EFFECT OF NANO- TO MICRO-SCALE SURFACE TOPOGRAPHY ON THE ORIENTATION OF ENDOTHELIAL CELLS

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ABSTRACT

A model vascular graft of cross-linked polydimethylsiloxane (PDMS) has been modified with protein and varied topographically to promote endothelial cell attachment as well as to guide cell-substrate interactions. PDMS with a smooth surface (RMS roughness ~ 0.5 nm) and grating-textured surfaces, having channel depths of 100 nm, 500 nm, 1 μm and 5 μm, and lateral width of 4 μm, are fabricated. While the pre-adsorbed fibronectin promotes cell adhesion, the underlying topographic features provide a contact guidance that influences cell morphology and cell orientation. Using phase contrast microscopy after seeding cells for 1, 4, 24 and 48 h, cell elongation and alignment parallel to the grating direction increases monotonically with increasing channel depth, reaches maximum orientation at 1 μm, and then slightly decreases at 5 μm. By fluorescence staining of F-actin and vinculin, cytoskeleton and focal contacts are observed to preferentially orient parallel to the grating direction on textured surfaces having depths of 1 and 5 μm. Confocal and scanning electron microscopies show that cell protrusions extend into channels and also along the side walls of the channels. Cell proliferation is found to be independent of surface topography. Relevant to tissue engineering applications, cell orientation is retained at confluence on textured PDMS surfaces. Using surface topography to create contact guidance provides an alternative pathway to obtain endothelial cell alignment, similar to flow in the natural blood vessel.

INTRODUCTION

Cell adhesion to biomaterials and tissue implants depends on chemical and topographical properties presented on a material's surface. Surface chemistry, provided by tethered proteins, peptides or chemical functional groups, has shown to promote the adhesive interaction between cells and their underlying substrate, while surface topography provides contact guidance [1] for cell movement and cell morphology [2]. By combining both chemistry and topography, the adhered cells can be simultaneously guided to move or to align in a certain orientation.

In this paper, a model PDMS graft with grating texture has been developed to investigate cell-substrate interaction in which cell morphology and cell orientation are strongly guided by surface topography. Endothelial cells are shown to recognize and response to grating texture by adopting an elongated shape and increase their orientation parallel to the grating direction with increasing channel depths. The optimum orientation is achieved when channel depth is 1 μm. Besides changes in cell morphology, cytoskeleton and focal contacts are also shown to align with the underlying grating direction.

MATERIALS AND METHOD

Substrate Preparation

All PDMS substrates were cast from Sylgard 184 Silicone Elastomer (Robert McKeown Company, Inc. Branchburg, NJ) which came as a kit of base resin and a curing agent. A 10:1 mixture of base resin to curing agent was thoroughly mixed prior to cast onto Si mold, fabricated by photolithography and reactive ion etch, for textured PDMS, and polystyrene Petri dish (Fisher) for smooth PDMS of 1 mm thick. The PDMS were cured at room temperature for 48 h and annealed at 65°C for 3 h to ensure complete crosslinking. The grating profiles on textured PDMS were examined by scanning electron microscope (SEM) as shown in Figure 1.

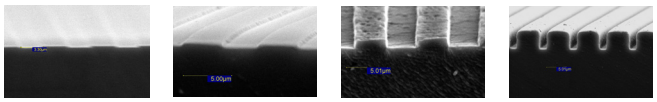

Figure 1. SEM micrographs of textured PDMS showing grating profile with varied channel depths from 100 nm to 5 μm

The cast PDMS substrates were cut into a circular shape to fit into a 12 well plate, and sterile with 70% ethanol under UV for 30 min before left dry in the culture hood overnight.

Fibronectin Adsorption

Both smooth and textured PDMS substrates were pre-adsorbed with 10ug/ml fibronectin (Fn) (Sigma, St. Louis, MO) in 1X phosphate buffered saline (PBS) solution at 37°C in a 12 well plate (Corning Inc., Costar NY). This Fn concentration was within the saturation range to provide a uniform coverage on a hydrophobic surface [3]. The surface density of Fn was quantitatively determine by using spectrophotometer reading on PDMS substrates that had been pre-adsorbed with fluorescence-labeled Fn (*Fn) [4]. For cell culture, smooth, 100-nm and 500-nm textured PDMS were incubated in Fn solution for 75 min while 1-um and 5-um textured PDMS were incubated for 15 h to ensure that all channels were saturated with adsorbed Fn. All pre-adsorbed PDMS substrates were washed twice with 1X PBS and then incubated with 1% bovine serum albumin (BSA) for 30 min at 37°C to block any non-specific adhesion. The substrates were then washed twice with 1X PBS and ready for cell seeding.

Cell Culture

Bovine aortic endothelial cells (BAECs) were house isolated and cultured to 80-90% confluent in gelatin (Sigma)-coated T75 flasks (Fisher) with Dulbecco's Modification of Eagle's Medium (Fisher) supplemented with 10% fetal bovine serum (FBS), 1 mg/ml glucose, 0.3 mg/ml L-glutamine, 10 μg/ml streptomycin, 10 U/ml penicillin, and 25 ng/ml amphotericins. Cell passages 9-15 were used in the experiment. BAECs were seeded on Fn-coated PDMS at 25,000 cells/well in DMEM without FBS, and incubated at 37°C with 95% air/CO_2. After 4 h of incubation, the supernatant was removed and each well was refreshed with DMEM containing 10% FBS. Cells were examined using phase contrast microscopy and fixed at 1, 4, 24 and 48 h after incubation.

F-actin and focal adhesion staining

The samples were washed twice with 1X PBS, followed by fixing in 4% formaldehyde (Fisher) for 30 min and rinsing twice in 1X PBS. The 1:200 (v/v) solution of mouse anti-vinculin (Chemicon, Temecula, CA) in 0.25% BSA was applied at 1 ml per sample and incubated for 45 min at room temperature. The samples were then washed 3 times, 10 min each, in 1X PBS. The fixed samples were then incubated in a solution of 1:1000 (v/v) FITC-conjugated goat anti-mouse (Chemicon) and 1:1000 (v/v) rhodamine-conjugated phalloidin (Sigma) in 1X PBS solution for 45 min at room temperature. The samples were washed 3 times, 10 min each, in PBS. Upon examining with fluorescence and confocal microscopy, the samples were mounted onto glass slides and covered with 10-20 ml of Vectashield (Vecta, Texas?) containing DAPI (Vector Laboratories, Burlingame, CA).

Scanning Electron Microscopy

PDMS samples were fixed in 2.5% gluteraldehyde in 0.1 M HEPES at room temperature for 1 h and at 4°C overnight. After removing gluteraldehyde, the samples were washed 3 times, 5 min each, in 0.1 M sodium cacodylate/HEPES solution, followed by rinsing with tissue culture water. The samples were then dehydrated in a series of 50%, 70%, 80%, 90% and 100% ethanol, 5 min immersion each. The dehydrated samples were critical point dried and examined by SEM.

RESULTS

Fibronectin Adsorption

All PDMS surfaces, as shown in Figure 2, yield similar *Fn coverage of ~ 300 ng/cm^2. This value is consistent with that on hydrophobic surface where the complete coverage, ~ 350 ng/cm^2, is obtained [3]. Fluorescence microscopy (data not shown) confirms the uniform fluorescence intensity on all surfaces.

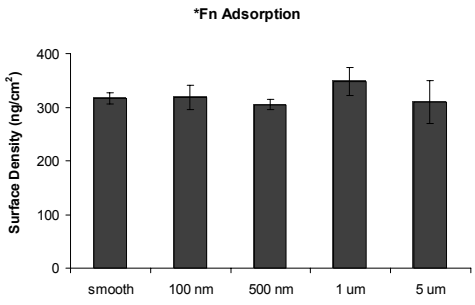

Figure 2. *Fn coverage shows similar surface density on both smooth and textured PDMS surfaces

Cell Orientation

The ability of elongated endothelial cells to align correspondingly to the grating direction on textured PDMS is characterized by their orientations. Elongated cells are defined as oriented if their alignments are within 20° with respect to the grating direction and as partially oriented if their alignments are beyond 20°. Cells that spread equally in all directions are defined as isotropic. Cell orientation at

early time of adhesion, 1 and 4 h, as well as at longer time, 24 and 48 h, on all 5 different Fn-coated textured PDMS surfaces is shown in Figure 3.

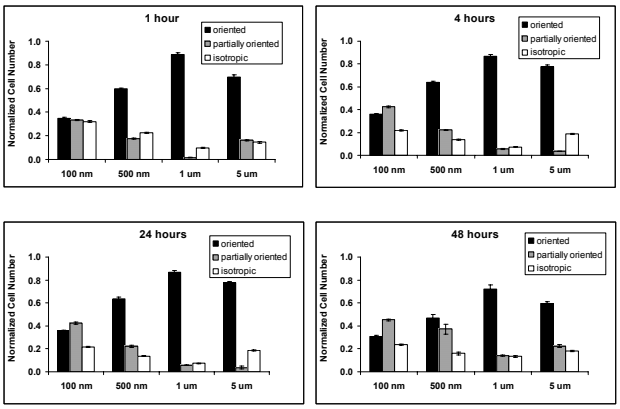

Figure 3. Cell orientation on textured PDMS surfaces as a function of incubation time: 1, 4, 24 and 48 h

The degree of orientation increases monotonically with increasing channel depth. Cells are most elongated and directed along the grating pattern on the 1-μm PDMS surfaces at all times of incubation. For 100-nm PDMS surfaces, cells are less sensitive to their underlying topography during the first hour of adhesion as presented by similar magnitudes of oriented, partially orientated and isotropic. At longer incubation time, cells become more elongated in response to the grating texture, resulting in a slightly higher number of cells being oriented and partially oriented.

SEM micrograph (Fig. 4) also show cell protrusions reach into the channel as well as spread over the step height. This confirms that the oriented cells are able to spread over the texture contour and are not confined only on the ridges or the channels.

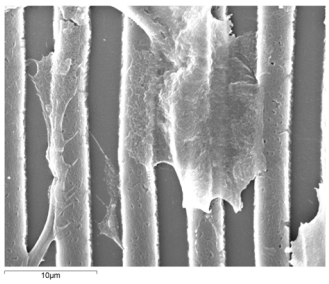

Figure 4. SEM micrograph of BAEC seeded on 1-μm PDMS for 24 h

Organization of F-actin and focal contacts

Besides the orientational effect on cell morphology, topographic texture also influences the alignment of F-actin and focal adhesion sites. Figure 5 shows confocal images of stained F-actin, vinculin and cell nuclei from cells seeded on smooth, 100-nm and 1-μm PDMS surfaces for 24 h.

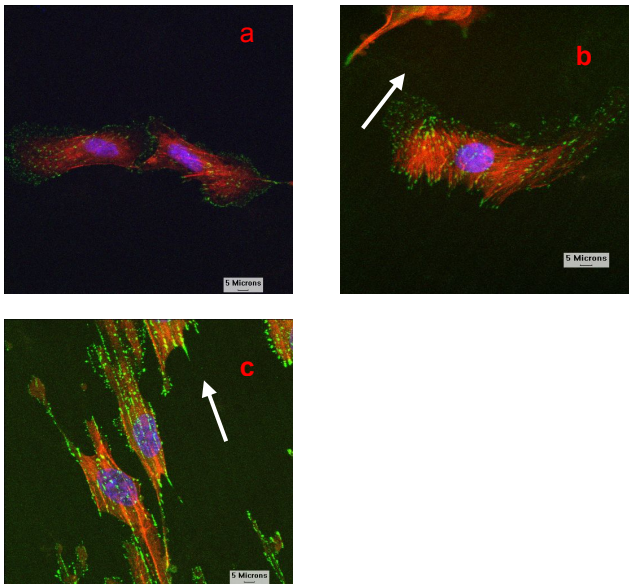

Figure 5. Confocal images of BAECs on a) smooth, b) 100-nm and c) 1-μm PDMS surfaces fixed at 24 h. White arrows show the grating direction on the textured surface.

Comparing to smooth surface, F-actin and vinculin at focal contacts are more oriented on textured PDMS. Even though the cell shown on 100-nm PDMS (Fig. 5b) is partially oriented by stretching itself across the grating texture, some of F-actin is aligned parallel to the grating direction, having the elongated shape of focal contacts at the termination of stress fibers. On 1-μm PDMS, however, the majority of F-actin and focal contacts are directed along the grating texture. More elongated shape of focal adhesion plaques are also observed from stained vinculin which are preferentially located along the step height. Similar orientation of F-actin and focal contacts are also observed on 5-μm PDMS.

DISCUSSION

Besides surface chemistry or presentation of active sites on protein [3] that play important role in cell adhesion and cell function, surface topography has clearly shown its effect on cell morphology and cell orientation. Contact guidance provided by

topographic texture on the surface has shown to influence cell orientation in many cell types [2,5,6] with similar effect on cell morphology and cell orientation. In this paper, given that all surfaces are uniformly covered by Fn to promote endothelial cell adhesion, the slight variation, in the sub-micron scale, has shown to largely effect cell orientation. This length scale of channel depth can provide insights into the critical interaction distance between cell-surface receptor and ligand in the integrin-mediated adhesion and also the relationship between the topographic texture provided by the surface and the stiffness of F-actin which results in the oriented alignment of F-actin. This ability of cells to response to topographic guidance can be useful in cell migration and in engineering cells onto special pattern on the surface. Further investigation on cell function such as kinase activity as a response to topographic cue is needed.

CONCLUSION

The effect of contact guidance provided by surface topography is shown in the elongated morphology and orientation of endothelial cells on grating-textured fibronectin-coated PDMS. Cell orientation is shown to increase with increasing channel depth, reaches maximum orientation at 1 μm. The F-actin and focal contacts are also directed along the grating direction where focal contacts are preferentially located at the step height on 1- and 5-μm PDMS. This textured PDMS provides both aspects of biomaterial and biomechanic environment for endothelial cells.

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