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Protein Adsorption on Chemically Modified Block Copolymer Nanodomains: Influence of Charge and Flow

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Abstract

Understanding the interactions of biomacromolecules with nanoengineered surfaces is vital for assessing material biocompatibility. This study focuses on the dynamics of protein adsorption on nanopatterned block copolymers (BCPs). Poly(styrene)-block-poly(1,2-butadiene) BCPs functionalized with an acid, amine, amide, or captopril moieties were processed to produce nanopatterned films. These films were characterized using water contact angle measurements and atomic force microscopy in air and liquid to determine how the modification process affected wettability and swelling. Protein adsorption experiments were conducted under static and dynamic conditions via a quartz crystal microbalance with dissipation. Proteins of various size, charge, and stability were investigated to determine whether their physical characteristics affected adsorption. Significantly decreased contact angles were caused by selective swelling of modified BCP domains. The results indicate that nanopatterned chemistry and experimental conditions strongly impact adsorption dynamics. Depending on the structural stability of the protein, polyelectrolyte surfaces significantly increased adsorption over controls. Further analysis suggested that protein stability may correlate with dissipation versus frequency plots.

Keywords

Quartz Crystal Microbalance; Atomic Force Microscopy; Block Copolymer; Protein Adsorption; Nanopattern

1. INTRODUCTION

Throughout their operational lifetime, medical devices often contact blood and other physiologic fluids. Devices that experience incidental blood contact for short periods include catheters, blood bags, and angioplasty balloons. Long term blood contacting medical devices

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range from stents, vascular grafts, pacemakers, and defibrillators. Regardless of contact duration, the foreign body response after injury can cause biofouling by proteins, small molecules, and various cellular phenotypes that may result in diminished effectiveness or adverse events.¹

Nanoengineered materials are increasingly investigated for use in biomedical devices, imaging, *in-vitro* diagnostics, and drug-delivery systems.^{2–4} Manipulation of topographical features has been considered as a technique to increase the biocompatibility of biomedical coatings.⁵ However, the difficulty of nanomaterial testing remains a concern for academics, industry, and regulators and was highlighted in recent reviews.⁶ Nanoscale surface roughness was previously shown to increase protein adsorption on metallic surfaces, yet decreased adsorption was observed on poly(ethylene oxide) based BCP surfaces.^{7,8} The comparison of results from dissimilar materials and proteins may explain conflicting results from distinct studies, and a study of systematically controlled nanostructures would be beneficial to identify critical parameters.

While clinical data remains central for biocompatibility testing as highlighted in ISO-10993,⁹ *in vitro* methods are gaining traction due to cost economy and high throughput methods. The development of proper protocols is imperative to determine whether a surface's nanostructure explicitly enhances its biocompatibility. Since medical device applications include both dynamic and static blood contact, a study with nanostructures under both these conditions would be beneficial.

The interplay between plasma proteins, platelets, and material characteristics determine the physiological response to an implant.¹⁰ After injury, protein adsorption is one of the primary events establishing implant biocompatibility and is key in determining whether an implant will be successfully integrated into the tissue or result in an adverse reaction.¹¹ The adsorption of pro-coagulant proteins such as fibrinogen or von Willebrand's factor have been established to increase platelet activation and adhesion on synthetic polymer surfaces.¹² During this process, conformational changes within fibrinogen expose motifs that can initiate coagulation.¹³ This is of major concern since thrombosis on medical device surfaces may result in catastrophic failure and patient mortality.¹⁰ Thus, the characterization of plasma proteins adlayers on synthetic polymer surfaces could be applied as models for biocompatibility.

Despite decades of research, there is still uncertainty describing material effects on protein adsorption.¹¹ Methods such as labeling, spectroscopy, immunosorbent assays (ELISAs), surface plasmon resonance sensors (SPR), and the quartz crystal microbalance (QCM) have been used to characterize protein adsorption to varying degrees of success.^{10,14–17} Those requiring fluorescent or radioactive labeling can increase protein hydrophobicity or present health hazards, respectively.¹⁸ Spectroscopy techniques often require high vacuum conditions and may be unable to provide quantitative information about adsorbed layers. ELISAs can provide vital information about competitive protein binding, but they may inaccurately assume the antigen binding site is available.¹⁹ Since SPR only provides qualitative information, QCM is advantageous due to its ability to quantify adsorbed layers.

QCM gravimetry uses the Sauerbrey equation, which linearly relates frequency loss to increased mass on the surface of quartz.²⁰ In vacuum, the QCM is capable of detecting masses less than 1 ng/cm² and the sensitivity decreases to approximately 20 ng/cm² in liquid.²¹ The sole use of frequency loss in QCM has limited its applicability for biological samples, which are viscoelastic and typically contain bound hydration shells. Rodahl, Hook, and coworkers developed a method to measure the signal dissipation and frequency simultaneously by rapid switching of the QCM driving circuit.^{21–23} This permits adlayer quantification through the fitting of frequency and dissipation data to established Voigt or Maxwell viscoelastic models.²⁴

QCM with dissipative monitoring (QCM-D) can provide real-time monitoring of adlayer formation and has been used to describe protein adsorption on self-assembled monolayers, block copolymers (BCPs), metals, and biopolymers.^{8,17,25–27} Furthermore, QCM-D can be used to infer the conformation, solution, and mechanical properties of polyelectrolyte multilayers, polymer brushes, lipids, and nanoparticles.^{28,29} QCM-D has been shown to depict adsorption more accurately in liquids than QCM due to consideration of an adlayer's viscoelastic properties.³⁰ QCM-D also permits the study of proteins both statically and under flow, which allows experimental freedom to represent the physiological environment.

A model system consisting of styrene-butadiene BCP nanopatterned surfaces modified with acid, amide, amine, and captopril moieties was characterized using contact angle measurement and atomic force microscopy (AFM). A quartz crystal microbalance with dissipation monitoring (QCM-D) was used to measure adsorption under both dynamic and static conditions of proteins with distinct size, charge, and rigidity. The wettability of the BCP surface patterns was significantly increased by thiol grafting and AFM imaging suggested this originated from selective block swelling of modified BCP domains. Protein adsorption measurements by QCM-D confirmed that both the BCP nanopatterns and protein characteristics strongly influence the nature of adlayers. In this study, disproportionate increases in protein adsorption under flow were observed for many nanopatterned surfaces containing polyelectrolyte moieties compared to experiments performed under static conditions.

2. EXPERIMENTAL SECTION

2.1. Materials

Poly(styrene)-block-(1,2-butadiene) (PS/PB) BCPs were purchased from Polymer Source (Montreal, Canada). Untreated poly(styrene) (PS) petri dishes, 30% ammonium hydroxide (NH₄OH), tetrahydrofuran (THF), dichloroethane, acetone, isopropanol, and dimethyl formamide (DMF) were purchased from Fisher Scientific (Pittsburgh, PA). Poly(ethylene-co-acrylic acid) (PEAA) was purchased from Allied Signal Advanced Materials (Sunnydale, CA). 1X phosphate buffered saline pH 7.4 (PBS), 30% hydrogen peroxide (H₂O₂), sodium dodecyl sulfate (SDS), propylene glycol monomethyl ether acetate, immunoglobulin g (IgG) (human serum, 95%), bovine serum albumin (BSA) (protease free, 99%), cytochrome c (CytC) (bovine heart, 95%), and fibrinogen (human plasma, fraction I, type I, 65%, 85% clottable) were purchased from Sigma Aldrich. Test grade silicon wafers with a 〈100〉 orientation were purchased from University Wafers (Boston, MA). After cleaving, they were

cleaned using an isopropyl alcohol/acetone wash, followed by drying in a clean nitrogen stream.

Stock protein solutions were prepared by dissolving each respective protein at 0.5 mg/ml in PBS and were stored at 4 °C for no longer than one week, with the exception of IgG, which was stored at −20 °C at 0.5 mg/ml, as suggested by the manufacturer. Working concentrations of protein were prepared by 10-fold dilutions of the stock solutions to 50 $\mu\text{g/mL}$ in PBS pH 7.4 and stored at 4 °C prior to use.

2.2. Nanopattern Processing on QCM-D Crystals

The PS/PB BCPs were modified by thiol-ene photochemistry to include boc-cysteamine, thioglycolic acid, 2-diethylaminoethanethiol, or captopril moieties. The synthesis of these functionalized polymers are described elsewhere.³¹ Briefly, a 63 kDa-block-33 kDa PS/PB BCP was modified using various thiols and Irgacure 819 photoinitiator. The percent functionalization for each modified BCP was determined to be 79, 78, 93, and 69%, respectively. These polymers are subsequently referenced by the R-group grafted onto the PS/PB BCP, eg. PS/PB-Acid in the case of poly(styrene)-block-poly(butadiene-graftthioglycolic acid). The codes for these BCP surfaces are PS/PB-Amide, PS/PB-Acid, PS/PB-Amine, and PS/PBCaptopril, respectively. For convenience, the structure of the grafted polymers is shown in Figure 1. After purification and drying, the BCPs were dissolved at 0.5 wt% using propylene glycol monomethyl ether, dichloroethane, THF, DMF, or combinations containing these solvents. Thin films were spin coated using a Laurell Technologies NPP-Lite (North Wales, PA) at 2000 rpm onto either silicon wafers or gold coated QCM crystals. PS was dissolved at 0.5 wt% in toluene and PEAA was dissolved at 1 wt% in THF and spin coated at 2000 rpm. Films were dried overnight under vacuum and baked at 80 °C for 30 minutes to promote film adhesion before protein adsorption experiments. The BCP film thicknesses were determined to be approximately 50–100 nm using the QCM-D apparatus and the Sauerbrey equation.

Tapping mode atomic force microscopy (AFM) was used to image the surface topography of spin coated BCP films using an Asylum MFP-3D system. VistaProbe AFM tips were purchased from Nanoscience Instruments with a nominal tip radius less than 10 nm, spring constant of 48 N/m, and resonant frequency of 190 kHz. Scans $2 \times 2 \mu\text{m}$ in dimensions were completed at 1 Hz with a resolution of 512×512 pixels. AFM was performed with the same scanning parameters in PBS after equilibration for 2 hours, using Olympus PSA400 tips with a nominal radius less than 20 nm, 0.08 N/m spring constant, and resonant frequency of 11 kHz.

2.3. Static Contact Angle Measurement

Water contact angles were measured with a Ramé Hart goniometer using 18.2 M Ω deionized water. Prior to experimentation, films were acclimated to PBS solutions for 2 hours according to previous procedures for similar systems).³² After incubation, films were flash dried using compressed nitrogen before placing a $\sim 1 \mu\text{L}$ deionized water drop onto the film via needle for measurement. At least 10 measurements were made on each polymer surface

and water contact angles were quantified using Drop Image software. The data represents the mean static contact angle and includes the standard error of the mean.

2.4. Quartz Crystal Microbalance with Dissipation (QCM-D)

QCM-D measurements were recorded using a QSense Auto E4. An Ismatec multichannel peristaltic pump dispersed buffer and protein solutions at 100 $\mu\text{L}/\text{min}$ over AT-cut 4.95 MHz Au-coated quartz crystals obtained from QSense (Sweden). An automated program was produced with the AutoE4 controller in the QSoft software to obtain a stable baseline in PBS, followed by a one hour adsorption, and completed with a 30 minute PBS wash to obtain a new baseline.

Due to the experimental set-up for static adsorption, real-time monitoring was not feasible without submitting proteins to dynamic conditions. Each run still consisted of a 30 minute PBS wash, one hour adsorption, and a final PBS rinse as in the dynamic experiments. Frequency and dissipation were measured before and after adsorption and data files were stitched together in the QTools software. QCM-D data was fit through the QTools software provided from QSense using a one layered Voigt viscoelastic model. A minimum of 4 adsorption curves were recorded for proteins on each respective material. Dissipation versus frequency changes are plotted every 60 points. Deviations from linear behavior were determined using regressions to determine the slope, intercept, and the R^2 value.

Gold QCM-D crystals were reused after each experiment using a cleaning technique recommended by the manufacturer. After a 2% SDS rinse within the sample loop, QCM-D crystals were washed with deionized water, dried with nitrogen, and exposed to ultraviolet light/ozone (UVO) for 20 minutes in a Bioforce Nanosciences Procleaner (Ames, IA). The crystals were cleaned with an $\text{NH}_4\text{OH}/\text{H}_2\text{O}_2/\text{water}$ (1/1/5 v/v/v) piranha solution at 75 $^\circ\text{C}$ for 10 minutes. The crystals were again washed with deionized water, dried with a stream of nitrogen, and baked in UVO for 20 minutes. QCM-D flow cell modules were washed with 2% SDS, deionized water, and dried with nitrogen between experiments before starting a new experimental run.

2.5. Statistical Analysis

Water contact angle and protein adsorption values were compared to determine statistical significance using ANOVA combined with Tukey's multiple comparisons test. Statistically significant data are reported using 95% confidence intervals after inputting raw data into Origin software. Within each comparison test, the seven different polymers were compared and their significance as related to the PS, PS/PB and PEAA controls are depicted with symbols above each respective data point.

3. RESULTS AND DISCUSSION

3.1. Characterization

As described previously, thiol-ene chemistry was used to graft functional groups onto the pendant vinyl group of PS/PB, including an acid, amide, amine, and captopril. Quasi-cylindrical BCP nanopatterns were formed on QCM crystals or silicon wafers using suitable

solvents previously established.³¹ Figure 2 displays dry AFM images of the surfaces, not including the PS films due to insignificant topographical variation. These cylindrical patterns had center to center distances in the range of 80 nm, with the PS/PB stock BCP having slightly smaller domains. Films consisting of PS and a PEAA random copolymer served as controls for determining the mechanism of protein adsorption on nanopatterns. Though it is not featureless, the COOH moieties in PEAA are randomly distributed throughout the polymer backbone, whereas the BCP functionalities are constrained within the BCP domains.

Initial static measurements from dry films showed little contact angle differences between the thiol grafted BCPs and the control groups (not shown). Xu et al. has shown that buffer equilibration significantly decreased water contact angles for amphiphilic BCP systems after flash drying to vitrify microdomains).³² Since subsequent experiments conducted in this work were measured at pH 7.4 in PBS after swelling, determination of the contact angles under these conditions was deemed more relevant and conducted in this manner.

Table I exhibits the water contact angles of the films used in this study. The contact angles of the hydrophobic polymers such as the PS, PEAA, and PS/PB BCP were found to be 81, 98, and 98°, respectively. Despite PBS equilibration of these surfaces, contact angles remained generally unchanged and agreed with literature values.^{33–35} Contact angles for the amide, acid, captopril, and amine modified BCPs were 67, 37, 30, and 56°, respectively. The PBS equilibration of the BCPs and controls before contact angle measurement confirmed that only surfaces with modified moieties were sensitive to this handling. Compared to unmodified PS/PB, the thiol grafted BCPs showed large decreases in the contact angle, suggesting increased hydrophilicity. The contact angle of the acid modified polymer resembled values obtained from a poly(styrene)-block-poly(acrylic acid) (PS/PAA) BCP described previously).³² While not strictly equivalent, PS/PB-Amine surface films showed similar contact angles to plasma polymerized poly(allyl amine).³⁶

Figure 3 shows AFM characterization of the patterned and unpatterned films after PBS equilibration. The PS/PB, PS/PB-Amine, and PS films generally maintained their structure soaking in buffer. In the case of PS/PB-Acid, Amine, and Captopril, varying surface structure changes were observed after PBS incubation due to swelling. The PS/PB-Acid surface swelled enough that little resemblance to the initial dry pattern remained after equilibration. The contact angles previously mentioned confirmed that wettability was considerably increased after acid grafting. The decreased contact angles from selective block swelling suggests the formation of an acid enriched surface layer after buffer equilibration.³⁷ The possibility of an acid enriched surface layer agrees with the disappearance of the microphase separated morphology on PS/PB-Acid film surfaces after both lateral and vertical swelling.

The PS/PB-Captopril films exhibited swelling that somewhat distorted the initial quasi-cylindrical pattern and resulted in dimple-like structures. The degree of swelling for the PS/PB-Captopril films may be smaller than PS/PB-Acid, which could explain the retention of microphase separated morphologies in swollen films. Lower amounts of swelling in PS/PB-Captopril nanostructured surfaces may occur due to the lower degree of functionalization

during the synthesis process or lower mobility of the side chains due to the ring structure on Captopril. The PS/PB-Amine polymer displayed minor swelling in AFM and resulted in a decreased contact angle relative to the PS/PB BCP. Decreased swelling and smaller contact angle changes in PS/PB-Amine, as compared to PS/PB-Acid and Captopril, may occur due to the hydrophobic alkyl groups surrounding the tertiary amine. Based on the pKa's of the attached functional groups relative to the solution pH and the contact angle measurements, the PS/PB-Acid, PS/PBCaptopril, and PS/PB-Amine are postulated to be charged surfaces.³⁸

3.2. Static Protein Adsorption

The isoelectric point (pI) and molecular weight of the proteins used in this study are summarized in Table II. Proteins far from their isoelectric points typically exhibit charge-dependent behavior, which applies to BSA, fibrinogen, and CytC.³⁹ BSA, fibrinogen, and IgG are negatively charged proteins at pH 7.4, while CytC is positively charged. Based on electrostatic interactions, the PS/PBAcid and Captopril surfaces would be expected to reduce BSA, fibrinogen, and IgG adsorption, while the PS/PBAmine polymer would increase deposition. Conversely, PS/PB-Acid and Captopril would electrostatically attract CytC, while the PS/PB-Amine surface would repel CytC. Electrostatics would not be expected to affect the adsorption of charged proteins on the neutrally charged PS/PBAmine polymer.

Figure 4 shows the average protein adsorption densities completed under static conditions. BSA adsorption on the various polymer surfaces was in the range of 1000–2500 ng/cm² based on a planar crystal. PS/PBAmine showed statistically increased BSA adsorption over the PS/PB, PS, and PEAA controls. Otherwise, BSA static adsorptions were fairly consistent between the various polymer surfaces. BSA adlayers on PS/PB-Amine increased relative to uncharged and negatively charged controls, potentially due to electrostatic attraction to the positively charged surface. Additionally, the PS/PB-Amine surface adsorbed statistically higher amounts of BSA compared to the negatively charged PS/PB-Acid and Captopril.

Fibrinogen adlayers were found to vary from approximately 2000–3000 ng/cm². Statistically increased adsorption was observed for the PS/PB-Acid polymer in comparison to PS/PB and PEAA. Fibrinogen adsorption statistically decreased on PS/PB-Captopril and PS/PB compared to PS. However, adsorption increased after contact with PS/PB-Acid, which contradicts the electrostatic repulsion expected between a negatively charged surface and protein.

While there is no statistical variance between some of the negative and positively charged BCPs, electrostatic forces clearly influenced adsorption on the nanopatterned surfaces. Marginal differences were seen for both CytC and IgG adlayers, with ranges between 500–1200 ng/cm² and 1200–2400 ng/cm², respectively. The adsorption values for both CytC and IgG were similar between most of the surfaces. The exception was statistically higher IgG adsorption on PS/PB-Amine compared to the captopril modified surface.

The theoretical monolayer adsorption limit of BSA is 360 ng/cm² for side-on type and 900 ng/cm² for end-on adsorption.⁴⁰ The theoretical limit of fibrinogen adsorption is approximately 180 ng/cm² for side-on type and 1700 ng/cm² for end-on type adsorption.⁴¹ The monolayer densities of IgG and CytC are both approximately 300 ng/cm².^{42,43} The

close-packed densities for BSA and fibrinogen are larger than IgG and CytC due to higher asymmetry in their spatial dimensions.

The results from static adsorption experiments show that large concentrations of protein adsorbed to some of the polymer surfaces. Within experimental errors, BSA typically formed a monolayer on the polymer surfaces, with the exception of PS/PB-Amine. The measured static adsorption of fibrinogen increased in excess of a monolayer on PS/PB-Acid, Amide, and PS. In the case of CytC, approximate monolayers were formed on all surfaces within experimental error. Finally, adsorption beyond an IgG monolayer was measured on all the surfaces investigated.

Despite swelling of the modified BCPs, its extent is not significant enough to increase the close-packed density of a monolayer according to AFM measurements. However, these BCPs often adsorbed more protein than one monolayer. Polyelectrolyte chains have been hypothesized to repel one another and increase the porosity of the swollen polymer layer, which may not necessarily manifest itself during AFM imaging.⁴⁴ The porosity may allow for proteins to penetrate through interstitial polymer spaces and increase the measured adsorption beyond a theoretical monolayer.

3.3. Adsorption Under Flow

In order to describe protein adsorption on material surfaces that encounter dynamic blood flow, QCM-D experiments were conducted to investigate protein adlayers under dynamic conditions. Representative raw QCM-D data are shown in Figure 5 for BSA on the polymer surfaces. All overtones were utilized in the viscoelastic models with the exception of the 1st overtone. The oscillatory frequency decreases upon protein adsorption and the dissipation shifts depending on the viscoelastic properties of the adlayer. Results from dynamic studies indicate two subsets of adsorption kinetics for BSA. The hydrophobic polymer surfaces (PS/PB, PS/PB-Amide, PS, PEAA) demonstrated a frequency decay which saturated within minutes of protein introduction, shown in Figure 5(a). The polyelectrolytes (PS/PB-Acid, Captopril, Amine) exhibited large frequency reductions in comparison to the control polymers.

Dissipative changes on the BCP polyelectrolytes were an order of magnitude higher for BSA adsorption compared to hydrophobic and uncharged polymers. Dissipative changes on the order of 10^{-5} were observed for BSA adsorption on the polyelectrolytes, depicted in Figure 5(b), compared to the hydrophobic and uncharged polymers which showed dissipative changes along the order of 10^{-6} . The exception for the polyelectrolytes was the PS/PBAmine surface, which showed significantly decreased dissipation during the adsorption process. The data suggests that BSA adlayers on the hydrophobic polymers were fairly rigid in comparison to the highly viscoelastic BSA adlayers on PS/PB-Acid and Captopril. The physical mechanism and discussion of the adsorption induced dissipation shift will be discussed in the succeeding section.

After data collection, these points were entered into the Voigt viscoelastic models within the QTools software to obtain adsorption densities. Figure 6 displays fitted results for the BCPs, PS, and PEAA for BSA, fibrinogen, CytC, and IgG experiments, respectively. BSA

adsorption on PS/PB-Acid, Captopril and Amine BCPs were significantly increased to 1000–3000 ng/cm², compared to ~300 ng/cm² for both PS and the stock BCP controls. The PS/PB-Amide, Captopril, PS/PB, PS, and PEAA surfaces consisted of a monolayer or less within experimental error. However, the PS/PB-Acid and Amine surfaces adsorbed in excess of a monolayer. Fibrinogen adsorption significantly increased for the acid modified polymer to a level of 5500 ng/cm² compared to densities of 1500–2000 ng/cm² for the stock BCP and PS. PS/PB-Amine and Captopril BCPs showed slight decreases in fibrinogen adsorption compared to controls and the amide BCP. The surfaces consisted of fibrinogen monolayers with the exception of PS/PB-Acid, whose density was significantly higher.

CytC adsorption remained fairly constant and was in the range of 175–300 ng/cm². The exception was the aminated polymer, which showed a significant increase to ~430 ng/cm². Considering the experimental error, these densities fall within the range of CytC monolayers for all surfaces.

IgG adsorption on the polymer surfaces were in the range of 230–1750 ng/cm². The aminated polymer exhibited the lowest density, which significantly varied from the PS/PB control. The PS/PB-Captopril, Amine, and PS control surfaces adsorbed IgG within a monolayer, while PS/PB-Amide, Acid, PS/PB, and PEAA all consisted of adlayers in excess of a monolayer.

Now that the adsorption densities for each respective surface and protein pair were introduced, these data will be discussed along with potential implications of the results. Thus far frequency and dissipation data were exclusively used to describe relative gravimetric differences between polymer surfaces for a given protein. However, these parameters can also provide qualitative information about adlayer structure. The succeeding section will describe some of the possible mechanisms occurring during the adsorption process.

3.4. Dissipation versus Frequency Plots

Dissipation versus frequency plots (D-f plots) detail time independent processes and have been used to determine whether multiple kinetic regions occur during adsorption.²¹ The slope of D-f plots describes the relative rigidity or viscoelasticity of adlayers. Large D/f values suggest that the adsorbed layer is highly dissipative or viscoelastic. Likewise, small D/f are indicative of rigid adlayers. Due to the non-specificity of QCM-D, these measurements could exclusively refer to the protein layer or contain some contribution from the polymer coatings.

Figure 7 depicts the D-f plots for the surfaces during BSA adsorptions. The hydrophobic polymers such as PS/PB, PS/PB-Amide, PS, and PEAA displayed linear relationships between dissipation and frequency. Linear regressions for these relations exhibited $R^2 > 0.95$. Additionally the slope, or D/f , for these three surfaces was all approximately 10^{-5} Hz^{-1} , suggesting that the adlayers have similar properties. The PS/PB-Acid surface exhibited a large increase in dissipation with a slope $5 \cdot 10^{-4} \text{ Hz}^{-1}$ during BSA adsorption. Conversely, PS/PB-Amine exhibited a significant decrease in dissipation.

Liner D-f plots are typical for BSA adsorptions on hydrophobic polymers. After the frequency saturated, marginal dissipation increases occurred which may be due to small conformational changes or denaturation of the adlayer. The partial denaturation of hydrophobic proteins such as BSA on hydrophobic surfaces has been confirmed by complimentary techniques, where protein subdomains become exposed.³⁹ BSA dehydration and partial denaturation may occur from bonding of complimentary hydrophobic domains on each respective structure.⁴⁵

For the cases of the acid and amine modified PS/PB BCPs, the landscape becomes more complex due to the charged nature of the nanostructured surfaces. As the frequency of PS/PB-Acid decreased, a large increase in dissipation occurred, suggesting that the adlayer became more viscoelastic with time and may be caused by denaturation of BSA upon its adsorption.

The PS/PB-Amine polymer exhibited a sharp decrease in dissipation as frequency decreased for BSA adsorption. Decreased dissipation during protein adsorption may suggest either dehydration of the adlayer, collapse of the swollen polymer, or some combination of the two. With a water contact angle of 56°, the PS/PB-Amine surface still maintains some hydrophobic character along with its suspected positive charge. BSA is both sensitive to denaturation on hydrophobic surfaces and may be electrostatically attracted to the cationic surface. This attraction may induce collapse of the polymer-protein complex but would require a complimentary technique to confirm this.

The D-f plots for fibrinogen are shown in Figure 8. Similar to BSA, linear relationships between dissipation and frequency were observed for fibrinogen adlayers on hydrophobic PS/PB, PS/PB-Amide, PS, and PEAAs surfaces. An inflection in the dissipation was present for these surfaces when frequency leveled, which is suggestive of partial denaturation.²¹ The stability of fibrinogen against denaturation is lower compared to BSA due to structural heterogeneity in the tripeptide. The preferential affinity of one chain for a surface has been suggested as the cause of lower fibrinogen stability during adsorption processes.⁴⁶

Deviations from typical adsorption isotherms were also observed for the PS/PB-Acid and PS/PB-Amine surfaces after fibrinogen adsorption. The PS/PB-Acid exhibited a sinusoidal like dissipation versus frequency relationship. The first phase of this adsorption mimics that of hydrophobic controls and may be approximated as a monolayer. Subsequently, either fibrinogen or PS/PB-Acid partially dehydrates before continuing to adsorb more protein with increased dissipation. PS/PB-Amine exhibited a slightly decreased dissipation, which may be due to dehydration processes occurring during the adsorption process.

The D-f plots for CytC demonstrated behavior more typical of rigid protein adlayers, as shown in Figure 9. The hydrophobic polymers had nearly linear profiles that smoothed around a monolayer. The PS/PB-Acid and Amine polymers both exhibited approximate monolayers, but both of their dissipations decreased during the adsorption process. For the negatively charged PS/PB-Acid, perhaps the opposite charge of CytC induces collapse of the protein-polymer complex, but this may require complimentary spectroscopy techniques for confirmation.

The dissipative versus frequency relationships for IgG were all generally linear, as shown in Figure 10, with the exception of PS/PB-Amine. PS/PB-Amine exhibited the dissipation loss observed with every protein on this surface. Despite the linear relationships, adsorptions greater than a monolayer were measured onto most surfaces under investigation. Since no inflection was present in the D-f plots, little can be inferred from these relationships in terms of qualitative discussions of its conformation. The mechanism of IgG adsorption is still under investigation.

3.5. Implications of Protein Adsorption on BCPs

The presence of charged groups significantly increased BSA and fibrinogen adsorption over uncharged polymers. BSA and fibrinogen values were especially high on the PS/PB-Acid polymer despite both the surface and proteins containing negative charges. Soft proteins such as BSA and fibrinogen can bind to polymer surfaces regardless of charge implications due to their low stability, even resisting electrostatic repulsion.^{45,47} This behavior was hypothesized to result from protein secondary structure changes after irreversible binding that exposes motifs not typically present on the protein's surface.⁴⁸ Another theory about large depositions on like-charged polymer protein complexes focuses on the charge anisotropy inherent in proteins.⁴⁹ Computational models from other studies indicated that uniformly charged surfaces could not predict the behavior of like-charged proteins and polymers and that charge on proteins most likely exists in patches.⁵⁰

From these data, some conclusions can be drawn about the role of electrostatic charge when confined within a BCP nanopattern. While electrostatic interactions did not necessarily guide protein adsorption on the nanostructured surfaces studied, they certainly affected the formation of protein layers. Under static conditions, protein adsorption often occurred in excess of monolayers without regard for electrostatics. Electrostatic interactions seemed to play a more significant role under dynamic conditions, where the polyelectrolyte BCPs adsorbed excess amounts of BSA and fibrinogen.

The consequences of both albumin and fibrinogen adsorption on biomedical polymer surfaces are imperative to understand since they are the most abundant plasma proteins in circulation. Albumin coated surfaces are long known to act as thrombo-resistant passivation layers, which may be beneficial for biomedical coatings.⁵¹ Conversely, fibrinogen was previously discussed to be an essential component of the coagulation cascade. In fact, the denaturation of adsorbed fibrinogen may enhance the coagulation response, which may be beneficial for hemostatic applications. Considering the large variety of biomedical applications discussed, the state of adsorbed protein layers may be a key predictor for surface biocompatibility.

4. CONCLUSION

A model system for determining the effects of nanopatterned chemistries on protein adsorption was developed through modular thiol-ene chemistry. Morphological control of the BCP surface patterns was confirmed by AFM characterization. Surface charge and relative hydrophobicity was related through contact angle measurements. The introduction of charge caused selective swelling of charged blocks and decreased water contact angles.

QCM-D experiments suggested that some polyelectrolyte BCPs disproportionately increased protein adsorption of proteins with low stability when compared to uncharged and/or unpatterned controls. The mechanism of increased adsorption is believed to result from protein adsorption to the interstitial space between swollen polymer chains. Qualitative frequency and dissipation analysis suggested that these charged BCPs may have larger denaturing effects on proteins with low solution stability, such as BSA and fibrinogen, compared to more rigid proteins such as CytC.

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Figure 1. Structures of grafted block copolymers originating from the following monomers. (a) Boc-cysteamine. (b) Thioglycolic acid. (c) 2-diethylaminoethanethiol. (d) Captopril.

Figure 2. Atomic force micrographs of nanopatterned block copolymers and unpatterned controls. (a) PS/PB ($z = 10$ nm). (b) PS/PB-Amide ($z = 10$ nm). (c) PS/PB-Acid ($z = 10$ nm). (d) PS/PB-Amine ($z = 15$ nm). (e) PS/PB-Captopril ($z = 15$ nm). (f) PEAA ($z = 250$ nm). Scale bars: 500 nm.

Figure 3. Atomic force micrographs of nanopatterned block copolymers and unpatterned controls imaged in PBS buffer. (a) PS/PB ($z = 15$ nm). (b) PS/PB-Amide ($z = 15$ nm). (c) PS/PB-Acid ($z = 25$ nm). (d) PS/PB-Amine ($z = 25$ nm). (e) PS/PB-Captopril ($z = 25$ nm). (f) PEAA ($z = 250$ nm). Scale bars: 500 nm.

Figure 4. Protein adsorption values for the polymeric surfaces done under static conditions. (a) BSA. (b) Fibrinogen. (c) CytC. (d) IgG. *⁻PS/PB; #⁻PS; †⁻PEAA are $p < 0.05$ for $n = 4$.

Figure 5.

Raw QCM-D data from BSA adsorptions (5th overtone). (a) Frequency changes from protein injection show mass deposition on polymer surfaces. (b) Dissipative element shows increased viscoelasticity for the majority of the surfaces after BSA adsorption.

Figure 6.

Protein adsorption data from QCM-D done at 100 $\mu\text{L}/\text{min}$ on various polymer surfaces. The fitted results are the saturated values fit to a Voigt viscoelastic model. (a) BSA. (b) Fibrinogen. (c) CytC. (d) IgG. *-PS/PB; #-PS; †-PEAA are $p < 005$ for $n = 4$.

Figure 7. Dissipation versus frequency plots of bovine serum albumin adsorption on the BCP patterns and controls.

Figure 8.
Dissipation versus frequency plots of adsorbed fibrinogen on the BCP patterns and controls.

Figure 9. Dissipation versus frequency plots of adsorbed cytochrome c on the BCP patterns and controls.

Figure 10. Dissipation versus frequency plots of adsorbed immunoglobulin g on the BCP patterns and controls.

Table I.

Static water contact angle measurements of the polymeric sur-faces described in this study.

Polymer	Contact angle $\pm$ SEM
PS/PB-Amide	66.9 ± 1.4
PS/PB-Acid	36.5 ± 1.5
PS/PB-Captopril	30.1 ± 0.8
PS/PB-Amine	56.0 ± 0.9
PS/PB	98.3 ± 0.7
PS	81.3 ± 0.5
PEAA	98.1 ± 2.7

Table II.

Property summary of the proteins investigated in this study. The isoelectric points (pI) and molecular weight (MW) are shown to distinguish between the large size and charge differences.

Protein	Source	pI	Charge at pH 7.4	MW (kDa)
Serum albumin	Bovine	4.9	(-)	66
Fibrinogen	Human	5.5	(-)	340
Cytochrome C	Bovine	10.5	(+)	12.2
Immunoglobulin G	Human	6.5	(-)	150