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Superhydrophobic effect on the adsorption of human serum albumin

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Abstract

Analytical protocol greatly influences the measurement of human serum albumin (HSA) adsorption to commercial expanded polytetrafluororethylene (ePTFE) exhibiting superhydrophobic wetting properties. Degassing of buffer solutions and evacuation of ePTFE adsorbent to remove trapped air immediately prior to contact with protein solutions are shown to be essential. Results obtained with ePTFE as a prototypical superhydrophobic test material suggest that vacuum degassing should be applied in the measurement of protein adsorption to any surface exhibiting superhydrophobicity. Solution depletion quantified using radiometry (^{125}I-labeled HSA) or electrophoresis yield different measures of adsorption, with nearly 4-fold higher surface concentrations of unlabeled HSA measured by the electrophoresis method. This outcome is attributed to the influence of the radiolabel on HSA hydrophilicity which decreases radiolabeled-HSA affinity for a hydrophobic adsorbent in comparison to unlabeled HSA. These results indicate that radiometry underestimates the actual amount of protein adsorbed to a particular material. Removal of radiolabeled HSA adsorbed to ePTFE by 3× serial buffer rinses also shows that the remaining "bound fraction" was about 35% lower than the amount measured by radiometric depletion. This observation implies that measurement of protein bound after surface rinsing significantly underestimates the actual amount of protein concentrated by adsorption into the surface region of a protein-contacting material.

Keywords: Protein adsorption; Interphase; Surface; Radiometry; Depletion

1. Introduction

Surface engineering has had a long and successful role in the development of biomaterials for a wide variety of medical devices to improve compatibility with blood and tissue [1–14]. A recent trend in surface engineering has been texturing surfaces at the micro- to nanoscale to influence important interfacial events such as protein adsorption [15,16] and cell adhesion [17,18]. A particular subgroup of textured materials of interest to this work are poorly water-wettable (hydrophobic) materials exhibiting "superhydrophobicity" or ultrahigh water repellency [19–29] (also occasionally termed ultrahydrophobic [19,22] and super anti-wetting [23] in the literature). The superhydrophobic effect ultimately arises from air trapped within the interstices of a rugose surface (patterned or random) that water fails to wet or wick into, so that water is partially riding on a cushion of air, yielding observed water contact angles much greater than the inherent contact angle of the smooth material—sometimes in excess of 150°. The superhydrophobic effect is common in nature, accounting for the water-repellent properties of bird feathers and plant leaves (the "lotus effect") [21,22,30–32] as examples. Certain

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materials in widespread biomedical application such as expanded polytetrafluororethylene (ePTFE) are superhydrophobic as commercially prepared because of the inherent hydrophobicity of PTFE (advancing water contact angle of about 114°) [33] and a fibrillated texture introduced by biaxial stretching (see Fig. 1) [34,35]. For example, we observe an advancing contact angle of about 138° on the ePTFE used in this work. The blood compatibility of ePTFE vascular grafts [36,37] has stimulated interest in understanding, and improving upon, biocompatibility of the general class of superhydrophobic materials.

A fundamental aspect of biocompatibility is protein adsorption [38–43]. Consequently, it is important to fully characterize protein adsorption to superhydrophobic materials. The general literature on protein adsorption is controversial and inconsistent [44,45], so it should be of no particular surprise that there is considerable diversity of opinion regarding protein-adsorption properties of superhydrophobic materials, with some investigators reporting protein-adsorbent properties [16,36] and others non-adsorbent properties [26,32,46].

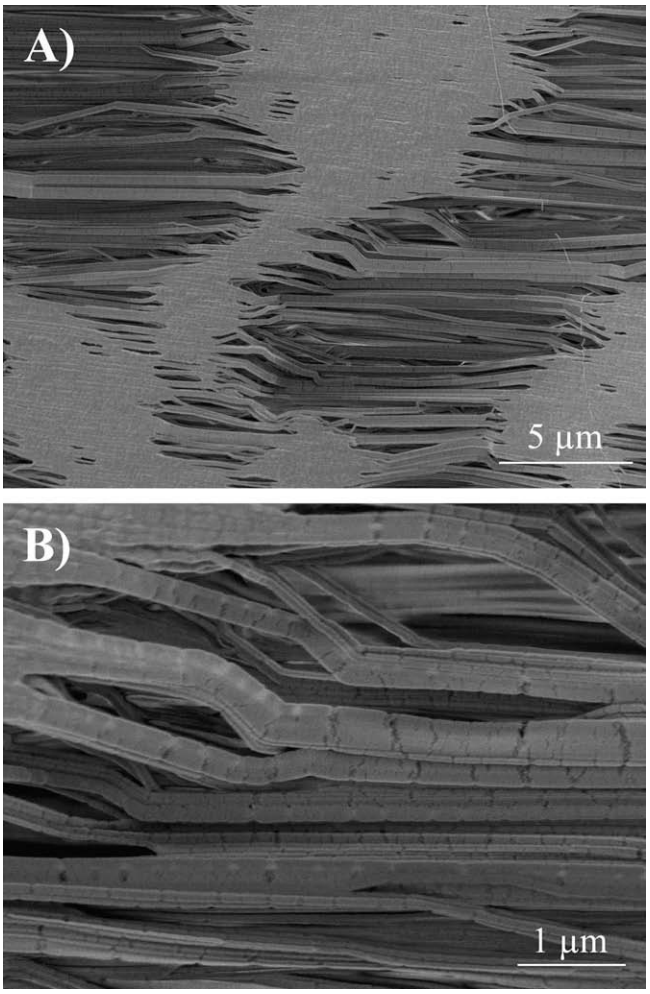

Fig. 1. Field emission scanning electron microscopy images of ePTFE at 5000× (A) and 20,000× (B) showing the fibular structure of the expanded PTFE matrix. ePTFE exhibits superhydrophobic wetting properties with water contact angles in excess of 150°.

We have initiated a program investigating a novel jet-blowing method of producing nanofibrous PTFE for biomedical applications [47,48] and have interest in relating protein adsorption to material properties and processing conditions, using commercial ePTFE (Fig. 1) as a reference material. Herein, we report that analytical protocol greatly affects measurement of the adsorption of human serum albumin (HSA) to ePTFE and show that different methods of calculating the amount adsorbed (bound vs. solution depletion) give rise to quite different impressions of ePTFE adsorbent capacity.

2. Materials and methods

2.1. Protein solutions

Fraction V HSA (MW = 66.3 kDa, 96–99%, lyophilized powder) was used as received from Sigma Aldrich (St Louis, MO) with no further purification. Solutions were prepared in phosphate-buffered saline (PBS, 0.01 M, prepared in 18 MΩ deionized water) degassed under reduced pressure obtained by evacuating the headspace with a rotary vacuum pump (approximately 500 torr) for 5 min. HSA was labeled using the Chloramine T method [49–51] for 30 s to yield a specific activity of 36.4 μCi μg^{-1}. We estimated that an average of ~3 iodine molecules were incorporated into each molecule of HSA (see Appendix A). HSA radioactivity (cpm) was measured by gamma counting in a Wallac 1470 Wizard Automatic Gamma Counter (PerkinElmer). Free iodine was separated from the labeled protein using a G50 Sephadex column (Sigma Aldrich). Labeled protein was stored at 2 °C and used within 2 weeks, over which time protein degradation was regularly assessed by chromatography on a G100 Sephadex column (Sigma Aldrich) to detect protein fragments or aggregation. Analysis of chromatographic peak area revealed that less than 10% of labeled protein was affected by radiolysis over the 2 week storage period. Test protein solutions were prepared by mixing 3 μl of stock labeled protein solution with 1.5 ml unlabeled protein solution at the desired concentration to yield approximately 0.09 μCi in each 200 μl sample.

2.2. ePTFE adsorbent preparation

Expanded ePTFE was a gift from Atrium Medical Corp (Hudson, NH). As illustrated in the left-hand side of Fig. 2, 20 mg portions were weighed into glass screw-top glass vials VWR (West Chester, PA) using a precision microbalance (XS 105, Mettler Toledo). Each vial was fitted with a rubber septum lined with PTFE with an evacuation port. Air was continuously evacuated from the vial through a syringe needle connected to a rotary vacuum pump (Precision, 1/8 HP) during subsequent wetting steps in which ePTFE was first pre-wet with 1 ml of 100% ethanol (EtOH,