A Bio-Inspired Cell-Microsystem to Manipulate and Detect Living Cells

Jung-Min Lim*, Sang-Won Byun*, Tai-Hyun Park, Jong-Mo Seo***, Young-Suk Yoo***, Hum Chung*** and Dong-il "Dan" Cho***

Abstract - In this study, we demonstrate for the first time a bio-inspired Cell-Microsystem to manipulate and detect living cells. Cultured retinal pigment epithelial cell line (ARPE-19) was directed to grow in a pre-defined Cell-Microsystem. The three-dimensional micropillars of 5 μm in height and diameter of the Cell-Microsystem were fabricated. Inhibited DNA synthesis and transformed cell morphology were observed throughout the culture period. The demonstration of manipulating and detecting living cells by the surface topography is a new approach, and it will be very useful for the future design of cell-based biosensors and bioactuators.

Keywords: Cell Morphology, DNA Synthesis, Micropillar, Microsystem, Retinal Pigment Epithelial Cells.

1. Introduction

Recently, innovative microfabrication and microfluidics technologies are beginning to transform the medical diagnostic field, allowing multiple chemical reactions to take place on small plastic microchips [1-3]. Previous studies have indicated that microfabrication technology is essential not only for the construction of a micro total analysis system but also for the investigation of fundamental cell biology [4-5]. With scientific requirements firmly established, we now need a robust infusion of cell-based biosensors and bioactuators for health, environment, and defense applications [6-8]. However, no previous studies have delved into the Bio-Inspired Cell-Microsystem to directly manipulate and detect living cells [9-10]. In this paper, to aid in the investigation of cell-based microchips, we will show aspects of manipulated living cell response in a Bio-Inspired Cell-Microsystem.

2. Design and Fabrication

To illustrate this concept, we designed a micropillar structure of disrupting cell-substrate contact area (focal contact) to manipulate living cells. A schematic diagram of Cell-Microsystems and SEM of 3-dimensional micropillars composed of polydimethylsiloxane (PDMS) substrates are shown in Fig. 1.

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3. Methods

3.1 Fabrication process

The micropatterned PDMS specimens were composed by an impression of a PDMS cast on the wafers in collaboration with the Digital BioTechnology Corp. (Seoul, Korea).

3.2 Cell culture and preparation of co-culture

The human retinal pigment epithelial cell line (CRL 2303; ARPE-19) was obtained from the ATCC collection at passage 10. The cells were maintained in a 1:1 mixture of Dulbecco's modified Eagles medium and Ham's F12 medium (DMEM/F12, Gibco) with HEPES buffer containing 10% fetal bovine serum (Gibco, Gaithersburg, MD), a 56 mM final concentration of sodium bicarbonate (Sigma, St. Louis, MO) and 2 mM L-glutamine (Gibco), and incubated at 37℃ in 5% CO_2. Passages up to passage 20 were used for the experiments. No changes in the growth characteristics or the morphology were observed during this time. The cells in the routine culture had the typical appearance of an epithelial, which exhibit morphological and functional polarity.

The cells were harvested from a routine culture and seeded on PDMS substrates in the full growth medium, which included serum proteins. The samples were either uncoated or pre-adsorbed with human fibronectin for a minimum of 30 min at 37℃, then rinsed 5 times with the tissue culture medium. The bulk fibronectin concentration was 5 μg/mL at 3.2 mL/well, corresponding to 1.3 μg of fibronectin per square centimeter. Fluorescence microscopy

was performed using an Olympus IX 51 microscope.

3.3 Scanning Electron Microscopy

The three-dimensional pillar PDMS surfaces were examined prior to the cell culture by scanning electron microscopy (SEM, XL30FEG (Phillips)). SEM was used to evaluate the microstructures at an accelerating voltage of 7kV.

3.4 Cell Cycle Analysis

ARPE-19 cells were plated at a density of 1×10^5 cells/cm^2 in 1% fetal bovine serum containing medium on tissue culture plates (TCP). For cell cycle synchronization, cells were incubated in serum free medium for 16 hours and were then incubated for 2 hours with the specific inhibitor of DNA polymerase aphidicolin (20 μM) (Sigma). The cells were seeded on smooth and micropatterned PDMS in a 60-mm dish and incubated for 1 day as described above. The cells were then washed briefly in PBS by centrifugation and re-suspended. A 1/10 volume of BrdU labeling solution (Roche, Mannheim, Germany) was added to the culture medium in which the cells were growing. The cells were incubated for 30-60 min at 37℃ in a humidified atmosphere (5% CO_2). The optimum incubation time in the presence of BrdU (labeling period) (Roche) was selected for each individual experiment. The labeling culture medium was removed by either aspiration or tapping. The cells were washed 3 times in PBS.

In order to fix the cells and perform immunostaining, the following procedure was adopted (In Situ Cell Proliferation Kit, Roche): The cells were washed in 0.5 ml PBS and incubated with 5 ml of the fixative. The cells were incubated for 30 min at 4℃ and were re-suspended in a 500 μl HCl-denaturation solution (Roche) and incubated for 10-20 min at RT. After denaturation, 2 ml PBS was added and washed for 10 min. In order to block the unspecific binding, the cells were incubated with a 500 μl incubation buffer for 10 min at RT. The cells were incubated in 50 ml of an anti-BrdU-FLUOS antibody working solution (Roche). The cells were incubated for 45 min at 37℃ in a humid chamber, washed twice in PBS and then re-suspended in 0.5 - 1 ml PBS. The resulting samples were subsequently analyzed by fluorescent microscopy (488 nm for excitation and a 515 nm bandpass filter for detection).

3.5 Immunocytochemistry

The cells were first rinsed in a 1M phosphate-buffered saline (PBS) solution at pH 7.4. A 20 minute fixation step in 4% paraformaldehyde (Sigma) in PBS was followed by further washing in PBS. The cells were then blocked with 2% bovine serum albumin (Gibco) in PBS for 45 min at 37℃. A 1:100 dilution of the primary antibodies (vinculin; mouse monoclonal anti-human vinculin, Sigma) was incubated overnight at 4℃. The samples were then rinsed with PBS and incubated for 1 h and 20 min at 4℃ in Texas Red-conjugated goat anti-mouse secondary antibodies (Molecular Probe, Eugene, OR) and Rhodamine-Phalloidin (Molecular Probe). The control samples were treated in the same way except that the primary antibodies were omitted. After washing several times in PBS, the cells were mounted on glass slides, the cover slips were sealed at the edges, and the cells were examined using an Olympus IX51 fluorescence microscope.

4. Results and Discussions

4.1 SEM of the Patterned Microstructure PDMS Surfaces

A surface inspection by SEM demonstrated that the pattern of micropatterned PDMS surfaces showed no significant defects or irregularities (Fig. 1C). The micropatterned PDMS surfaces did not exhibit a significant geometrical variation with respect to the Si master. However, there were occasional minor defects at the tip of the pillar, which might have been induced during the release of the PDMS from the Si wafer. The circular pillars were similar to the specified dimensions (5 μm).

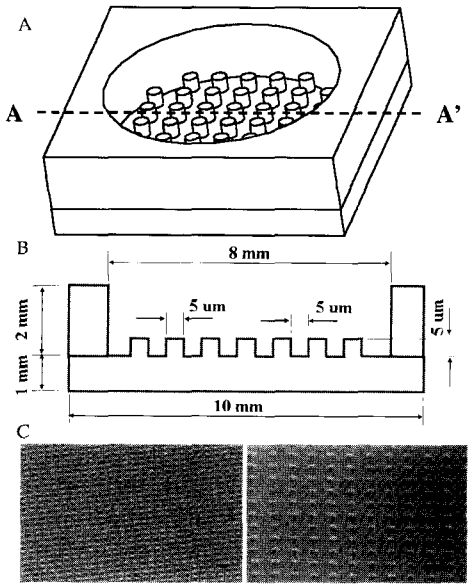

Fig. 1 Schematic diagram of a Cell-Microsystem. (a) Overhead view (b) Cross-section view (c) SEM pictures of micropatterned surfaces (Bar = 10 μm).

4.2 Cell-substrate Contact Area as a Regulator of Focal Contact

By labeling cells for vinculin, the intimate association of cell-to-substrate focal contact was demonstrated. Arrowheads denote vinculin at focal contact assembly (Fig. 2E). In contrast, the immunostained pattern of vinculin on the micropatterned surfaces showed less apparent distribution (Fig. 2F). These results clearly indicated that a disturbed early focal contact on the micropatterned surfaces gave way to a less distinct and diffused distribution of vinculin and F-actin compared to the cells on the smooth surfaces. The corresponding phase contrast and nucleus images were shown in Fig. 2A-D.

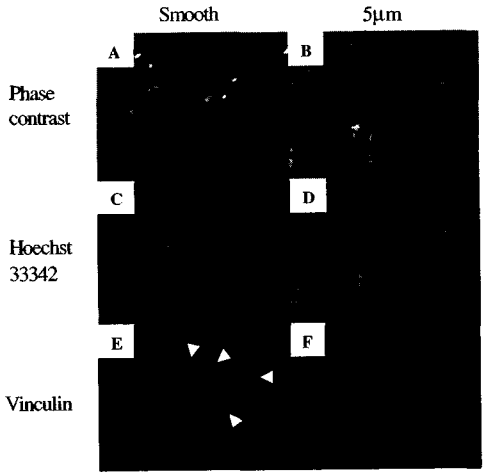

Fig. 2 Micropillar structure in a Cell-Microsystem disrupted the early focal contact formation (arrow head) of ARPE-19 cells. The phase contrast (A, B) and immunostained images of Hoechst 33342 (C, D: nucleus) and vinculin (E, F: focal contact) are shown.

4.3 Cell cycle analysis

We have synchronized ARPE-19 by the treatment of aphidicolin. The use of BrdU enabled the cell cycle assessed by the nucleus to be measured readily either on the smooth or micropatterned PDMS surfaces. RPE cell cycle progression was assessed after one day of culture. As shown in Fig. 3B, significant cell cycle inhibition was observed between the smooth and the micropatterned PDMS surfaces. Also, cells were stained with Hoechst 33342 as described in Methods.

4.4 Cell morphology

Finally, in order to realize the effect of micropatterned substrates on cell phenotype, we cultured ARPE-19 cells over three weeks. To characterize cell morphology, actin stress fibers were visualized by rhodamine-phalloidin. Overall, the integrity of the actin on the micropatterned surfaces was disrupted throughout the culture period. Cells on the smooth surfaces were well spread (Fig. 4A), whereas cells on the micropatterned were mostly disturbed (Fig. 4C). Cells were co-stained with Hoechst 33342.

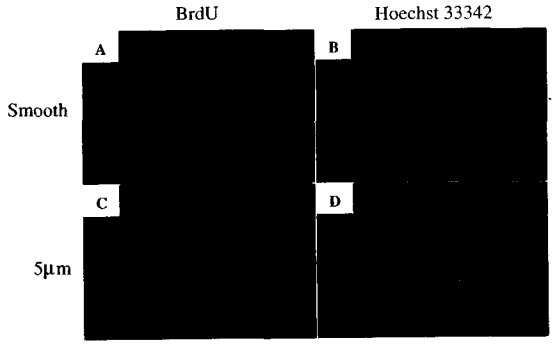

Fig. 3 Inhibited DNA synthesis as detected by BrdU labeling in Cell-Microsystem. BrdU labeled cells of synchronized ARPE-19 on the (A) smooth and (C) 5 μm micropillar in a Cell-Microsystem. The nucleus was detected by Hoechst 33342.

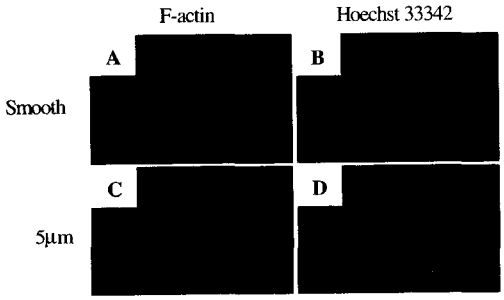

Fig. 4 Micropillar transformed cell phenotype in a Cell-Microsystem as detected by immunostaining with Rhodamine-Phalloidin (F-actin). The nucleus was detected by Hoechst 33342.

5. Conclusions

The data presented in this study support the hypothesis that the amount of surface area with which the cell is in contact alters the cellular responses in cultured ARPE-19 cells. The concept of modifying the cell-substrate contact area was implemented by the microfabrication of PDMS surfaces. Inhibited cell cycle and disturbed morphology of ARPE-19 were observed in the presence of a disturbed early focal contact.

The detailed investigation of RPE on a Cell-Microsystem can be used to manipulate RPE in such a manner that the desired cell responses can be obtained on

tissue engineering at the molecular level [11-12]. The experimental system of this study may also find use in applied cell culture including the development of supports for the substrates of tissue engineering [13-14].

The bio-inspired Cell-Microsystem provides a unique way to manipulate and probe living cells. As these approaches continue to advance, the process of integrating cells and microchips will provide new insights into how cells can be manipulated in microchips for biosensor and bioactuator applications.

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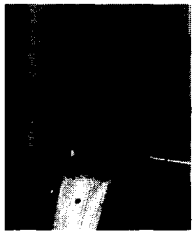

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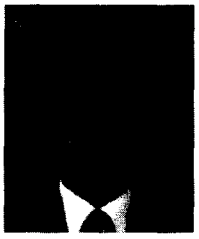

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