Functional Changes in Adsorbed Fibrinogen Measured by Adhesion Mode AFM

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Statement of Purpose: Platelet adhesion to a biomaterial surface requires fibrinogen adsorption and conformational changes that expose the platelet binding epitope in the γ-chain dodecapeptide (γ400-411). Atomic force microscopy (AFM) has emerged as a useful tool to study biological interactions due to its nanoscale resolution and its ability to image in physiologically relevant aqueous environments. Previous studies in our lab used AFM to study the conformational changes in single fibrinogen molecules [1] and map their locations in complex protein films by a technique known as adhesion mapping [2].

In this study, the adhesion mapping technique was extended to time-dependent protein activity. A monoclonal antibody that recognizes fibrinogen γ392-411, including the γ-chain dodecapeptide, was coupled to the end of an AFM probe. Adhesive interactions between the modified probe and the surface are used to measure the antibody-antigen interactions, creating a map which illustrates time-dependent functional changes of the surface-adsorbed fibrinogen, schematically shown in figure 1.

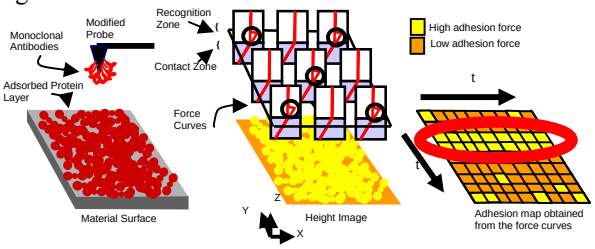

Figure 1: Schematic illustration of the measurement. Monoclonal antibody is attached to the probe, measurements are made across the sample and each individual measure is linked to the time at which the force curve was acquired. In this way, the spatial distribution is coupled to the time when the measure was made.

Methods: Triangular silicon nitride cantilevers with integral probes (k~0.06N/m) were modified with monoclonal antibodies recognizing γ-392-411, a region which includes the chain dodecapeptide, through a standard protocol [2]. Bovine serum albumin (BSA) (76μg/ml) and human fibrinogen (86μg/ml) were adsorbed onto muscovite mica for time periods of 5-60 minutes in a fluid cell. Protein solution was exchanged for fresh buffer by a steady flow of PBS at 0.3ml/min.

All data was collected using a Nanoscope III Multimode AFM (Digital Instruments, CA) under buffer conditions with varying scan sizes (500nm^2-5000nm^2) and scan rates (0.5Hz – 1 Hz). Images were collected in 32x32 pixel format with 256 data points per force curve. Adhesion values were determined from the maximum deflection of the force curve multiplied by the appropriate spring constant. Interactions between fibrinogen and the antibody were then characterized as either specific or nonspecific using a 95% confidence interval calculated from BSA data generated for each probe. Adhesion map data from multiple runs were compiled and the probability of the antibody encountering the antigen at any post-adsorption time (± 30 sec) was calculated.

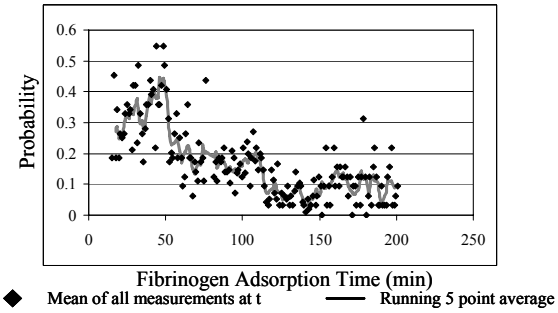

Figure 2: Probability of adhesion between a monoclonal antibody and its antigen on fibrinogen. The gray line shows a running 5 point average to guide the eye.

Results / Discussion: Figure 2 illustrates the time-dependence in the probability of antigen recognition of the region that includes the platelet binding domain. Data shows that the maximum likelihood of adhesion occurs in the range of 30-50 minutes post-fibrinogen adsorption, although very short time frames (< 15 mins) have been difficult to measure for technical reasons. At longer adsorption times, the probability of adhesion decreases dramatically. Furthermore, experiments performed with 60 minutes protein adsorption prior to initiating measurements showed similarly low probabilities of adhesion at corresponding time points, confirming that this is a time-dependent surface process rather than an artifact of antibody degradation after extended periods of measure. We continue to perform experiments and refine data handling of the many measures collected (~15,000 individual force curves). Force distributions of BSA and fibrinogen show sufficient differences to demonstrate that the monoclonal antibody specifically recognizes its antigen (data not shown) Thus, the monoclonal antibody modified probe can potentially map both the spatial location as well as the time-dependent functional activity of absorbed fibrinogen at AFM type scales.

Summary: A monoclonal antibody recognizing a region that includes the platelet binding epitope of the γ chain dodecapeptide (γ400-411) was used to measure fibrinogen functional activity following adsorption. The probability of adhesion is highest at ~ 40 minutes after adsorption and then declines. This data can provide us the means to assess time-dependence in platelet binding at AFM scales.

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References: (1)Agnihotri, Langmuir, 88, 2004.
(2)Agnihotri, Ultramicroscopy, 102, 2005.