Reconfigurable microfluidic device with discretized sidewall

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Various microfluidic features, such as traps, have been used to manipulate flows, cells, and other particles within microfluidic systems. However, these features often become undesirable in subsequent steps requiring different fluidic configurations. To meet the changing needs of various microfluidic configurations, we developed a reconfigurable microfluidic channel with movable sidewalls using mechanically discretized sidewalls of laterally aligned rectangular pins. The user can deform the channel sidewall at any time after fabrication by sliding the pins. We confirmed that the flow resistance of the straight microchannel could be reversibly adjusted in the range of 10^1 – 10^5 Pa s/ μ l by manually displacing one of the pins comprising the microchannel sidewall. The reconfigurable microchannel also made it possible to manipulate flows and cells by creating a segmented patterned culture of COS-7 cells and a coculture of human umbilical vein endothelial cells (HUVECs) and human lung fibroblasts (hLFs) inside the microchannel. The reconfigurable microfluidic device successfully maintained a culture of COS-7 cells in a log phase throughout the entire period of 216 h. Furthermore, we performed a migration assay of cocultured HUVEC and hLF spheroids within one microchannel and observed their migration toward each other. *Published by AIP Publishing.*

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INTRODUCTION

Microfluidic devices can mimic *in vivo* environments, which helps to identify ways of exploiting cells or cellular systems for diagnostic or therapeutic use. As described in recent reviews,^{1–8} many advanced cell culture systems, such as single cell culture, coculture, and 3D culture, have been developed, in addition to microfluidic cell culture systems for *in vivo*-like perfusion culture. To accomplish complex handling of flows, cells, and cell culture surfaces in microfluidic systems, various microscaled structures have been used, including meanders,⁹ pillars,¹⁰ branching and confluence of microchannels for cell sorting,¹¹ stimulation,¹² patterning,¹³ and traps. Among these structures, traps are frequently used to precisely position appropriate cells or adjust flow velocity around cells.^{14–24} These structures have different names but can be comprehensively described as partial modifications of the cross-sectional area depending on their function and appearance. Furthermore, various implementations of spheroid (co)cultures^{25–29} in microfluidic systems use traps or wells to aid spheroid formation and hold them in place during cultivation.

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FIG. 1. Discretization of a microfluidic channel sidewall. (a) Typical microchannel with a ceiling and two vertical sidewalls. (b) Reconfigurable microchannel feature comprising an elastomeric half-channel and small machine elements, referred to as “pins”. One sidewall in (a) has been replaced with the packed end faces of lined machine elements. Each pin can move longitudinally, guided by adjacent pins.

However, traps and other microfluidic features limit the velocity and the rate of flow in the microfluidic system, even after their desired functions are no longer needed. For example, a trap that helps cells settle onto the microchannel surface can prevent fresh medium exchange near the cells at a high flow rate. Ideally, the size and the position of microfluidic channels would be adjustable to accompany the timely progress of cell attachment, migration, proliferation, and other procedures required, for example, by performing additional cell seeding or live cell staining, removing dead cells or air bubbles, or harvesting several cell aggregates. However, it is difficult to temporarily add, adjust, or remove features in microfluidic channels fabricated by micromachining or soft lithography because these features are fixed at the time of fabrication.

Herein, we developed a highly reconfigurable microfluidic system with a discretized sidewall that comprises small movable elements (see Figure 1). These elements, called pins, are configured in line so that their flat ends form one of the channel sidewalls. The user can deform the channel sidewall at any time after fabrication by sliding the pins. We have developed a cell-culture-compatible microfluidic channel with a discretized sidewall that suppresses leakage and other unwanted flow through gaps between the pins. We evaluated the flow rate adjustment ability of this channel by simply moving several pins. Furthermore, we demonstrated the ability of the reconfigurable microfluidic channels by patterning of cells, gradually widening culture surfaces that support clonal to near-confluent growth, and migration assay of cocultured cell spheroids.

EXPERIMENTAL

Microfluidic device design

Figure 2 shows the overview of a microfluidic device containing one sidewall with a variable shape. As shown in Figure 2(a), the volume defined by a poly(dimethylsiloxane) (PDMS) channel (ceiling and one sidewall), a glass substrate (floor), and the ends of ten stainless steel rectangular pins (the other sidewall) formed the microfluidic channel. Each pin could be moved longitudinally by applying a downward force with a blade tip to create grip and then sliding the blade tip along the adjacent pins. Moving the end face, which is always parallel to the PDMS sidewall, virtually widens or narrows a segment of the channel, displacing fluid in the channel to impede flow or obstruct the channel.

Unfortunately, gaps between the pins allowed liquid to leak from the channel by capillary effects, and the meniscus force caused adjacent pins to stick together. To eliminate these problems, the pin gaps were filled with a mixture of a medical-grade paraffin wax and polytetrafluoroethylene powder, referred to as a filler. To prevent the depletion of the filler, the center of the pins was wet etched into a dog-bone shape to make the gaps large enough to accommodate the filler. As the filler itself could leak out of the gap into the channel under the same capillary action, the filler was enclosed in a barrier made of silicone elastomers. Figure 2(b) illustrates

FIG. 2. Reconfigurable microfluidic channel with a discretized sidewall. (a) Top view of a microfluidic device with a reconfigurable microchannel, comprising a microfluidic channel with one straight fixed sidewall and a reservoir at each end. The device has typical soft lithography microfluidic features, such as a glass bottom and a poly(dimethylsiloxane) (PDMS) channel, but has one sidewall formed by the ends of ten stainless steel pins. The paraffin wax-based filler prevented liquid in the channel from leaking through the pin gaps, and a silicone adhesive barrier prevented filler leakage. An air vent channel was placed between the microchannel and the barrier edge to prevent shear flow around the moving pins from entering the microchannel. (b) Assembly process for the microfluidic device shown in (a): (1) dispensing paraffin wax-based filler and silicone elastomer on a glass substrate with a robotic dispenser; (2) bonding the PDMS channel with reservoirs to a glass slab. Rectangular pins are inserted in the gap between glass and PDMS, and the PDMS-pins-glass assembly is bonded to the substrate; and (3) dispensing more silicone elastomers and fillers to fully embed the middle of the pins.

the processes used to form the channel sidewall from pins and to construct the filler region surrounded by a silicone elastomer barrier.

Moving the pins generated strong displacement flow that was effective for flow generation and handling non-liquids, such as air bubbles and cell aggregates. However, pin movement also generated small but undesired shear flow from the microchannel to the gaps near the ends of the pins. To reduce transient flow from/to the pin gaps, the pressure in the pin gaps was

moderately discharged through an air vent channel in the middle of the pin gaps. No continuous leakage occurred through the air vent channel to outside the device because the back pressure was high due to its hydrophobicity.

Microfluidic device fabrication

Etching of rectangular pins

SUS316L stainless steel rectangular pins ($0.3 \times 0.3 \times 7$ mm, Micro Giken, Tokyo, Japan) were placed on a glass slide (76×52 mm No.1, Matsunami Glass, Osaka, Japan) with a groove (4 mm wide, 0.5 mm deep) with the middle of the pins aligned with the groove. Both the ends of the rectangular pins were covered with silicone adhesive (KE-41, Shin-etsu Chemical, Tokyo, Japan), leaving the center exposed. After curing the adhesive, the pin centers were etched by covering with an etchant (5 M HCl + 0.5 M HNO₃, Wako Chemicals, Osaka, Japan) and heating at 65 °C for 40 min. The pins were then rinsed by immersing in 0.8 M Na₂CO₃ for 30 min, followed by sonication in acetone for 15 min. The pins were passivated by immersion in 10% HNO₃ at 65 °C for 30 min.

Microchannel features formed in PDMS

PDMS slabs comprising the ceiling, a sidewall of the reconfigurable microfluidic channel, and an air vent channel were fabricated by typical soft lithographic methods. A negative mold of the PDMS slab features was fabricated by patterning a 270- μ m-thick layer of photoresist (SU-8 3050 Nippon Kayaku, Tokyo, Japan). Photoresist was spun on a glass slide three times, twice at 1000 rpm followed by drying at 95 °C for 15 min and once at 2000 rpm followed by drying at 95 °C for 30 min. The photoresist layer was exposed to 450 mJ/cm² of 365-nm UV light through a 12 700 dpi photoplotted film (Unno Giken, Tokyo, Japan), baked at 95 °C for 15 min, and then developed by spraying with 2-methoxy-1-methylethyl acetate (Cat #130-10505, Wako Chemicals). The prepolymer of two-part clear PDMS (KE-106, Shin-Etsu Chemicals) was poured onto the photoresist mold to a thickness of 3 mm and cured at 65 °C for 1 h. The PDMS slab was peeled from the mold, and holes for the inlet/outlet ports were perforated into the slab. The main channel and air vent channel ports had diameters of 2 mm and 1 mm, respectively.

A 450- μ m-thick coverglass (No.4, Matsunami Glass) cut to 13×30 mm was bonded to the microfluidic channel features in the PDMS slab. Cut coverglasses were degreased with acetone, cleaned by immersing in 1% detergent (TMSC, Tama Chemicals, Kanagawa, Japan) in deionized water at 60 °C for 15 min, rinsed twice with ionized water for 10 min, and then dried at 120 °C for 1 h. Both the channel side of the PDMS slab and the cut coverglass were treated with air vacuum plasma (20 Pa, 20 mA, 30 s) using a sputter coater (SC-708, San-yu Electron, Tokyo, Japan) and immediately bonded with edges aligned. After bonding, ten pins were inserted into the open side so that their ends formed the other sidewall of the microchannel.

Device assembly

An overview of the assembly processes is illustrated in Figure 2(b). The pin gap filler was prepared by mixing paraffin wax (Sun White P-1, Nikko Rica, Tokyo, Japan) and poly(tetrafluoroethylene) powder (Microflon II, Power House Accel, Tokyo, Japan) in a 2:1 ratio (w/w). A line segment pattern of the pin gap filler (length, 11 mm; width, 800 μ m) was dispensed on a glass slide using a robotic dispenser (Ez-ROBO3, Iwashita Engineering, Tokyo, Japan and ML-606GX, Musashi Engineering, Tokyo, Japan). An 800- μ m-wide contour pattern of silicone adhesive surrounding the filler segment was also dispensed automatically. A coverglass (No. 4, 13×30 mm) was laid on the glass slide, adjacent to one of the two longer edges of silicone adhesive. The microchannel assembly described in the subsection on “Microchannel features formed in PDMS” was then placed on the glass slide next to the opposite longer edge of the adhesive contour. Another filler segment and an adhesive contour of the same size and position were dispensed again to cover the pins and pin gaps. The device was cured for two days at

room temperature. Finally, each pin was held down and moved longitudinally with a scalpel tip. Once released from the adhesive, the pins were able to move individually.

Evaluation of flow resistance control

By moving one or several pins constituting the sidewall, the fabricated microfluidic channel was expected to dynamically change the flow resistance. However, possible changes in variable flow resistance would be limited by the sizes of the pins, the whole channel, and even the inlet/outlet ports. We evaluated changes in flow resistance in the microfluidic channel when narrowing the channel by displacing one pin towards the fixed wall. High flow rates were measured by monitoring droplets at the channel outlet, whereas low flow rates by diffusion of a fluorescent dye.

Measurement of flow resistance at a high flow rate

The experimental setup and measured parameters used to calculate the flow resistance are shown in Figure S1, [supplementary material](#). Initially, all pins were positioned to maintain a width of 400 μm throughout the channel. The microfluidic channel was primed with deionized water to bring water levels of both the inlet and the outlet up to the top surface. The pin nearest the outlet was then pushed toward the fixed wall to produce widths of 300, 150, 75, 50, and 25 μm . A 4- μl water droplet was placed on the inlet of the water-primed microchannel. Side views of both the inlet and the outlet were video recorded using a digital camera (Nikon 1 J4 with a telecentric lens, Nikon, Tokyo, Japan) at 1200 frames/s. The flow rate, Q , was then calculated from the difference in the outlet volume derived from the curvature diameters of the air-water interface measured on the frame images

$$Q = \frac{\Delta V}{\Delta t},$$

where Δt is the interval of the two frames and ΔV is the change in the volume of the droplet placed on the top surface during the interval Δt . The volume of the droplet, V , was calculated as

$$V = \frac{\pi h}{6}(3r^2 + h^2),$$

where h is the height of the droplet measured from the top surface of the device and r is the diameter of the inlet/outlet holes.

The pressure differential between the outlet and the inlet was calculated using

$$\Delta p = \gamma \left(\frac{1}{R_o} - \frac{1}{R_i} \right) + \frac{\rho(V_o - V_i)}{\pi r^2},$$

where γ and ρ are the surface tension and the density of water (72.75 mN/m and 0.997 g/cm³ at 25 °C, respectively), R_o and R_i are the droplet curvature radii at the outlet and the inlet, and V_o and V_i are the initial and final droplet volumes.

Flow resistance was computed from the pressure differential and the flow rate, as follows:

$$R = \Delta p / Q.$$

Measurement of flow resistance at a low flow rate

The microfluidic channel width was adjusted to 300 μm , and the channel was primed as described in the subsection on “Measurement of flow resistance at a high flow rate.” The pin nearest the outlet was then pushed toward the fixed wall to completely close the pin-wall gap.

A 4 μl drop of 10 μM Alexa Fluor 488 free dye (Thermo Fisher Scientific, Waltham, MA, USA) solution was added to the water-primed microfluidic channel. Fluorescence intensity profiles were obtained under a stereomicroscope with a camera (MZ10F, Leica and CoolSnap K4, Photometrics, Tucson, AZ, USA) every 5 min. The time evolution of dye concentration was evaluated by fitting the intensity profile with a steady state solution of the convection-diffusion equation with a continuous point source³⁰

$$\frac{I(t, x)}{I(0, 0)} = \frac{1}{2} \left(\operatorname{erfc} \left(\frac{x}{2\sqrt{D_y t}} - \frac{v\sqrt{t}}{2\sqrt{D_y}} \right) + \exp \left(\frac{vx}{D_y} \right) \operatorname{erfc} \left(\frac{x}{2\sqrt{D_y t}} + \frac{v\sqrt{t}}{2\sqrt{D_y}} \right) \right),$$

where I is the fluorescence intensity as a function of time t and position x , and v is the flow velocity. The apparent diffusion coefficient, D_y , is given as follows:

$$D_y = D \left(1 + \frac{1}{210} \left(\frac{vH}{D} \right)^2 f \right),$$

where D is the molecular diffusion coefficient of the fluorophore (435 $\mu\text{m}^2/\text{s}$ for Alexa Fluor 488³¹), f is the sidewall factor (~ 1.76 for the rectangular cross-section),³² and H is the channel height ($= 300 \mu\text{m}$). Fluid resistance was calculated as previously described.

Cell culture

COS-7 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; SH30021.01, GE Health Care, Pittsburgh, PA, USA) supplemented with 5% fetal bovine serum (Biowest, Nuaille, France) and 1% antibiotic-antimycotic (15240-062, Thermo Fisher). Cell suspensions of all cell species were prepared by trypsinization.

Human umbilical vein endothelial cells (HUVECs, C2517A, Lonza, Basel, Switzerland) and human lung fibroblasts (hLFs, CC-2512, Lonza) were maintained in EBM-2 BulletKit (CC-3162, Lonza) and FGM-2 BulletKit (CC-3132, Lonza). Spheroids were formed by seeding 1.0×10^4 cells into each well on an ultra-low attachment 96-well plate (7007, Corning, NY, USA) and were statically cultured for 3 days. The diameter of the resultant spheroids was approximately 300 μm .

The inside of the microfluidic channels was coated with 100 $\mu\text{g}/\text{ml}$ human fibronectin (354008, Corning) to promote cell adhesion or 1% blocking agent containing the 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer (N102, Nichiyu, Tokyo, Japan) for adhesion inhibition.

Imaging

HUVECs were stained with 10 $\mu\text{g}/\text{ml}$ rhodamine-labeled *Ulex Europaeus* agglutinin I (RL-1062, Vector Laboratories, CA, USA) in phosphate-buffered saline (PBS) for 40 min, and hLFs were stained with 2 μM calcein-AM (PK-CA707, PromoCell, Heidelberg, Germany) in PBS for 30 min. Phase contrast and fluorescence images were obtained using an inverted microscope (DMI8, Leica) and a charge-coupled device (CCD) camera (Retiga 2000R, Q-Imaging, Surrey, BC, Canada). The resulting images were analyzed using Fiji imaging software (<https://fiji.sc/>). Plugins for linear stack alignment and manual tracking are used to obtain the movement of cell spheroids.

Cell handling in a reconfigurable microchannel

Figure 3 shows the pin manipulation steps for handling various cells using the reconfigurable microchannel.

FIG. 3. Microchannel reconfiguration for in-channel cell manipulation. (a) Patterning one type of cell into multiple segments in a straight microchannel: (1) cells are seeded to a straight microfluidic channel; (2) pins are displaced to obstruct the channel; and (3) pins are returned to the straight position after cell adhesion. (b) Patterning two types of cells (human lung fibroblasts, hLFs, and HUVECs) into two segmented regions: (1) hLFs are introduced to the channel and obstructed by a "wall" using pin displacement; (2) the culture area of hLFs is defined by closing the pin upstream. HUVECs are then introduced at the opposite end of the channel and also obstructed by the wall; (3) excess HUVECs are driven from the channel; the culture area for HUVECs is defined; (4) the channels are returned to the straight position after adhesion of both cell types is observed. (c) Gradual cell culture area expansion in a reconfigurable microchannel. (1) Cells are seeded to a microfluidic channel. (2) Cells (between one and five) are confined within a space equal to one pin-width, and cells outside are aspirated. (3) After proliferation of the confined cells, the confined space is expanded. (4) Cell culture is kept nonconfluent by continuing to expand the culture area. (d) Spheroid coculture in a reconfigurable microchannel. (1) A HUVEC spheroid is introduced into the channel after narrowing the region near the outlet. (2) The spheroid settles before the narrow region, and another pin is moved to narrow the channel on the other side of the spheroid. (3) An hLF spheroid is introduced and settles before the pin actuated in step (2). (4) Another pin is moved to enclose both the settled HUVEC and LF spheroids between narrow regions, giving a coculture.

Forming segmented cell patterns

All the pins were adjusted so that the width of the entire microchannel was $400\ \mu\text{m}$. The microchannel was coated with fibronectin for 90 min and rinsed twice with PBS.

A $20\text{-}\mu\text{l}$ drop of COS-7 cell suspension was introduced into the inlet of the microfluidic channel. As illustrated in Figure 3(a), several pins were pushed until closed to give three culture areas ($300\ \mu\text{m} \times 500\ \mu\text{m}$) spaced by 300 or $600\ \mu\text{m}$. The microchannel was then incubated at 37°C in 5% CO_2 for 3 h to promote adhesion. Pins pushed in the previous step were retracted to form a straight channel. The cells were imaged by phase contrast microscopy every 24 h using an inverted microscope.

Patterning of cocultured cells

The entire microchannel was configured to be $300\ \mu\text{m}$ wide and coated with fibronectin, as described in the subsection on "Forming segmented cell patterns." As shown in Figure 3(b), to prevent cells from passing through, two pins in the channel center were pushed toward the fixed

sidewall to achieve a gap smaller than $10\text{ }\mu\text{m}$. hLFs were stained with $2\text{ }\mu\text{M}$ calcein-AM, incubated at $37\text{ }^{\circ}\text{C}$ in 5% CO_2 for 1 h, and suspended in EBM-2. A $10\text{-}\mu\text{l}$ drop of hLF suspension adjusted to 3.0×10^6 cells/ml was introduced to the channel inlet. The hLFs were confined within a $400 \times 600\text{ }\mu\text{m}$ area by pushing the two pins nearest the inlet. The device was incubated at $37\text{ }^{\circ}\text{C}$ in 5% CO_2 for 3 h.

After attaching the hLFs in the channel, a $10\text{-}\mu\text{l}$ drop of HUVEC suspension, adjusted to 1.5×10^6 cells/ml, was introduced into the other channel inlet. The HUVECs were confined within a $400 \times 600\text{ }\mu\text{m}$ area by pushing the two pins nearest the inlet. The device was incubated at $37\text{ }^{\circ}\text{C}$ in 5% CO_2 for 1 h. The pins pushed in the previous steps were then retracted to form a straight channel. The medium in the channel was replaced with 4% paraformaldehyde in phosphate buffer, fixed for 10 min, and rinsed with PBS. HUVECs in the channel were selectively stained with rhodamine-agglutinin, as described in the section on “Imaging.” The cells were imaged by fluorescence microscopy using an inverted microscope.

Cell culture with a variable culture area

The entire $400\text{-}\mu\text{m}$ -wide microchannel was filled with a suspension of COS-7 cells adjusted to 1.5×10^6 cells/ml. As shown in Figure 3(c), the pins were manipulated to seed one to five cells in the area defined by one retracted pin (cross-sectional size, $300\text{ }\mu\text{m} \times 300\text{ }\mu\text{m}$). The culture area was then adjusted by further pin movement to reach a cell density in the range of $10\text{--}20$ cells/ mm^2 . All the cells expelled from the cell culture area were aspirated from the channel. The device was incubated at $37\text{ }^{\circ}\text{C}$ in 5% CO_2 and observed using an inverted microscope every 24–48 h. The cell culture area was expanded by retracting one pin every time 70% confluence was observed. We did not add or exchange medium during cultivation on the chip. Enough supply of nutrients was made by diffusion into the culture area, as shown in Figure S2, [supplementary material](#).

For comparison, the same cell suspension was diluted with a medium to obtain cell densities comparable to the cell culture in the microfluidic channel. Diluted suspension ($100\text{ }\mu\text{l}$) was added to a well in a 96-well cell culture plate (3861-096, AGC Techno Glass, Tokyo, Japan) and incubated at $37\text{ }^{\circ}\text{C}$ in 5% CO_2 for 1 h. Live cells were counted under an inverted microscope. The resultant cell seeding density was 10.2 ± 1.34 cells/ mm^2 ($n=3$, mean $\pm$ standard deviation (SD)). Cells were observed using an inverted microscope every 24–48 h and counted every 3–4 days.

Coculture of spheroids

A coculture of endothelial cell and fibroblast spheroids within one channel was performed, as shown in Figure 3(d). The reconfigurable microchannel was adjusted to be $500\text{ }\mu\text{m}$ wide throughout. The microchannel was coated with the blocking agent to prevent cell attachment. The channel was then filled with the culture medium, and the pin nearest the outlet was actuated to narrow the channel width to $50\text{ }\mu\text{m}$. A HUVEC spheroid was seeded and confined within a $600\text{ }\mu\text{m} \times 500\text{ }\mu\text{m}$ area defined by pins. An LF spheroid was then seeded and confined into a separate $600\text{ }\mu\text{m} \times 500\text{ }\mu\text{m}$ area. The device containing both the spheroids was incubated at $37\text{ }^{\circ}\text{C}$ in 5% CO_2 and observed every day using an inverted microscope. The incubating device was either kept static or vibrated by placing on a polypropylene box containing a 0.4 W DC motor (FA-130, Mabuchi Motor, Chiba, Japan) with a 0.6-g eccentric weight rotating at approximately 26 Hz .

RESULTS AND DISCUSSION

Flow resistance

We evaluated the dependence of flow resistance on the channel width by moving the side-walls. Generally, in microfluidic cell culture, a fast flow is needed to supply the fresh medium, quickly change culture conditions, and apply appropriate shear force to cells, while a slow flow keeps cells within an appropriate area in the channel and generates a concentration gradient of

test factors. Therefore, one advantage of a movable sidewall in a microfluidic channel is the ability to adjust flow resistance on demand to balance convection and diffusion in the channel.

Figure 4 shows the flow resistance in the 300- μm -wide reconfigurable microchannel containing a 300- μm -long narrowed region produced by displacing one pin. The relationship between the width of the narrowed region, w , and the flow resistance, R , calculated from changes in the droplet volume, was fitted from the experimental values as follows:

$$R = 3.699 \times 10^6 W^{-3} + 5.3397.$$

These measured flow resistances were slightly deviated from the theoretical formula of flow resistance for a channel with a rectangular cross-section³³

$$R = \frac{12\eta L}{H^3 W} \left[1 - \sum_{n,\text{odd}} \frac{192H}{(n\pi)^5 W} \tanh \frac{n\pi W}{2H} \right],$$

where η is the viscosity of water ($\sim 9 \times 10^{-4} \text{ Pa s}$ at 25°C), and L , H , and W are the channel length, height, and width, respectively. This difference between theoretical and measured resistance was due to the length of the narrowed region (300 μm), which was too small relative to the width and the height (both about 300 μm), while the nonuniform channel widths of the discretized sidewall may also have contributed.

The observed increase in flow resistance was inversely proportional to the cube of W , the distance between the end of the displaced pin and the fixed sidewall. A possible range of flow velocities with the reconfigurable microchannel would be around 1–10 000 $\mu\text{m/s}$ if 10 Pa of pressure differential was applied between the inlet and the outlet. This indicated that a simple operation, such as moving only one pin during cell handling, easily enabled reversible switching between introducing, settling, migrating, perfusing, or harvesting cells.

To take advantage of the microfluidic approach to cell culture over conventional cell culture surfaces, various microfluidic channels have been carefully designed to settle, grow, and maintain cells or cell aggregates in desired regions to ensure that they experience desired flow/diffusion conditions and are compatible with various biological microscopy methods. Typical microfluidic channels fabricated by micromachining or soft lithographic techniques have difficulties in settling cells or cell aggregates in place, continuing cultivation with perfusion and harvesting within one type of microchannel. In contrast, the reconfigurable microchannel can dynamically change its shape and flow resistance on demand by simple operation and can flexibly alter the flow rate as needed.

FIG. 4. Flow resistance of a reconfigurable microchannel with the channel segment width varied by displacing one pin. A 300- μm -long region of the 3-mm-long channel was narrowed relative to the remaining channel (set width of 400 μm) by displacing one pin. As the width narrowed, flow resistance increased in inverse proportion to the cube of the width. Measurements were performed three times under each condition and are presented as mean $\pm$ SD.

Cell patterning

Patterning cells in microfluidic channels has many applications, such as studying cell migration or cell–cell interactions and their effects on soluble factors. This technique also controls the number and position of cells, allowing researchers to follow and monitor all cell populations under the desired microfluidic conditions. Coculture of two or more types of cells in the same straight channel would be a preferable cell patterning method because all cell populations could be kept under the same stream, allowing the fluidic conditions experienced by all cell types to be easily understood and predicted. Therefore, we attempted to pattern one or two types of cells into segments of a microfluidic channel with a discretized sidewall.

Figure 5 shows segmented cell patterns within one straight microchannel obtained by operating the pins as shown in Figure 3. Multiple segments of COS-7 cells, a typical cell line (Figure 5(a)), were generated by closing the channel immediately after seeding. At 2 day and 4 day, most cells had not migrated from the area that had been originally exposed. This limited migration might be caused by the fibronectin coating, a cell attachment factor on the glass, being removed by the pin movement. Another example for cell patterning was the coculture of HUVECs and hLFs grown in one segment each (Figure 5(b)). Seeding HUVECs and hLFs from opposite inlet ports yielded a well-defined coculture without the different cell type mixing, as confirmed by observing segments containing only green fluorescence (hLFs), red fluorescence (HUVECs), and no fluorescence.

Several methods for patterning cells have been developed. Most involve prior patterning of cell adhesion-related molecules, while others use stencils,^{34,35} multi-layered laminar flows,^{36,37} and inkjet printing.³⁸ These methods generally require difficult handling of microfluidic channel features and involve labored cell seeding protocols or require materials for selective cell adhesion that provide uneven environments for the adhering cells. Furthermore, multiple branched channels or other microfluidic configurations for cell patterning remain, even after cell patterning is completed, and interfere with perfusion or other microfluidic operations that are important in cell culture. The movable walls of the reconfigurable microfluidic device can pattern cells by physically preventing them entering the channel and then retracting the barriers to form a straight channel for proceeding with long-term cultivation or other patterning. Moreover, cell density in the pattern, which is often unpredictable before introducing the cell suspension into the microchannel, can be adjusted by varying the cell density in microflows.

FIG. 5. Cell patterning in a reconfigurable microchannel with a movable discretized sidewall. (a) COS-7 cells patterned in a microchannel. The cells had adhered after 1 day, at which point all pins were retracted to open the channel. Pins that had already been retracted before 1 day were further retracted to examine the extent of cell migration into previously obstructed areas (indicated by white arrows). (b) Patterned coculture of human umbilical vein endothelial cells (HUVECs) and human lung fibroblasts (hLFs) in a reconfigurable microchannel. hLFs were stained with calcein AM (green fluorescent dye that stains live cells) before introducing into the microchannel, while a rhodamine-labeled lectin (red fluorescent dye that binds only to HUVECs) was added into the channels after both hLFs and HUVECs had been seeded.

Microfluidic cell culture with steady proliferation rates

We examined the cell growth of various densities in the reconfigurable microchannel, starting with confining a few cells in a small area insulated by the deformed sidewall and then continuing to support proliferating cells by gradually expanding the cell culture area. As shown in Figure 6, five COS-7 cells seeded into a confined segment in the reconfigurable microchannel grew to a larger population at steady exponential rates.

As shown in Figure 6(a), the growth of a small number of cells was supported in a small area, whereas a larger cell population was supported in an expanded area. The small and large cell culture areas were adjusted based on the cell count, successfully allowing cells to proliferate by minimizing stress caused by subculture and overconfluency. Cells are known to slow or stop proliferation at low cell densities. Therefore, configuring a small culture area successfully triggered proliferation. It should be noted that no cells remained at the channel inlet to support cell growth. At 9 day, the cells had proliferated but remained underconfluent approximately five times the original culture area.

Figure 6 shows that COS-7 cells continued proliferating in a gradually widening culture area. As shown in Figure 6(b), the growth curve (change in cell count over time) was exponential, referred to as a log phase. The mean doubling time of three seven-day culture experiments similar to Figure 6 was 39.3 ± 5.61 h ($N=3$, mean $\pm$ SD), while that of the same COS-7 cells in a 96-well plate was 53.4 ± 10.4 h ($N=3$, mean $\pm$ SD). The faster steady growth rate in the microfluidic culture was due to the smaller initial culture area and the adjustment of the cell culture area on days 2, 5, and 6 to make more room for actively proliferating cells. Maintaining a log phase for a long period would benefit cell health and experimental results obtained from the cells.

Maintaining underconfluent cell culture for a long period requires a low cell density (cell count per unit culture area) to be maintained and a decreasing cell density growth rate over time. Figure 6(b) shows that cell density was low at the start (14.0 cells/mm²) and gradually approached 100 cells/mm², a general benchmark for cell seeding density. The cell density exceeded 100 cells/mm² on day 7 of the culture. The doubling time varied within a range of 25.3 h– 36.4 h as the culture area expanded. The shortest doubling time during 47 h– 119 h may

FIG. 6. Progressive and continuous cell growth with variable cell culture areas in a reconfigurable microchannel. (a) COS-7 cell growth in a cell culture area confined by moving sidewalls. On the seeding day (0 day), only two cells were present in the microfluidic channel. Pins were actuated to form a small culture area confining the cells. The cell culture area was gradually expanded by moving the pins at 2, 6, and 7 days. (b) Growth curve and time evolution of the density of COS-7 cells confined in variable-size culture areas in the reconfigurable microchannels shown in (a). A growth curve of COS-7 cells cultured on immediately open microchannels is shown as a dotted line fitted to measured average cell counts ($N=3$, mean $\pm$ SD). Three vertical arrows denote the expansion of the cell culture area at 2, 6, and 7 days, respectively. In addition to cell count, cell densities are shown for the same culture areas, fitted individually with each exponential growth curve and used to estimate the local doubling time (t_d [h]) shown in the frames.

be due to the largest culture area expansion ratio at 47 h (see Figure 6(b)), which accelerated the cell culture growth.

Figure 6(b) also shows that the cell density growth curve of the entire open channel (i.e., no reconfiguration of the culture area) reaches 150 cells/mm² on Day 4, whereas that of gradually expanded culture was Day 8. This means that the reconfiguration of the cell culture area can extend the duration of cell culture at least four days. The comparison of the growth curve of cells on a gradually expanded microchannel area and that on a straight microfluidic channel showed that the expansion of the culture area enabled starting cell culture from a much smaller cell number and extending the duration of cell culture while ensuring an appropriate doubling time.

Cells condition their environment by producing factors needed for their normal growth, known as the paracrine effect. The role of the paracrine effect in cell health has been reviewed and examined using a microfluidic device that can control the cell seeding density.³⁹ The reconfigurable microfluidic device enabled the paracrine effect to be controlled for a longer cultivation period by adjusting cell seeding density and controlling the cell culture area using pin movement. Reconfiguring the channel also met the changing requirements for doubling time; a short doubling time is preferable when initiating cell culture at low densities, while a long doubling time becomes desirable to prevent congestion of the cell culture area and exhaustion of the medium in the microfluidic system.

Migration assay of cocultured spheroids

As movable sidewalls of the reconfigurable microchannel could be used to directly displace fluids, solids, and air bubbles without pressurization through the channel inlet, to demonstrate handling difficult-to-flow objects, we introduced different kinds of cell aggregates (spheroids) into a channel and achieved in-line coculture. Figure 7 shows that HUVEC and hLF spheroids were successfully introduced, set in place, and cultured in a reconfigurable microchannel for at least five days. As the size of a spheroid is 10–50 times larger than an individual cell, transporting and trapping spheroids, and generating flow around them, require higher flows than those required for cell manipulation. The reconfigurable microchannel successfully manipulated spheroids by generating displacement flow by moving the wall. Moving walls were also effective at settling spheroids in a desired area, insulating each spheroid and maintaining a small fluidic connection. Spheroid handling is an example of powerful and flexible displacement-mediated handling in the reconfigurable microchannel.

As shown in Figure 7(a), on day 2 of the static culture, both HUVEC and hLF spheroids approached each other, began to attach strongly to pins, and did not subsequently detach. Although it was possible that the MPC polymer, a cell repellent, had not coated the SUS316L stainless steel surface owing to weak adsorption, cells strongly attaching to the metal surface might be evident for the device having high compatibility with cell culture. In contrast, Figure 7(b) shows that spheroid attachment to pins was weakened by placing the microfluidic device on a box vibrating at a small displacement amplitude. Figure 7(c) shows that, under static conditions, both the HUVEC and hLF spheroids had a tendency to migrate toward the middle point of the segmented channel. In contrast, under vibration conditions, only hLF spheroids migrated with positive directionality. It was possible that both cell species responded to soluble attractant factors secreted from the other spheroid. However, vibration showed that hLFs migrated with stronger attachment to the surface compared to HUVECs. These results indicated that a static culture was better suited to evaluating migration with high sensitivity. Vibrating the device, however, would prevent spheroid adhesion to pins and facilitate a long-term culture of spheroids to be harvested from the microfluidic system for further investigation.

HUVECs and hLFs have been adopted in a microfluidic system to replicate vascular networks *in vitro*.⁴⁰ The hLFs induce *in vitro* lumen formation by secreting several extracellular matrix components.⁴¹ The migration assay indicated that coculture of hLFs and HUVECs might enhance *in vitro* angiogenesis and vasculogenesis at early stages (typically done within one week) by physically approaching together. However, mix-up of both cell species could affect

FIG. 7. Migration assay of cocultured single spheroids spaced in a reconfigurable microchannel. (a) Static coculture of HUVEC (left) and hLF (right) spheroids in a reconfigurable microchannel for 5 days. At 0 day, each spheroid was introduced to a different cultivation area. The cultivation areas were connected by a narrow channel segment defined by a pin that also acted as a wall. (b) HUVEC (left) and hLF (right) spheroids cocultured for 5 days on a vibrating table placed on a CO_2 incubator. (c) Migration vectors of spheroids presented as polar plots of data obtained in (a) and (b). The zero degree was defined by the direction from a spheroid to the middle point of the segmented channels (shown in (a)). Data are representative of three similar experiments.

vascular remodeling processes by invasion of fibroblasts to lumens.⁴² The reconfigurable microchannel could be applied to build coculture systems to study long-term vessel remodeling processes utilizing interactions between spheroids with controlled migration.

CONCLUSION

We developed a microfluidic channel containing a discretized sidewall comprising a row of miniature rectangular pins. No power was needed to maintain the shape of the channel sidewall, and it was easily changed by hand. An oil-seal-like structure and air vent channels successfully suppressed the adverse effects of capillary action at the gaps between pins. As a result, this microfluidic channel facilitated internal cell culture and achieved reconfigurability, allowing the spatiotemporal adjustment of the flow velocity and flow rate on demand. We successfully controlled flow resistance in the range of $10\text{--}100\,000\text{ Pa s}/\mu\text{l}$ by adjusting the channel width.

We applied this reconfigurable discretized sidewall to create cell patterns within single straight channels that were stable for at least three days and supported log phase growth by postponing confluent situations. Furthermore, we cocultured two different types of spheroids within one microchannel at a defined, but adjustable, distance. We observed the attractive migration of HUVEC and LF spheroids cultured in the same channel.

We have used a razor blade to manually drive the pin. A razor blade could make large friction to successfully grip the pin surface with relatively small force. However, precise and automated pin position control systems are needed to be developed in the near future. Possible future prototypes include a motorized pin manipulator with visual feedback or connecting pins to a miniature linear actuator array.

These systems can be utilized for simple cell culture and can introduce, manipulate, and harvest any difficult-to-flow objects within one microfluidic channel. These reconfigurable microfluidic devices could also accept various raw samples or bioproducts not yet conceived of by the authors. In the future, we expect this type of microfluidic channel to be used to interface typical microfluidic systems and the outside world.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for the measurement setup for flow rates (Figure S1) and diffusion into a region enclosed by pins (Figure S2).

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