Effect of substratum morphology on cell behavior

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ABSTRACT

Contact guidance induced by the surface topography of the substrata influences the direction of cells with artificial material. Mouse bone marrow stromal fibroblast was examined on grooved substrata of varying dimension (0.3 μm~1.2 μm groove width, 0.5 μm depth and 0.5 μm ridge). It was found that the repeat space had an effect in determining cell alignment. Measurement of cell alignment and examination by scanning electron microscopy showed the fibroblast interacted grooved substrata in monomodal responses and alignment to the direction of the grating.

Keywords: contact guidance, stromal, cytoskeletal, plasma etching, microfabrication

1 INTRODUCTION

Several in vitro studies have documented the ability of microgrooved substrata to produce cell orientation and directed migration. But the role of the texture of materials in inducing cell and tissues responses is still unclear. In spite of several publications and reviews have reported the effect of microtextured surface, little is known about the exact mechanism [(1-5)]. So controlling cell responses to a material is of interest with regard to tissue culture in the laboratory or implantable devices in body. Because the surface is in direct contact with cells, both chemical and topographical properties of the material surface can play a crucial role in determining cell responses. A number of physicochemical surface properties, including surface composition, surface charge, surface energy, surface oxidation, solidity, curvature, and surface morphology, have been shown to affect cell attachment and behavior [(6)].

Microfabrication technology employed to create substrates with topography is photolithography that produce features with controlled dimensions and specific shape [(7)]. Using this technique features in the range of micrometer could be etched precisely and uniformly on a variety of substrata such as silicon and glass, and this technique is flexible enough to allow a wide variety of textures and feature size. So this surface has facilitated systematic in vitro experiments to study influence of surface morphology on diverse cell physiological aspects such as adhesion, growth, and function. A common topographical feature investigated is a uniform multiple parallel grooves in the study of the effects of surface structure on cells. Typically, the grooves are often of the square ware, or V-shape. In general, grooved surface revealed that cells aligned to the long axis of the grooves, often with organization of actin, and other cytoskeletal elements in an orientation parallel to the grooves [(8)]. So we would examine how parallel grooved substrata with different space width affect the alignment to mouse bone marrow stromal fibroblast cell.

2 MATERIAL AND METHODS

2.1 Production of substrata

Oxide films were deposited at 700 ℃ to the thickness of 500 nm by LPCVD. Afterwards, trench-line patterns were defined on these oxide films by a g-line stepper (ASM PAS 2500). Subsequently, patterned films were etched in a CF_4/CHF_3 mixed plasma using a helicon wave plasma system (ANELVA ILD-4100). The line patterns are 40 μm in length, groove widths ranging from 0.3 μm to 1.2 μm and separated by 0.5 μm ridge.

2.2 Cell culture

Mouse bone marrow stromal fibroblast are performed in culture that is in a RPMI 1640 medium supplemented with 10 % fetal bovine serum (FBS), 1 % antibiotics (100 units/ml penicillin + 100 ug/ml streptomycin + 50 ug/ml nystatin). Then incubate at 37 ℃ 5 % CO_2/air for routine culture.

2.3 Cell seeding

The day before the test, all samples were immersed into 70 % ethanol and using an ultrasonic bath, subsequently, they were rinsed in double-distilled water and store overnight in cell culture medium at 4 ℃. Fibroblast cells, approximately a concentration $5x10^4$ cell/ml were harvested from routine culture, were seed onto grooved substrata in 35 mm petri dish and incubated for 2 day at 37 ℃, 5 % CO_2/air.

2.4 Scanning electron microscopy

Cells on patterned substrata were fixed in 2.5%glutaraldehyde in phosphate-buffered saline (PBS) at 4 ℃ for 1 hr and two 15 min rinse in PBS at 4 ℃. Then samples were dehydrated in alcohol, dried by the critical

point method using CO_2, sputtered with gold and viewed with JEOL JSM-5600 scanning electron microscopy.

3 RESULTS

Fig. 1 shows the microstructured substrata surface texture, SiO_2 on Si wafer. The depth of the grooved was 0.5 μ m with a serial of width (0.3 μ m,0.4 μ m,0.5 μ m,0.6 μ m,0.7 μ m,0.8 μ m,0.9 μ m,1.0 μ m,1.2 μ m), and 0.5 μ m ridge.

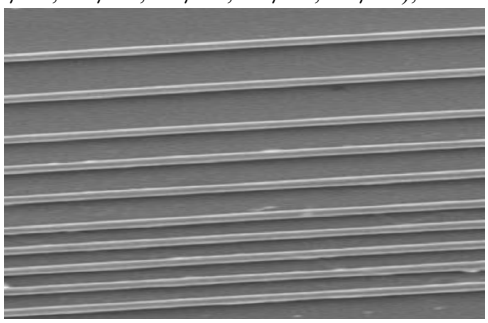

Figure 1: The scanning electron micrograph of microstructured surface, SiO_2 on silicon wafer.

Fig. 2 shows the cells on the unpatterned substrata (planar silicon dioxide surface) were extremely flattened and randomly distributed.

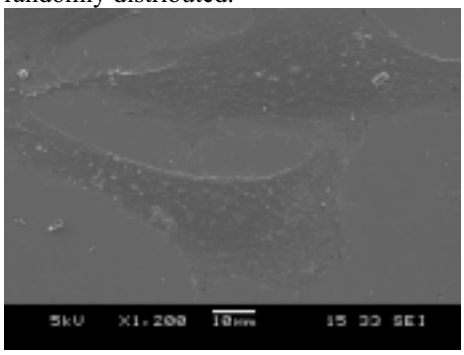

Figure 2: Mouse bone marrow fibroblast after 2 day incubated on the planar silicon oxide surface.

Fig. 3 and Fig. 4 were the cell growth on the grooved substrata, the cell was spindle shapes and both ends being drawing out into long, fine processes. The growth and alignment of fibroblast on grooved substrata was dependent on groove width, and being inversely proportional to groove width, the cells on the shorter grooved substrata were highly elongated and aligned but no cells on the wider parts.

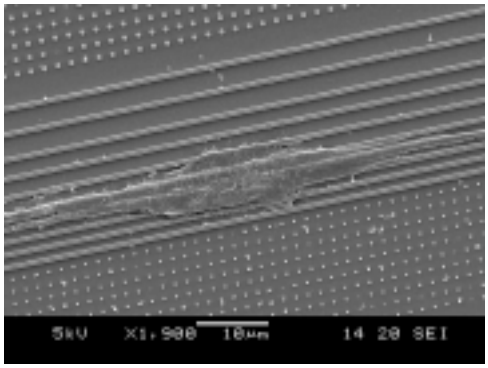

Figure 3: The scanning electron micrograph of mouse bone marrow fibroblast after 2 day of incubated on a series of groove width, 0.5 μ m deep, 0.5 μ m ridge of SiO_2 on silicon wafer.

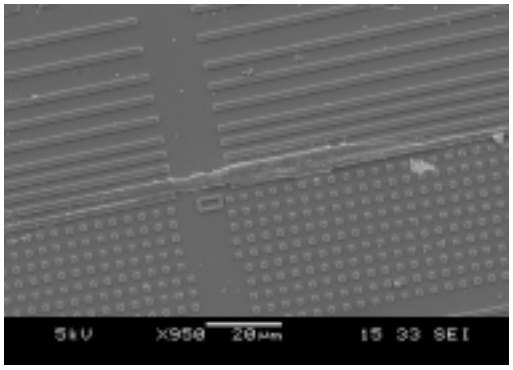

Figure 4: The scanning electron micrograph of mouse bone marrow fibroblast after 2 day of incubated on a series of groove width, 0.5 μ m deep, 0.5 μ m ridge of SiO_2 on silicon wafer.

The cells may have attempted to conform to the substrata, which their long axes cross and span the grooves and their lamellar regions cling intimately to the underlying planar substrata (Fig. 5).

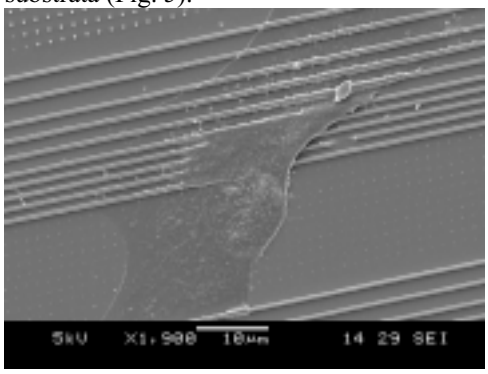

Figure 5: The scanning electron micrograph of mouse bone marrow fibroblast after 2 day of incubation on a series of groove width, 0.5 μ m deep, 0.5 μ m ridge and planar SiO_2 on silicon wafer.

must be straight, not bent. So that cells align to the substrata micromorphology to avoid a distortion of their cytoskeleton.

Further studies are required to clarify the ridge width and depth of grooves to cell behavior, and examined how various parallel grooved substrata affect the alignment of different cell type.

4 DISCUSSION

It has been known that photolithographic microfabrication techniques have been applied to address the problems to what extent and what mechanism topography influences animal cell behavior [5, 9, 10, 11, 12]. They examined some parameters of the grooved substrata to alter cell shape and guide cell movement. Most attention was the ridge width and depth of grooves. Dunn shown that the shape change and alignment of fibroblast on grooved substrata was most dependent on ridge width, alignment being inversely proportional to ridge width [11].

Our studied revealed that fibroblast migrated along the major axis of surfaces with parallel ridges/grooves of various line space, this phenomenon was called "contact guidance" by Weiss [13].

Contact guidance of fibroblasts on grooved substrata has reported by some laboratories. M. Abercrombie [14, 15] is the first to detect the fibroblast adhered to the substrata surface in specialized zone that was focal contact. They show a characteristic structure [16] with transmembrane integrin receptor, which are linked by several cytoskeletal proteins to intracellular fibers. Some experimental revealed that the stiffness of the cytoskeleton is probably a reason for cellular alignment [17] and their intracellular actin fibers must be straight, not bent. So that cells align to the substrata micromorphology to avoid a distortion of their cytoskeleton.

Further studies are required to clarify the ridge width and depth of grooves to cell behavior, and examined how various parallel grooved substrata affect the alignment of different cell type.

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