Molecular Detection of Proteins on Polymer Materials by Atomic Force Microscopy

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Statement of Purpose: Blood contacting medical devices play an important role in modern healthcare. Many of these devices are constructed from polymeric materials, and although they function adequately for a number of applications, complications including surface-induced thrombosis still exist. Thrombosis arises from complex interactions between protein and cellular elements of blood and the synthetic material surface. The ability to study these interactions has been hindered by a lack of methods to measure single-molecule interactions with materials. Recent advances in atomic force microscopy allow detection of proteins on model surfaces using antibody-coupled probes that measure antibody-antigen interactions.[1] In this study, we continue to develop techniques for molecular identification and seek to extend these tools to polymeric systems including poly-(dimethylsiloxane) (PDMS) with a goal of developing techniques for single molecule analysis on a variety of complex polymers used in medical applications.

Methods: Long-thin triangular silicon nitride cantilevers with integral probe ($k=0.06N/m^2$) were modified with polyclonal antibodies against human fibrinogen as described previously[1]. Protein patterns were prepared using PDMS stamps consisting of an array of pillars having heights of 0.7μm and diameter of 0.7μm. PDMS stamps were incubated with bovine serum albumin (BSA, 250μg/mL) for 1 hr. BSA was patterned onto plasma-cleaned PDMS or onto a hydrophilic mica surface by microcontact (μCP) printing. The patterned sample was subsequently incubated in 100μg/mL fibrinogen solution for 1 hour to fill in the patterned areas. Measurements were made using a Nanoscope IIIa Multimode AFM (Digital Instruments, CA). Topographic images and phase images (to measure mechanical properties) were made at 512 x 512 pixels, while adhesion maps were collected over an area of 2.5 X 2.5 μm with a resolution of 32 x 32 pixels.

In a second set of experiments, Sulfo-N-Hydroxy-Succinimido Nanogold (Nanoprobes) was conjugated to polyclonal rabbit anti-fibrinogen using the manufacturer supplied protocol. Patterned samples were incubated with gold-labeled antibodies for 30 minutes and conjugated nanogold particles were visualized by phase imaging.

Results / Discussion: Standard images of PDMS samples stamped with BSA showed no specific patterns (Fig 1a). This is attributed to the high surface roughness (~14nm) of the PDMS materials (data not shown). The sample was backfilled with fibrinogen and adhesion maps were taken with modified probes. Fibrinogen-antibody interactions were detected on the sample but patterns were not easily visualized, primarily due to increased background forces seen with these polymer materials (Fig 1b).

Nanogold particles conjugated to anti-fibrinogen were used as a second protein detection technique. As a test system, BSA was again patterned, this time on mica and adsorbing fibrinogen onto the mica was found to result in a smooth protein layer (Fig 2a). Following infusion of anti-fibrinogen conjugated nanogold, μCP pattern were easily detected in the phase image (Fig 2b). Current efforts are focused on application of these techniques to PDMS to verify the existence of patterns on the surface for use in immuno-AFM development.

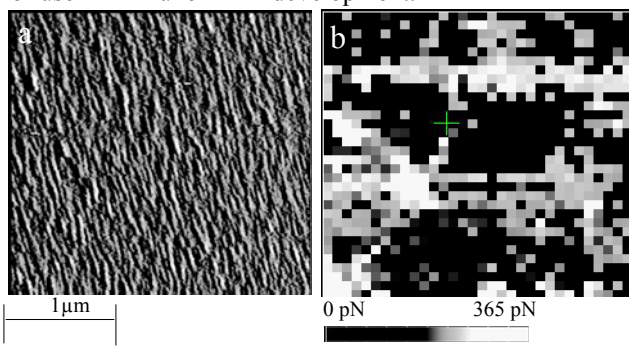

Figure 1: (a) Phase image of BSA stamped PDMS sample with fibrinogen. (b) Adhesion map of sample in (a)

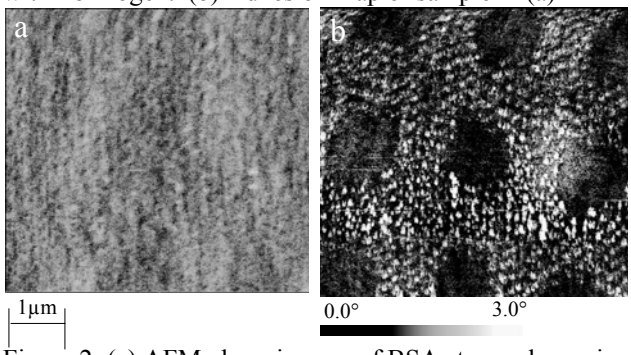

Figure 2: (a) AFM phase images of BSA stamped on mica with fibrinogen backfilling before, and (b) after addition of nanogold anti-fibrinogen conjugates. Patterns of fibrinogen are clearly visualized.

Summary: Molecular detection of individual proteins in multi-protein films on polymers is important for understanding blood material interactions. Adhesion mode AFM can be used to detect a specific protein type in a dual protein layer created by microcontact printing. Patterns were not as easily visualized on the polymers as they were previously on mica due to difficulties in μCP on polymers as well as large background forces.

Mica was used to test the utility of gold conjugated anti-fibrinogen as an additional technique for protein detection. Phase images showed clear patterns following immunolabeling and can be obtained at higher resolution compared to the adhesion techniques.

References:

1. Agnihotri A., *Ultramicroscopy,* 102, pg 257, 2005.