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The effect of micronscale anisotropic cross patterns on fibroblast migration

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ABSTRACT

Cell movement on adhesive surfaces is a complicated process based on myriad cell–surface interactions. Although both micron and nanoscale surface topography have been known to be important in understanding cell–materials interactions, typically only simple patterns (e.g., parallel lines or aligned posts) have been used in studying cell morphology, migration, and behavior. This restriction has limited the understanding of the multidirectional aspects of cell–surface response. The present study was performed to investigate cell morphology and motility on micronscale anisotropic cross patterns and parallel line patterns having different aspect ratios (1:2, 1:4, and 1:∞), grid size (12-, 16-, and 24-μm distance neighboring longer side ridges), and height of ridges (3- and 10-μm). The movement characteristics were analyzed quantitatively with respect to cell migration speed, migration angle, persistence time (P) and motility coefficient (μ). A significant effect of the 1:4 grid aspect ratio cross patterns and parallel line patterns on cell alignment and directionality of migration was observed. Cell motility was also dependent on the patterned surface topography: the migration speed was significantly enhanced by the 1:2 and 1:4 cross patterns when the grid size was smaller than the size of individual cells (i.e., ~16 μm). In addition, the migration speed of cells on lower patterns was greater than on higher ridges. Overall, cell morphology and motility was influenced by the aspect ratio of the cross pattern, the grid size, and the height of ridges.

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1. Introduction

Cell migration plays a critical role in a variety of physiological and pathological phenomena. Migration of leukocytes mediates phagocytic and immune responses. Migration of fibroblasts, vascular endothelial cells, and osteoblasts contributes to wound healing and tissue regeneration. Tumor cell migration is essential to metastasis [1]. In some cases, migration can lead to implant failure as in the "marsupialization" of percutaneous devices and dental implants due to epithelial downgrowth on the implant surface [2]. As migration and other cell responses, such as adhesion and proliferation, are dependent on cell–materials interactions, numerous reports have examined the influence of both surface chemistry and topography on cell behavior in the absence of soluble chemotactic signals [3,4].

Cell responses to chemically patterned surfaces can be effectively categorized with respect to the patterning scale of specific chemistries, while cell responses to topography are classified based on the pattern topography (isotropic or anisotropic) [4]. Chemically patterned surfaces on the micronscale have been exploited for some time to spatially define the position and shape of cells, and to study these perturbations on cell function such as protein and gene expression, differentiation, and mechanotransduction [5–7]. Furthermore, nanoscale patterned surfaces have been used to regulate more collective cell functions such as proliferation, differentiation and molecular arrangement [8,9]. In contrast to the chemical patterning, uniformly or randomly textured surfaces on the micron-and nano-scale have been used to study cell adhesion, spreading, migration and phenotype [10,11]. Anisotropic topographic patterns induce cell alignment and dictate the degree of migration along grooves and ridges [12–17]. Kaiser et al. studied the effect of topography on cell shape, cell orientation, migration angle, and velocity with 10 differently structured parallel line patterns and concluded that the reaction of the surface structure might be far more complex than generally assumed [12]. In spite of the importance of the topographic anisotropy, however, more often parallel straight line patterns have been exploited for understanding the interaction between cells and topographic surfaces. Recently, Mai et al. reported a microfabricated grid-patterned surface could enable topographically multidirectional cell stimulation [18]. They demonstrated cell response to the multidirectional

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stimulation and intracellular signal transduction on grid patterns. However, they still did not examine cell response to varying anisotropy of topographically patterned surface. Multidirectional grid-patterned substrates were also reported to control directional migration of cells [19]. Inspired by these observations, we investigated how the topographic anisotropy can affect fibroblast cell behavior.

In this study, we demonstrate anisotropic cross patterns and parallel line patterns with ridges in a range of 1- to 2-μm width can be fabricated by two-photon induced polymerization with a photosensitive organic–inorganic hybrid polymer. The patterns can exert contact guidance stimulation in multiple directions with different efficiency, depending on the grid aspect ratio. We examined the behavior of NIH3T3 fibroblasts cultured on the surfaces with three different aspect ratios of 1:2 grid, 1:4 grid, and parallel line patterns (1:∞). The morphology of the cells was characterized on different aspect ratio and grid patterns with respect to their orientation and elongation, based on fluorescent images acquired using epifluorescent confocal microscopy. The motility of the cells was analyzed quantitatively with respect to migration angle and speed by long-term monitoring of cell migration. General migration parameters, cell motility coefficient (μ) and persistent time (P), were calculated by using the persistent random walk model. Moreover, the cell migration enhancement due to cross pattern shape and size was studied.

2. Materials and methods

2.1. Fabrication of scaffolds using two-photon polymerization

The micronscale cross patterns and parallel line patterns were fabricated with photosensitive organic–inorganic hybrid polymer ORMOCER® US-S4 on glass substrates using two-photon polymerization technique. The ORMOCER® US-S4, which is also referred to by the trade name ORMOCOMP® (Micro resist technology GmbH, Berlin, Germany), is ultraviolet (UV) curable, non-toxic, and optically transparent over a 400–1600 nm wavelength range [20–23].

In order to prepare a precoated substrate, the photosensitive resin (PR) was coated on a glass cover slip by a spin coater at 8000 RPM for 100 s, prebaked at 80 °C for 2 min on a hot plate, cured with a UV lamp for 30 min, and hard baked at 140 °C for 1 h (Fig. 1(a,b)). The PR was spin coated again at 6000 RPM on the precoated surface and prebaked with the same conditions (Fig. 1(c)). Femtosecond laser pulses (<500 fs, 1 MHz, 1045 nm, FCPA μJewel D-400, IMRA America, inc.) were frequency-doubled to the wavelength of ~523 nm and focused onto the interface between precoated surface and uncured PR through the glass cover slip using an M plan apo 50× N.A. = 0.55 microscope objective (Mitutoyo), Kawasaki, Japan (Fig. 1(d)).

The power of the laser beam emitted downstream of the objective lens was measured by a power meter and controlled by a half wave plate and a polarizing beam splitter. The exposure duration was controlled by a mechanical shutter. The sample was placed on a motorized X–Y stage. The desired micropattern was fabricated by a mechanical shutter and computer controlled stages. The laser power of 0.5 mW and scanning speed of the stages of 0.2 mm/s were set to fabricate ~3-μm high ridge patterns. The laser power of 1 mW and scanning speed of the stages of 0.5 mm/s were set to fabricate ~10-μm high ridge patterns, as detailed in a previously published parametric study [23].

After the laser irradiation, the samples were baked at 110 °C for 10 min, and uncured resin was removed by immersing in developer (ORMODEV, Micro resist technology) for 30 min. Thereafter, the samples were rinsed successively with isopropyl alcohol (IPA) and deionized (DI) water three times (Fig. 1(e)). Finally, samples were placed on a hot plate at 140 °C for 1 h for hard baking and dipped in 70% ethanol for sterilization prior to use.

2.2. Characterization of patterned surface

Considering the high intensity level of femtosecond laser irradiation, femtosecond laser-cured patterns might experience unwanted alterations in surface molecular structure and pattern morphology, which can critically affect the cell behavior. It is important to confirm that the chemical characteristics of laser-cured surface are similar to those of UV lamp-cured film. In this context, surface molecular structures were analyzed for both laser-cured patterns and UV lamp-cured surfaces by a micro ATR-FTIR spectrometer system, combined with an FTIR spectrometer (FT/IR-4100, JASCO Corp., Tokyo, Japan), an MCT detector, and an IR microscope (IRT-1000, JASCO Corp., Tokyo, Japan) with a ZnSe prism. A total of 64 scans were integrated for each spectrum at 4 cm^{-1} wave number resolution and ~80 × 80 μm spatial resolution over the range of 600–5000 cm^{-1}. For ATR-FTIR measurements, a sample with dense ridges at 5 μm pitch was fabricated using laser power of 2.5 mW and scanning speed of 1 mm/s.

2.3. Cell culture and imaging

NIH3T3 cells were maintained in Dulbecco's Modified Eagle Medium (Gibco Invitrogen), 10% fetal bovine serum (FBS) (Gibco Invitrogen) and 100 units/ml penicillin (Gibco Invitrogen) in 75-cm^2 culturing flask (Corning) at 37 °C and 5% CO_2 culture incubator. Before experimentation, confluent cells were detached from the flask by 0.05% Trypsin-EDTA (Gibco Invitrogen) and seeded onto the patterned substrate in sterile polystyrene well plates and placed in an incubator maintained at 37 °C and 5% CO_2. After one day, the cells were fixed with 3.7% formaldehyde (Fisher Scientific) for 10 min, and permeabilized with 0.1% TritonX-100 (Fisher Scientific) for 5 min. Actin cytoskeleton was stained with 165 nM Alexa Fluor® 488 phalloidin (Invitrogen) for 20 min, and subsequently nucleus was stained with 300 nM DAPI (Invitrogen) for 4 min. Samples were kept in PBS and turned over for inspection by

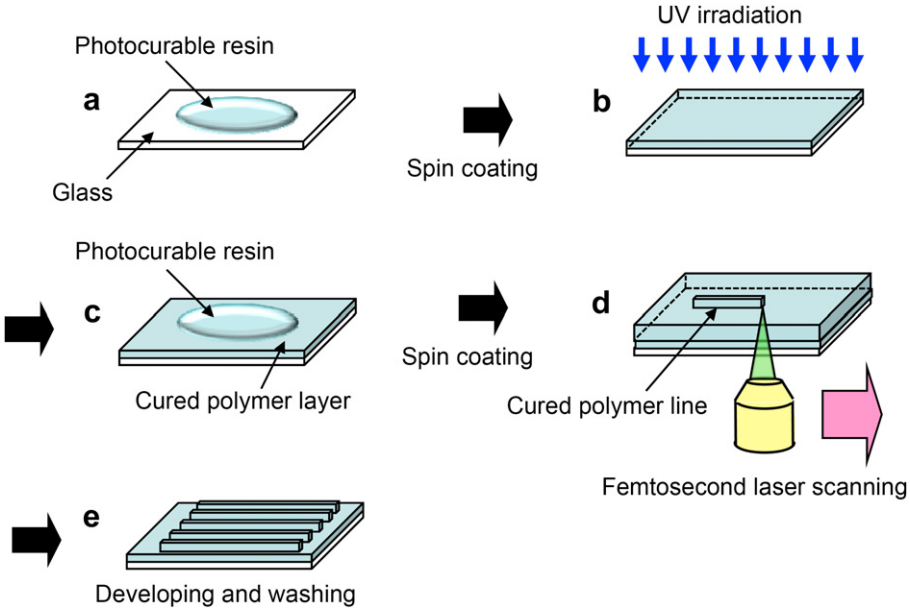

Fig. 1. Illustration of fabrication procedure.