Molecular Detection of Fibrinogen on Poly (dimethyl siloxane) by Atomic Force Microscopy

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Statement of Purpose: The success of long-term blood-contacting devices is largely dependent upon the interaction of the blood components with the device biomaterial surface. The ability to study these interactions has been hindered by a lack of methods to measure single-molecule interactions in complex multi-protein environments similar to those found in-vivo. Atomic force microscopy methods typically utilize sub-monolayer distributions of a single protein type from dilute solutions onto model material surfaces. In vivo however, multiple proteins can adsorb onto rough polymeric biomaterial surfaces thereby making AFM identification of specific proteins at the molecular level a difficult task.

Previous experiments in our lab have used AFM in conjuction with gold nanolabels to map out fibrinogen in a dual-protein layer on mica without the topographical clues usually necessary for high resolution visualization. In this work, the technique has been extended to a model polymer – Poly (dimethyl siloxane) (PDMS) as well as to non-patterned substrates with the future goal of investigating competitive protein adsorption on more complex polymers.

Methods: A PDMS stamp consisting of an array of pillars (nominal height – 0.7µm and spacing – 0.7µm) was incubated with bovine serum albumin (BSA) (1mg/mL) for 1 hour. This pattern of BSA was stamped onto plasma-cleaned PDMS by microcontact printing (µCP). Fibrinogen solution was added to the patterned sample (1mg/mL), backfilling the holes to form a uniform layer. Sulfo-N-Hydroxy-Succinimido Nanogold (diameter-1.4nm) (Nanoprobes Yaphank, NY) was conjugated to polyclonal rabbit anti-Fibrinogen. Nanogold solution was flown onto the PDMS sample (flow rate – 1ml/hr) for 1 hour. A Nanoscope III Multimode AFM (Digital Instruments, CA) was used to directly visualize the location of fibrinogen by phase imaging in buffer.

A mixed protein solution (1mg/ml - 90% fibrinogen + 10% BSA by volume) was adsorbed to a PDMS sample, labeled with Nanogold-antibody and imaged by AFM in buffer. The phase images were converted into an intensity map using image processing tools in matlab. A histogram of all the intensity values was plotted and a 95% confidence interval cut-off limit was established using guassian fitting.

Results / Discussion: Nano-gold particles conjugated to anti-fibrinogen were used as a detection technique to map out fibrinogen from a dual protein layer on PDMS. BSA was stamped onto PDMS (fig.1a) and backfilled with fibrinogen which successfully filled the height difference between the two proteins (fig.1b). After infusing the sample with anti-Fibrinogen conjugated Nanogold, µCP pattern can be easily detected in the phase image (fig.1c). The nanogold-conjugated antibody binds to the surface-adsorbed fibrinogen and is identified from the lower phase angle shift induced by the presence of the hard nanogold particles. The phase image shows little non-specific binding in region between the circular fibrinogen regions. In order to use this technique to study competitive protein adsorption, mixed protein solutions of fibrinogen and BSA were absorbed onto PDMS and Nanogold-antibodies were added. Phase image in figure 2a shows darker regions with lower phase angles potentially identifying fibrinogen on the sample. The histogram of the intensity values of the phase images (fig. 2b) clearly shows two distinct peaks, the lower intensity peak as fibrinogen and the higher intensity peak as BSA. The cut-off limit was calculated from fitting Gaussian distribution to the BSA peak and will be used to detect specific fibrinogen recognition events. We continue to optimize the methods to characterize specific recognition of proteins in mixed protein experiments using varying ratios of proteins.

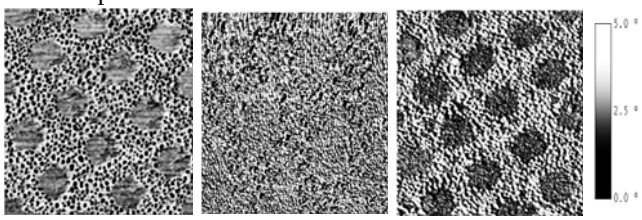

Figure 1: Phase image (a) showing BSA stamped PDMS sample, (b) after fibrinogen backfilling & (c) showing direct visualization of fibrinogen using Nanogold particles. Scan size – 5 x 5 µm^2

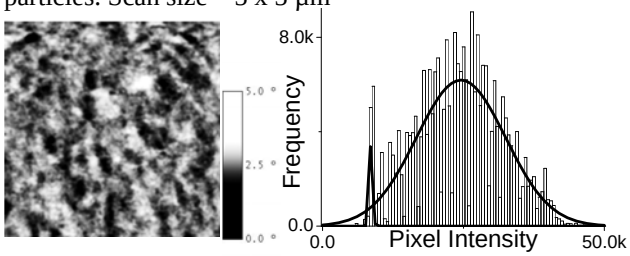

Figure 2: (a) Phase image of mixed protein solution (90% Fibrinogen+10% BSA) on PDMS (scan size-2x2µm^2) (b) Histogram of intensity values of the phase image in (a).

Summary: This technique identified fibrinogen in a mixed protein layer in aqueous environment on a clinically relevant polymer. Gold nano-labels were conjugated to an anti-fibrinogen polycolonal antibody to produce a unique identifier tag for fibrinogen. AFM mechanical property imaging showed that the gold-conjugated labels specifically identify the regions of the sample corresponding to fibrinogen with little to no nonspecific labeling. This technique was also extended to fibrinogen in a non-patterned mixed protein layer.

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