

Albumin Adsorption Behavior on Polystyrene and Glass Surfaces

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Statement of Purpose: Protein adsorption is of great interest in the biomaterials community. When a material is implanted *in vivo*, the surface is completely covered in protein within seconds to minutes. This adsorbed protein layer controls further interaction with surrounding tissue.¹ Surface chemistry and hydrophobicity are two of the many material properties influencing protein adsorption. In these studies, polystyrene and glass were used as model surfaces to study the adsorption of albumin, the most abundant protein in blood. Due to its high concentration and small size, albumin is likely among the first proteins to adsorb onto an implanted biomaterial surface.² The goal of this work was to determine the amount of albumin adsorbed onto the two surfaces and how the protein was packed, two important characteristics that influence tissue interaction with an implanted biomaterial.

Methods: Two types of substrates were used: polystyrene (PS) and glass. X-ray photoelectron spectroscopy (XPS) was used to determine surface elemental compositions, with nitrogen as a marker of protein. For XPS, bovine serum albumin (BSA) solutions were incubated with PS petri dishes and glass coverslips for 2h at 37°C. For radiolabeled adsorption, BSA was labeled using Na¹²⁵I. PS substrates were made by spin-coating 3wt% PS in toluene onto glass coverslips. PS and glass substrates were incubated with ¹²⁵I-BSA solutions for 2h at 37°C. BSA surface concentration was calculated from retained radioactivity, specific activity of the protein solution, and surface area. A previously developed model was used to relate XPS and ¹²⁵I-BSA data to determine protein coverage and thickness.³ Surface concentration in ¹²⁵I-BSA experiments is the product of thickness, fractional coverage, and BSA specific weight. Nitrogen signal in XPS is dependent on thickness, fractional coverage, photoelectron cross sections, elemental concentrations, electron inelastic mean free paths, and instrument parameters. For a given sample, there is one solution of thickness and fractional coverage that satisfies both ¹²⁵I-BSA and XPS relationships.

Results: XPS showed increasing nitrogen content on both substrates as the solution concentration of BSA increased from 0.0001mg/ml to 50mg/ml, to a maximum of ~8 atomic % nitrogen (Fig. 1). A moderately higher nitrogen content was observed on PS samples compared to glass. ¹²⁵I-BSA results showed a similar trend (Fig. 1). The surface concentration increased on both substrates as the solution concentration of BSA increased. Maximum surface concentration was ~400ng/cm² for glass substrates and ~500ng/cm² for PS. Relating XPS and ¹²⁵I-BSA data, we observed that on PS the BSA coverage remained ~0.4 for all solution concentrations tested (Fig. 2). Thickness increased from ~2nm to ~10nm as solution concentration increased. In contrast, thickness remained relatively constant (~5nm) on glass while coverage increased from 0.1 to 0.7.

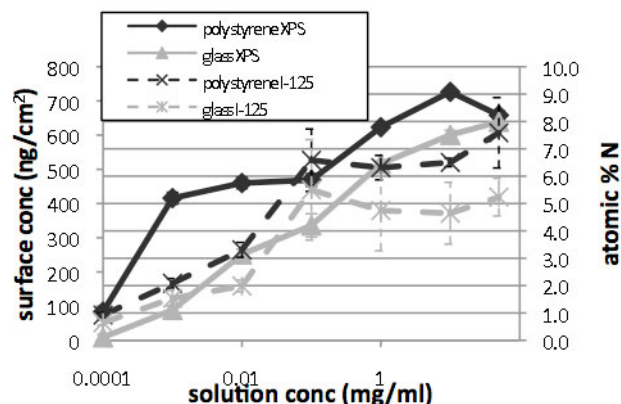


Figure 1. Quantity of BSA adsorbed XPS (solid lines) and ¹²⁵I-BSA results (dashed lines) from protein adsorption onto PS (black) and glass (gray). XPS n = 3; ¹²⁵I-BSA n = 4; error bars = SEM.

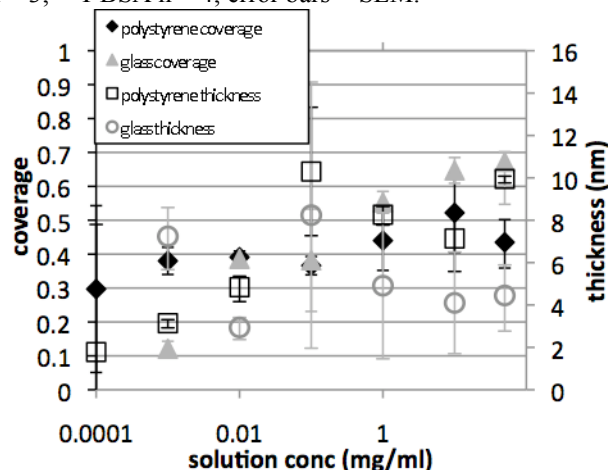


Figure 2. Coverage and thickness of BSA on surfaces Using XPS and ¹²⁵I-BSA data, coverage and thickness of BSA was calculated on each surface. Error bars = SD.

Conclusions: In determining how adsorbed protein will interact with its environment, both quantity and protein packing are important factors. These studies show that the quantity of BSA adsorbed onto two different surfaces is similar, but the protein packing on the surface is different. On glass, the relatively constant thickness suggests that the protein retains a similar shape on the surface at all solution concentrations. In contrast, on PS, thickness increases as the solution concentration increases. This suggests that the protein might be more denatured on the surface at lower solution concentrations.

References:

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