Fibrinogen Adsorption on Ti-6Al-4V Studied With Atomic Force Microscopy

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Introduction: The process of Fibrinogen (Fb) adsorption is a key indicator of thrombus formation on medical devices. Ti-6Al-4V is used in a variety of medical device applications and is one of the most biocompatible metallic biomaterials available. It is therefore important to study how Fb adsorbs on the surface of Ti-6Al-4V thus affecting its biocompatibility. Atomic Force Microscopy (AFM) can directly image Fb molecules adsorbed on relatively flat surfaces allowing qualitative and quantitative assessment of protein adsorption. Thus, the goal of the current study was to understand the conformation and time dependent adsorption behavior of Fb on the surface of Ti-6Al-4V using AFM.

Methods: Fraction I, type I-S Fibrinogen from bovine plasma (Sigma-Aldrich) was suspended in 0.154M phosphate buffered saline (PBS), pH 7.4 at a concentration of 5.0 μg mL^{-1}. Circular disc shaped samples of Ti-6Al-4V (n=3) were polished using a mechanical-chemical polishing technique (10% w/v H_2O_2 with colloidal silica suspension) to obtain relatively flat surfaces.[3] A Digital Instruments multi-mode AFM-2 with nanoscope IIIa scanning probe microscopy controller (Veeco Instruments) was used for imaging. Ex situ experiments were performed wherein the samples were immersed in protein solution for the desired time, removed, rinsed with DI H_2O, dried in vacuum and imaged. Tapping Mode AFM in air was used for imaging purposes. High concentration experiments were performed (1 min immersion time at a concentration of 100 μg mL^{-1}, Contact Mode AFM) to confirm the presence of Fb. Sample immersion time was varied from 0 to 15 min and all the experiments were carried out at ambient room temperature. At least three images were obtained for each immersion time for each of the three samples (n_{total}=9 for each immersion time). AFM bearing and section analysis software was used to determine area fraction covered by Fb and height of Fb molecules respectively.[2] A Langmuir adsorption kinetics equation was fit to the data using a least squares regression method; $\theta(t) = \theta(\infty)[1-\exp(-t/\tau)]$, where t is the time in min, $\theta(t)$ is the area covered by Fb in time t, $\theta(\infty)$ is the equilibrium area coverage and τ the is time constant.

Results: Relatively flat surfaces were obtained by the polishing technique used to clearly distinguish the substrate from adsorbed Fb, R_{RMS}<0.8nm. High concentration experiments confirmed that Fb adsorbed in a porous network-like structure [Fig. 1]. Monolayer coverage was observed with an average height of 2.7±0.7 nm. Figure 2 presents a series of images showing Fb adsorption on Ti-6Al-4V over time at 5 μg mL^{-1} concentration. A network-like assembly pattern can be observed to form over time. Similar images were used to analyze area coverage and create adsorption kinetics plots which were fit with the Langmuir model [Fig. 3].

Figure 1. AFM height image of Fb adsorbed onto Ti-6Al-4V (immersion time: 1min @ 100 μg mL^{-1}). Center region was scraped off to confirm presence of Fb. Scan size: 3 μm x 3 μm. The contrast in z direction is 15 nm.

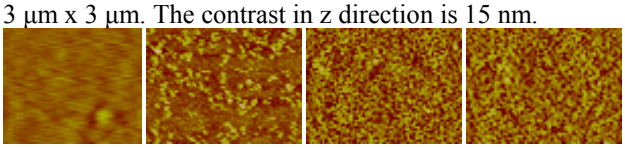

Figure 2. A sampling of AFM height images used to create kinetics curves (Fig. 3). Scan size (all images): 1 μm x 1 μm. The contrast in z direction is 8 nm. Fraction area covered by Fb increases with time.

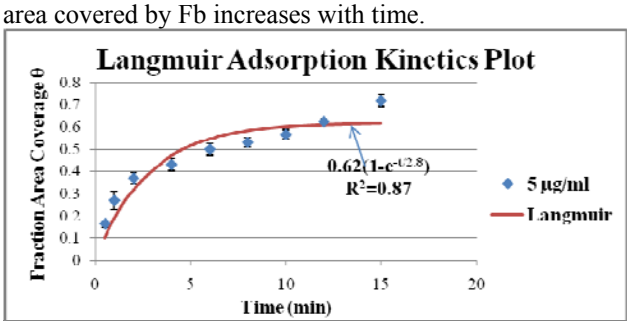

Figure 3. Graph showing Avg. Fraction Area Covered v/s Time and the Langmuir Curve Fit to the raw data. Error bars represent ±SD(n=9).

Conclusions: This study demonstrates that AFM image analysis can both qualitatively and quantitatively analyze protein adsorption. Fb forms a porous network structure on the surface of Ti-6Al-4V which was not the case with 316L SS reported earlier.[1] A Langmuir kinetics approach was used to determine the behavior of Fb on Ti-6Al-4V. The Langmuir model fit quite well with an R^2 value of 0.87. Maximum area fraction coverage of 0.62 was observed with a time constant of 2.8 min for a concentration of 5.0 μg mL^{-1}. Future work will include looking at different concentrations and immersion times. In situ experiments will be carried out and results will be compared to those obtained with ex situ imaging method. The formation of porous network structure will be explored further.

References: 1. Gettens RTT, JBMR A 2006; 465-473.
2. Gettens RTT, JBMR A 2005; 72:246-257.
3. Van De Keere, Langmuir 2008; 24(5):1844-52.