Selective Protein Adsorption Influenced by Exposure of Electrical Charges Along Grain Boundaries on Metals

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Introduction. Interaction of human plasma proteins with the vascular biomaterial surfaces is the initial step in the chain of events leading to tissue incorporation of endovascular devices. Electrostatic forces have been known to play a significant role in the interaction of proteins and blood cells with the vascular wall as well as vascular biomaterial implants. Electrostatic forces associated with metal surfaces of implants such as vascular stents are known to influence the adsorption of charged plasma proteins. This study explores a unique approach to exploit localized surface electrical charges associated with grain boundaries to influence the adsorption of selective plasma proteins.

Materials and methods. The stent materials tested included differently finished 316L stainless steel (SS) and L605 coupons. SS (Fort Wayne Metals, IN) was degreased (as received, AR), mechanically (MP) or electrochemically polished (EP); and chemically etched (CE). L605 (High Temp Metals, CA) was degreased, electropolished (EP) and electrochemically etched (CE). In both cases the CE process was chosen such that the grain structure of the material was revealed. These surfaces were characterized using optical and fluorescent microscopy, AFM (Veeco Nanoscope IIIa, CA) and XPS. Cationic (LissamineTM rhodamine B) and anionic fluorescent dyes (Fluorescein-5-(and-6)-sulfonic acid) and were used to gain qualitative information about the charge state of the specimens. The AFM characterization included determination of the adhesion forces at the surface (force volume measurement).
Fluorescent and ^{125}I labeled human albumin (Bayer, US), fibronectin, vitronectin (Sigma/Aldrich, US) and fibrinogen (Molecular Probe and MP Biomedicals) PBS solutions of physiological concentrations of were incubated on the SS specimens. Adsorbed plasma protein distribution was detected using primary and secondary antibodies. Quantitative assessment was carried out by measuring the adsorption of radiolabeled (^{125}I) proteins on the surfaces.

Results. Figure 1 shows adhesion between a negatively charged Si_3N_4 AFM tip and different SS specimens, as evaluated from force volume data. Note the high attractive force at the grain boundaries. Figure 2 shows AFM data obtained on a CE L605 specimen. Figure 3 shows selective protein adsorption results. The differences between CE and EP specimens are significant for all proteins ($P<0.05$).

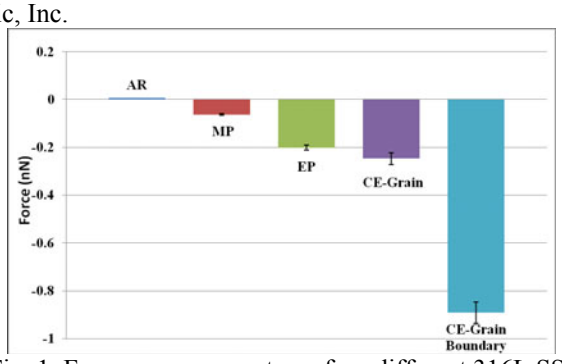

Fig. 1. Force measurements on four different 316L SS substrates using a 5 nm silicon nitride tip; 0.01M NaCl medium pH 7.4.

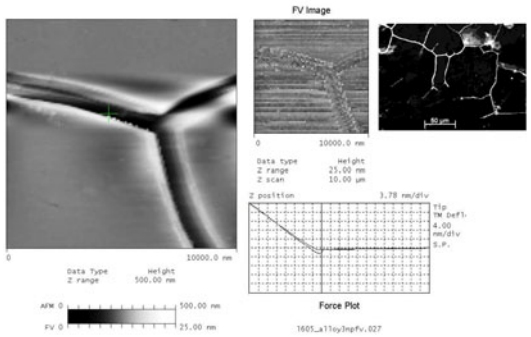

Fig. 2. AFM height (left) and force (center, FV) images on grain boundary etched L605 specimen. Right: fluorescence microscopic image of adsorbed anionic dye (20X). Bottom: A force-distance curve taken at the cross in the height image).

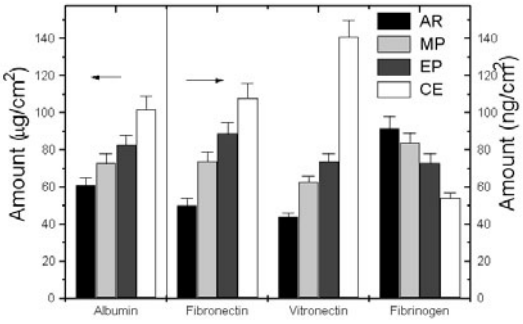

Fig. 3 Adsorbed protein amounts on SS specimens after 120 min incubation.

Conclusions. Due to their RGD sequence, fibronectin and vitronectin play key roles in the attachment of endothelial cells, while fibrinogen adsorption is a key indicator of thrombus formation on medical devices. The preferential adsorption observed on CE specimens is likely to be due to the electrical charges observed and could be utilized to improve stenting outcomes.