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Effect of bi-directional microfabricated topographical cues on cellular behavior of mammalian cell line

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ABSTRACT

The study of cell migration is valuable for the understanding of various physiological and pathological processes. Microgrooved/microridged substrates have been intensively studied to reveal the effect of single topographical cues on cellular behavior. However, cells in vivo are usually surrounded by multidirectional topography signals. This study investigated the effect of bi-directional topographical cues on cellular behavior of mammalian cells. In this study, bi-directional pit patterned poly(dimethylsiloxane) (PDMS) substrates were fabricated with different sizes but same depth (1 μm). The fabricated patterns were then used to study cellular response to bi-directional cues of mammalian cell line, in this case HeLa cells. After seeding cells for 48 h, the PDMS substrates were examined by SEM. Cell alignment angle and aspect ratio were measured from the SEM images. The results show that the cells on the patterned substrates with square pits did not align along either *x*- or *y*-axis. However the patterns with rectangular pits could promote cell alignment along the longer sides of pits. Moreover, comparing to the cell elongation on the control flat substrate, the elongation of cells was decreased on all the patterned substrates. These results are helpful for a deeper understanding of the mechanism of bi-directional topographical cues on cellular behavior of mammalian cell line.

1. Introduction

Cells in human body are stimulated by the surrounded extracellular matrix (ECM) and they tend to migrate toward the more favorable orientation [1]. The study of cell migration is required for the understanding of various biological processes including embryonic development, tissue regeneration, and immune response [2]. In addition, the knowledge of cell migration is also important for tissue engineering in various medical applications including medical implants and pharmaceuticals [3]. The in vivo cell migration can be affected by various factors, such as chemoattractants, temperature, topography, and mechanical and tensile properties of ECM [3]. Among all these factors, the topographical property is spatially confined, visible, and stable. The pioneered work of Paul Weiss in 1958 reported the phenomena 'contact guidance' that cell migration responded to the underlying topography on micrometer and sub-micrometer scale [4,5]. Thus cell alignment can be guided by structures that mimic extracellular matrix (ECM) processes [6]. A recent study attributed contact guidance to cellular signaling processes [7]. Contact guidance is an essential regulator in cell migration for individual or groups of cells [8,9]. Consequently, the study of the effect of topographical cues on cell migration is critical for developing the fundamental knowledge of in vivo cell behavior.

Nowadays, different studies using artificial nano- or microstructured surfaces have been conducted to reveal the effect of topographical signals on cellular behavior. Among these structures, nano- or microgrooved/microridged surfaces are being used intensively for studies. They have been shown to exhibit great influences on cellular contact guidance of mammalian cells [10–12]. Studies of nanogratings reported that various cell types (endothelial progenitor cells (EPCs), bovine aortic endothelial cells (BAECs), human embryonic stem cells (HESCs), human mesenchymal stem cells (HMSCs), human embryonic kidney cells (HEK-293), and human foreskin fibroblasts) aligned and elongated in the direction of the grating axis [13–18]. The investigation of poly (dimethylsiloxane) (PDMS) substrates engraved with nano- and

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microgrooves showed the alignment and elongation of BAECs along the groove directions [19]. Microgrooves on polystyrene substrates also have been reported to cause rat dermal fibroblasts (RDF) to align along the groove direction [20].

These previous studies were limited to stimulate cells along a single direction [3,21,22]. However, cells in vivo are subject to complex three-dimensional topographical features of ECM which cannot be fully represented by the single directional patterns [10]. It was recently found that bi-directional stimuli studies are important in understanding the fundamental mechanisms of how topography signals influence cellular behavior in vivo [21,22]. In this work, pit patterned PDMS substrates were fabricated and used to study cellular response of mammalian cell line, in this case HeLa cells. The size of each pit was smaller than a single cell. As a result, each cell could simultaneously experience topographical stimulation in two directions. This paper aimed to investigate the effect of bi-directional topographical cues on migration of HeLa cells.

2. Materials and methods for experiment

2.1. Fabrication of pit patterned PDMS substrates

In this study, PDMS substrates with pit patterns were fabricated by photolithography technology. The pits were separated by intersecting microridges and the patterns were defined by $R_X \times R_Y \times P_X \times P_Y$ as illustrated in Fig. 1(A). Seven types of pit pattern with the same depth (1 μm) were fabricated: $10 \times 10 \times 10 \times 10$, $5 \times 5 \times 5 \times 5$, $10 \times 10 \times 5 \times 5$, $5 \times 10 \times 5 \times 5$, $5 \times 5 \times 10 \times 5$, $5 \times 10 \times 10 \times 5$, and $5 \times 10 \times 5 \times 10$. All the units are micrometer (μm). In addition, a flat PDMS substrate was fabricated as control.

A simplified schematic diagram of the fabrication process is shown in Fig. 1(B). The pre-cleaned silicon wafers were spin coated with photoresist 5214E (AZ Electronic Materials). Then the photoresist was soft baked on a hotplate. In this study, photolithography was performed by SF-100 Xcel Platform (Intelligent Micro Patterning LL). The inverse patterns drawn by software AutoCAD (AutoDesk) were loaded into the system as image masks. After exposure and developing by AZ400K (AZ Electronic Materials), the inverse patterns were transferred to the photoresist layers. The resulting inverse patterned photoresist layers were then used as mask molds to fabricate the PDMS molds by casting. The PDMS molds, together with the master molds, were baked at 90 °C for 165 min. After cooling down, the PDMS molds were peeled off and used as the cell culturing substrates with pit patterns on the surface. One flat PDMS substrate was also fabricated by the same method using a pre-cleaned silicon wafer without photoresist.

2.2. Cell seeding

All the PDMS substrates were fixed into a 24-well cell culture cluster (Costar) and sterilized in 75% ethanol (EMSURE) for 1 h at room temperature. Afterwards, the ethanol was discarded and the substrates were rinsed with phosphate buffered saline (PBS, Gibco) to wash away residual ethanol. HeLa cells (American Type Culture Collection) at passage 16 were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% Fetal Bovine Serum (FBS, Gibco) and 1.1% Penicillin Streptomycin (Gibco), at 37 °C and 5% CO_2. In this experiment, HeLa cells were dissociated with 0.25% Trypsin–EDTA solution (Gibco) at room temperature for 5 min and seeded onto the PDMS substrates with a concentration of about 1×10^4 cells/cm^2. The cells were then incubated at 37 °C and 5% CO_2.

2.3. Cell fixing and imaging

Cells were cultured for about 48 h prior to fixing. After discharge the DMEM, the substrates were rinsed in PBS to remove all the unattached and dead cells. Then, the cells were fixed with 2.5% glutaraldehyde (GTA, Electron Microscopy Sciences) for 1 h and then washed three times with buffer Sodium Cacodylate Trihydrate (Electron Microscopy Sciences). Then, the cells were dehydrated by ethanol with gradually increasing concentration (25%, 50%, 70%, 90% and 100%, respectively), followed by critical-point drying (Bal-tec CPD030 critical point dryer). After sputtering with gold and gallium (Bal-Tec SCD005 sputter coating), the PDMS substrates with HeLa cells were examined by scanning electron microscope (SEM, Hitachi S-3400N Variable Pressure Scanning Electron Microscope). SEM images were evenly taken from various parts of each substrate and analyzed using software ImageJ (National Institute of Health). Cell alignment angle and aspect ratio were measured. Cell alignment angle was measured as the intersecting angle between the long axis of a cell and the *y* axis of the pattern. While on the control flat substrate, the *y* axis was defined by a randomly chosen straight line. Due to the symmetry of the alignment, all the angles larger than 90° were symmetrically folded to 0–90° range. The aspect ratio of a cell was defined by the major cell axis

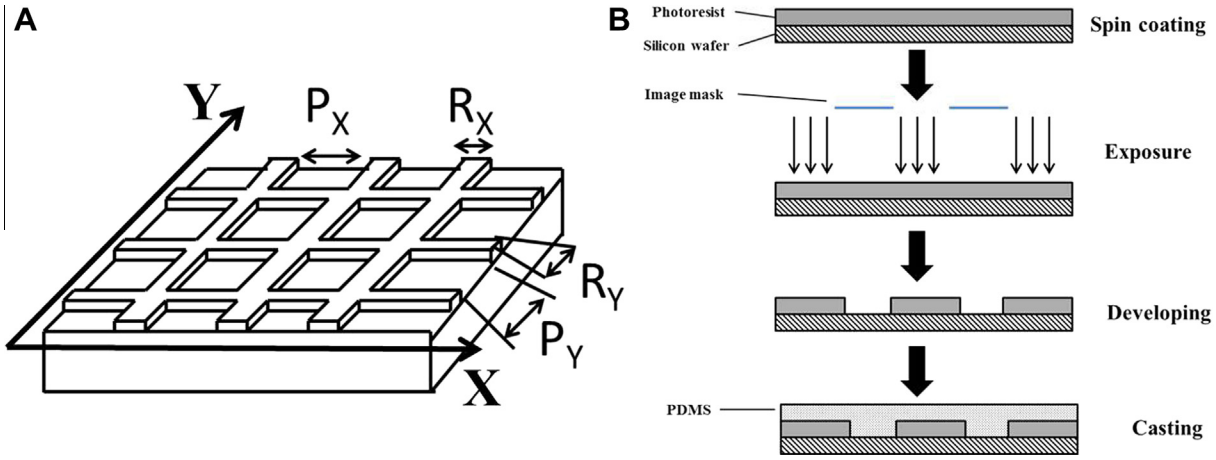

Fig. 1. Schematic diagrams of patterned PDMS substrates and fabrication process. (A) The pit patterned PDMS substrates were defined by $R_X \times R_Y \times P_X \times P_Y$ with R_X being the *x*-axis width of *y*-axis ridge, R_Y being the *y*-axis width of *x*-axis ridge, P_X being the *x*-axis width of pit, and P_Y being the *y*-axis width of pit. (B) Schematic diagram of the fabrication process of PDMS substrates. A pre-cleaned silicon wafer was spin coated with a uniform layer of photoresist. After exposure and developing, the inverse pattern from the image mask was transferred to the photoresist layer. Finally, PDMS substrate was fabricated by casting using the inverse patterned photoresist layer as the mask mold.