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A quantitative and selective chromatography method for determining coverages of multiple proteins on surfaces

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Abstract

Competitive protein adsorption plays a key role in the surface hemocompatibility of biological implants. We describe a quantitative chromatography method to measure the coverage of multiple proteins physisorbed to surfaces. In this method adsorbed proteins are displaced by CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) and then analyzed by high performance liquid chromatography to separate and quantify the individual proteins, in this case bovine serum albumin (BSA) and bovine fibrinogen (Fg). CHAPS displaced over 95% of the adsorbed proteins and was easily removed from solution by dialysis. This method was tested by measuring the coverage of BSA, 66 kDa, and Fg, 340 kDa, simultaneously adsorbed from solutions with concentration of 20 μg/ml, on bare and dextranized silicon. Relative to silicon, the dextranized surfaces were found to strongly inhibit protein adsorption, decreasing BSA and Fg coverages by 76 and 60%, respectively.

Keywords: Multiple protein coverages; SEC-HPLC; Biocompatible surfaces; Dextran

1. Introduction

Despite the numerous biomedical implants placed successfully into patients every year, a generally accepted single definition of biocompatibility for devices and indwelling biomaterials has not yet been established. Ambiguity persists especially in the definition of blood compatibility [1]. The surfaces of many biomaterials and biomedical devices have been attributed to provoking blood clotting, tissue inflammation, and infection, which contribute to their failure [2]. The initial event in the foreign body reaction stimulated by tissue contact with an implant is often a massive inflammatory response in which competitive protein adsorption takes place on the surface of the biomaterial. After blood contacts a biomaterial, plasma proteins rapidly adsorb onto the surface to form a monolayer of selected proteins. Albumin and fibrinogen are among those first molecules selectively adsorbed, however their concentrations, as well as the concentrations of other adsorbed proteins, change with time. These surface adsorbed proteins compete for occupancy of the biomaterial surface, and may become tightly packed, irreversibly bound, and immobile [2]. Adsorbed proteins also undergo limited conformational changes that may expose "receptor" amino acid sequences that are recognized by specific blood cells or bulk plasma proteins. Monolayers of densely packed immobile plasma proteins become arranged in undefined mosaics that vary spatially and temporally. This multi-protein layer determines all further events in coagulation and cellular adhesion [1,2]. Following protein adsorption, the principal phenomena are blood coagulation, platelet adhesion and aggregation, and complement activation leading to leukocyte aggregation.

Because of the importance and complexity of protein adsorption on implants, several techniques have been developed to identify and quantify the type and amount, respectively, of physisorbed proteins [3]. Traditional analytical methods to study protein adsorption typically use radioactive [4–11] or fluorescent [12,13] labeled compounds, gel electrophoresis and immunoblot analysis [14–18], total internal reflection fluorescence and in situ ellipsometry [19–21]. Radioactive and fluorescent labeling methods can be used to measure adsorption of two proteins simultaneously if different labels are used, but this technique is

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mainly used to detect a single protein adsorbing out of a mixture. One disadvantage is that labeling alters the protein structure and hence its adsorption behavior [22]. Recently, electron spectroscopy for chemical analysis and time-of-flight secondary ion mass spectrometry have been used to characterize surfaces containing multiple types of adsorbed proteins [23,24]. These methods are expensive, time consuming, and require specialized technical expertise and/or equipment. Another major limitation is their inability to quantify protein coverage from complex mixtures, rather they only provide semi-quantitative measures of competitive adsorption.

High-performance liquid chromatography (HPLC) is now firmly established as a premier technique for the analysis and purification of a wide range of molecules. HPLC is highly reproducible, easily manipulated for selectivity, and generally provides high species recovery [25]. One objective of this paper is to demonstrate the potential of HPLC as a tool to investigate protein adsorption from multicomponent mixtures onto biomaterial surfaces. HPLC chromatograms provide both the qualitative (i.e., retention time) and quantitative (i.e., peak area) information needed to analyze protein mixtures. With regard to separation and quantification of several proteins adsorbed on biomaterial surfaces, the main limitation of HPLC is that it is an ex situ technique. Namely, all proteins must be completely removed from the surface and then introduced into the HPLC system. An important finding in our approach, is that nearly 100% of the adsorbed proteins studied to date can be removed from surfaces by rinsing with CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), which is known as a surfactant that solubilizes membrane proteins while preserving their native structure [26]. Several other detergents, including SDS, were investigated; however CHAPS proved to be the most versatile and efficient detergent for displacing a range of proteins.

In this paper, we will show that CHAPS displaces over 95% of two major plasma proteins, bovine serum albumin (BSA) and fibrinogen (Fg) [6,27], simultaneously adsorbed onto bare and dextranized silicon wafer surfaces. Having established a successful approach for displacing and recovering adsorbed BSA and Fg on different surfaces, we then demonstrate a method to separate and quantify the displaced proteins by gel filtration HPLC. In toto, this novel CHAPS/HPLC approach is used to demonstrate that dextranized silicon surfaces strongly inhibit protein adsorption, relative to unmodified silicon, while preferentially adsorbing BSA. These results suggest that dextran coatings on blood-contacting biomedical implants provide a promising approach for reducing inflammatory response.

2. Experimental

2.1. Surfaces

We tested our CHAPS/HPLC method by measuring competitive protein adsorption on silicon wafers and biomimetic, dextranized silicon wafers prepared in our laboratory. The details regarding the preparation and characterization of these surfaces can be found in previous publications [28–30]. The dextranized surface for this experiment was obtained by oxidizing dextran for 0.5 h prior to grafting on amino-functionalized silicon [30]. Briefly, our dextran grafting procedure was developed on single side polished silicon wafers cut into squares of approximately 1 cm^2. However, this surface area is too small to adsorb enough protein to be detected by HPLC which has a sensitivity determined by the efficiency of the UV detector, namely about 1 μg. To overcome this issue, we therefore adapted our dextran grafting procedure [28–30] to double side polished silicon wafers which present a surface area of ∼40 cm^2 for protein adsorption.

Table 1
Physical properties of BSA and Fg

Protein	Plasma concentration (mg/ml)	Mass (kDa)	Size (nm)	Volume (nm^3)	p*I*	Reference
BSA	40	66	4 × 3 × 3	271	4.7	[13]
Fg	2–3	340	47 × 5 × 5	3645	4.3	[13]

2.2. Materials

To prepare protein solutions and clean the experimental apparatus only water from a Barnstead Mega-Pure® MP-1 water purification system was used. Bovine serum albumin (BSA), lyophilized, fatty acids- and globulin-free was obtained from Sigma Chemical Co., USA, as was bovine fibrinogen (Fg) (fraction I; >75% clottable protein). The proteins were used without further purification. Single and mixed solutions of concentrations ranging from 1 to 50 μg/ml of both BSA and Fg were prepared using 40 mM phosphate-buffer saline (PBS) at pH 7.0. Analytical grade chemicals for the buffer preparation were used without further purification. Physical properties relevant to the proposed method are listed in Table 1. CHAPS (Sigma C3023) and SDS (sodium dodecyl sulfate, Sigma L6026) were tested as surfactants to displace BSA and Fg from bare and dextranized silicon surfaces. CHAPS was selected because, unlike SDS, it did not interfere in the UV/HPLC detection of proteins.

2.3. Methods

2.3.1. CHAPS/HPLC method

A flow chart of the individual steps involved in the CHAPS/HPLC method is shown in Fig. 1. After preparing surfaces (step 1), proteins were adsorbed by immersing samples in a PBS solution containing a mixture of BSA and Fg. After immersion for 1 h on a shaker at 37 °C in PBS containing 20 μg/ml, each, of BSA and Fg, surfaces were then rinsed with PBS to remove loosely bound proteins (step 2). Adsorbed proteins were eluted from the surfaces by 1 h of immersion in 8 mM CHAPS solution on a shaker at 37 °C (step 3). The eluted protein samples (step 4) were then dialyzed for 24 h at 4 °C in PBS (pH ∼ 7.0) replaced four times to remove the CHAPS (step 5). The samples were frozen to −70 °C before being freeze-dried overnight (step 6). The displaced proteins were subsequently identified and quantified by HPLC (step 7), as described below.

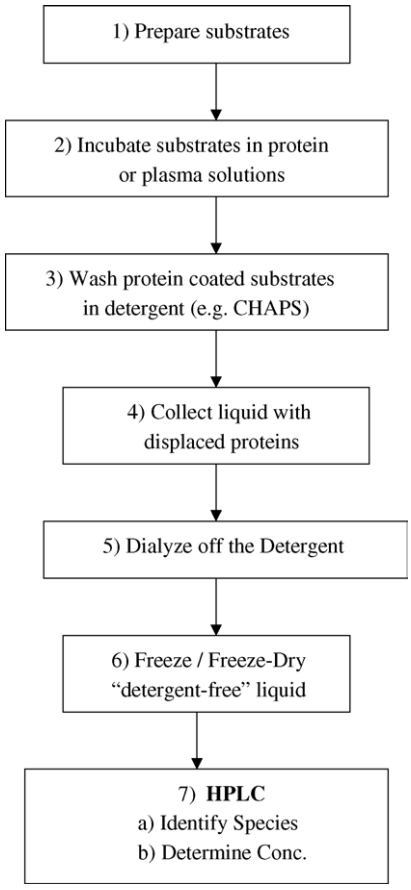

Fig. 1. Schematic of the newly developed HPLC method for the study of competitive protein adsorption onto the surface of materials.

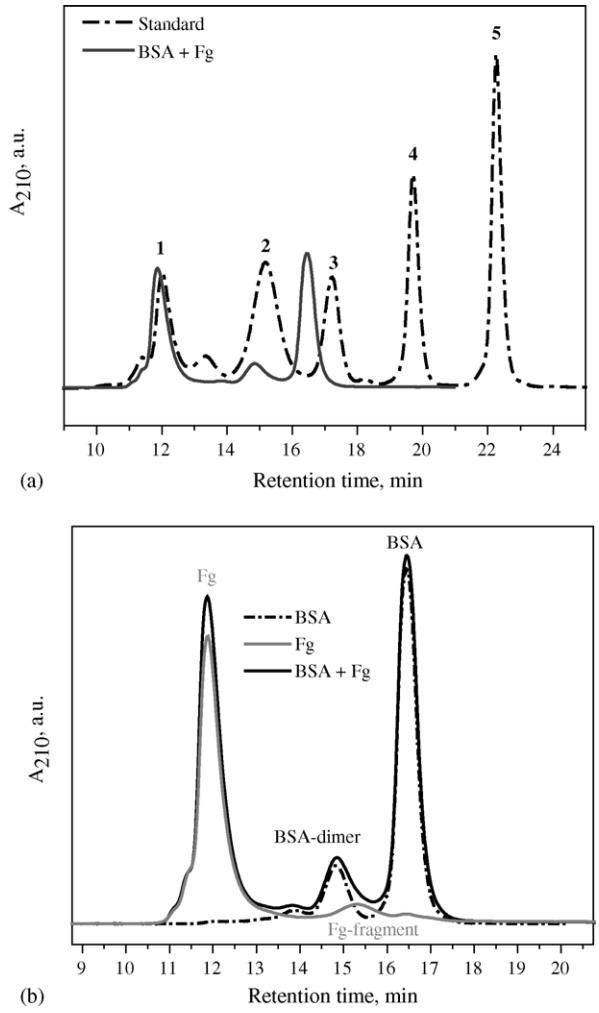

Fig. 2. SEC of a 50/50 mixture of BSA and Fg. (a) Comparison with the elution pattern of a mixture of five gel filtration standards ((1) thyroglobulin, M_w 670 kDa, (2) IgG, M_w 158 kDa, (3) ovalbumin, M_w 44 kDa, (4) myoglobin, M_w 17 kDa, (5) Vitamin B12, M_w 1.35 kDa). (b) Comparison with the elution patterns of solutions containing BSA alone and Fg alone. Column and conditions as described in the text.

Chromatography analysis was conducted on a Beckman Coulter System Gold® HPLC system running 32 Karat™ software. The apparatus was comprised of a 126 pump model solvent delivery module, a 168 programmable detector module, which is a diode array UV–vis HPLC detector set at 210 nm wavelength and a manual injector. Because BSA and Fg have similar isoelectric points, pI = 4.7 and 4.3, respectively, but significantly different molecular weights, 66 and 340 kDa (see Table 1), size exclusion chromatography (SEC) was used to identify and quantify the proteins. SEC separates biomolecules based on differences in their molecular size. To accomplish this we used a bonded silica Bio-Sil SEC 250 column (Bio-Rad). The eluent was a buffer composed of 0.05 M NaH_2PO_4, 0.05 M Na_2HPO_4, 0.15 M NaCl and having pH 6.8. The flow rate was set at 0.6 ml/min with a 200 μl. injection volume. The elution spectra of a 50/50 mixture of BSA and Fg are shown in Fig. 2. The data are plotted as absorbance at 210 nm, A_{210}, versus retention time in minutes. To identify the species, Fig. 2a compares the spectrum of the 50/50 mixture (solid line) with a mixture of five gel filtration standards (thyroglobulin, M_w 670 kDa; IgG, M_w 158 kDa; ovalbumin, M_w 44 kDa; myoglobin, M_w 17 kDa; vitamin B12, M_w 1.35 kDa). This comparison allows for the identification of the main peaks at 11.9 and 16.5 min as Fg (340 kDa) and BSA (66 kDa), respectively. The difference in retention time, Δt, between the two species is 4.6 min so, under these experimental conditions, the peaks for Fg and BSA are readily separated.

In Fig. 2b, the spectrum of the 50/50 mixture of BSA and Fg (dark solid line) is compared with that of pure Fg and pure BSA. Structurally, Fg is a dimer, each half of which is composed of three disulfide-bonded polypeptide chains designated Aα, Bβ, and γ [3], whereas BSA consists of a single peptide chain forming three small globular units [3]. We can assign the strongest peaks at a retention times of 11.9 and 16.5 min to Fg in its dimeric form and BSA in its monomeric form, respectively. The weaker peaks near retention time of 14.9 min are attributed to Fg molecules in monomeric or fragmental form and BSA molecules in dimeric form [31]. In the current work the secondary peak appearing at a retention time of 14.9 min will not be analyzed.

To quantify the amount of protein, A_{210} from known concentrations of Fg and BSA were measured. Fig. 3 shows calibration curves for both the Fg and the BSA main peaks. They were

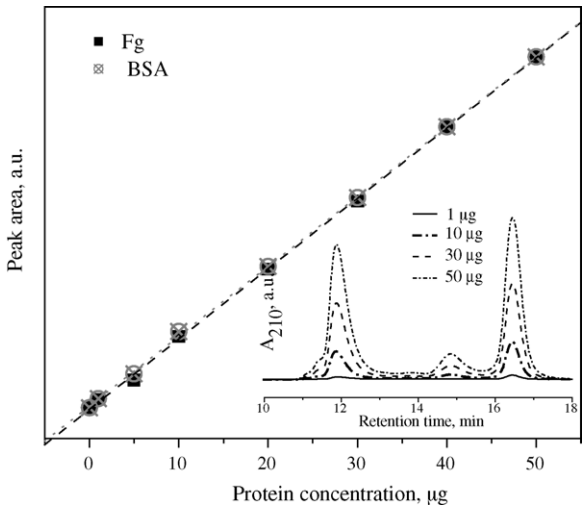

Fig. 3. SEC of a standard mixture of BSA and Fg: calibration curves. Column and conditions as described in the text.

obtained by injecting fixed volumes of 50/50 mixtures of BSA and Fg with concentrations ranging from 1 to 50 μg/ml and measuring the Fg and BSA peak areas. As an example, the elution spectrums of 50/50 mixtures with concentrations of 1, 10, 30 and 50 μg/ml are reported in the inset of Fig. 3.

Two factors determine the choice of detergent. As previously noted, the detergent must displace nearly 100% of adsorbed proteins. A second feature is that the detergent does not interfere with the chromatography measurements of the desired proteins.

After investigating several detergents, CHAPS was found to be the best choice because the concentration can be greatly reduced by extensive dialysis against PBS buffer. As demonstrated in Fig. 4a, SDS (one possible detergent candidate) solutions injected in the HPLC system show strong peaks between 13 and 17 min even after dialysis for 24 h at 4 °C in PBS (pH ∼ 7.0) replaced four times. In contrast, CHAPS solutions do not show any strong peaks between 10 and 17 min after dialysis under the same conditions (Fig. 4b). To allow a direct comparison of the CHAPS and SDS adsorption peaks with those from the displaced proteins, an elution pattern containing of both BSA and Fg displaced from dextranized silicon (presented later in Fig. 7) is included in Fig. 4a and b. As shown in Fig. 4a the peaks associated with BSA/Fg strongly overlap with the SDS peaks both before (solid line) and after (dashed line) dialysis. Fig. 4b shows that the CHAPS spectrum overlaps the BSA/Fg before dialysis (solid line). However, following dialysis, the main peaks of Fg and BSA (i.e., between 10 and 17 min) are easily distinguished from the CHAPS spectrum (dashed line). Moreover, the absorbance from CHAPS over this time range is flat allowing for easy subtraction. Although CHAPS and SDS have similar critical micelle concentrations (7–10 mM [32]), their micellar properties are significantly different. CHAPS has an aggregation number of 4–14 and an average micellar molecular weight of 6000 Da whereas SDS has significantly larger values of 62 and 18,000, respectively [32]. The large size of the SDS micelle compared to CHAPS may explain why SDS is difficult to remove by dialysis.

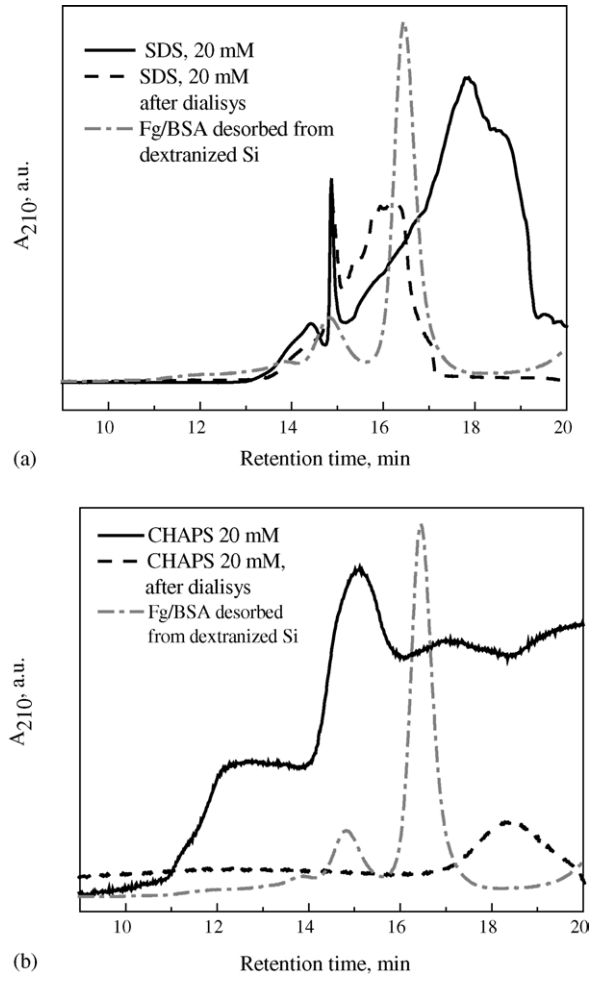

Fig. 4. Elution pattern of a 20 mM: (a) SDS and (b) CHAPS solution before and after extensive dialysis against PBS buffer. Columns and conditions as in Figs. 2 and 3.

2.3.2. Protein fluorescence measurements

To determine the efficacy of CHAPS solutions to displace proteins from surfaces, fluorescence measurements of proteins labeled with Oregon Green (OG) 488 dye (Molecular Probes, Eugene, OR) were performed in separate tests. Solutions containing either labeled BSA (20 μg/ml) or Fg (20 μg/ml) were adsorbed onto silicon and dextranized silicon surfaces which were placed in 12-well tissue culture plates (Corning Inc., Costar, NY) for 1 h on a shaker at 37 °C. The BSA and Fg labeling and the quantification of labeled proteins on the surfaces were performed according to the method described by Toworfe et al. [12]. The concentration and degree of labeling was determined by transferring 100 ml of the BSA and Fg into cuvettes (1 cm pathlength) for spectrophotometer readings of the conjugate solutions at 280 and 496 nn. The optimum degree of labeling for BSA and Fg is between 4 and 8 mol of Oregon Green 488 dye/mol of protein (Molecular Probes). BSA dimers, less than 10% of all the molecules, are not included in the evaluation of the degree of labeling. BSA and Fg concentrations were determined using $4.70 \times 10^4\ M^{-1}\ cm^{-1}$ and $5.12 \times 10^5\ M^{-1}\ cm^{-1}$, respectively, as the molar absorptivities of the proteins at 280 nm [33]. Fluorescence readings were per-

formed in a microplate reader (Chameleon™, Hidex, Finland) at 485 nm excitation and 535 nm emission wavelengths, so that the fluorescence of the solid surface, with the adsorbed labeled proteins on it, was directly determined. After incubation, initial fluorescence readings were recorded after washing substrates in PBS to remove loosely bound proteins and prevent rebinding. A second reading was recorded after the same substrates were washed by 8 mM CHAPS solutions for 1 h on a shaker at 37 °C. This procedure replicated the experimental conditions used in the protein displacement step of the HPLC method (see Fig. 1). A comparison between the two fluorescence readings allowed for the determination of the efficiency of CHAPS at displacing BSA and Fg from the surfaces.

3. Results

3.1. Protein displacement by CHAPS

Fig. 5 shows the surface density (Γ) of BSA (Fig. 5a) and Fg (Fig. 5b) adsorbed from pure solutions on silicon and dextran-modified silicon. Each value of Γ is the average of three fluorescence readings performed on five different surfaces. For each surface, these readings were within 10% of each other.

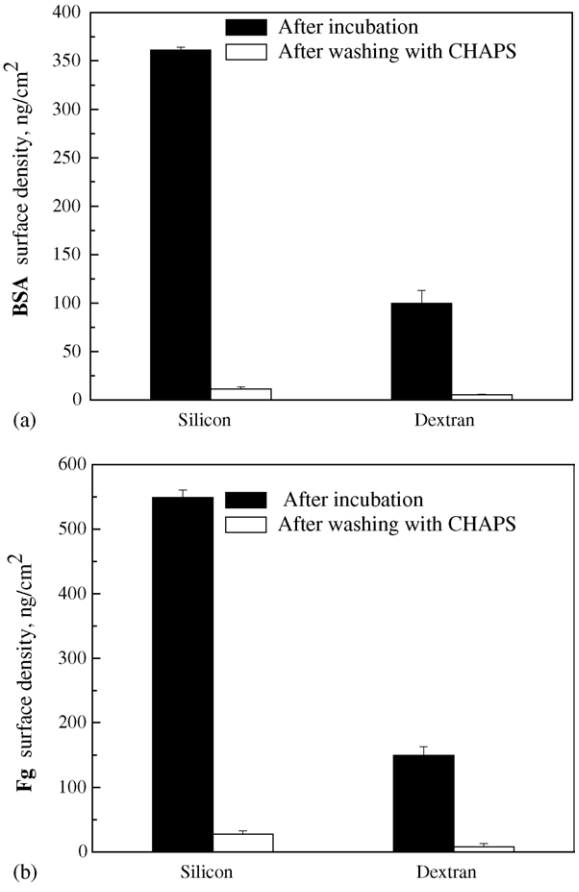

Fig. 5. Protein surface density Γ on bare and dextranized silicon surfaces (adsorption from 20 μg/ml solution) before and after washing by 8 mM CHAPS solutions for 1 h at 37 °C, obtained using fluorescence method. (Top) BSA and (bottom) Fg.

After incubation for 1 h, the surface coverage of BSA was 360 ± 40 ng/cm^2 on silicon and 100 ± 10 ng/cm^2 on dextran-coated silicon. The surface coverage of Fg was 550 ± 60 ng/cm^2 on silicon and 150 ± 15 ng/cm^2 on dextran-coated silicon following incubation. After washing with CHAPS, the surface coverage of BSA was reduced to 11 ± 1 ng/cm^2 on silicon and 5 ± 0.5 ng/cm^2 on dextran-coated silicon. The surface coverage of Fg decreased to 27 ± 3 ng/cm^2 on silicon and 7 ± 1 ng/cm^2 on dextran-coated silicon following CHAPS exposure. These results indicate that CHAPS exposure displaced 95.0–96.9% of adsorbed protein that remained after the initial PBS wash. These findings also demonstrate that the effect of the dextran surface coating reduces both BSA and Fg adsorption by approximately 72–73% with respect to bare silicon surfaces.

3.2. Surface coverage by CHAPS/HPLC method

Before analyzing competitive adsorption, CHAPS/HPLC was initially used to determine single protein coverage on both bare and dextranized silicon as shown in Fig. 6. Each experiment was repeated five times. The surface coverage of BSA was 400 ± 50 ng/cm^2 on silicon and reduced to 120 ± 15 ng/cm^2 on dextran-coated silicon. Similarly, the surface coverage of Fg was 500 ± 130 ng/cm^2 on silicon and reduced to 130 ± 16 ng/cm^2 on dextran-coated silicon. The protein resistance of dextranized silicon will be explored in an upcoming paper. These coverages are in excellent agreement with those obtained by the fluorescence method shown in Fig. 5, demonstrating the reliability of the CHAPS/HPLC method. Fig. 7 shows representative elution patterns of mixed solutions comprised of both BSA and Fg following displacement from both bare and dextranized silicon surfaces. The range of retention time (10–18 min) shown in Fig. 7a includes the appearance of both proteins. To accentuate the smaller Fg peak, a narrow range of retention time, from 10 to 13 min, is plotted in Fig. 7b. A standard chromatogram of a bulk mixture of Fg and BSA is also shown in Fig. 7a and b.

Using the calibration data (e.g., Fig. 3), the raw chromatography data were converted to BSA and Fg surface concentrations (Γ) for both substrates and plotted in Fig. 8. The values of Γ were

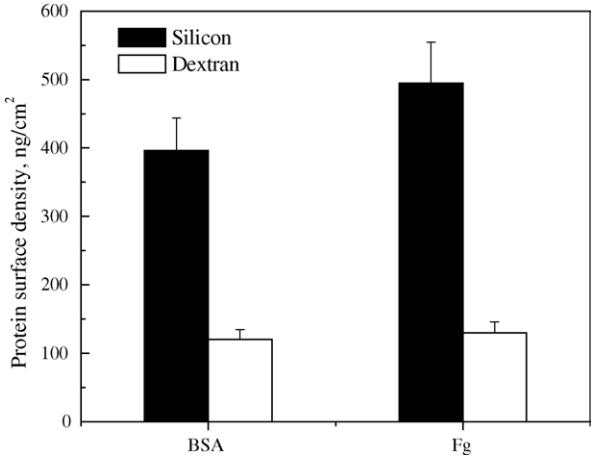

Fig. 6. Single protein surface density Γ on bare and dextranized silicon surfaces (adsorption from 20 μg/ml solution) obtained using CHAPS/HPLC method.

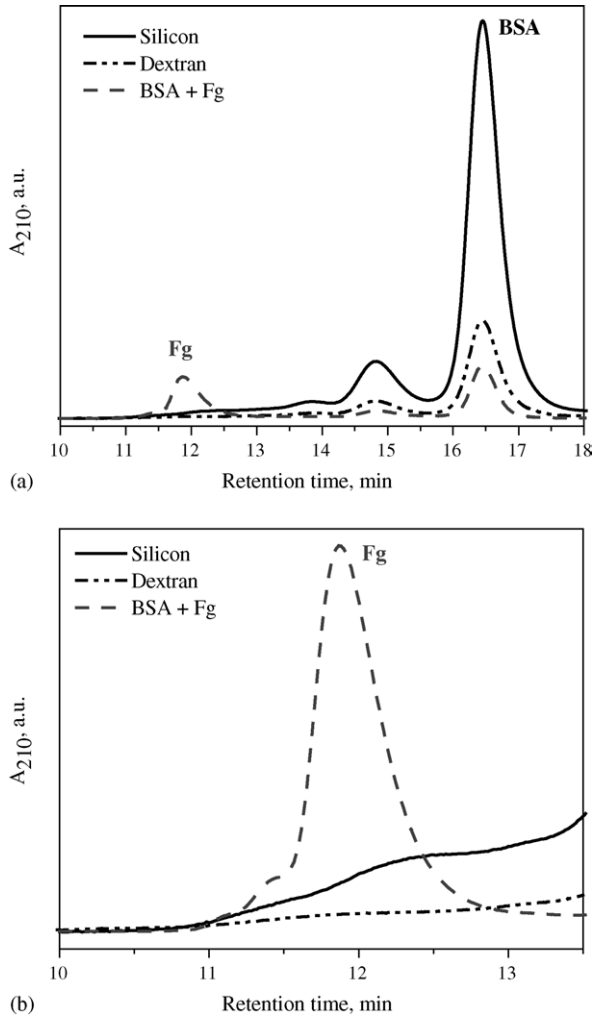

Fig. 7. SEC of mixtures of BSA and Fg displaced from bare and dextranized silicon surfaces (for details see the text). Column and conditions as in Figs. 2 and 3.

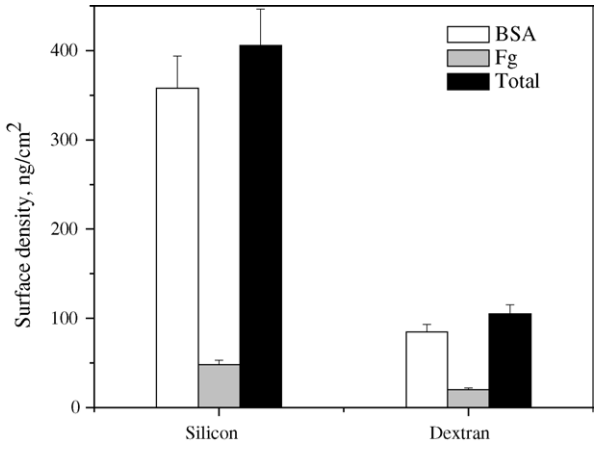

Fig. 8. Protein surface density on bare and dextranized silicon surfaces (competitive adsorption from a mixture of BSA and Fg both at concentrations 20 μg/ml). Data obtained from the chromatograms in Fig. 6 and the calibration curves in Fig. 3.

determined to be 360 ± 40 ng/cm^2 for BSA and 50 ± 5 ng/cm^2 for Fg on silicon and 85 ± 9 ng/cm^2 for BSA and 20 ± 2 ng/cm^2 for Fg on dextranized silicon. The experiments were repeated on five silicon and dextranized silicon surfaces.

From these competitive adsorption experiments, dextran coatings are found to decrease total protein adsorption by 74.4% compared to the bare silicon controls. The individual proteins are observed to decrease by similar amounts, namely 76.4% for BSA and 60.0% for Fg. Moreover, both surfaces after 1 h show preferential adsorption of BSA from the mixed BSA/Fg solution, with BSA accounting for 80.9 and 87.8% of the total protein adsorbed onto the bare and dextranized surfaces, respectively.

4. Discussion

Quantitative and selective methods are needed to interrogate competitive protein adsorption to biomaterials, especially vascular implants and other blood-contacting surfaces. Although considerable experimental data are available on the kinetics of irreversible protein adsorption, little is known about the selectivity of proteins adsorbed on surfaces. Our approach, called the CHAPS/HPLC method, provides quantitative analysis of multiple proteins that have been adsorbed onto solid substrates. Referring to the flowchart of CHAPS/HPLC illustrated in Fig. 1, a systematic discussion of the relevant experimental steps is proposed.

The CHAPS/HPLC method was tested using bare silicon as a control and dextran-grafted silicon surfaces synthesized and characterized in our laboratories [28–30]. The dextran grafting chemistry was extended to double-sided surfaces to provide a sufficiently large homogeneous sample that would nominally adsorb microgram quantities of proteins (i.e., sufficient for detection by UV absorption). Measurement sensitivity, but not surface shape, is then a limitation for application of this method, which is good for any geometry. Radioiodine labeling, one of the most sensitive and precise methods for measuring quantity of protein adsorbed, the detection limit is on the order of 1 ng/cm^2 [3]. Popular techniques such as gel electrophoresis and immunoblot analysis [14–18] provide only a semi-quantitative estimation of the protein coverage. In the current use, the sensitivity of CHAPS/HPLC is 25 ng/cm^2. This detection limit can be readily improved to $\sim$2 ng/cm^2 by displacing proteins from larger surface areas. Namely, the surface area of double sided silicon wafers was 40 cm^2 although wafers with significantly larger diameters are available. Including all experimental errors, the precision of the present CHAPS/HPLC method is $\pm$10 ng/cm^2. This value is similar to total internal reflection fluorescence, in situ ellipsometry and fluorescent labeling methods [12,13,19–21].

The physical properties of the particular proteins (BSA and Fg) chosen for testing the CHAPS/HPLC method dictated the use of SEC, which allows for the separation of the two proteins on the basis of their different molecular weights. We emphasize that SEC is one of several possible separation approaches offered by HPLC, including reversed phase chromatography,

ion-exchange and hydrophobic interaction chromatography, as well as affinity chromatography. In general, HPLC encompasses a highly versatile set of separation techniques that can be chosen depending on the properties of the proteins of interest.

A key step in the CHAPS/HPLC method was the identification of CHAPS as a facile, efficient detergent for displacing adsorbed proteins. At least 95% efficiency in displacing proteins was achieved without any demonstrable interference with the UV detection of the proteins after injection in the HPLC system. Although CHAPS shows adsorption peaks in the 10–18 min retention region (i.e., BSA + Fg region, see Fig. 4b), it is easily removed from solution by dialysis against PBS buffer. Fig. 4b shows that, following dialysis, CHAPS-related peaks are no longer present in the retention time range for the elution of BSA and Fg. Thus, the BSA/Fg spectrum will not be confounded by the presence of CHAPS. After ensuring removal of surfactant, the efficiency of CHAPS to displace BSA/Fg from both surface types needed to be demonstrated. Using an established fluorescence technique to measure protein adsorption from single-component solutions [12], the surface concentration of adsorbed BSA and Fg was quantified on both surfaces before and after washing with CHAPS. This protocol employed the same experimental conditions used for protein removal prior to HPLC quantification. The results from these parallel experiments confirmed that less than 5% of the total adsorbed protein remained bound to the solid substrate.

Although an extensive array of methods are available to study protein adsorption, clearly no single technique can provide a complete understanding of all relevant aspects of protein-surface interactions. Thus, a number of complementary methods are presently used to describe adsorption to the full extent possible. Hlady et al. [34] have summarized the attributes of an ideal method: quantitative, fast response, in situ, conformationally sensitive, applicable to all surface geometries and types and applicable to competitive adsorption. No available methods, including the CHAPS/HPLC method, have all these attributes. Whereas the advantages of CHAPS/HPLC as a method to study mixed protein surfaces have been discussed, two limitations are its inability for in situ analysis and the need to displace proteins. Because it is not in situ, the CHAPS/HPLC method is unsuitable for following rapid protein adsorption kinetics on the scale of 1 s or faster. Second, because proteins must be displaced prior to HPLC, surfaces must be rinsed with a surfactant able to displace all the adsorbed proteins. We have attempted to minimize any uncertainty in the displacement step by using a standard, constant washing procedure. We minimized the loss of protein due to adsorption to the dialysis membrane and/or the walls of the vessel during freeze-drying by repeated rinsing. We note that protein coverage values obtained by CHAPS/HPLC are in very good agreement with those obtained by the fluorescence methods as demonstrated by comparing Figs. 5 and 6. This agreement suggests that the amount of protein lost during the dialysis/freeze-drying steps is very small. Through parallel experiments involving fluorescently tagged proteins, the effectiveness of CHAPS at displacing BSA and Fg from surfaces has been demonstrated.

Despite these limitations, the CHAPS/HPLC method described herein is a powerful tool for studying the complex interactions between multicomponent mixtures of proteins and the surfaces of biomaterials and biological implants. Because this method provides a quantitative measure of protein adsorption, it can be used to screen the biocompatibility of material surfaces which are candidates for biomedical devices or indwelling materials. A major strength of CHAPS/HPLC is that it is the first technique that is both quantitative and selective. While the pilot studies in this paper demonstrate proof of concept using two proteins, CHAPS/HPLC has the potential to be developed for the study of competitive adsorption of mixtures containing three or four proteins (e.g., fibronectin, von Willebrand factor, vitronectin [4–8]), on virtually any surface of interest including polymers, metals, ceramics as well as glass. Note that other detergents may be necessary for new combinations of proteins and surfaces.

True understanding of blood compatibility requires tracking the adsorption of hundreds of proteins from a plasma over a broad range of time. No techniques can accomplish this task. However, because HPLC is able to identify and quantify proteins from a variety of synthetic and biological sources the CHAPS/HPLC method is inherently attractive for studying competitive adsorption from multicomponent systems and possibly body fluids. For example, CHAPS/HPLC can be used to follow the adsorption of selected proteins known to underlie the biocompatibility (namely, BSA, Fg fibronectin, von Willebrand factor, vitronectin, IgG [4–8]) directly from plasma. Platelet adhesion, as well as the surface activation of coagulation, has been observed to have a causal relationship to the composition of the adsorbed protein layer. Because synthetic surfaces tend to adsorb relatively large amounts of Fg, fibronectin, von Willebrand factor or IgG, the biocompatibility of implants may be largely determined by their “selectivity” towards specific adsorbed species. On the other hand, surfaces that preferentially adsorb BSA or (theoretically) no proteins are relatively blood compatible [3]. Thus, by using CHAPS/HPLC to track three- or four-candidate proteins from blood, this method can become an essential tool in testing the biocompatibility of surfaces found in biomedical devices. The CHAPS/HPLC method can provide quantitative measurements of selective adsorption of BSA and Fg on bare and dextranized silicon surfaces. The ability of dextranization to decrease total protein adsorption by 70% with respect to bare silicon has been demonstrated. Our studies also show that the dextranized surfaces preferentially adsorb BSA after immersion in a BSA/Fg solution comprised of these proteins at the same concentration (20 μg/ml). Even though this is the first quantitative study of simultaneous BSA and Fg adsorption on bare and dextranized silicon, a comparison with literature data on single protein adsorption is in particularly good agreement with our findings. Ortega-Vinuesa et al. [35] and Sharma et al. [36] report coverages of ∼400 ng/cm^2 for BSA and ∼700 ng/cm^2 for Fg for these proteins adsorbed from single protein solutions on silicon. These values agree both with our one component coverage studies on silicon which yield single 360 and 550 ng/cm^2 for BSA and Fg, respectively as well as the total coverage determined from CHAPS/HPLC in competitive adsorption studies.

By comparison, protein adsorption on dextranized silicon is greatly reduced. Dextrans are known to show very low non-specific interactions with proteins [37] and dextran coatings reduce protein adsorption at surfaces enhancing biocompatibility [38–40]. For the first time, this study shows that BSA preferentially absorbs to dextran, relative to Fg. This result is important because polymers capable of preferentially adsorbing BSA are thought to be less thrombogenic than those enriched in Fg. For example, albumin coated surfaces have been shown to be inert to platelets, whereas fibrinogen enhances platelet adhesion and aggregation [41]. The findings in our paper suggest that dextran-based coatings are advantageous for blood-contacting medical devices, especially when fibrinogen-inert surfaces are required such as in vascular applications [41].

5. Conclusion

Blood-borne proteins rapidly adsorb onto foreign materials that come into contact with blood or plasma, and the resultant adsorbed protein layer determines all further events in coagulation and cellular adhesion [41]. When testing the biocompatibility of candidate materials for blood-contacting medical devices, it is important to assess their behavior with respect to competitive protein adsorption. Among the many analytical techniques available to study protein adsorption onto surfaces, no technique has yet to be readily applied to quantitatively and selectively study competitive adsorption. We developed a HPLC-based method using CHAPS to displace proteins and SEC to quantify and identify proteins previously adsorbed to surfaces. This method uses a simple and convenient protocol, provides excellent precision and expands the experimental tools for assessing the biocompatibility of new and old materials. In developing the method, we evaluated the efficacy of a dextran coating on a silicon substrate to control non-specific protein interactions. Our competitive adsorption studies on bare and dextran-modified silicon surfaces indicate that dextranized films significantly reduce protein adsorption.

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