

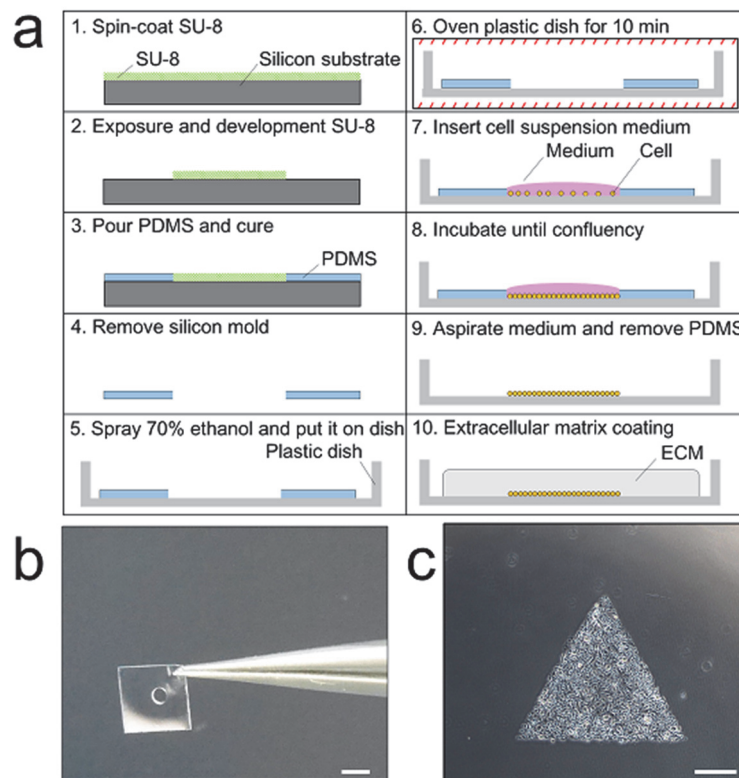
***In vitro–in silico* interface platform for spatiotemporal analysis of pattern formation in collective epithelial cells**

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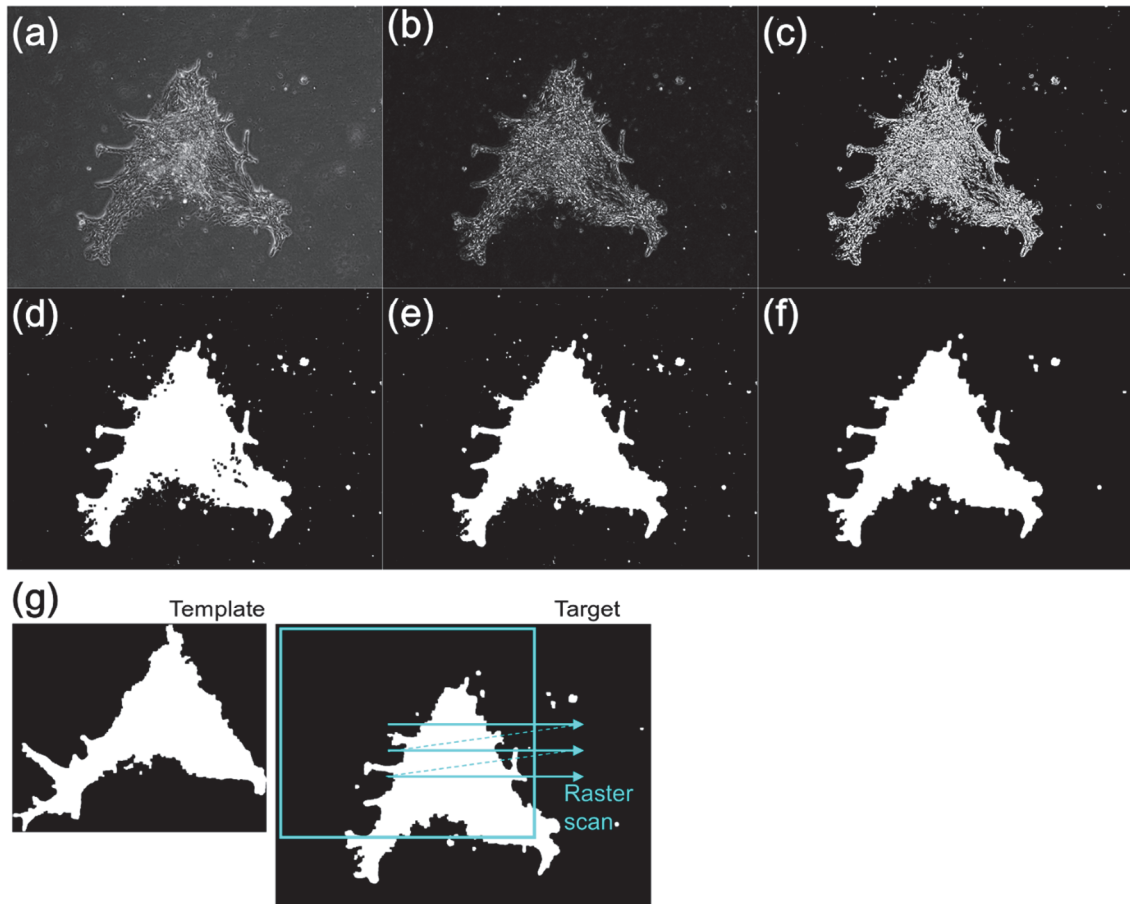
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Keywords: Collective cell migration, Pattern formation, Branching, Reaction-diffusion



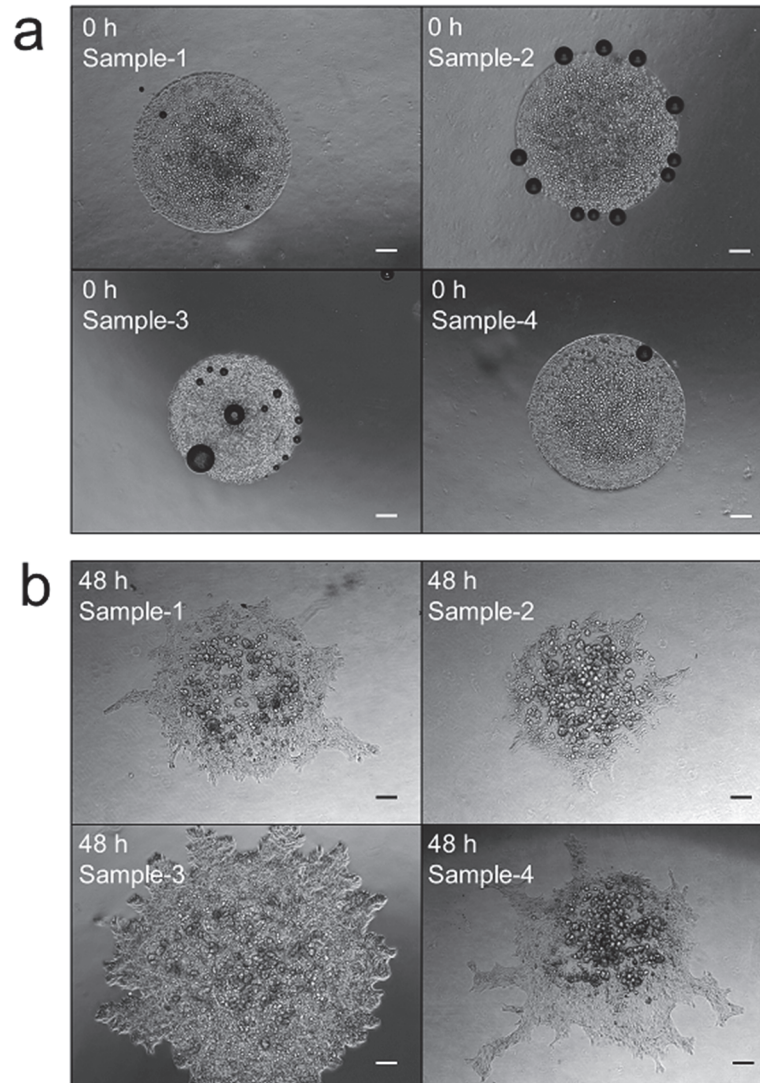
Supplementary Figure 1

Fabrication process to control initial cell conditions in 2D culture. (a) Fabrication flow. 1) SU-8 negative photoresist is spin-coated on the silicon substrate and 2) it is exposed and developed to produce a micropattern on the substrate. 3) PDMS is poured on the substrate and 4) produces the PDMS membrane with a micropatterned hole. 5) Ethanol is sprayed on the PDMS, which is placed on a dish. 6) After ethanol evaporation in an oven, the adhesion force increases. 7) Cell suspension medium is applied to the micropatterned hole and 8) incubated for cells to achieve confluency. 9) Excessive medium is aspirated and PDMS membrane is removed. 10) Cover cells with ECM to generate branching morphogenesis. (b) PDMS membrane with circular hole is generated. Scale bar, 2 mm. (c) Examples of initial cell conditions. Cell position and concentration were controlled. Scale bar, 200 μm .



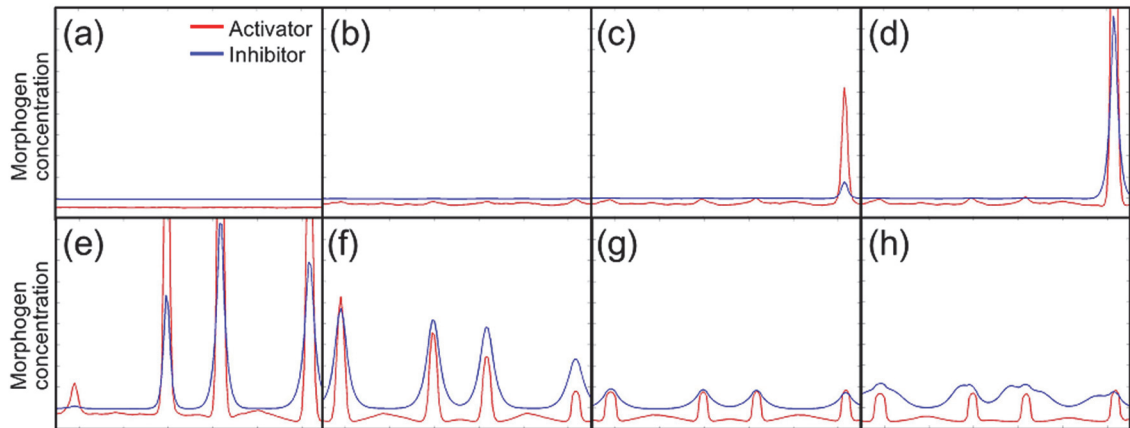
Supplementary Figure 2

Calculation method for branched pattern similarity. (a) Original gray scale image. (b) Edges of individual cells were detected to eliminate the lightening effect of the original image. (c) The image was changed to a binary image. (d) The edge line was dilated, followed by erosion. (e) The small holes were filled to identify the pattern area. (f) Erosion operation was conducted again, followed by dilation to reduce signal noise. (g) The template was moved to all areas of the target image with raster scan, and normalized cross correlation (R_{NCC}) was calculated at each position.



Supplementary Figure 3

Examples of branched pattern without initial culture control by microfabrication. A one-to-one mixture of cell suspension media (50,000 cells/ μl) and Matrigel was placed with 0.5 μl of the mixture on a dish. After 5-minute incubation, the extra Matrigel was covered. (a) The initial geometries of collective cells were all circular patterns even without microfabrication, but size and concentration varied. (b) The variations in initial cell conditions were amplified after 48 hours and the repeatability of the branched pattern decreased. Scale bars, 200 μm .



Supplementary Figure 4

1D simulation of morphogen dynamics at the edge of the collective cell boundary aligned with the straight line (detailed dynamics in Fig 5(e)). (a) The activator and inhibitor concentrations were both uniform at the start. (b) Inherent fluctuation in cell viability was enhanced by the autocatalytic function of the activator. (c) The positive feedback of the activator gave rise to a steep peak of activator concentration and at this point branching started developing. (d) The inhibitor was also augmented by high activator concentration. (e) The diffusion rate of the inhibitor was much higher than the diffusion rate of the activator and it decreased the activator concentration near the peak area. As a result, branches could not be generated near a generated branch. (f) Morphogen concentration has peaks with keeping certain space. (g) After branches were generated, the morphogen concentration started stabilizing. (h) Secondary and even tertiary branches from the initial branches generated occupied low inhibitor concentration areas.

Supplementary Video 1

Time-lapse imaging of NHBE culturing on a dish. Case 1: NHBE cells were placed at a high concentration gradient without any ECM. Case 2: NHBE cells were homogeneously distributed with ECM. Case 3: NHBE cells were placed with a high concentration gradient and covered by ECM. Under this conditions, NHBE formed 2D branch pattern on a dish.

Supplementary Video 2

Time-lapse imaging of branching morphogenesis in vitro, starting from the triangle shape. Cells migrated to the tips of the triangle shape at the beginning, and there were lesser number of cells at the center of the initial triangle shape. Then, the cells reconstructed the triangle shape and developed branches at tips

Supplementary Video 3

Simulated dynamics of biomarker Y value by RD model. The Y value was higher at the tips and the edge of the triangle at the beginning, which indicates that cells move to the edge at first. Then, the triangle center became higher and branching was initiated from tips.

Supplementary Video 4

Simulated dynamics of biomarker Y value by RD model when cells are placed without ECM. The differences in diffusion rates of the activator and inhibitor are too small to generate branches ($D_A = 0.19$, $D_H = 0.20$).

Supplementary Video 5

Simulated dynamics of morphogens by RD model when cells placed at a high-concentration gradient are covered by the ECM. Under this circumstance, branches could be generated ($D_A = 0.02$, $D_H = 0.18$). Activator concentration A, inhibitor concentration H, and biomarker Y value were overlapped to show the morphogen interactions.

Supplementary Video 6

Simulated morphogen dynamics by RD model starting from a long straight line. Morphogens secreted by cells diffused in the perpendicular direction since molecules diffuse more through a high-gradient path so that the cells grow in a perpendicular direction. Activator concentration A, inhibitor concentration H, and biomarker Y value were overlapped to show the morphogen interactions.

Supplementary Video 7

1D simulation of morphogen dynamics at the edge of the collective cell boundary aligned with the straight line. Inherent fluctuation of cell activity was enhanced by autocatalytic function of the activator to generate a steep activator concentration peak. The inhibitor was also amplified by the activator at the peak point; the activator concentration near the peak point was reduced due to long-range inhibition to regulate branching.

Supplementary Video 8

Activator and inhibitor concentration dynamics with corresponding biomarker Y value when cells were set in a triangle shape. Initially, inhibitor concentration was higher at the center of triangle but relatively lower at the tips of the triangles. The effect of activator then relatively increased to generate branches at the tips