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Control of highly migratory cells by microstructured surface based on transient change in cell behavior

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ABSTRACT

Cell migration control techniques have been proposed for cells with relatively low migratory activity, based on static analyses performed with cells that attain a temporally homogenous state after being exposed to a cell guiding stimulus. To elucidate new functions of substrate topography, we investigated the transient change in the behavior of highly migratory cells coming from a flat surface to a grooved surface on a silicon substrate covered with SiO_2. A single line groove (1.5 μm in width, 20 μm in depth) and intersecting grooves (1.5 μm in width, 5 μm in spacing, 20 μm in depth) functioned as an effective cell repellent. In the case of wider grooves, a single line groove (4 μm in width; 20 μm in width) had no specified function. In contrast, intersecting grooves (4 μm in width, 5 μm in spacing) functioned as a trap for the cells. Our findings yield a new design concept of cell repelling and trapping surfaces which are applicable to cell guiding methods and single or multiple cell confinement on cell culture substrates, and thus may contribute to development of more advanced biomaterials.

1. Introduction

Cell migration control is important for developing biomaterials that effectively exploit cellular functions, particularly in the fields of tissue engineering, and cancer diagnosis and treatment, and thus a wide variety of cell migration control techniques have been advanced. The techniques are based on the mechanism inherent in cells for changing migratory behavior adaptively in response to various external stimuli [1]. Chemoattractant and electric fields are effective directional cues that bias the migratory direction [2–5]. Cell–substratum interaction is one of most important and extensively studied factors that affects cell migratory behavior [1,6]. Advances in micro- and nanofabrication techniques have enabled the fabrication of well-defined model surfaces with various chemical, mechanical and topographical properties. For example, chemically modified asymmetric cell adhesive islands [7,8], substrates with different stiffness [9–11], parallel multiple grooves [12–18], micropillars [19,20], and square lattice pattern arrays [21] are effective for guiding cells in a desired direction.

Compared with the variety of functional substrates, methods to evaluate cell responses tend to show less variation. The existing techniques have mostly been developed based on the static analysis performed with cells that attain a temporally homogenous state after being exposed to a guiding stimulus. Some recent studies have focused on transient changes in cell migratory behavior in response to the stimulus [11,17,19,21]. Regarding cell type, slowly migrating cells, such as fibroblasts, are used as model cells, although with some exceptions [18]. Therefore, the present techniques are based on the characteristics of slowly migrating cells: a strong cell-extracellular matrix (ECM) adhesion, well organized stress fibers, and resulting traction force [8,11,17,19,21,22]. In contrast, rapidly migrating cell types, such as fish keratocytes, neutrophils, macrophages, and metastatic malignant cells, have fewer focal adhesions and poor stress fibers. Thus, dynamic analysis of transient change in the behavior of rapidly migrating cells will elucidate potential effects of the functional substrates, and lead to creation of more advanced biomaterials.

Substrate topography has been one of the extensively studied, but has great latent potential for application as a biomaterial. Topographical control is a purely physical and biologically non-invasive method without bio-molecules [6]. Furthermore, it would be stable against non-specific adsorption of proteins from the media and/or that secreted from the cells [17]. Static analyses with

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slowly migrating cells in a temporally homogenous state have shown that multiple parallel grooves are a simple and effective model surface to control cell migration. Cells orient and migrate predominantly along the anisotropic direction of the grooves, and the magnitude of the cell response is affected by the density of grooves, and probably by the flexibility of the cytoskeleton of cells [13–17,23,24].

Our purpose here was to explore new functions of micro-structured surfaces focusing on transient changes in the behavior of cells migrating from a flat surface to a grooved surface. Fish epidermal keratocyte was used as a model cell since its rapid migratory activity and simple shape [25] were considered to be suitable for strict and reproducible evaluation of the functions of the substrate. We first characterized the effects of a single line groove as a basic examination, and then investigated how the effects of the single line groove were altered when a cell was exposed to intersecting grooves. The analysis newly identified two functions of the grooved surfaces – as a repellant and a trap of migrating cells – and further provided a design concept for these functional surfaces.

2. Materials and methods

2.1. Substrate fabrication

Fig. 1a shows a schematic illustrating the substrate with a single line groove. In this paper, the abbreviation "L-" stands for a line groove, and the number following "W" indicates the groove width in micrometers. The width of the groove was 1.5 μm (L-W1.5), 4 μm (L-W4), or 20 μm (L-W20). On all surfaces with the single line groove, the groove depth was 20 μm. Fig. 1b shows a schematic illustrating that the substrate consists of a flat surface and a grooved surface with intersecting grooves. The abbreviation "IS-" stands for intersecting grooves, and the number following "W" indicates the groove width in micrometers. The width of the grooves was 1.5 μm (IS-W1.5), or 4 μm (IS-W4). On all surfaces with the intersecting grooves, the groove spacing was 5 μm, and the groove depth was 20 μm.

Grooved silicon substrates (Fig. 1) were fabricated as previously described [26,27]. Briefly, bare silicon wafer was patterned with a photoresist (AZ5214E, Hoechst Celanese, USA), and the silicon top surface was vertically etched to 20 μm depth using a deep reactive ion etching (DRIE) system (STS Multiplex ICP, UK). Then, the photoresist was removed using an oxygen plasma asher (Plasma-finish, Germany). The fabrication process was completed by growing SiO_2 using a thermal oxidation system (Sungjin Semitech, Korea) on the substrates. The fabricated samples were observed with a scanning electron microscope (SEM; JSM-6330F, JEOL, Japan). Fig. 1c–f show representative SEM images of the single line groove (Fig. 1c) and the intersecting grooves (Fig. 1d–f).

2.2. Cell culture

Fish epidermal keratocytes were cultured as described previously [28,29], with small modifications. Briefly, Black tetra (*Gymnocorymbus ternetzi*) keratocytes were cultured in 70% DMEM (30% purified water, Hepes modified, Invitrogen) supplemented with 15% (v/v) fetal bovine serum (Invitrogen) and penicillin–streptomycin (100 units/mL penicillin, 100 μg/mL, respectively, Sigma Aldrich). Fish scales were extracted with tweezers, placed external side up on the dry flat surface of the substrate, and allowed to adhere for ~60 s (until almost dry). Culture medium was then added and the scales were kept at room temperature for 12–24 h to allow the cells to spread from the scale to form a layer on the flat surface of the substrate. The layer was treated with 0.05% Trypsin/EDTA (Sigma Aldrich) to disaggregate the cells. After disaggregation, the cells were placed in the culture medium for recovery and subsequent observation.

2.3. Observation of cell migration

Migrating cells from a flat surface to a grooved surface were observed every 30 s using a 20×, 0.4NA Plan objective and a metallurgical microscope (ML8530, MEIJI) equipped with a digital camera (E330, Olympus). For detailed observation, reflective differential interference contrast (DIC) micrographs were obtained every 10 s using a 20×, 0.4NA Plan Apo objective and a microscope (BX51M, Olympus) equipped with a digital camera (E620, Olympus).

Cells migrating on the intersecting grooved surface were observed by fluorescent microscopy. For fluorescent microscopy, disaggregated keratocytes were incubated at 33 °C with Vybrant DiI cell-labeling solution (Invitrogen), diluted 1:100 in the cell culture medium. After 15 min, the cells were washed three times with the cell culture medium. Stained cells were imaged every 30 s using a 40×, 0.95NA Plan-Apochromat objective and a fluorescent microscope (IX81, Olympus) equipped with a CCD camera (iXon, Andor Technology). All these experiments were done at room temperature.

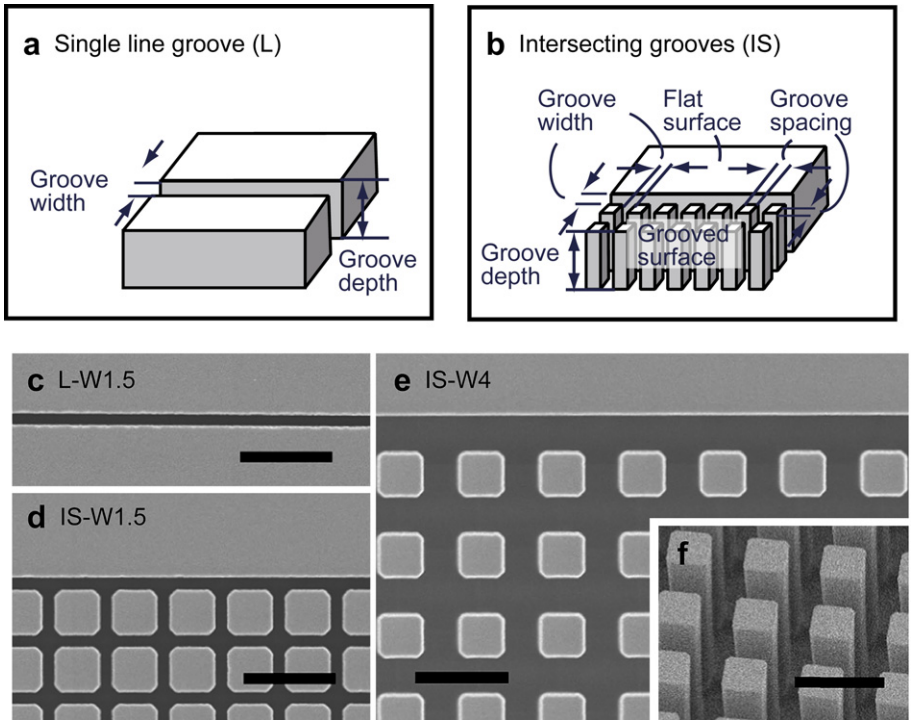

Fig. 1. Substrate topography. (a) Schematic illustrating the substrate with a single line groove. (b) Schematic illustrating that the substrate consists of a flat surface and a grooved surface with intersecting grooves. SEM images of substrates with (c) a single groove of 1.5 μm in width and 20 μm in depth (L-W1.5), (d) intersecting grooves of 1.5 μm in width, 5 μm in spacing and 20 μm in depth (IS-W1.5), and (e and f) intersecting grooves of 4 μm in width, 5 μm in spacing and 20 μm in depth (IS-W4). The scale bars correspond to 10 μm.