A high throughput method using electron microprobe analysis for quantification of protein adsorption

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Introduction: Protein adsorption is the initial event when biomedical devices are implanted into the body and plays a vital role in implants, biosensors and other biomedical applications. For example, fibrinogen adsorption is implicated in surface-induced thrombus formation and also plays an important role in inflammatory responses and wound healing. A high throughput approach to study protein adsorption onto metallic biomaterials of varying composition and roughness requires that the quantitative method be rapid and accurate while providing sufficient spatial resolution. Proteins contain substantial amounts of carbon, a fact that can be exploited by modern scanning electron microprobe (EMP) instruments able to detect carbon using wavelength dispersive spectroscopy. Our state-of the art EMP system is fully automated, making it additionally suitable for high throughput measurements. One key question is whether the EMP has sufficient sensitivity to detect carbon in a thin (monolayer or submonolayer) protein film. Here, we show that EMP can easily detect less than 1nm of sputtered carbon on a Si surface. We also show that the carbon signal can be used to measure the adsorption isotherm for fibrinogen on a Si surface.

Methods: Silicon wafer samples (20 mm x 15 mm) were cut from [100] Silicon wafers (B-doped, Silicon Inc., Idaho, USA) and cleaned ultrasonically in acetone, ethanol and nanopure water for 15 mins, respectively, prior to testing. Ultra-thin carbon films on these wafers were produced by sputter deposition using a Corona Vacuum Coaters V3T magnetron sputtering system. Short sputtering times of 12s, 21s, 33s, 43s and 61s, respectively were used to create films of 1.2, 2.1, 3.3, 4.3 and 6.3 nm of thickness, respectively. Thickness was determined from a previously calibrated sputtering rate (0.1±0.01 nm/s). Protein adsorption experiments were carried out by incubating silicon samples in fibrinogen solution (bovine plasma fibrinogen in phosphate buffered saline solution (PBS), pH7.4, Sigma, USA) for 1hr at different concentrations of 0.1, 1, 10, 50, 100, 500, 1000 and 2000 ug/ml. After adsorption, the samples were rinsed in PBS and flowing nano-pure water several times then dried in air. The carbon content of all samples on the Si surfaces was measured using a JEOL JXA-8200 Superprobe (JEOL, MA, USA) by wavelength dispersive spectroscopy (3kV, 200 nA and spot size of 50um) by peak height after background subtraction. At least three points on each sample were evaluated.

Results and Discussion: Figure 1 shows the x-ray counts per second versus wavelength for the series of sputtered carbon films. Data collected on a control sample (no sputtered carbon) is also shown. The strength of the x-ray signal clearly increases with the sample thickness. Furthermore, the signal is easily observed over background for the thinnest (1.2 nm) film.

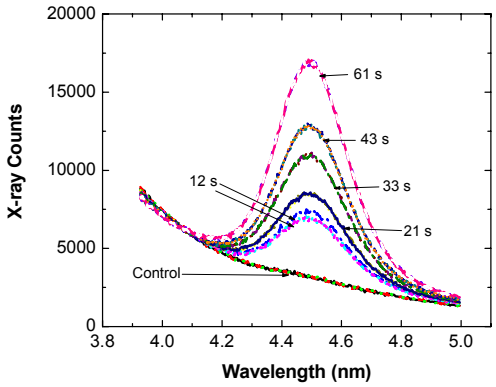

Figure 1. X-ray signal for sputtered carbon films

Fibrinogen is ~51% by weight carbon so it can be detected by EMP. Figure 2 shows the adsorption isotherm, reported as equivalent fibrinogen thickness (effective carbon thickness/weight fraction carbon) versus fibrinogen concentration, using Figure 1 as a calibration between x-ray count rate and carbon thickness.

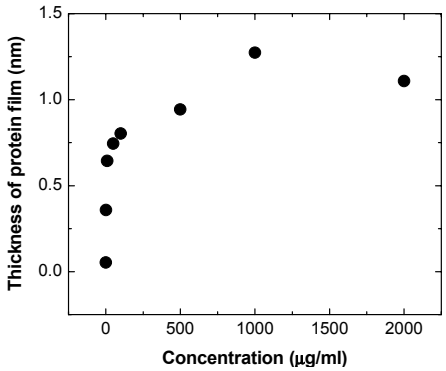

Figure 2. Adsorption isotherm of fibrinogen on Si.

The trend in Figure 2 is clear and indicates an apparent thickness of about 1 nm for the protein monolayer, which is smaller than that measured by AFM (2 nm) [1]. The relationship between EMP signal and absolute fibrinogen thickness will require further adjustment beyond that derived using the carbon film (graphite) data alone.

Conclusion: EMP analysis is a very sensitive technique for detecting carbon film thickness down to the sub-nanometer range, and can be used to quantitatively study protein adsorption on biomaterial surfaces.

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Reference

[1] R.T.Gettens,J.Biomed Mater Res 72A:246-257,2005