Fibrinogen Adsorption onto 316L Stainless Steel: Voltage Effects

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Statement of Purpose Fibrinogen adsorption has been proposed as an indicator of thrombus formation on medical devices.[1] Direct imaging using atomic force microscopy (AFM) provides the opportunity to qualitatively and quantitatively understand protein adsorption onto surfaces. We have developed methods to directly observe and quantify protein adsorption onto surfaces using AFM.[2] Research in the late 60s and early 70s using animal models showed how voltages at metallic surfaces can effect blood-surface interactions. The work showed that clotting occurred with more noble alloys, while little to no clotting occurred with less noble alloys.[3] The hypothesis was that anodic voltages caused fibrinogen to polymerize at the metallic surface inducing the blood clotting cascade. At the time, methods for direct observation of protein adsorption onto surfaces were not developed. Now that we have the tools to directly observe fibrinogen adsorption onto surfaces, we wish to revisit the effects of voltage on blood protein-metallic surface interactions. The purpose of this work is to quantify fibrinogen adsorption over a range of potentials onto a single alloy (316L stainless steel).

Materials and Methods

The substrate material used was 316L stainless steel (SS, Medtronic, Inc.) The steel was mechanically polished followed by electrochemical polish, passivation and plasma etching. The protein solution was prepared with bovine plasma fibrinogen (fraction I, Sigma-Aldrich), suspended in 0.154 M phosphate buffered saline (PBS), pH 7.4 (Sigma-Aldrich) at a concentration 2.0 µg/ml. All test samples were immersed in the protein solution for 30 min. in an electrochemical set-up, removed from solution, rinsed in de-ionized water and imaged using AFM in a dry state. Control samples were immersed in PBS solution devoid of protein using the same method. A model 263a potentiostat/galvanostat (Princeton Applied Research) was used with a Ag/AgCl reference electrode and a carbon counter electrode to apply the electrical potentials to the samples in a glass beaker. Samples were potentiostatically polarized between -1 V and 1 V (for 30 min.). A control sample with an open circuit potential (OCP) of 142 mV was analyzed.

A Multi-Mode AFM-2 with a Nanoscope IIIa controller (Veeco Instruments, Inc.) was used for imaging. Standard NP-S (Veeco, inc.) afm probes were used for imaging in contact mode.

Results and Discussion

A marked difference in fibrinogen surface area coverage was observed at potentials above 0 V (vs. Ag/AgCl, Fig. 1). At potentials between 300 and 700 mV the average height of the observed fibrinogen molecules increased greatly (Fig. 2). The observed protein molecules in this voltage range appeared to fall into discrete height ranges of ~3 nm and ~6 nm indicating possible multi-layer formation in this voltage range. Since areas of both ~3 nm and ~6 nm were observed the average height value in this region falls some where between these values. At 700 mV all protein molecules fell in the ~6 nm range. At potentials lower than 300 mV and at 900 mV the average fibrinogen molecule height was ~3 nm. The experiment was carried out at voltages lower than -300 mV, however a precipitate or hydrated surface clouded both the test and control images at these voltages (particularly between -400 mV and -600 mV) and conclusive quantitative analysis of fibrinogen adsorption is not yet possible. Figure 3 displays two representative AFM images of fibrinogen adsorption onto polarized 316L SS surfaces used to make the analysis.

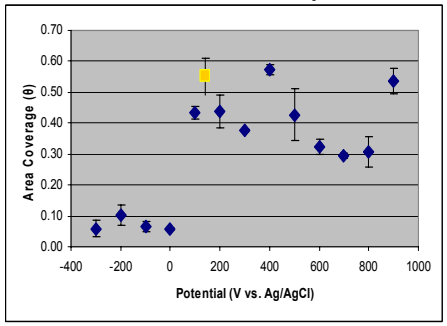

Fig. 1: Protein area coverage of polarized 316L SS. Diamonds indicate means. Error bars represent ± one standard deviation. The square represents the control sample.

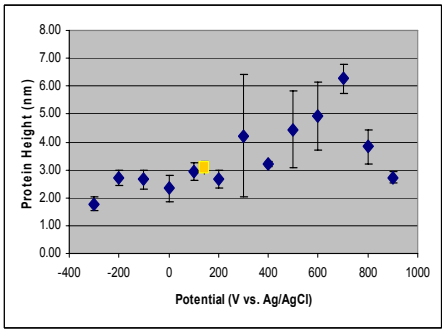

Fig. 2: Protein height analysis of polarized 316L SS. Diamonds represent means. Error bars represent ± one standard deviation. The square represents the control sample.

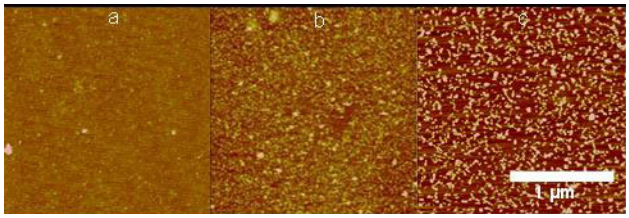

Fig. 3: Images of fibrinogen adsorption onto polarized 316L SS. Surfaces were polarized to a) -200, b) +100 and c) +600 mV. The scan height in all images is 10 nm.

Conclusions

A marked decrease in protein adsorption was observed in 316L SS samples polarized below 0 V. Evidence of multilayer formation or conformational change was observed in 316L samples polarized between 300 and 700 mV.

References

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3. Sawyer, P.N.: Nature. 1965: 206(989): 1162-3.