The Effects of SBS Tri-Block Copolymer Surface Roughness on Protein Adsorption

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Abstract:

Polymers can be used for a variety of different tasks including biomedical applications. However, most polymers are inherently incompatible with biomolecules such as proteins and DNA. There is growing evidence to suggest that surface topography of a polymer can affect the biocompatibility, due to the effects that the surface morphology has on the protein adsorption. Greater protein adsorption to the surface is useful in applications such as orthopedic implants. However, since immune reactions are common in contact medical devices, ways to reduce protein adsorption are valuable to explore for blood or tissue contact devices. Here we aim to develop a biocompatible polymer surface using a polystyrene (PS)-block-polybutadiene (PB)-block-polystyrene (PS) triblock copolymer (SBS). SBS is not biocompatible on its own, but allows us to change the surface topology by changing the annealing times of the triblock copolymer or etching.

We prepared SBS thin films (60-80 nm thick) on both silicon substrates and glass disks. For comparison purposes, PS and PB homopolymer thin films were also prepared on the substrates. Atomic force microscopy images verified the concept that changing annealing time effects the surface topography. Protein experiments using fluorescent BSA protein were conducted to determine the effects of controlling surface topography on protein adsorption. The results evidenced that the PS and PB homopolymers show very minimal protein adsorption, while the SBS triblock copolymer show much more protein adsorbed on the surface. In addition, we found as the annealing times increased, less protein adsorbed onto the polymer surface.

Keywords: Tri-block copolymer, protein adsorption, annealing, atomic force microscopy

1 Introduction

Polymers are widely used in everyday life in products such as plastic bags, bottles, rope, piping, packaging and coatings. Additionally, they have an important role in research for use in medical devices. Research is being done to see if modifications to polymers can either increase or decrease protein adsorption on their surfaces. Many have studied surface microtopography, and its ability to influence cell behavior, and the effects that changing these surface micropatterns have on the amount of protein adsorbed on the surface [2][8][9]. There are many ways that surfaces can be modified in order to be patterned, including spincasting, molding, and sonication. Each of these methods have their advantages in surface patterning and can create different sized surface structures. Previous literature concludes that patterned surfaces adsorb more protein on their surfaces than flat surfaces [2]. Figure 1 shows this principle; the pattern surface adsorbed 46% more protein than the flat surface with only an 8% increase in the surface area [2].

Many polymers are not biocompatible and therefore cannot be used for medical devices because they may be harmful to living tissue [7]. Two polymers
used for the present study are polystyrene (PS) and polybutylene (PB). In order to use either of these two polymers with less biocompatibility for uses in the body, they must be modified for the specific use of the medical device. For applications with orthopedic implants, a surface that adsorbs protein is necessary; however, less protein adsorption is desirable for uses in blood or tissue contact devices. Protein adsorption is important for designs of biosensors, medical diagnostics devices, and drug delivery vehicles.

Although some other polymers are naturally biocompatible, they are often very expensive; PS and PB are both fairly inexpensive polymers and would be ideal for uses where high protein adsorption is needed. A proposed solution to this problem would be the use of a PS-block-PB-block-PS triblock copolymer (SBS) as the coating for biomedical devices. Ideally, the block copolymer will adsorb proteins better than the PS and PB homopolymers. Block copolymers are appealing for this application because of their ability to self-assemble into ordered microdomains [1]. The way that these copolymers pattern could be taken advantage of in order to obtain the highest possible protein adsorption [1].

Block copolymers have been studied extensively due to their repulsion between unlike blocks, which leads to the microphase separation. Most research in this field involves thermal annealing for long periods of time in order to minimize surface-thermodynamic effects, remove volatile solvents, and maximize the chances of the surface reaching thermodynamic equilibrium morphology. Block copolymers can be used as a template for surface patterning, and there are many methods for changing their surfaces. One study, “Fabrication of nanopatterns using block copolymer and controlling surface morphology,” tests surface morphology changes through several methods [3]. This study shows that differences in polymer solution and spin coating speeds affect the thickness of the polymer layer, which in turn affects the overall surface pattern. Additionally, different sonicating solvents created different surface pattern, and a combination of solvents can create fingerprint regions along the surface. The study also showed the ways that polymer surfaces can be modified, and the different methods for removing different substances on the surface [3]. Research has also shown that SBS can maintain periodicity of spheres, cylinder, or lamellae [5].

While the chemical composition of different polymers or copolymers affects the ability of proteins to adsorb to a surface, the surface itself can affect protein adsorption. Previous research has shown that surface topography on the nanoscale level can have an effect on protein adsorption. There are a few ways these surface patterns can be created. Some of these techniques are photon ad electron based lithography, nano-imprint lithography, and etching and glancing angle deposition [4]. The combination of polymers and topography can enhance or diminish protein adsorption on surfaces, resulting in better surfaces for many medical applications.

The goal of this experiment is to test the effects of surface roughness via a thermal
annealing process on protein adsorption. We hypothesize that greater the surface roughness (i.e., annealing times) less proteins will adsorb on the surface.

2. Materials and Methods

2.1 Materials

290k molecular weight polystyrene was purchased from Sigma Aldrich. 38k molecular weight polybutylene was purchased from Polymer Source Inc. Toluene was purchased from VWR. 85k molecular weight SBS was purchased from Asahi-Kasei Chemical Corporation. 30 mg/mL FITC-BSA was purchased from Protein Mods. PBS was purchased from Invitrogen. Glass disks were purchased from Ted Pella, Inc. Silicon wafers were purchased from University Wafers. 9” Glass Pasteur Pipet was purchased from VWR.

PS, PB, and SBS were all dissolved in toluene and left overnight before spincasting the polymer films. Samples were annealed in a vacuum oven for varying durations of time at 130 °C. 30 mg/mL FITC-BSA was diluted with PBS to the desired concentration of 1mg/mL before performing protein incubation.

2.2 Experimental Methods

2.2.1 Spincasting

We first spincasted the polymers onto substrates. The polymers used were PS, PB, and SBS. The polymers were spincasted (Headway Research, Garland, TX) onto both glass disks and silicon wafers so they could be imaged through optical microscopy as well as atomic force microscopy. Before spincasting, glass disks and silicon wafers were cleaned. The glass disks were cleaned by sonication (Ultrasonic Cleaner, Branson 200) in ethanol for one minute. Silicon wafers were cleaned using a 1:1:1 solution of water, hydrogen peroxide, and ammonium hydroxide for 20 minutes followed by using a 1:1:1 solution of water, hydrogen peroxide, and sulfuric acid. After cleaning the glass disks and silicon wafers, they were placed on the spincasting apparatus and spun until excess liquid was removed, generally 30 seconds. Once the excess liquid was removed, the polymer was spincasted onto the substrate for 30 seconds at 2500 rpm.

The silicon wafers were cleaned prior to us working with them. The polymers were spincasted onto the silicon wafers so that the thickness of each polymer film could be recorded using the ellipsometer. The number of samples spincasted was based on how many different annealing times were going to be tested. For each annealing time that was going to be tested, both a glass disk and silicon wafer were spincasted.

2.2.2 Annealing

Once the substrates were spincasted, they were annealed. They were annealed in a vacuum oven (Sheldon Manufacturers, Cornelius, OR) at 130 °C. Samples were annealed for different time periods so that the effects of annealing time on surface topography could be observed. The time periods they were annealed for were 0 hours, 8 hours, 24 hours, and 48 hours. High temperature annealing of polymer thin films improves their uniformity [6].

2.2.3 FITC BSA Protein Experiment

After samples are annealed, the protein incubation was completed on the glass disks. The protein incubation had to be performed in a BSL-2 laboratory, which we were all trained for. A 1 mg/mL solution of fluorescein conjugate (FITC) Bovine Serum Albumin (BSA) protein in PBS was diluted from a 30 mg/mL stock solution. 100 microliters of this dilute solution were pipetted onto a glass plate that had been covered in parafilm. On top of each drop the annealed spincasted sample was placed with the surface side down, so the polymer directly interacted with the BSA.
protein. Each sample was incubated for 20 minutes, then rinsed in water and dried with compressed air. The dry samples could then be analyzed through optical microscopy.

2.3 Analytical Methods
2.3.1 Ellipsometer

The ellipsometer (Rudolph Research AutoEL, Hackettstown, NJ) was used to determine the thickness of the polymer film. In order to find the thicknesses of the films, the polymer must be spincasted onto silicon wafers, as opposed to glass, because the laser used to identify the thickness is refracted off of the silicon surface. The index of refraction used for SBS is 1.530.

2.3.2 Optical microscope

After the samples were rinsed and dried with compressed air, images were taken using a fluorescent optical microscope. Since the type of BSA used was fluorescein conjugated, areas glowed bright green where protein adsorbed to the surface. The microscope was set to FITC with a magnification of 10x. The sample was placed onto the center of the microscope with the protein side up. The microscope was adjusted so that the image on the computer is clear. Once the image was clear, images at three different locations on the sample were taken. On the computer program, the color was adjusted to be fluorescent green.

2.3.3. AFM

Atomic force microscopy was used to show the surface topography of each sample. AFM was taken at a variety of different specifications including wide and zoom magnification.

3. Results and Discussion:

First, PS and PB homopolymers were spincasted and protein adsorption was tested using the FITC BSA protein experiment. The results of the protein experiment are shown below in Figure 2. The PS and PB films were spincasted to be 41 nm and 227 nm respectively.

3.1 PS and PB Homopolymers

Figure 2. Optical microscope images of PS (annealed 24 hrs at 130°C: 40.8 nm thickness) and PB (annealed 24 hrs at 80°C: 227.7 nm thickness) at different incubation times compared to glass disks with and with BSA. untreated w/o BSA (I), untreated w/ BSA 20 min (II), PS 10 min (III), PS 20 min (IV), PB 10 min (V), PB 20 min (VI)

The results from the protein incubation of PS and PB both show that less proteins adsorb to the substrates when there is a polymer layer, as opposed to the untreated glass disk, which does not have polymer layer on it (see Fig. 2). These images were used a basis for the next test of PS-block-PB-block-PS triblock copolymer.

3.2 Thick Out-of-Period SBS thin films (78 nm thick)

3.2.1 AFM
AFM images were taken of the 78 nm SBS thin films at three different annealing times. We made samples with no annealing, 8 hour annealing, 24 hour annealing, and 48 hour annealing. The sample with no annealing had dust particles on it, therefore we could not get a good AFM image. The other three AFM images are shown in Figure 3.

![Figure 3. AFM images of SBS (annealed at 130°C: 78 nm thickness) at varying annealing times. 8 hrs (I), 24 hrs (II), 48 hrs (III)](image)

These AFM images show dewetting, or the rupture of the thin film, which is caused by the surface energy differences between the surface and the film. In order to determine whether the film dewetted down to the silicon wafer surface, a scratch test was completed and the AFM is shown in Figure 4.

3.2.2 Scratch Test

![Figure 4. Scratch test AFM height image of SBS (annealed at 130°C: 78 nm thickness) with 40 μm scale.](image)

The height AFM image shown in Figure 4 shows that the film did not dewet down to the silicon wafer surface, based upon the cross-sectional analysis. The cross-sectional analysis shows that vertical distance between the scratched and unscratched surfaces is 37 nm, and the total film thickness is 78 nm. Since the vertical distance is only 37 nm, which is not the total height of the polymer film, we can conclude that the polymer film did not dewet down to the silicon surface. Rather, the SBS film show a terrace structure, as previously reported [10]. We take advantage of this “rough” structure for the study.

3.2.3 FITC BSA Protein Experiment on the SBS 78 nm film

The FITC BSA protein experiment was completed on 78 nm SBS thin films at four annealing times. The results are shown in Figure 5.

![Figure 5. Optical microscope images of SBS (annealed at 130°C: 78 nm thickness) on glass disks at varying annealing times. 0 hr (I), 8 hr (II), 24 hr (III), 48 hr (IV)](image)

The images shown indicate that are changes in protein adsorption when introduced to varying annealing times. The bright fluorescent areas show greater protein adsorption. The problem with this method is that the filter used on fluorescent microscopy makes the entire image a green hue, making it hard to determine which areas of the film actually have adsorbed protein. From these images, the no annealing and 8 hour
annealing (Fig. 5, I and II) seem to have adsorbed the most protein, as seen by their bright green areas. By that same logic, the darkness of the 24 and 48 hour samples (Fig. 5, III and IV) show less protein adsorption.

3.3 Thinner In-Period SBS thin film (60 nm)

3.3.1 AFM

We first characterized the surface structures with AFM. 60 nm SBS films were spincasted, and the AFM images are shown in Figures 6 and 7.

![Figure 6](image)

**Figure 6.** Wide AFM images of SBS (annealed at 130°C: 60 nm thickness) at varying annealing times. 0 hrs (I), 24 hrs (II), 48 hrs (III)

![Figure 7](image)

**Figure 7.** Zoom AFM images of SBS (annealed at 130°C: 60 nm thickness) at varying annealing times. 0 hrs (I), 24 hrs (II), 48 hrs (III)

As seen with the 78 nm films, different annealing times lead to a topological change in the films. From here, we tested the protein adsorption to the 60 nm SBS films to see if there was a correlation between the surface morphology and the amount of protein adsorbed.

3.3.2 FITC BSA Protein Experiment on the SBS 60 nm film

The FITC BSA protein experiment was completed on the 60 nm thick films. The samples were incubated for 20 minutes each. The results can be seen in Figure 8 below.

![Figure 8](image)

**Figure 8.** Optical microscope images of SBS (annealed at 130°C: 60 nm thickness) on glass disks at varying annealing times. 0 hr (I), 8 hr (II), 24 hr (III), 48 hr (IV)

Figure 8 shows the protein adsorption on the 60 nm films annealed at the four different times. The exposure time for the samples was kept consistent at 31 seconds. The bright fluorescent green spots on the images indicate protein adsorption to the surface. The brighter spots on an image indicates that more protein was adsorbed to the polymer surface. Figure 8 images I and II have more protein than images III and IV.

3.3.3 AFM for the SBS thin films on GLASS

In order to view the FITC BSA under fluorescent microscopy, the thin films needed to be spincasted onto glass disks. However, to make the AFM imaging easier and to test the thickness on the ellipsometer, the films needed to be spincasted onto silicon wafers. Since silicon wafers and glass have differing chemical formulas (SiOₓ vs. SiO) we could not logically assume that the interaction between the polymer and the surface is the same for the two. In order to test this, AFM of the 60 nm SBS samples were tested on glass disks, seen below in Figure 9.

![Figure 9](image)

The patterning of the SBS film is similar on both the silicon wafers on glass disks, as seen in Figures 7 and 9. Although there are some differences in the 8 hour, the
24 and 48 hour samples are very similar in morphology.

3.4 Discussion

From analysis of the AFM image in Fig. 4, we concluded that the surface did not dewet down to the silicon. This conclusion allows us to confirm the fact that we have created the rough surfaces for the proposed protein adsorption experiments.

The AFM images of both 78 nm and 60 nm samples show that as annealing time progresses, surface morphology changes. Less annealing time creates more island-like structures, while greater annealing times shows these islands merging together into a conglomerate. The darker areas of the AFM images show the valleys in the film, while the lighter areas show the peaks.

SBS has a domain spacing of 30 nm, as clarified by small-angle x-ray scattering performed by the Koga group. This spacing refers to the distance between PB blocks in the SBS triblock copolymer. Films that follow the rule of being a multiple of the domain spacing will show symmetrical wetting. Since the 78 nm sample did not follow this rule, the surface pattern could not have symmetrical wetting, which caused such a terrace structure to occur. Due to this, we decided to try to make 60 nm SBS films to note the difference in surface morphology.

There was a visible difference in morphology between the 60 nm and 78 nm films. The differences in morphology contributed to protein adsorption. Figure 5 and Figure 8 show the protein adsorption on the 78 nm and 60 nm films, respectively. While it appears that about the same amount of protein adheres to the samples based on the annealing time, the patterns at which they anneal are different. The protein adsorption that occurs in Figure 8 images I and II adheres in bands while in Figure 5 images I and II still have the same amount of protein, they do not seem to have any type of bands of proteins. This occurs because of the actual surface. The surfaces of the 60 nm films are less random leading to the proteins to adhere in the same way on the films.

Figures 6 and 7 show the AFM images for the 60 nm SBS samples with white dots across the frame. We attribute these small dots to dust particles on the sample, or small defects in the sample, but we are assuming they had no effect on the protein adsorption on the surface.

Our hypothesis stated that changing the annealing time of polymer films would change the amount of protein that adhered to the surface. More specifically, the amount of FITC BSA that adsorbed to SBS films would change based on the annealing times the films were subjected to. Our results show that the longer a sample is annealed, the less protein will adhere to the sample.

4. Conclusion

Changing the surface topography of a polymer film is one way to achieve the biocompatible properties that a scientist or researcher may want. Surface topography can be changed by varying the length of annealing times. The longer the SBS films were annealed in the vacuum ovens, the less protein adsorption to the polymer surface occurred.

Biocompatible properties are important in the study of medical devices and applications. Coatings are needed to assure that the devices being introduced into the body are not rejected. Some devices need to have the ability to adsorb proteins while other
devices do not want to adsorb proteins. The different characteristics depend on where the device will be going into the body and what its purpose is. In order to create these coatings, polymers are used on the devices. However, many of the polymers that are currently available are expensive and could be further optimized. Our research showed that controlling the surface topography has a direct effect on the protein adsorption.

Future researchers can examine a variety of different things. Researchers can perform the experiment with different proteins. Using different proteins will allow for this polymer film to be used on medical devices entering different parts of the body. An ideal annealing time can also be observed by performing the experiment at more annealing times.

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