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Cell Adhesion Control Using Nanostructure Fabricated on Polymer Surface

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We studied the adhesion control of cells cultured on nanometer-scale patterned poly (dimethylsiloxane) (PDMS) structures with various shapes. Although cell cultures on microstructures and nanostructures with random shapes have been studied so far, cell cultivation on well-defined nanopatterned PDMS has not been reported. PDMS was molded into stripes and pillars with nanometer- or micrometer-scale widths using a patterned glass template. Human stomach cancer cells (Kato-III) were cultured on the nanopatterned PDMS sheets. The cells adhered to the nanometer-scale stripe and became elongated as structures. They extended along the direction of the nanostructures. When the surface was thickly coated with a protein (*e.g.*, extracellular matrix (ECM) gel), cell extension was not observed. Cultured cells selectively recognized the direction of the nanostructure. The findings in this work could lead to future developments in whole-cell-based biosensors.

1. Introduction

A variety of biosensors have been developed using biological elements (e.g., antibodies, enzymes, and nucleic acids) with a physicochemical component.[1,2] Recent studies using a whole cell as a new biological element have been applied to environmental analysis,[3] neural networks,[4] and drug screening.[5] Moreover, there is a

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growing interest in the development of micro-total-analysis systems (μTAS)[6,7] utilizing whole cells for biological assay,[8] drug screening,[9,10] and quantitative biology.[11] Generally, on the cell-based μTAS the following are integrated: (a) a microfluidic chip containing microchambers and channels for liquid handling; (b) living cells located inside the microchambers, which sense and respond to target substances; (c) a transducer converting the signal into electronic information for further processing.

To maintain sufficient cell activity on the sensors, control of the cell adhesion is very important, because the cell function is regulated by signals produced by cell adhesion. To control cell adhesion on the target surface, micrometer- and nanometer-scale patterns have been developed with chemical and biochemical surface modifications to control protein and cell adsorption. In many cases, the physical adsorption of extracellular matrix (ECM) proteins is used for the selective attachment and patterning of adherent mammalian cells. Cell patterning in a particular region in the microchannel without ECM coating has not been realized.

In this study, we fabricated nanometer-scale structures on a polymer surface to achieve the control of cell adhesion without any coating materials. Maheshwari *et al.* has reported[12] that cell adhesion depend on the clustering density of contact molecules. The nanometer-scale structure might determine the chance of the focal contact and regulating cell adhesion. The nanometer-scale structures in this study might be suitable for cell adhesion and extension. If the control of cell adhesion and extension by regulating of the structure scale were achieved, easy handling of cell patterning and high performance biosensor could realize.

2. Experimental

2.1 *Reagents and materials*

Poly(dimethylsiloxane) (PDMS) elastomer (Sylpot 184) was purchased from Dow Corning Asia (Tokyo, Japan). Fibronectin and laminin from mice were purchased from Asahi Techno Glass (Tokyo, Japan). ECM gel was purchased from Sigma-Aldrich (MO). Phosphate-buffered saline (PBS) was purchased from Invitrogen Japan K. K. (Tokyo, Japan). Stomach cancer cells (Kato-III) were obtained from the Institute of Development, Aging, and Cancer, Tohoku University (Miyagi, Japan).

2.2 *Nano- and microfabrication*

A schematic image of the fabrication of nano- or micrometer-scale structures is shown in Fig. 1. Nano- or micrometer-scale structures were fabricated on glass substrates by electron-beam lithography and reactive ion etching. We successfully fabricated nano- (210 to 1070 nm) and micro- (3 to 9 μm) stripes and dots. The fabricated substrates were used as a nano- or micromold. A PDMS prepolymer was cast on the patterned mold.[13] After heating (70°C, 1 h) and peeled from the mold, the PDMS sheet was cut to a moderate size.

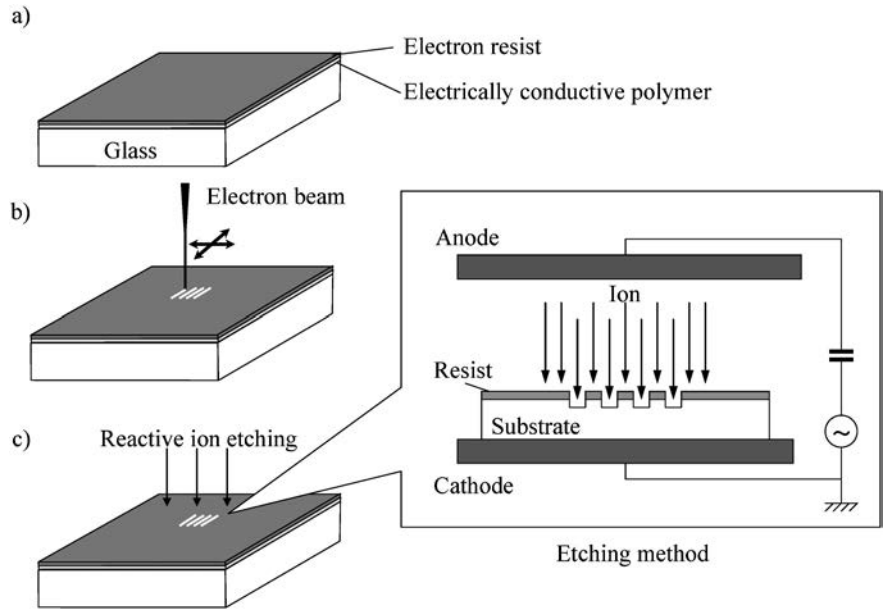


Fig. 1. Schematic image of the fabrication of nano- or micrometer-scale structures.

2.3 *Cell culture*

A PDMS sheet containing the nanometer- or micrometer-scale structure, which was a transparent film, was washed with PBS and coated with/without 1 μg/ml fibronectin, laminin, or ECM gel in a PBS set in a 6-well plate. Kato-III was cultured in the well at 37°C with 5% CO_2 in an RPMI-1640 medium supplemented with penicillin, streptomycin, and 10% fetal bovine serum. The standard morphology of most of the cells is round and myeloma-like, while a few cells are epithelial-like and spindle-shaped. (Fig. 2)

2.4 *Analysis of cells*

A morphological observation was performed using an inverted microscope, IX71 (Olympus, Japan), equipped with a CCD camera (C-7070 WZ; Olympus, Japan). The placement and number of cells on each structure were observed using the microscope with a ×10 objective lens as described above. The cell morphology was classified into three types by measurement of the cell dimensions, as shown in Fig. 3. We defined the lengths along and across the stripe direction as A and B, respectively. The counted cells were then classified by calculating $a = A / B$: $a>1$: parallel ellipse, $a=1$: globular, $a<1$: perpendicular ellipse. Most cells were globular and only epithelial-like cells were classified as ellipses.

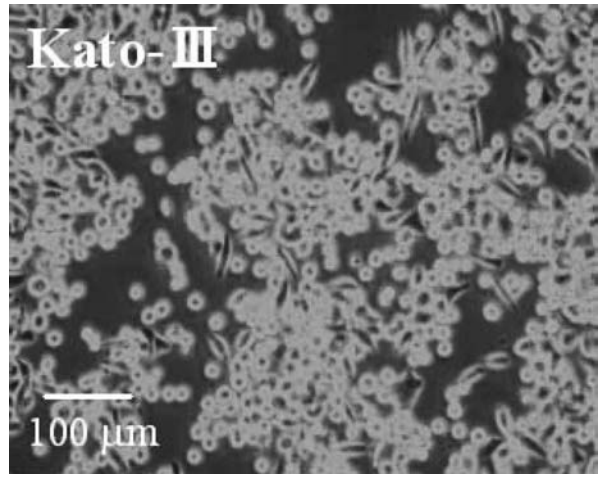
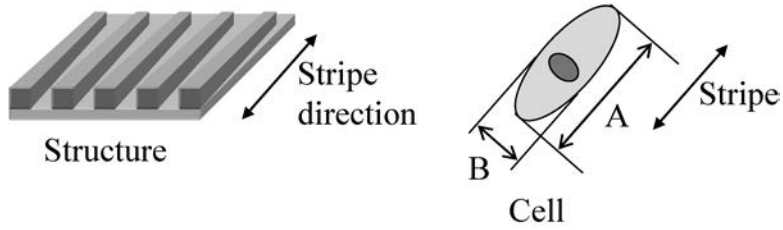


Fig. 2. Micrographs of Kato-III cells on a conventional plate dish.



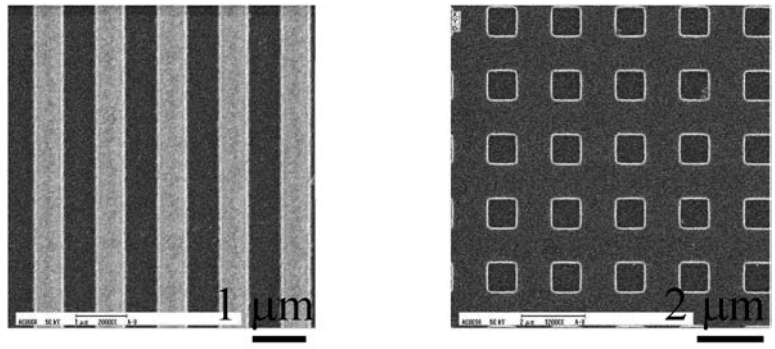
a=A／B
a＞1 : Parallel ellipse
a=1 : Globular
a＜1 : Perpendicular ellipse

Fig. 3. Analysis of cell morphology on the patterned surface.

3. Results and Discussion

3.1 *Characterization of the structures*

We first evaluated the structures fabricated on the glass substrate. Representative scanning electron microscope (SEM) images of (a) stripes and (b) dots on the glass substrate are presented in Fig. 4. The nanometer-scale stripes were structured with five patterns with the following widths and intervals between adjacent stripes, respectively: (a) 210 and 240 nm, (b) 400 and 470 nm, (c) 660 and 590 nm, (d) 890 and 1050 nm, and



(a) (b)

Fig. 4. SEM views of a nanometer-scale patterned surface. (a) shows a stripe structure and (b) shows a dot structure.

(e) 1070 and 1350 nm. Nanometer-scale dot shapes were also structured with 550 nm widths and 850 nm intervals. Micrometer-scale stripe patterns were structured with 3 to 9 μm widths and intervals, and dots were also structured with 6 μm widths and 6 μm intervals. The depth of all structures was 250 nm.

3.2 *Quantification of cell adhesion*

To investigate the cell adhesion on the patterned-polymer structures, the cell morphology was observed using the inverted microscope. Figure 5(a) shows typical micrographs of the Kato-III cells on a nanometer-scale stripe (240–1350 nm widths) without coating on day 3 of the culture. Individual cells attached to the nanopatterned surface preferentially, and some cells on the nanometer-scale stripe extended along the stripe direction, as shown in Figs. 5(b) and 5(c). For the nanometer-scale pillar, although the cells were attached to the surface, only a few cells were elongated. The result that few cells were attached to the nonpatterned PDMS surface showed that the nanometer-scale structure on PDMS realized the control of cell adhesion on the surface.

Figure 6 shows the statistical results of the cell extension on nanometer-scale stripes (590 nm width), pillar (850 nm width) and micrometer- scale stripes (5 μm width). The cells extending in the parallel and perpendicular directions were counted. Cell elongation along the stripe direction was only observed in the nanometer-scale stripe. Therefore, a nanometer-scale stripe seems to induce elongation and alignment of the cells.

Variations in cell shape were quantified in terms of the stripe width in Fig. 7. The change in the cell morphology became more pronounced as the line width increased, whereas the cells elongated in the stripe direction for all cases.

Moreover, the variations in the cell shape were also quantified for various surface conditions on the stripe structures (590 nm width) as follows: noncoated PDMS surface or laminin-, ECM gel-, or fibronectin-coated surfaces (Fig. 8). The perpendicular

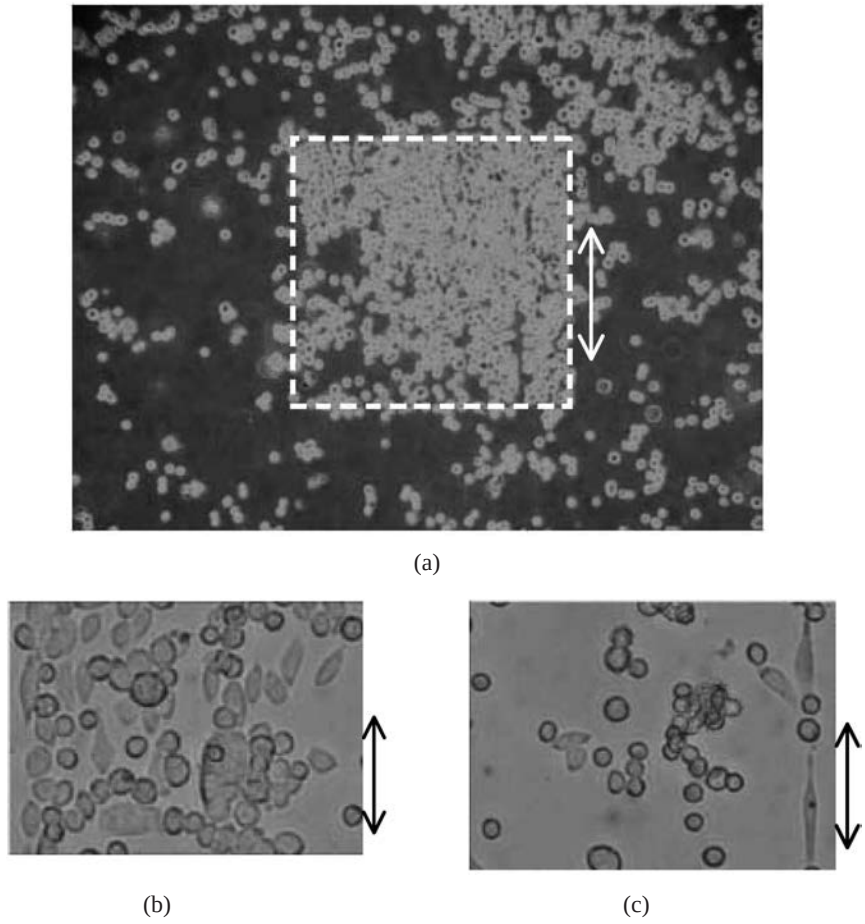


Fig. 5. Typical micrographs of the Kato-III cells on a nanometer-scale stripe (within the dotted line) without coating on day 3 of culture (a), and representative images of elongated cells (b, c). The arrow indicates the stripe direction.

ellipse cell (a<1) was not observed on the structures with fibronectine coating. When the surface was coated with laminin, the cells tended to extend in the stripe direction, similarly to the case of the noncoated PDMS surface, whereas these phenomena were not observed in the cases of ECM gel and fibronectin. These results indicated that a thick coating of ECM proteins might bury the nanostructure entirely. Using a noncoated nanometer-scale structure is a possible approach to achieve cell adhesion control.

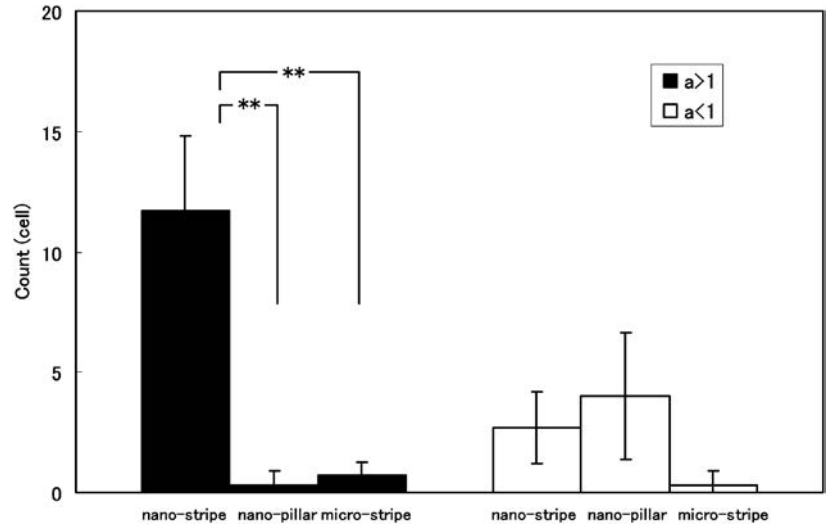


Fig. 6. Statistical results of the cell extension as a structural variation on a nanometer-scale stripes, pillar and micrometer-scale stripes.

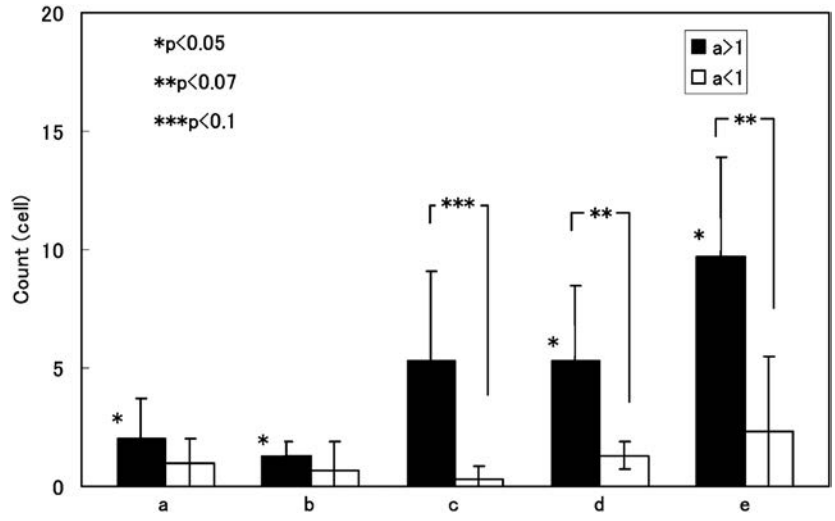


Fig. 7. Quantification of cell-shape variations in terms of the stripe width. (a to e) The stripes were structured in five patterns with the following widths: (a) 240 nm, (b) 470 nm, (c) 590 nm, (d) 1050 nm, and (e) 1350 nm.

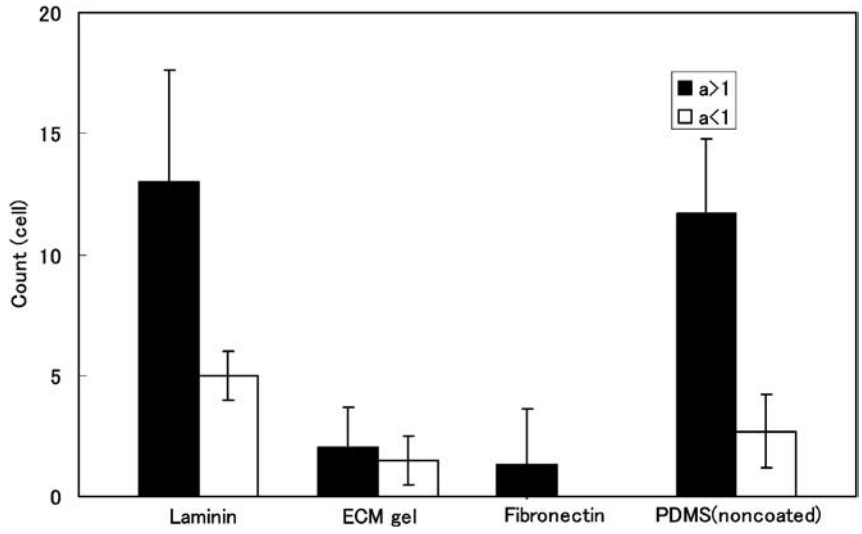


Fig. 8. Quantification of cell-shape variations for various surface conditions on the stripe structures.

4. Conclusions

We studied the interaction between a cultured cell and a patterned PDMS structure. We first developed a nanostructure scaled at more than 200 nm on a glass template and molded it onto a PDMS sheet. Adhesive cancer cells were then cultured on the patterned PDMS sheet. The cells selectively adhered to the patterned structure and became elongated along the stripe direction. Moreover, the cells extended on the noncoated patterned surface of the nanometer-scale stripe as much as they did on a surface coated with an ECM protein. Therefore, a nanometer-scale structure fabricated on a polymer surface can be used for the control of cell adhesion in optional positions and will be useful for the development of whole-cell-based biosensors.

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