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**QUANTITATION OF LAMININ ADSORBED
ONTO POLYDIMETHYLSILOXANE SURFACES USING
VARIOUS TREATMENT PROTOCOLS**

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INTRODUCTION

There is considerable interest in how cells respond to mechanical stimuli, from the ligands used to transmit the stimulus to the signaling pathways initiated and the proteins expressed upon phenotype change [1]. Previous work focused on the evaluation of the quality of the extracellular matrix (ECM) coating and cell proliferation [2]. Our focus is the characterization of a flexible polymeric substrate, treated with ECM, used to induce tensile strain on cells. In this work, we expand our physical characterization of the protein modified polydimethylsiloxane (PDMS) surface by quantifying the coverage of laminin on PDMS, plasma-treated PDMS, and PDMS treated with plasma and aminopropyltrimethoxysilane (APTMS) (Silane_70 protocol) using X-ray reflectivity.

In short, we subjected PDMS substrates to oxidation via plasma. Then the surfaces were subjected to one of the following treatments using fibronectin or laminin: physical adsorption, adsorption via polar interactions (chemisorption), and covalent bonding. We performed physical characterization of relative amounts of protein coverage, uniformity, roughness and hydrophilicity. Attachment and proliferation of rat aortic smooth muscle (A10) cells were also evaluated. It was found that one of the chemisorption protocols, silane_70, provided the highest amount of protein coverage and uniformity. Cell attachment between all methods was comparable, while the silane_70 protocol yielded the most cells after 4 d.

Specular X-ray reflectivity (SXR) is a well established technique used to characterize film thickness, average film density, and interfacial roughness. Recently, our group developed a new metrology utilizing SXR to profile the patterned structure through the film thickness direction in sub-nanometer resolution [3]. Here, X-rays average the density of both the patterns (filled with materials) and space (filled with air) regions as long as physical dimensions being

investigated are smaller than the coherence length of incident X-ray beams. The averaged density is reduced proportionally by volume fraction not covered by materials (in this study, protein). Using this approach, the coverage of protein adsorbed on the smooth substrate can be extracted by fitting SXR data with a multilayer recursive model [4].

EXPERIMENTAL

Materials and Methods

Three types of samples were made: laminin on PDMS, laminin on plasma-treated PDMS, and plasma treated PDMS with APTMS and laminin. For all samples, a 5 % by volume solution of Sylgard 184 elastomer base and curing agent (Dow Corning, MI) [5] in heptane was spun onto a silicon wafer. The wafer was heat treated at 70 °C for 45 min in air. For two methods, the wafers were subjected to oxygen plasma treatment for 30 s at 40 W. For one method, the plasma treated PDMS was exposed to hydrolyzed APTMS for 10 min at 25 °C and 10 min at 70 °C. All samples were then washed with 100 % ethanol and dried with nitrogen gas. The wafers were incubated overnight in 10 µg/ml laminin (Sigma, St. Louis, MO). The surfaces were then washed with water to remove unbound protein, dried with nitrogen, and stored for 24 h at ambient conditions before analysis.

Specular X-ray Reflectivity

The SXR measurements were accomplished using a modified high-resolution X-ray diffractometer in a $\theta-2\theta$ configuration at the specular conditions with the incident angle equal to the detector angle. A finely focused Cu K_{α} X-ray source with a wavelength, λ , of 1.54 Å was conditioned with a four-bounce Ge (220) monochromator and focused onto the film of interest. A three-bounce Ge (220) channel cut crystal was also used to direct the reflected X-rays into the detector. The sample and detector goniometer have an active servo feedback

system to provide an angular reproducibility of $\pm 0.0001^\circ$ [6]. Typically, the specular reflected intensity was collected as a function of incident angle between 0.1° to 1.0° . The detailed SXR measurements and data analysis were performed in a manner reported elsewhere [7]. The sampling area ranges from 100 mm^2 to 140 mm^2 .

RESULTS AND DISCUSSION

Results from immunochemistry (IHC) relative fluorescence intensity experiments and pooled variance t-tests have shown qualitatively that the amount of laminin and fibronectin deposited using APTMS on plasma treated PDMS is significantly higher than for laminin only on plasma-treated PDMS. Confocal fluorescence microscopy was also performed on the fibronectin-treated surfaces and showed identical trends [2]. IHC of laminin deposited on PDMS without plasma treatment was not performed in previous work. This sample was included in the X-ray reflectivity studies to illuminate the effect of plasma treatment on the PDMS surface.

We conducted SXR measurements on this multi-layer system to quantify the protein coverage on PDMS using various treatments. Figure 1 shows the experimental reflectivity data from laminin adsorbed on PDMS surfaces with various surface treatments. The reflectivity collected over a range of incident angles is plotted as a function of $\text{Log}(R, \text{reflectivity})$ versus the scattering vector q , where $q = 4\pi/\lambda \sin(\theta_i)$ where λ and θ_i are wavelength and incident angle of the X-ray beams, respectively. The reflectivity ratio R is defined as the ratio of the reflected (I) to the incident (I_0) beam intensity. At low q , the $\text{Log}(R)$ is zero from total external reflection occurs. Eventually, a critical wave vector is encountered at which the reflected intensity exhibits a sharp decrease as the X-rays start to penetrate the film and beyond which oscillations (with q) of the reflected intensities are observed. The magnitude of q_c^2 (or scattering length density, SLD) is proportional to the total electron density ρ_e of the film through the expression: $q_c^2 = 16\pi r_0 \rho_e$ where r_0 is the classical radius of an electron. The electron density profile perpendicularly through the plane of the film can be deduced by modeling the SXR data as mentioned earlier [4]. Models used for fit the experimental data include single or multi-layers that contain the quantitative information of thickness, electron density, and roughness of each layer. In this case, up to four distinct layers including protein layer, oxidized PDMS layer, non-oxidized bulk PDMS layer, and Si substrate are used to achieve the best fits for each sample.

Several insights were found with this analysis. For the untreated PDMS, there is about a 50 \AA layer of material that is about 70 % less dense than the bulk. The plasma treatment induces a densified layer about 100 \AA thick and results in a decrease of film thickness of about 200 \AA . Approximately 10 \AA of silanized layer appears after treatment with the APTMS. By comparing the experimental protein layer density with the known protein density, one can deduce the coverage of protein. For example, if the protein layer shows 50 % of density compared to the known density of the protein, this indicates a protein coverage of 50 % on the substrate. We calculate the density of laminin (850 kDa) to be 1.42 g/cm^3 [8]. If a density of 1.3 g/cm^3 is assumed, then all surface coverages are 3 % higher.

The detailed structural characteristics determined from SXR are summarized in Table I. All the protein layers have the same thickness, within experimental error. Protein molecules are adsorbed most effectively on the functionalized PDMS with APTMS, following the trend from plate reader results. The PDMS layer with plasma treatment results in second best adsorption, while PDMS with no treatment displays the least coverage of laminin.

CONCLUSIONS

We have shown that we are able to extract quantitative information about multi-layer protein structures on PDMS surfaces. The thickness of laminin on PDMS for all three treatment protocols is statistically the same. Using protocols similar to those in the literature, we have surfaces covered anywhere from one-third to one-half with laminin.

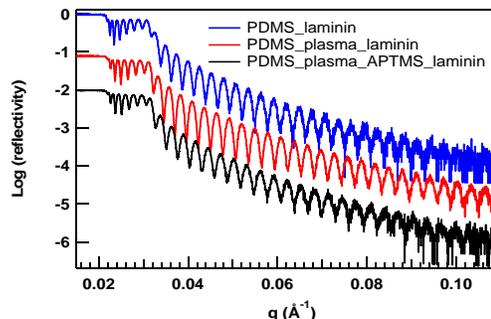


Figure 1. SXR data collected from protein samples adsorbed on PDMS/Si substrate treated with varying surface treatment.

Table I. The detailed structural characteristics determined from fits to the SXR experimental data. The relative standard uncertainties of the scattering length density (SLD), film thickness, and density are $\pm 0.01 \times 10^{-3} \text{ \AA}^{-2}$, $\pm 10 \text{ \AA}$, and $\pm 0.01 \text{ g/cm}^3$ and respectively.

| Substrate | SLD/ 10^{-3} (\AA^{-2}) | Thickness (\AA) | Density (g/cm^3) | Surface coverage (%) |
|-------------------|--------------------------------------|----------------------------|-----------------------------|----------------------|
| PDMS | 0.218 | 30 | 0.479 | 34 |
| PDMS/plasma | 0.281 | 20 | 0.617 | 44 |
| PDMS/plasma/APTMS | 0.329 | 22 | 0.723 | 51 |

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- Certain commercial materials, equipment, and software are identified in this paper in order to specify adequately the experimental and analysis procedures. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology (NIST) nor does it imply that they are necessarily the best available for the purpose. Official contribution of the National Institute of Standards and Technology; not subject to copyright in the United States.
- The data throughout the manuscript and in the figures are presented along with the standard uncertainty (\pm) involved in the measurement.
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