Nanoscale adhesion, friction and wear of proteins on polystyrene

THESIS

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By

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Abstract

Protein layers are routinely deployed on biomaterials and biological micro/nanoelectromechanical systems (bioMEMS/NEMS) as a functional layer allowing for specific molecular recognition, binding properties or to facilitate biocompatibility. In addition, uncoated biomaterial surfaces will have uncontrolled protein layers adsorbing to the surface within seconds of implantation, so a pre-defined protein layer will improve the host response. Implanted biomaterials also experience micromotion over time which may degrade any surface protein layers. Degradation of these protein layers may lead to system failure or an unwanted immune response. Therefore, it is important to characterize the interfacial properties of proteins on biomaterial surfaces. In this study, the nanoscale adhesion, friction and wear properties of proteins adsorbed to a spin coated polystyrene surface were measured using atomic force microscopy (AFM) in deionized (DI) water and phosphate buffered saline. Adhesion, friction and wear have been measured for bovine serum albumin (BSA), collagen, fibronectin and streptavidin (STA) in DI water and PBS as a function of protein concentration. These proteins were chosen due to their importance and widespread application in the biotechnology field. Adhesion and friction were also measured for BSA and STA at two different temperatures and different pH values to simulate a biological environment. Based on this study, adhesion, friction and wear mechanisms of the different proteins are discussed.

Dedicated to my family and friends

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Fields of Study

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Chapter 1: Introduction

Upon implantation into the body, every biomaterial will have some amount of protein directly adsorbing to the surface (Anderson, 2001; Wilson et al., 2005; Roach et al., 2007). Protein layers initially coated on biomaterial surfaces ex vivo may then be displaced by other proteins with a higher affinity for the substrate surface (Ratner et al., 2004). Figure 1 shows a schematic of the different stages involved in the foreign body reaction of a biomaterial in vivo (Bridges, 2008). The far left portion of the figure represents the initial stage when the proteins directly adsorb to the biomaterial surface. This initial adsorption of protein is fundamental for the body's immune response and determines the type of immune response that occurs (Wilson et al., 2005; Anderson et al., 2008). The interaction that occurs between the cells and the biomaterial is then mediated by the surface layer of proteins (Wilson et al., 2005).

Next, in a process known as exudation, fluid, proteins, and cells move from the blood stream to biomaterial surface (Anderson, 2001). Most biomaterials will come into contact with vascularized tissue forming a provisional extracellular matrix at the implantation site (Ratner et al., 2004). Initially, neutrophils are the primary immune cells found at biomaterial/body interface during the first several days following implantation (Ratner et al., 2004). Over time, other immune cells such as macrophages, foreign body giant cells, and mononuclear leucocytes migrate into the area to begin to repair any tissue damage (Ratner et al., 2004).

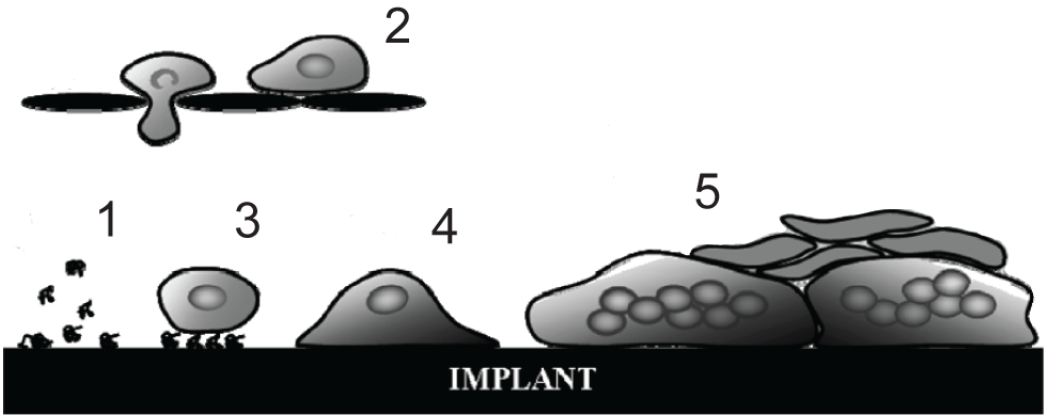

Figure 1. Schematic showing the steps involved in the foreign body response from left to right. (1) Initially protein adsorption occurs upon biomaterial implantation. (2) Exudation of fluids, proteins and cells occur from surrounding blood vessels. (3 and 4) Cell attachment to the biomaterial is then mediated and influenced by the adsorbed proteins. (5) Eventually tissue repair and remodeling occurs. Adopted from Bridges (2008).

The provisional extracellular matrix is then converted over time into granulation tissue primarily by fibroblasts and vascular endothelial cells (Anderson, 2001; Ratner et al., 2004).The final step usually consists of fibrous encapsulation in an attempt by the body to wall off the biomaterial (Anderson, 2001). This entire process, from implantation to fibrous encapsulation, is initially dependent on the properties of the proteins that adsorb to the biomaterial surface. Therefore, in order to rationally design an improved biomaterial, the nanoscale adhesion, friction, and wear of protein layered surfaces should be well characterized.

Protein layers have been used in biological micro/nanoelectromechanical systems (bioMEMS/NEMS) which include sensors, immunoisolation capsules, and drug delivery systems (Park, 1997; Grayson et al., 2004; Bhushan, 2010). In addition, coating material

surfaces with a protein layer facilitates biocompatibility along with implant functionality (Black, 1999; Wise, 2000). Both the implanted biomaterial and the layer of protein will experience a range of physical and chemical assaults over the life of the device (Ratner et al., 2004). These include degradation by serum or tissue proteases, biochemical dissociation from surfaces, chemical modification, and abrasion by tissue elements (Turbill et al., 1996). In addition, an implanted biomaterial will experience micromotion over time leading to wear and degradation of the protein layer (Bhushan at al., 2006). These can significantly affect and degrade the performance of the biomaterial eventually leading to failure (Ratner et al., 2004). Optimizing the adhesion, friction and wear on the adsorbed protein layer is therefore crucial to prevent device failure.

Previous studies have focused on the adhesion of biomolecules to various substrate surfaces (Lee et al., 1994; Sagvolden et al., 1998; Wang et al., 2004; Bhushan et al., 2005; Bhushan et al., 2009; Palacio et al., 2011; Palacio and Bhushan, 2012). Also the nanoscale adhesion, friction and wear of proteins on silicon based surfaces have been studied (Bhushan et al., 2006). Nanoscale adhesion, friction, and wear were shown to be dependent on protein concentration, pH of the surrounding liquid medium, and the method used to attach the proteins to the substrate surface. Substrate hydrophobicity and adsorption time of collagen were investigated showing a time and surface dependence on the degradation of the protein film (Pamula et al., 2004). Adhesive forces have also been studied between bovine serum albumin, collagen, and fibronectin deposited on various block copolymer surfaces (Palacio et al., 2011). Extensive work has been conducted on protein adhesion, and several proteins have been characterized on different surfaces

individually, but no study has yet been conducted to compare and contrast the nanoscale adhesion, friction, and wear of different proteins individually.

The objective of this study was to characterize the nanoscale adhesion, friction and wear behavior of four proteins - bovine serum albumin (BSA), collagen, fibronectin and streptavidin (STA) - adsorbed to a spin coated polystyrene surface. The measurements were made using atomic force microscopy in deionized water and phosphate buffered saline (pH 5.0, 7.4 and 9.0). Adhesion and friction were investigated for all four proteins by varying the concentration of the protein solution from 1 to 100 µg/mL in DI water and PBS (pH 7.4). Nanoscale wear was also studied at a protein concentration of 100 µg/mL in PBS (pH 7.4). In addition, adhesion and friction were investigated by varying the pH and temperature of the liquid medium for both BSA and STA at 100 µg/mL.

Chapter 2: Experimental

2.1 Protein and Substrate Selection

In this study, bovine serum albumin, collagen, fibronectin and streptavidin were chosen based upon their use in the biotechnology field. Bovine serum albumin is a homolog of human serum albumin and its primary physiologic functions include helping to maintain blood oncotic pressure, the binding and transport of important ligands, and several antioxidant functions (Francis, 2010). Type I collagen is the most abundant protein in the human body and is the primary load bearing component of connective tissue (Di Lullo et al., 2002). Fibronectin is a major extracellular component required for clot formation and wound healing found throughout the body (Pankov and Yamada, 2002). Finally, streptavidin is used extensively in the biotechnology field for protein purification, biosensors, and the immobilization of biomolecules due to its interaction with biotin (Green, 1990). All of the proteins used in this experiment were purchased from Aldrich.

Figure 2 shows an overview of the different protein structures. Bovine serum albumin, fibronectin and streptavidin are all globular proteins. Collagen is the only protein with a linear structure used in this experiment. Table 1 compares and contrasts the masses and isoelectric points of the four proteins used. The isoelectric point is the pH at which the protein will be electrically neutral in solution. Changing the pH of the solution above or below the isoelectric point will change the net charge on each protein.

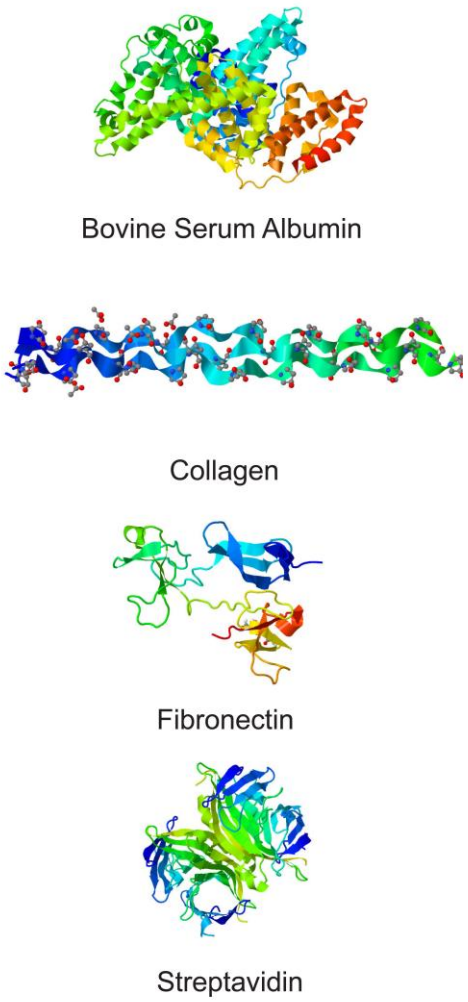

Figure 2. Images showing the physical structure of the bovine serum albumin, collagen, fibronectin and streptavidin. Proteins are not shown to scale relative to one another. All figures based off of the protein data bank's files.

All of the proteins used in this experiment have an acidic isoelectric point giving them a net negative charge in DI water. Streptavidin and BSA have masses of 60 and 67 kDa respectively with fibronectin and collagen being an order of magnitude more massive.

Polystyrene was selected as the substrate surface due to its extensive use in the biotechnology field. Examples include petri dishes, tissue culture trays, respiratory care

equipment, and syringe hubs (Sastri, 2010). Additionally, polystyrene nanoparticles have been tested as a potential drug delivery system (Soppimath et al., 2001), and modified polystyrene has also been investigated to study cell-material interactions (Van Kooten et al., 2004).

Table 1. Summary of the different protein masses and their isoelectric point.

Protein	Molecular Weight (kDa)	Isoelectric Point
Bovine Serum Albumin	~67[a]	4.7[e]
Collagen	~300[b]	4.7[f]
Fibronectin	~500[c]	5[g]
Streptavidin	~60[d]	5.5[d]

(a) Hirayama et al., 1990 (b) Di Lullo et al., 2002(c) Pankov and Yamada, 2002(d) Green, 1990(e) Peters, 1980(f) Hattori et al., 1999(g) Proctor, 1987

2.2 Sample Preparation

Approximately 1 x 1 cm^2 sized samples were created from a silicon wafer. These samples were cleaned by sonication for 5 minutes in deionized water, acetone and isopropyl alcohol to remove any debris from creating the sample. Samples were then blown dry with filtered nitrogen to remove any remaining solvent. Polystyrene (350,000 MW, Aldrich, St. Louis, MO) was dissolved in reagent grade toluene to form a 10% w/w solution. Spin coating occurred by flooding the silicon substrate with approximately 100 µl of polystyrene solution. The samples were then spun at 2000 rpm for 1 minute and then baked in an oven at 50ºC for 4 hours. Baking the samples evaporated any remaining solvent and weakly annealed the polystyrene film. Once cooled, 50 µl of protein solution

was added to the surface, and the samples were allowed to sit overnight. Before beginning any experiment, the samples were rinsed with deionized water to remove the electrolytes and any unbound protein. PBS (pH 7.4) was purchased from Aldrich, and the two remaining pH levels were created by combining monosodium phosphate (Aldrich) and disodium phosphate (Aldrich) in DI water to create the proper phosphate buffered solution.

2.3 AFM Measurements

These experiments were conducted with atomic force microscopy in a liquid environment to simulate a natural biological environment. The atomic force microscope (AFM) is a versatile instrument to characterize surface properties at the nanoscale (Bhushan, 2010). Atomic force microscopy can measure adhesive forces in the pico- to nanonewton range and allows for single asperity contact to occur between two different interfaces making it the ideal instrument to use for these experiments (Bhushan, 2011). The effects of protein concentration on adhesion, friction and wear were investigated in DI water and PBS (pH 7.4). In addition, the pH and temperature of the liquid medium was altered to simulate conditions potentially encountered in vivo.

2.3.1 Adhesion Force Measurements

Adhesive force measurements were conducted in two different liquid environments (DI water and PBS) using a commercial Multimode AFM (Bruker, Santa Barbara, CA). A custom fluid cell was used to contain the liquid on the sample surface.

For these experiments, silicon nitride pyramidal probes (Applied NanoStructures, Inc., Santa Clara, CA) with a nominal spring constant of 3 N/m were used.

The adhesion measurements were made in force calibration mode using a bare silicon nitride AFM tip. The adhesive force for each force calibration plot was obtained by multiplying the maximum deflection of the cantilever in the retract position with the spring constant of the cantilever. Adhesion measurements were conducted with a bare silicon nitride AFM tip against a bare silica surface, a control polystyrene surface, and polystyrene surfaces coated with the protein at different concentrations. The applied protein concentrations used were 1 and 100 µg/mL bound to the polystyrene surface through direct physical adsorption. Adhesive forces were measured on at least three different points on the surface to allow for a statistical comparison between the different samples.

Next, adhesive force measurements as a function of pH were conducted at 22ºC for BSA and STA at 100 µg/mL. All measurements were made using a commercial AFM (Agilent Technologies, 5500 Scanning Probe Microscope, Chandler, Arizona) with silicon nitride pyramidal probes (Applied NanoStructures, Inc., Santa Clara, CA). The spring constant for these probes was 3 N/m. The four pH levels tested were 5.0, 7.0, 7.4, and 9.0. To vary the pH level, the liquid cell was drained of PBS and then refilled several times with new solution at the pH level of interest. The adhesion measurements were then made in the same fashion as before.

Finally, the temperature was raised to 37ºC to simulate nominal human body temperature. Again, adhesive force measurements as a function of pH were measured for

both BSA and STA at 100 µg/mL using the Agilent system. To vary the pH level, the liquid cell was drained of PBS and then refilled several times with new solution at the pH level of interest and allowed to thermally equilibrate before measurements were taken.

2.3.2 Friction Measurements

The experimental procedure for measuring the coefficient of friction was based upon that developed by Ruan and Bhushan (1994). In this method, the sample is scanned perpendicular to the long axis of the cantilever beam and the output of the two horizontal quadrants of the photodiode-detector is measured. In this arrangement, as the sample moves under the tip the friction force will cause the cantilever to twist. Therefore the light intensity between the left and right detectors will be different. The differential signal between the left and right detectors is denoted as FFM signal. This signal can be related to the degree of twisting, hence to the magnitude of friction force. Because of a possible error in determining normal force due to the presence of an adhesive force at the tip-sample interface, the slope of the friction data (FFM signal vs. normal load) must be taken for an accurate measurement of the coefficient of friction. The tests were repeated at least 5 times to determine the average value of the coefficient of friction. The scan size was 2 µm and the scan rate was 1 Hz. Normal forces were kept in the range of 1-10 nN. The samples that were created were tested in both DI water and PBS at different temperature and pH levels in the same manner and with the same AFM systems as the adhesion measurements.

2.3.3 Wear Tests

Wear tests were conducted in contact mode of the Multimode AFM in PBS (pH 7.4). For these experiments, square pyramidal Si3N4 probes (DNP-S10, Bruker, Camarillo, CA) with a nominal 10-40 nm tip radius mounted on triangular Si3N4 cantilevers with a spring constant of 0.06 N/m were used. A lower spring constant was chosen in order to better control the normal loads being applied to the sample surface. For the wear tests, a 1 x 1 μm2 area was scanned three times at various normal loads (3, 6 and 9 nN), and then a 3 x 3 μm2 area was imaged at the minimum possible normal load in order to image the wear marks. The scan rate used was 1 Hz. The approximate applied normal load in contact mode can be obtained by multiplying the amplitude set point voltage (V), the sensitivity (nm/V) obtained from the AFM settings and the cantilever stiffness (nN/nm)

Chapter 3: Results and Discussion

3.1 Adhesion Measurements

First, the effect of protein concentration on the adhesive force was investigated for all the proteins. Figure 3(a) shows the adhesive force data between the bare silicon nitride AFM tip and the various proteins. The adhesive force increases between the protein film and the bare silicon nitride AFM tip as the concentration of protein is increased. This is in agreement with what was previously reported by Bhushan et al. (2006). This increase in adhesive force is believed to be in part due to multiple interactions occurring between the protein film and the AFM tip (Bhushan et al., 2006). This is also consistent with the observation that increasing the concentration of protein solution increases the density of the immobilized protein on the polystyrene surface. This increase may also be due to the more hydrophobic domains of the protein interacting with the polystyrene surface leaving the hydrophilic domains to be exposed to the AFM tip. Previous studies have shown that near maximum binding will occur at roughly 2 hours, and that more protein will directly adsorb depending on solution concentration (Browne et al., 2004; Pamula et al., 2004). It has also been reported that above a concentration of 10 μg/ml, certain proteins will begin to form higher order structures not present at lower concentrations (Bhushan et al., 2006).

Figure 4 lists the basic interactions that may occur during protein adsorption and is only meant to show some of the interactions that can occur. The strongest interaction occurs between the hydrophobic domains of the protein and a hydrophobic surface.

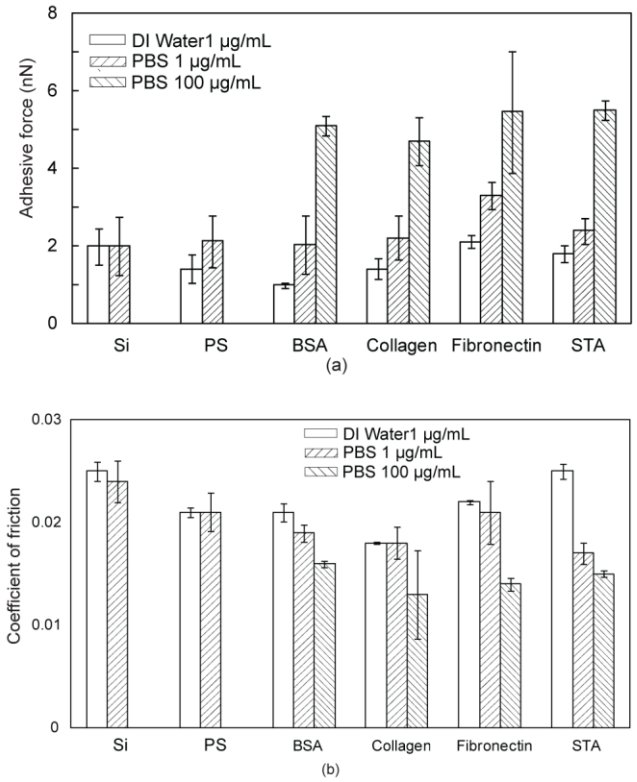

Figure 3. Effect of protein concentration on (a) the adhesive force and (b) the coefficient of friction between the protein film and bare AFM tip. All experiments conducted at ambient temperature 22±1°C unless otherwise noted. DI water (pH 7.0) and PBS (pH 7.4) were used for all experiments unless otherwise noted. Error bars represent ± 1 standard error.

Next, moderate adsorption occurs between charged protein domains and charged surfaces. Finally, weak adsorption occurs between hydrophilic protein domains and hydrophilic surfaces. Protein adsorption may involve several of these interactions occurring on the same surface and with the same protein.

Given that polystyrene is a hydrophobic surface, it would be expected that the hydrophobic domains of the proteins would interact with the surface during adsorption. The strength of the hydrophobic interaction is strong enough to induce a conformational change in the protein exposing the inner hydrophobic domains to the surface. This allows for the hydrophilic and charged domains to interact with solution.

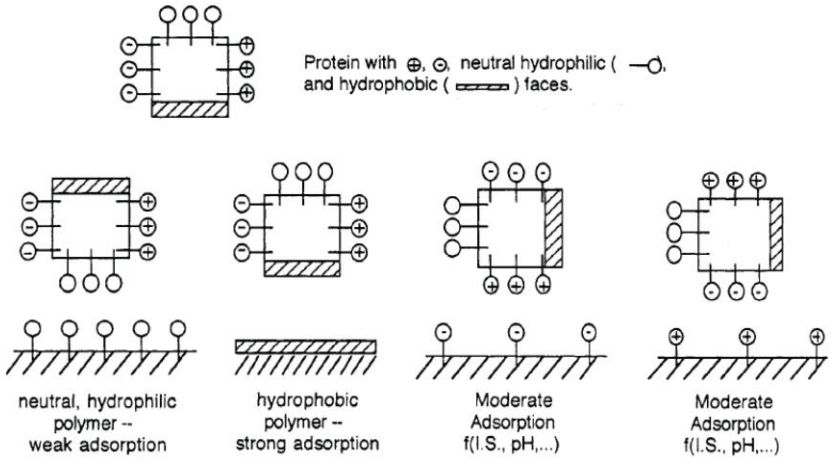

Figure 4. Schematic showing the different protein domains and the potential interactions with the substrate surface (Andrade et al., 1992).

It is also well known that by varying the pH of the solution, proteins can be made to denature from their native structure, thus allowing the inner hydrophobic domains to interact with the polystyrene surface. Previous studies have shown that chemically modifying the polystyrene surface has an effect on the amount of adsorbed protein suggesting that the four proteins used in this study have a higher affinity for hydrophobic surfaces (Browne et al., 2004; Pamula et al., 2004). This hydrophobic interaction is believed to be the dominant mechanism for protein adsorption to polystyrene; however we do not have the data to show the orientation or packing density of the protein films

(Browne et al., 2004). The data from this experiment demonstrates no clear trend to distinguish the proteins from one another. However, what is clear for all of the selected proteins is that an increase in protein concentration leads to an increase in adhesive force between the AFM tip and the protein layer.

Next, the effect of pH on BSA and STA at 100 µg/mL concentration was investigated. Figure 5(a) shows the adhesive force data as a function of pH at 22ºC. The general trend is that the adhesive force increases for each protein as the pH of the solution becomes more basic. Both proteins exhibit higher adhesive forces than the polystyrene control surface over the range of pH values. As the protein layer is exposed to a more basic solution, the surface will have a much larger net negative charge. Given that silicon nitride has an isoelectric point of pH 6-7, this may lead to a stronger interaction between the surface and the silicon nitride AFM tip (Kosmulski, 2001). For lower pH values (< pH 7.4), STA displays higher adhesive forces than BSA but this trend does not hold for the most basic solution.

Finally, the effect of temperature over the range of pH values was tested for BSA and STA at 100 µg/mL concentration. Figure 5(b) shows the adhesive force data as a function of pH at 37ºC. Adhesive forces for both proteins are much lower than at 22ºC and are not much different than the polystyrene control surface. This decrease in adhesive force at 37ºC may be due in part to the fact that more protein-protein and protein-surface interactions are occurring that result in a lower adhesive force with the AFM tip.

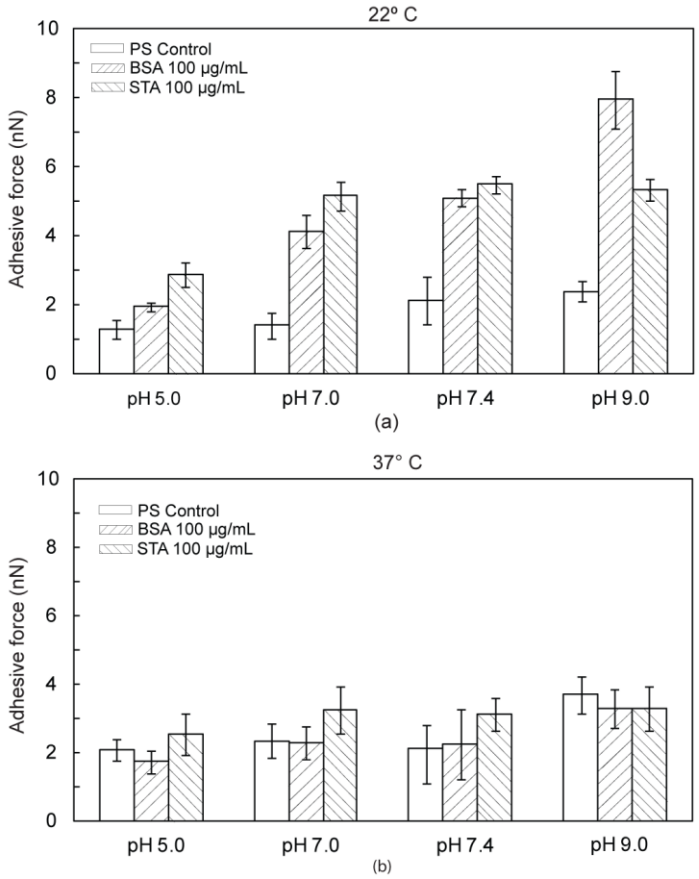

Figure 5. Effect of pH on adhesive forces (a) at 22ºC and (b) 37ºC. Error bars represent ± 1 standard error.

The decrease in adhesive force could also be due to a temperature related conformation change in the protein which then interacts more weakly with the AFM tip. However, a slight increase occurs for all samples as the solution becomes more basic. This trend is consistent with the data that was obtained at 22ºC. As before, no clear trend is present to distinguish BSA from STA. This may be in part due to their similar protein structure, isoelectric point and mass.

3.2 Friction Measurements

The coefficient of friction was measured for all proteins as a function of protein concentration. Figure 3(b) shows the coefficient of friction between the bare silicon nitride AFM tip and the various proteins. The friction measurements that were taken show a decreasing coefficient of friction as the protein concentration is increased from 1 µg/mL to 100 µg/mL. This decrease is believed to be due to the proteins acting as a lubricant film between the polystyrene surface and the silicon nitride AFM tip. At a higher protein concentration, a continuous lubrication film is formed due to a more uniform protein layer being formed on the substrate surface (Bhushan et al., 2006). Like the adhesion data, there is no clear trend to distinguish the proteins from one another. However, the general trend is that by increasing the concentration of these proteins leads to a decrease in the coefficient of friction for each protein tested.

Next, the coefficient of friction was measured for BSA and STA at 100 µg/mL concentration as a function of pH at 22ºC. Figure 6(a) shows the coefficient of friction between the bare silicon nitride AFM tip and two different protein coated surfaces. Both proteins exhibit lower friction than the polystyrene control, but the data appears to show that the coefficient of friction is fairly constant for all of the samples as the pH of the solution is altered. Also, no clear trend is present to distinguish BSA from STA when the pH varied, but the data from this experiment is in good agreement with what was found earlier by varying the protein concentration.

Finally, the effect of temperature was investigated by heating the sample and solution to 37ºC. Figure 6(b) shows the coefficient of friction between the bare silicon

nitride AFM tip and two different protein coated surfaces. At an elevated temperature the coefficient of friction increases slightly or stays the same relative to the 22ºC data. Again, the coefficient of friction is lower for the protein coated samples relative to the polystyrene controls, but the coefficient of friction does not significantly change from one pH level to the next.

From these experiments, the coefficient of friction appears to be more significantly affected by the concentration of the adsorbed protein film and not by the pH or temperature of the surrounding medium.

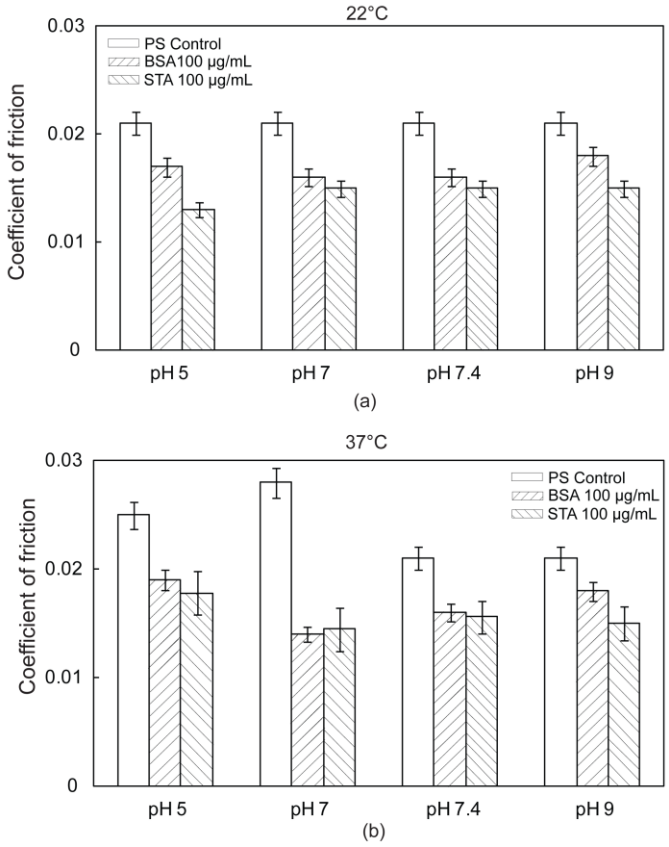

Figure 6. Effect of pH on the coefficient of friction (a) at 22ºC and (b) 37ºC. Error bars represent ± 1 standard error.

As reported earlier by Bhushan et al. (2006) and Fan et al. (2011), and confirmed with these experiments, protein concentration appears to be the primary factor in the formation of a continuous lubrication film. Solution pH and temperature appear to have secondary effects on the friction force experienced by the AFM tip.

3.3 Wear Tests

Figure 7 shows the wear images for each protein and a bare polystyrene control surface. Each figure shows the height image and cross-sectional profile of a wear mark after conducting the wear scans. For these tests, the protein concentration was kept constant at 100 µg/mL with the normal load increasing from 3-9 nN. These tests show that the depth of wear increases with an increasing normal load for all of the samples imaged. Several key differences become apparent on comparing the wear marks between the different protein coated surfaces. The surface coated with BSA shows the most significant wear relative to all of the other samples. This may be in part due to the smaller mass of BSA and lower isoelectric point. At a pH of 7.4, BSA will have a net negative charge which will interact more strongly with the AFM tip. When the AFM tip comes into contact with the protein film, the net negative charge and small mass allows for more wear to occur relative to the other samples. Streptavidin has approximately the same mass as BSA, but it does not exhibit the degree of wear that BSA undergoes. Therefore, protein mass appears to have a secondary effect on wear over the range of normal forces tested. As expected, the wear loads are of the same order of magnitude as the adhesive forces found in this experiment.

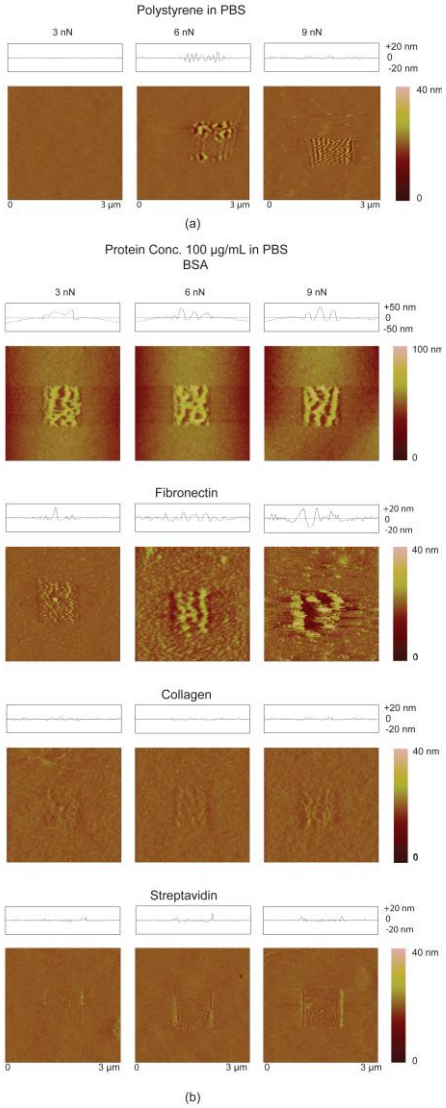

Figure 7. AFM height images and corresponding cross-sectional profile of a wear mark in contact mode of (a) bare polystyrene and (b) four proteins at a concentration of 100 µg/mL in PBS. The normal loads used were 3, 6, and 9 nN.

A trend becomes apparent by comparing the wear of the three globular proteins. As the protein isoelectric point becomes more basic and closer to the pH of saline (pH 7.4), the wear marks become smaller. The most significant wear occurs with BSA which

has the lowest isoelectric point (pH 4.7). The surface coated with fibronectin (isoelectric pH 5) displays less wear than the BSA sample, but greater wear than the streptavidin (isoelectric pH 5.5) sample. The difference in wear marks for the globular proteins may be in part due to charge-charge repulsion that occurs when the pH of the solution is different than the isoelectric point of the protein.

The most interesting surface of all however appears to be the one coated with collagen. This surface appears to not undergo any significant wear as compared to the other surfaces on the length scale in which we are interested. Collagen is the only protein tested that is linear in structure compared to the other proteins, and it has the ability to strongly self associate with itself (Silver et al., 2003). Collagen also contains more hydrophobic amino acid groups than other proteins, which results in a stronger interaction with the polystyrene surface (Xu et al., 2007). As the protein solution is allowed to sit overnight, slow rearrangements take place in the adsorbed collagen creating a denser mesh with an increased resistance to wear (Pamula et al., 2004). Given that collagen is the primary load bearing protein in the body and can be found in tissues in which significant mechanical wear can occur, it seems logical that it could be the most wear resistant protein (Di Lullo et al., 2002). However, as shown by Pamula et al. (2004), and confirmed in this study as well, the collagen surface will still undergo slight degradation at higher loads. Although fibronectin has the ability to self associate, it does not appear to form the wear resistant mesh that collagen does due to its globular structure.

Figure 8 shows a schematic of the hypothetical wear mechanism for the globular proteins at a higher protein concentration.

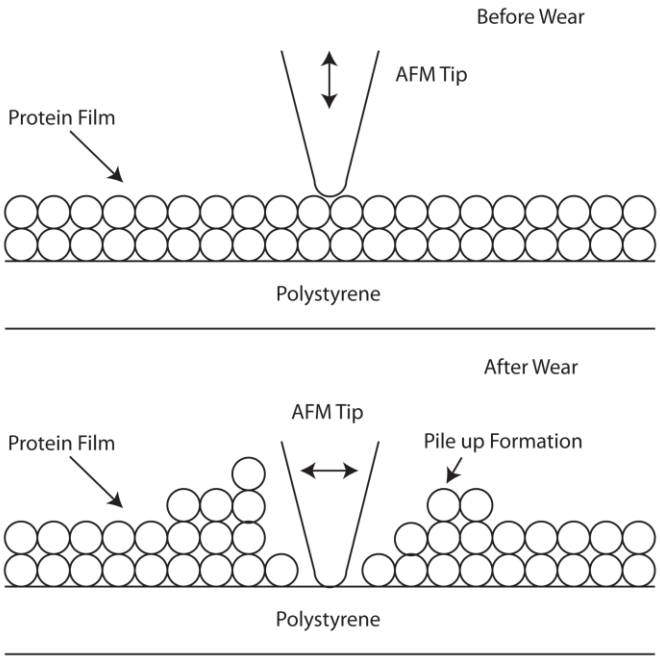

Figure 8. Schematic of hypothetical wear mechanism of the adsorbed protein film at higher concentration

Due to the motion of the AFM tip during scanning, the protein molecules displace laterally and begin to interact with the remaining protein molecules. Protein aggregation results leading to a pile up formation that keeps the molecules attached to the surface. The increased normal force causes more protein molecules to displace, leading to an increased degradation of the surface.

Chapter 4: Conclusions

In this study, the nanoscale adhesion, friction and wear properties of four proteins adsorbed to a spin coated polystyrene surface were measured using atomic force microscopy in deionized water and phosphate buffered saline by varying the concentration of the protein solution, pH, and temperature of the liquid medium.

Under mild operating conditions, all of the proteins appear to have similar adhesion and friction behavior compared to one another making a direct comparison between proteins difficult. The adhesive force between each protein and the AFM tip has been shown to increase with an increasing protein concentration for all four proteins tested. This increase in adhesive force is believed to be in part due to the formation of multiple interaction sites between the protein film and the AFM tip. Adhesive forces also increase for BSA and STA as the solution is altered from acidic to basic. This is possibly due in part to the larger net charge of the protein interacting with the AFM tip. Adhesive forces then decrease when the temperature is raised to 37ºC. No clear trend is present to distinguish BSA from STA possibly due to their similar structure, isoelectric point and mass.

The coefficient of friction has been shown to decrease as the protein concentration is increased for all proteins tested in PBS (pH 7.4). This is thought to be due to the protein layer acting as a continuous lubrication film at higher protein concentrations. The coefficient of friction also decreases for BSA and STA when each pH level was tested at 22ºC as compared to the polystyrene control. The coefficient of

friction remains fairly constant across the range of pH values for each protein tested. Elevating the temperature to 37ºC kept the coefficient of friction fairly constant across the range of pH levels as compared to the polystyrene control surface for BSA and STA. From these experiments it appears that the existence of a lubrication film is most important in affecting the coefficient of friction. Solution pH and temperature appears to only have secondary effects. No clear trend is present to distinguish BSA from STA when the pH and temperature of the solution are varied.

The distinction between the different proteins becomes clear only during the wear tests. The wear images show increased degradation as the normal force is increased for all of the samples imaged. The depth of wear marks increases with an increase in normal load for all of the proteins tested. The globular proteins appear to degrade in order of their isoelectric points with bovine serum albumin degrading the most, and the remaining proteins degrading in the order fibronectin > streptavidin > collagen. Collagen is suggested to be more wear resistant due to its linear structure and ability to self associate. Protein mass appears to have a secondary effect on wear for the normal forces tested with the primary wear mechanism resulting from protein structure and isoelectric point.

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