary antibody (Mouse anti-human CD31) solution for 90 minutes, followed by immersing into secondary antibody (Goat anti-human IgG AlkP Conjugate) solution for 90 minutes. After washing with DI water, the sample was placed in BCIP solution for 5-10 minutes, followed by the observation within 10-30 minutes.

Application of Strain to Fiblrin Gels

Using a NC laser cutting machine, we fabricated an acrylic resin based device that can apply continuous strain to hydrogel sheet. The both ends of the formed fibrin gel sheet was clamped to each holder of the device and a continuous tensile strain was applied to the gel by adjusting the distance between the holders.

Confocal Microscopy

Cultured cells present in the fibrin gels were imaged using an inverted microscope (TE300, Nikon, Tokyo, Japan) with a confocal scanner unit attached (CSU21, Yokogawa Electric Corporation, Tokyo, Japan) and a digital CCD camera (Luca, Andor Technology, South Windsor, CT) connected to a computer. MetaMorph software (Molecular Devices, Sunnyvale, CA) was used to construct composite side-view images of gels made from sequential series of horizontal confocal images.

Confocal-imaged side view of gel/HUVEC/glass systems

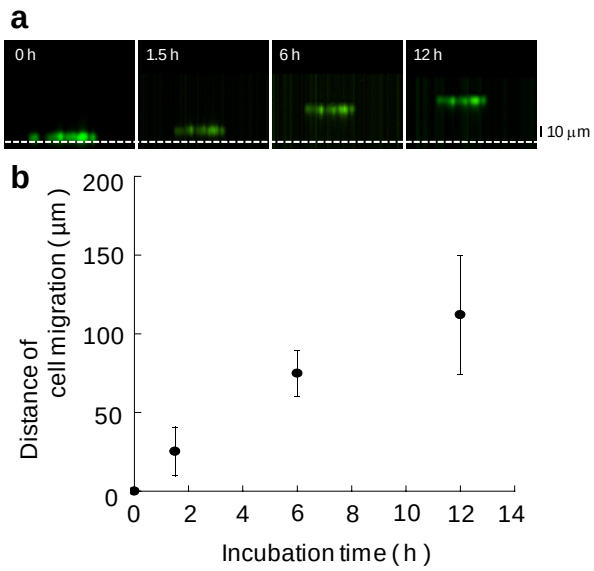

Fig. S1 (a) Confocal-imaged side-views of a fibrin gel covering a HUVEC-patterned substrate just after gelation and after incubation period of 1.5 h, 6 h, and 12 h. The dotted lines indicate the positions of the substrate surface. (b) Plots of cell migration distance from the substrate surface and into the gel versus the incubation time. The cell migration distance is defined as an average distance of the individual cells. The value of each point is the mean ± SD of at least 8 samples.

To understand the cell transfer process, we preliminary investigated how the cells behaved when in contact with the fibrin gel. Figure S1 shows confocal-imaged side view of gel/HUVECs/glass systems and plots of

cell migration distance versus the incubation time. Just after fibrin gelation, the cells reside on the substrate surface. With time, the cells migrate into the gel in a direction that is perpendicular to the substrate surface and the migration distances of individual cells are almost the same during a 12 h-incubation. By this reason, cell populations transferred into the gel could retain their original 2D patterns (see Figs. 2a,b). The cell migration speed in the gel is 13±4 μm h^{-1}, while that on 2D substrate surface is 4±1 μm h^{-1}.[3] Such a faster migration in 3D ECM has been understood by the fact that less of the cell is in contact with the surface, so more of its machinery is available to drive the cell forward.[4] With longer incubation times, the cells migrate more deeply into the gel. However, the breakdown of the pattern occurs in twenty-four hours due to the proliferative outgrowth and the invasion of cells into neighboring regions.

Reference

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