Research Article

Fibronectin Adsorption to Nanopatterned Silicon Surfaces

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The possibility of using surface topography for guidance of different biological molecules and cells is a relevant topic that can be applied to a wide research activity. This study investigated the adsorption of fibronectin to a diffraction grating silicon surface. The rectangular grating profile featured a controlled surface with 350 nm period and a corrugation depth of 90 nm. Results demonstrated that the controlled surface had a significantly positive effect on the fibronectin binding. Thus, nanoscale surface topography can enhance fibronectin binding.

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1. INTRODUCTION

The possibility of using surface topography for guidance of different biological molecules and cells is a relevant topic that can be applied to a wide research activity [1]. One important subject is the attachment of various plasma and extracellular proteins to biomaterial surfaces [2]. Fibronectin (FN) is a well defined extracellular protein consisting of two dimer subunits, each is about 250 kilodaltons (kD) and an elongated shape with dimensions 45 nm × 9 nm × 6 nm [3]. FN plays a key role in cell adhesion and mediating cell response, thus it will be used as a model for protein adherence in our study. Although FN adsorption on different materials has been investigated very actively, it has not been identified how FN adherence is altered in response to nano- and micropattern roughness. We used diffraction grating on silicon substrate as controllable roughness to investigate alterations in FN adsorption. Diffraction grating fabrication technologies are well developed, thus it is possible to fabricate diffraction gratings with a wide range of grating periods, corrugation depth, and grating profile.

Many engineering applications have focused on biomimetic sensors based on waveguide technology. Considering new advances in microelectromechanical systems/na-

noelectromechanical systems (MEMS/NEMS) fabrication, soft lithography, and the development of smart adhesives, integration of complementary metal-oxide-semiconductor (CMOS) and MEMS/NEMS should be further explored to provide the infrastructure for integration of the whole silicon-based sensory system especially in controlling host-biomaterial interactions. Any attempt to make a sophisticated, functional surface for biointeractions must take into account the highly developed ability of biological systems to recognize specially designed features on the molecular scale [4]. The materials used in BioMEMS/BioNEMS devices must exhibit desirable micro-/nanoscale tribological and mechanical properties [5]. From the cellular perspective, the interactions of cells with each other and extracellular materials (proteins, matrices, solid surfaces, etc.) are of vital importance to proper cell functioning. These interactions have major effects on the proliferation, differentiation, migration, and organization of cells [6, 7]. When designing novel biomaterials properties, one must understand that when an implant surface comes into contact with physiological solutions, proteins adsorb immediately on material surface. This adsorption is known to cause conformational changes in the native protein structure with the possibility of subsequently promoting or inhibiting nearby cells to interact with material,

FIGURE 1: Attachment model for different diffraction grating profiles.

thus leading to implant integration or rejection [4, 6, 8]. Recent studies on protein/material surface interactions have increased the knowledge base on this topic and this relationship appears to be mediated by a class of high molecular weight glycoproteins that are involved both in these interactions and in the actual structure of extracellular matrices. Some of the most intensively studied glycoproteins are FN, laminin, Willebrand protein, thrombospondin, and vitronectin [9–11]. The general structural outline of FN consists of a dimer of two subunits, each is about 250 kilodaltons (kD) [12]. Each subunit is folded into an elongated and flexible arm 60 nm long, and the two subunits are joined by disulfide bonds very near their C-termini. Within each subunit, there is a series of tightly folded globular domains; each specialized for binding to other molecules such as collagen, glycosaminoglycans, transglutaminase, or to cellular membrane receptors [13, 14]. Since it is known that cells may never see the native biomaterial, the configuration of the absorbed proteins is of utmost importance in cell activation and response. By optimally designing a surface for a specific protein conformational change, we must take into account how the protein 3D topography and chemical structure will affect its absorption onto the material surface. To further investigate the phenomenon of protein adsorption and the effect of nanoscale modulation of the surface, we chose to examine how nanoscale modulation affects FN binding to silicon surfaces.

2. GRATING CHARACTERIZATION

As mentioned, the mechanisms of protein absorption to patterned structures are not clear yet. We chose diffraction grating technology for two reasons. Firstly, there are several results with regards to the role of periodic structures positively affecting the attachment of biological objects, with emphasis on cells [15, 16]. Secondly, diffraction gratings are one of the most widely used optical instruments that are very well inves-

FIGURE 2: Diffraction efficiency at 1st order dependence versus the corrugation depth.

tigated both theoretically and practically. Diffraction grating technologies are recognized to permit for a defined grating period, corrugation profile, and corrugation depth.

There are three main practical diffraction grating profiles: sinusoidal, trapezoidal, and rectangular. Figure 1 presents a simplified model for optimal grating profile and hypothesized protein attachment based on assumptions described in [17].

The next parameter to determine is corrugation depth. We expect that optimal corrugation depth will correspond to the maximum of diffraction efficiency. Figure 2 presents results of calculations of diffraction efficiency versus the corrugation depth made by modified C-method [18].

In total, we decided to use nanopatterned surfaces with a period about 350 nm (175 nm plateaus and 175 nm valleys), a corrugation depth about 90 nm and rectangular grating profile to explore protein adsorption onto silicon surfaces.

3. TECHNOLOGY AND EXPERIMENT

3.1. Grating fabrication

P(boron)-type silicon wafers from Silicon Quest International, (Santa Clara, Calif, USA), with $\langle 1-0-0 \rangle$ orientation with thickness equal to 510–540 μm ; material resistivity was 4–20 $\Omega\text{-cm}$, were utilized for this study. Diffraction gratings were fabricated by optical holography. As a laser source, we used Coherent INNOVA 300C FReD Ar laser with frequency doubling. The diffraction grating was fabricated on the silicon substrates by holography with UV5 photoresist as a mask material. The mask structures were etched by RIE DryTek system at the following conditions: C_2F_6 —40 sccm; O_2 —8 sccm; RF power—120 W; pressure—223 mTorr. These conditions resulted in diffraction gratings with rectangular profile having size of 3 mm $\times$ 5 mm within the total 10 mm $\times$ 10 mm silicon substrate.

FIGURE 3: AFM image of fabricated diffraction grating used as controllable rough surface and the sectional height profile along the line.

3.2. Atomic force microscope (AFM) and scanning electron microscope (SEM) measurements

In order to assess surface topography of the grated surfaces, AFM (Nanoscope III, Digital Instruments/VEECO) was used. All the AFM images were obtained using an E scanner with maximum scan area $14.2 \times 14.2 \mu\text{m}^2$. Height, deflection, and friction images were obtained in contact mode in ambient air with silicon nitride tips (NP, VEECO). The scan rate used was 0.8–1 Hz. Integral and proportional gains were approximately 2.0 and 3.0, respectively. Figure 3 presents an example of AFM measurements for the fabricated gratings. It is very important that our samples have highly homogeneous nanopatterned structure. Five different areas of the diffraction grating were analyzed to check the periodicity and the depth of the grating. Nanoscope software was used to analyze the images. Using sectional analysis, the periodicity of the grating was found to be $355 \text{ nm} \pm 0.08 \text{ nm}$ and the height was found to be $87 \text{ nm} \pm 3 \text{ nm}$ (Height information might not be very accurate as may be the tip is not reaching the bottom most point of the grating).

In order to verify AFM measurements, we made SEM image of the fabricated grating. These measurements confirmed the high uniformity of the fabricated gratings; this is an important factor for our biomedical research.

In summary, the grating was found to be highly uniform in periodicity and height at various places. A 2D image of a two-dimensional grating is shown in Figure 5. The homo-

FIGURE 4: SEM image of the fabricated grating.

geneity was demonstrated to be 0.02% for the grating period and 2.0% for the grating corrugation depth.

3.3. Protein adsorption assay

Prior to protein absorption, all silicon samples were cleaned via the RCA cleaning procedure. After which, human FN (Sigma, St. Louis, Mo, USA) was reconstituted to a final concentration of $10 \mu\text{g}/\text{ml}$ in phosphate buffered saline (PBS). Protein was adsorbed onto experimental silicon surfaces by immersion in the prepared FN solution for 2 hours at room temperature with gentle rotation. Additional samples were also immersed in PBS solution without protein to be used as control surfaces. After incubation, the solutions were removed and the samples were carefully washed 3 times with PBS to eliminate any unbound protein. Care was taken in order to prevent the drying of the protein-coated surfaces before further analysis.

3.4. Immunodetection of adsorbed proteins

Patterned and control surfaces were removed from PBS and incubated with 2% bovine serum albumin (BSA; Sigma, St. Louis, Minn, USA) solution for 2 hours in room temperature in order to block later nonspecific antibodies binding. Immunostaining procedure was performed with FN chicken antihuman antibodies (Invitrogen, Chicago, Ill, USA) diluted 1 : 1000. Following a PBS wash, samples were then incubated for 2 hours with an Alexa Fluor 488 goat antichicken IgG (H+L) (Invitrogen, Chicago Ill, USA) diluted 1 : 200. Both primary and secondary antibodies were individually diluted in PBS with 1% BSA. Between each step of the immunostaining procedure, samples were repeatedly washed with PBS. For each assay, an additional control was prepared consisting of a protein-coated sample submitted to the same described procedure but instead of incubating with the primary antibody, PBS was used. Thus, the protein-coated samples were exposed to the secondary antibody only as a control. Subsequently, the silicon wafers were mounted on glass slides using ProLong Gold Antifade reagent (Invitrogen,

FIGURE 5: (a) Fluorescence micrograph of the border between the patterned surface (right) and the nonpatterned surface (left) (200x). (b) Graph depicts the average pixel density from the fluorescence microscopy analysis.

Chicago Ill, USA). Immunostaining results were observed and recorded using fluorescent microscope (Nikon Eclipse TE2000-U). Ten digital images of each sample ($n = 3$) were captured and analyzed for average pixel intensity on both the patterned and nonpatterned areas using Image J Software. The results demonstrated a significantly higher ($P = .01$) level of fluorescence on the patterned area as compared to the nonpatterned surface (Figure 4). These results strongly indicate a higher level of FN protein attachment occurring on the patterned surface as compared to the nonpatterned surface.

3.5. Further development

The mechanisms of why there was an increase in protein attachment on the patterned surfaces are not clear yet. Thus it will be interesting to investigate variations in nanopatterned structures (size and shapes) which could be effective for alterations in protein attachment. After fabrication of 1D diffraction grating by deep UV lithography, we also fabricated 2D

FIGURE 6: 2D diffraction grating with grating period $\Lambda = 351.2$ nm.

gratings with the same period $\Lambda = 351.2$ nm in both coordinates (Figure 5).

To fabricate such structures, we simply exposed the surface twice. Prior to the second exposure, we rotated structure on 90° . We expect that 2D gratings will give another prospective structure for examining changes in protein adsorption. It has been shown that 2D periodical structure is a good candidate for using of tuning localized plasmons for the surface-enhanced Raman scattering [19]. Future studies will compare protein adhesion of 1D and 2D nanopatterned surfaces.

4. DISCUSSION

FN is a representative of a cell adhesion protein that is present in both plasma and the extracellular matrix. Altering the attachment of this protein suggests that our nanopatterned structures may lead to changes in the acceptance level of biomaterials by the host. Rechendorff et al. proposed that protein shape effects its interaction with biomaterial surface [20]. They created random nanosize rough surface by evaporation of tantalum films, with surface roughness in the range between 2.0 and 32.9 nm. They determined that fibrinogen, due to of its elongated shape, is much more sensitive to the surface roughness as compared to bovine serum albumin, a protein which has a nearly globular shape.

5. CONCLUSIONS

We proposed a simple model for protein attachment regarding grating corrugation profile. From our model, we examined diffraction gratings with rectangular grating profile.

We found that diffraction grating could serve as a controlled rough surface for FN. Our results strongly indicate a higher level of FN attachment occurring on the patterned surface. Thus the nanopatterned surface has a significant positive effect on the binding of FN.

Such a positive result for FN, which plays a key role in cell adhesion and mediating cell response, proves that

cell attachment could be improved on investigated nanopatterned structures.

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