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A Highly Stable Nonbiofouling Surface with Well-Packed Grafted Zwitterionic Polysulfobetaine for Plasma Protein Repulsion

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An ideal nonbiofouling surface for biomedical applications requires both high-efficient antifouling characteristics in relation to biological components and long-term material stability from biological systems. In this study we demonstrate the performance and stability of an antifouling surface with grafted zwitterionic sulfobetaine methacrylate (SBMA). The SBMA was grafted from a bromide-covered gold surface via surface-initiated atom transfer radical polymerization to form well-packed polymer brushes. Plasma protein adsorption on poly(sulfobetaine methacrylate) (polySBMA) grafted surfaces was measured with a surface plasmon resonance sensor. It is revealed that an excellent stable nonbiofouling surface with grafted polySBMA can be performed with a cycling test of the adsorption of three model proteins in a wide range of various salt types, buffer compositions, solution pH levels, and temperatures. This work also demonstrates the adsorption of plasma proteins and the adhesion of platelets from human blood plasma on the polySBMA grafted surface. It was found that the polySBMA grafted surface effectively reduces the plasma protein adsorption from platelet-poor plasma solution to a level superior to that of adsorption on a surface terminated with tetra(ethylene glycol). The adhesion and activation of platelets from platelet-rich plasma solution were not observed on the polySBMA grafted surface. This work further concludes that a surface with good hemocompatibility can be achieved by the well-packed surface-grafted polySBMA brushes.

Introduction

Nonbiofouling surfaces have an important medical application as blood-compatible materials for antithrombogenic implants.[1,2] However, only a few candidates are regarded as nonbiofouling materials or superlow biofouling materials. It is generally acknowledged that hydrophilic surfaces are more likely to reduce protein adsorption, but those surfaces are often not sufficient to prevent the undesirable adhesion of cells, bacteria, or other microorganisms.[3] Even a small amount of proteins on a surface can lead to the adhesion and propagation of unwanted biofouling.[4] For example, for those surfaces in contact with blood, even 10 ng/cm^2 of adsorbed fibrinogen or 1 ng/cm^2 of adsorbed von Willebrand factor (vWF) may introduce a full-scale blood platelet adhesion and then lead to thrombosis and embolism at the blood contact side of implant devices from the blood stream.[5,6] Moreover, stability of antifouling characteristics is another key issue that needs to be considered for the performance of nonbiofouling surfaces in the human body.

Two common types of polymeric segments, poly(ethylene glycol) (PEG)- and phosphotidylcholine (PC)-based polymers, have been extensively employed for surface grafting to create nonbiofouling surfaces.[7–11] PEG-based materials are most commonly used as synthetic surfaces for resisting protein adsorption. However, it has been recognized that PEG decomposes in the presence of oxygen and transition metal ions found in most biochemically relevant solutions.[12–14] Although PEG exhibits an excellent nonfouling capability, it faces the problem of long-term stability for implantation. Therefore, materials containing zwitterionic PC headgroups have become one of the popular synthetic materials for developing nonbiofouling surfaces.[9] Recently, numerous reports have shown that material surfaces containing zwitterionic structures similar to that of phosphorylcholine, such as phosphobetaine, sulfobetaine, and carboxybetaine, are ideal for resisting protein adsorption when the surface density and chain length of zwitterionic groups is controlled.[15–21] In previous research, Jiang and co-workers showed that the surfaces grafted

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with poly(sulfobetaine methacrylate) (polySBMA) or poly(carboxybetaine methacrylate) (polyCBMA) reduced the fibrinogen adsorption to a level comparable with the adsorption on PEG-like films.[16,18,21] They also grafted brush-like polySBMA on a gold surface via atom transfer radical polymerization (ATRP)and detected only 3 ng/cm^2 fibrinogen adsorption by surface plasmon resonance (SPR) measurement. Despite the superlow biofouling property of zwitterionic groups, their blood compatible stability is still unclear from the limited literature.

In the present work, a well-packed polySBMA grafted surface could be controlled via surface-initiated ATRP, and the effects on the stability of antifouling characteristics, especially for resisting plasma protein adsorption, are investigated. A cycling protein adsorption is carried out for testing the antifouling properties of the prepared surface. The effects of a wide range of various salt types, buffer compositions, solution pH levels, and temperatures on their antifouling characteristics are also discussed for detailed evaluation. This study also demonstrates the adsorption of plasma proteins and the adhesion of platelets on the polySBMA grafted surface from human blood plasma.

Materials and Methods

Materials. [2-(Methacryloyloxy)ethyl]dimethyl(3-sulfopropyl)-ammonium hydroxide (sulfobetaine methacrylate, SBMA) macromonomer was purchased from Monomer-Polymer & Dajac Laboratories, Inc. in the United States. Copper(I) bromide (99.999%), 2-bromoisobutyryl bromide (BIBB, 98%), pyridine (98%), 2-hydroxyethyl acrylate (97%), 2,2′-bipyridine (BPY, 99%), triethylamine (99%), tetrahydrofuran (THF, HPLC grade), and ethanol (absolute 200 proof) were purchased from Sigma-Aldrich. 1-Undecanethiol (99+%), (1-mercapto-11-undecyl)tetra(ethylene glycol) (99+%), and 11-mercapto-1-undecanol (99+%) were purchased from Asemblon Inc. Fibrinogen (fraction I from human plasma), γ-globulin (fraction II, III, 99%), and human serum albumin (HSA, 96–99%) were purchased from Sigma Chemical Co. Water used in experiments was purified using a Millipore water purification system with a minimum resistivity of 18.0 MΩ·m. THF for reactions and washings was dried by sodium before use. ω-Mercaptoundecyl bromoisobutyrate was synthesized through reaction of BIBB using a method published previously.[21] ^{1}H NMR (300 MHz, CDCl$_3$): 4.15 (t, J) 6.9, 2H, OCH$_2$), 2.51(q, J) 7.5, 2H, SCH$_2$), 1.92 (s, 6H, CH$_3$), 1.57–1.72 (m, 4H, CH$_2$), and 1.24–1.40 (m, 16H, CH$_2$).

Preparation of Self-Assembled Monolayers. In this study, three self-assembled monolayers (SAMs) were formed on the substrates: (1) methyl-terminated (CH$_3$), (2) oligo(ethylene glycol)-terminated (OEG), and (3) initiator ω-mercaptoundecyl bromoisobutyrate (Br) SAMs. Glass chips were first coated with an adhesion-promoting chromium layer (thickness 2 nm) and a surface plasmon active gold layer (48 nm) by electron beam evaporation under vacuum. Before SAM preparation, the gold-coated glass substrate was cleaned by washing with pure ethanol and distilled water in sequence, dried with N$_2$, then left in an UV light cleaner for 20 min at the source power of 110 W followed by rinsing with distilled water and ethanol, and finally dried by N$_2$. For preparation of CH$_3$- or OEG-SAMs, the cleaned chip was soaked in a 2 mM ethanol solution of 1-undecanethiol or (1-mercapto-11-undecyl) tetra(ethylene glycol) thiols for 24 h to form SAMs on the gold surface, and the chip was rinsed in sequence with ethanol and water and then dried in a stream of N$_2$. For the preparation of an initiator SAM on a gold surface, the cleaned chip was soaked in a 2 mM ethanol solution of ω-mercaptoundecyl bromoisobutyrate for 24 h to form Br-SAMs on the gold surface, and the chip was rinsed with pure ethanol followed by THF and then dried in a stream of N$_2$.

Preparation of PolySBMA Brushes. Well-packed polymer brushes of polySBMA on the SPR sensor chip were achieved via the surface-initiated ATRP method and were prepared following the method reported previously.[21] PolySBMA brushes were polymerized on the gold substrates with immobilized initiators of Br-SAMs based on our previous reports. The reaction solution of CuBr and BPY

were first placed in a sealed glass reactor in a dry box under a nitrogen atmosphere. A 200 mM degassed solution (pure water and methanol in a 1:3 volume ratio) of SBMA monomers was transferred to the reactor, and the gold surface with immobilized initiators was then placed in the reactor under nitrogen. After polymerization, the substrate was removed and rinsed with ethanol and water, and the samples were kept in water overnight. The prepared substrates were usually rinsed with PBS buffer to remove unbound polymers before any experiments. The thickness of the substrates was measured by ellipsometry. For reaction with a SBMA concentration of 200 mM, the thickness of the polySBMA film was controlled to a level of about 7 nm. The details regarding polySBMA film thickness as a function of polymerization time for different SBMA concentrations was already reported in our previous work.[21]

Plasma Protein Adsorption. In this work, a custom-built surface plasmon resonance (SPR) biosensor based on wavelength interrogation with a four-channel Teflon flow cell was used to monitor protein adsorption on the coated substrate. A SPR chip was attached to the base of the prism, and optical contact was established using refractive index matching fluid (Cargille). A protein solution of 1.0 mg/mL human fibrinogen, γ-globulin, or HSA in phosphate-buffered saline (PBS, 0.15 M, pH 7.4) was delivered to the surfaces at a flow rate of 0.05 mL/min. In this study, platelet-poor plasma (PPP) solution containing plasma proteins was also tested with the coated substrate. A surface-sensitive SPR detector was used to monitor protein−surface interactions in real time. The wavelength shift was used to measure the change in surface adsorption amount (mass per unit area). The calibration of the wavelength shift from SPR data associated with the amount of adsorbed protein was calculated on the basis of the quantitative mathematical formalism established by Campbell and co-workers.[22] The calibration follows the standard calculation for the same custom-built SPR system resulting a 1 nm wavelength shift in the SPR response equivalent to about 15 ng/cm^2 of adsorbed proteins.[19,22]

Blood Platelet Adhesion. The chips with CH$_3$-SAMs, OEG-SAMs, and PolySBMA of 1 cm^2 surface area were placed in individual wells of a 24-well tissue culture plate, and each well was equilibrated with 1000 μL of PBS for 2 h at 25 °C. Blood was obtained from a healthy human volunteer. Platelet-rich plasma (PRP) containing about 1 × 10^5 cells/mL was prepared by centrifugation of the blood at 1200 rpm for 5 min and then at 3000 rpm for 10 min. The platelet concentration was determined by microscopy (NIKON TS 100F). A 200 μL sample of the platelet suspension plasma was placed on the chip surface in each well of the tissue culture plate and incubated for 120 min at 37 °C. After the chips were rinsed twice with 1000 μL of PBS, they were immersed into 2.5% glutaraldehyde in PBS for 48 h at 4 °C to fix the adhered platelets and adsorbed proteins, then rinsed 2 times with 1000 μL of PBS, gradient-dried with ethanol in 75% v/v PBS, 50% v/v PBS, 25% v/v PBS, 5% v/v PBS, and 0% v/v PBS for 20 min in each step, and dried in air. Finally, the samples were sputter-coated with gold prior to observation under JEOL JSM-5410 SEM operating at 7 keV. The number and morphology of adhering platelets on the substrates was observed from scanning electron microscopy (SEM) images at a 1000 magnification from five different places on the same chip.

Results and Discussion

Well-packed polySBMA grafted surfaces via surface-initiated ATRP were studied for the nonbiofouling stability of their resistance to plasma protein adsorption and blood platelet adhesion. Preparation and characterization of these prepared surfaces were reported in our previous work.[21] Numerous reports have shown that the nonbiofouling stability of an implant surface is crucial to its blood compatibility for preventing thrombosis and embolism from the blood stream.[1,2,4] For comparison, the self-assembly method is also used to create two well-packed surfaces with CH$_3$- and OEG-SAMs as references. It is generally

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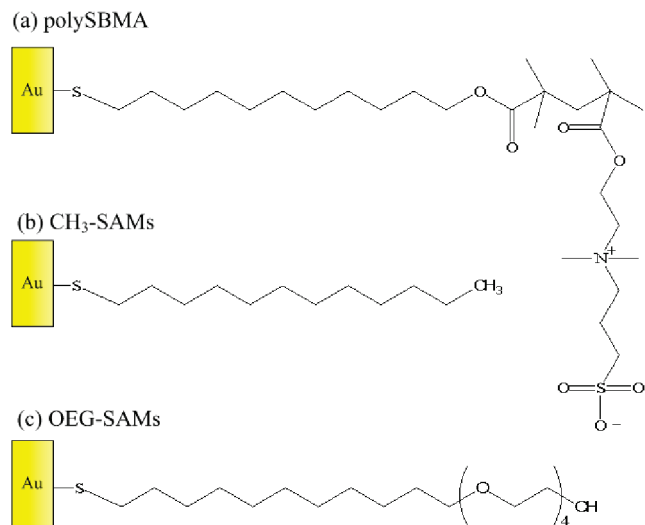
Figure 1. Chemical structures of (a) polySBMA grafted surface, (b) CH$_3$-SAMs, and (c) OEG-SAMs.
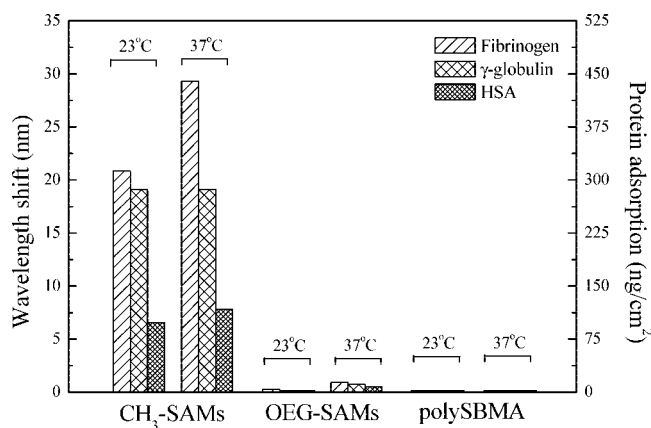

Figure 2. Adsorption of 1 mg/mL fibrinogen, γ-globulin, and HSA in PBS buffer on CH$_3$-SAMs, OEG-SAMs, and polySBMA grafted surfaces at 23 and 37 °C. A wavelength shift of approximately 1 nm in SPR is equivalent to 15 ng/cm^2 adsorbed proteins.

acceptable that SAMs presenting hydrophobic methyl groups usually induce a large amount of protein adsorption. A SAMs surface terminated with OEG ((EG)$_n$OH, $n = 3-6$) groups is used as standard against protein adsorption when new protein-resistant surfaces are judged. Chemical structures of polySBMA grafted surface and SAMs with CH$_3$ and OEG terminated groups used in this study are shown in Figure 1. This work is aimed at addressing two important issues for polySBMA stability: (i) protein adsorption on polySBMA surfaces at different ionic strengths, solution pH values, and temperatures and (ii) polySB-MA blood compatibility in the human body temperature.

Single Protein Adsorption on PolySBMA. Protein adsorption on the various substrates of CH$_3$-SAMs, OEG-SAMs, and polySBMA was measured by SPR. Three of the major plasma proteins, human fibrinogen, γ-globulin, and HSA, were selected for the testing in this study. SPR results for the model protein adsorption on different surfaces are shown in Figure 2. It was found that polySBMA and OEG-SAMs were highly resistant to nonspecific adsorption for each model protein at 23 °C (room temperature), whereas hydrophobic CH$_3$-SAMs showed high protein adsorption, especially for fibrinogen. The results are consistent with previous findings of protein adsorption on these surfaces.[18,22–25] However, OEG-SAMs lost their protein repulsive
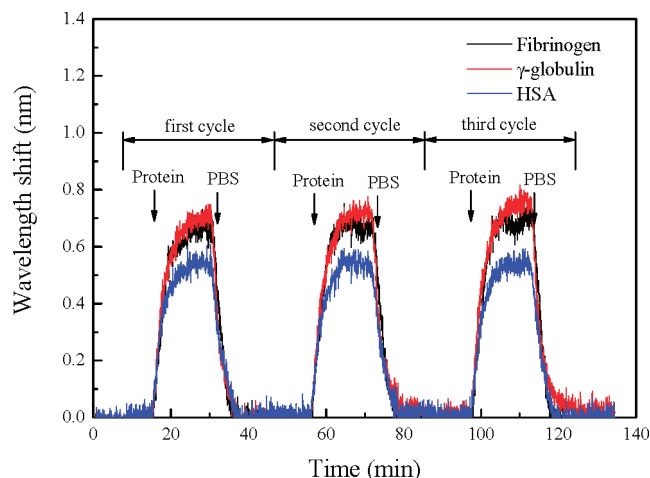

Figure 3. Cycling adsorption of fibrinogen, γ-globulin, and HSA on polySBMA grafted surfaces at 23 °C. The final wavelength shift in each cycle for each case is indicated inside the brackets. A shift of approximately 1 nm in the SPR response is equivalent to 15 ng/cm^2 of adsorbed proteins.

properties at 37 °C while polySBMA grafted surfaces still kept their good protein resistance. This indicates that polySBMA might provide a better nonbiofouling surface at human body temperature than OEG-SAMs. The main reason that we could identify is the hydrophobic interaction between the proteins and OEG-SAMs that is promoted as the temperature is increased. In the early studies, it was also shown that grafted PEG brushes exhibited protein resistance at room temperature, but lost their protein repulsive properties above 35 °C.[23,26] Moreover, it is now recognized that the chemical structure of PEG is unstable in most biochemically relevant solutions, but the chemical stability of polySBMA is still not fully understood. In this study, the effects of a wide range of various salt types, buffer compositions, solution pH levels, and temperatures on the nonbiofouling stability of polySBMA grafted surfaces were further tested, and a detailed discussion is in the following sections.

Surface Cycling Protein Adsorption. The biofouling characteristics of the polySBMA grafted surface were evaluated by cyclic protein adsorption tests. To begin the test, PBS buffer was first passed through the flow cell until a steady SPR signal was obtained, and protein solution was then delivered to the subsequent fluid flow. The amount of protein adsorption is defined as the difference between the two SPR baselines established before and after protein adsorption. Figure 3 shows the change in SPR signal from cyclic protein adsorption as a function of time on a well-packed grafted polySBMA surface. It was found that the baseline change for the adsorption of proteins on the polySBMA surface from PBS buffer (0.15 M and pH 7.4) containing 1 mg/mL fibrinogen, γ-globulin, and HSA, respectively, was below 0.02 nm in wavelength shift and almost identical in the three cycles, which means there was no further irreversible biofouling by proteins after the first cycle. The adsorbed amount of fibrinogen even remains lower than 2 ng/cm^2 after three cycles. The polySBMA grafted surface possesses excellent nonbiofouling properties and stability from the surface cycling of protein adsorption.

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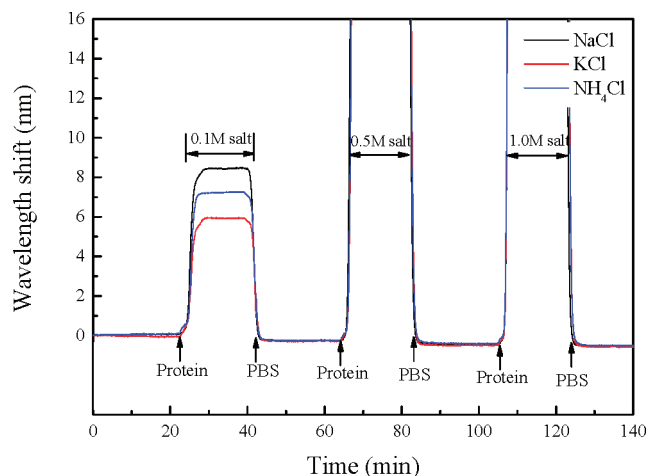(26) Leckband, D.; Sheth, S.; Halperin, A. *J. Biomater. Sci., Polym. Ed.* **1999**, *10*, 1125.

Figure 4. Irreversible adsorption of fibrinogen to polySBMA surfaces as a function of time performed by increased ionic strength cycling at 23 °C from SPR measurements. The PBS buffer was 0.01 M phosphate (pH = 7.4), and the ionic strength was adjusted by dissolving NaCl, KCl, and NH₄Cl, respectively, in the appropriate concentrations from 0.1 to 1.0 M. The SPR signals corresponding to the different ionic salts are indicated on the plots with various color curves.

Effects of Ionic Strength and pH on Protein Adsorption In this study, the effects of the ionic strength and pH of the buffer on the amount of protein adsorbed to polySBMA brushes were further tested to present the surface biofouling characteristics. Three types of ionic salt (NaCl, KCl, and NH₄Cl) were used in this work, and the ionic strength of buffer medium was adjusted by dissolving salt into PBS buffer (0.01 M and pH 7.4) in the appropriate concentrations from 0.1 to 1.0 M. Figure 4 compares the amounts of protein that adsorbed to polySBMA grafted surface at three different ionic strength cyclings. Human fibrinogen solutions of 1.0 mg/mL in PBS buffer medium with various types of salt and ionic strengths were delivered in sequence to the polySBMA grafted surface at a flow rate of 0.05 mL/min and at room temperature (23 °C). The remarkable changes of wavelength shift of SPR during the flow of protein solution can be mainly attributed to the refractive index changes of the bulk solution with salt. However, the surface characteristics of the reversible/irreversible adsorption behavior of protein are elemental properties that need to be understood for nonbiofouling materials development. Based on the SPR measurements, the amount of irreversible protein adsorption can be revealed by the differences between the two baselines established before and after protein adsorption for each cycle. SPR results showed that the amounts of irreversibly adsorbed fibrinogen at the polySBMA surface are minimal and do not depend on the ionic strength of the protein solution, even in a high ionic strength of NH₄Cl solution. These results also prove that electrostatic interactions are important for the polySBMA surface with proteins and the interactions are reversible. In general, it is a typical observation that proteins tend to adsorb on a hydrophobic surface at high ionic strength and the reasons are manifold. In high ionic strength solution, the protein molecule is exposed to a high surface tension and protein conformation may change and cause the exposure of hydrophobic segments of the protein molecule. Hydration of the exposed hydrophobic segments makes the solvation of the protein possible. However, if a hydrophobic surface is provided, dehydration of water molecules from the protein surface resulting in the protein adsorption onto the hydrophobic surface becomes spontaneous due to the entropy gain from the water molecules. Therefore, the results in Figure 4 indicate that the high resistance of fibrinogen adsorption on the polySBMA surface is due to intrinsically strong
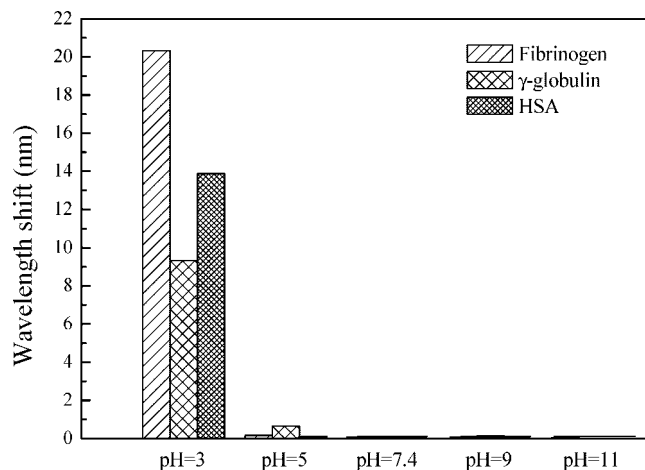

Figure 5. Adsorption of 1 mg/mL fibrinogen, γ-globulin, and HSA on polySBMA grafted surfaces as a function of solution pH at 23 °C from SPR measurements.

hydration via electrostatic interactions for polySBMA brushes. Based on the hypothesis of preferential exclusion to protein-resistant surfaces proposed by Whitesides et al.,[27] greater amounts of adsorbed protein will be expected as the ionic strength or hydration capability of ionic salt increases. In this work, the dependence of the contact angle on various ionic strengths of NH₄Cl from 0.0 to 1.0 M was also measured on the polySBMA surface. PolySBMA brushes display a very hydrophilic surface with a water contact angle of about 12°. As the ionic strength increased, almost fully wetted droplets on the polySBMA surface with undetected contact angle were observed. The results demonstrate that a preferential hydration surface can be formed by the polySBMA brushes with nonbiofouling surface characteristics to irreversible fibrinogen adsorption even at high ionic strength solution.

Figure 5 compares various proteins (human fibrinogen, γ-globulin, and HSA) adsorption onto the polySBMA surface in buffers with pH values from 3 to 11, while ionic strength was kept at a low salt concentration (0.01 M). These results point out that there are effects of pH on the single protein adsorption to the polySBMA surface. Previous study showed that proteins would have denatured structures and cause different protein–protein interactions at lower pH.[28] As shown in Figure 5, fibrinogen adsorbed on the surface of polySBMA at pH = 3 with a SPR signal shift similar to that observed on CH₃-SAMs at pH 7.4. HSA adsorbed on the polySBMA surface, on the other hand, with a much larger SPR shift compared with the monolayer HSA adsorption on CH₃-SAMs in Figure 2. The SPR signal shift of HSA may be caused by a denser packing or multilayer of HSA adsorption resulting from the protein–protein interactions between denatured HSA at pH 3. On the basis of the contact angle measurement with pH values from 11 to 3, it was found that the polySBMA surface keeps a nearly constant contact angle of about 12 ± 1°. That means the polySBMA brushes even display a very hydrophilic surface at low pH. This indicates the significant increase in fibrinogen adsorption at low pH might not be due to the attribution of hydrophobic interaction to the polySBMA surface. We regard that the increase in the amount of protein that adsorbed to the polySBMA surface at values of pH below 5.0 reflects the electrostatic dipoles at the protein–polySBMA

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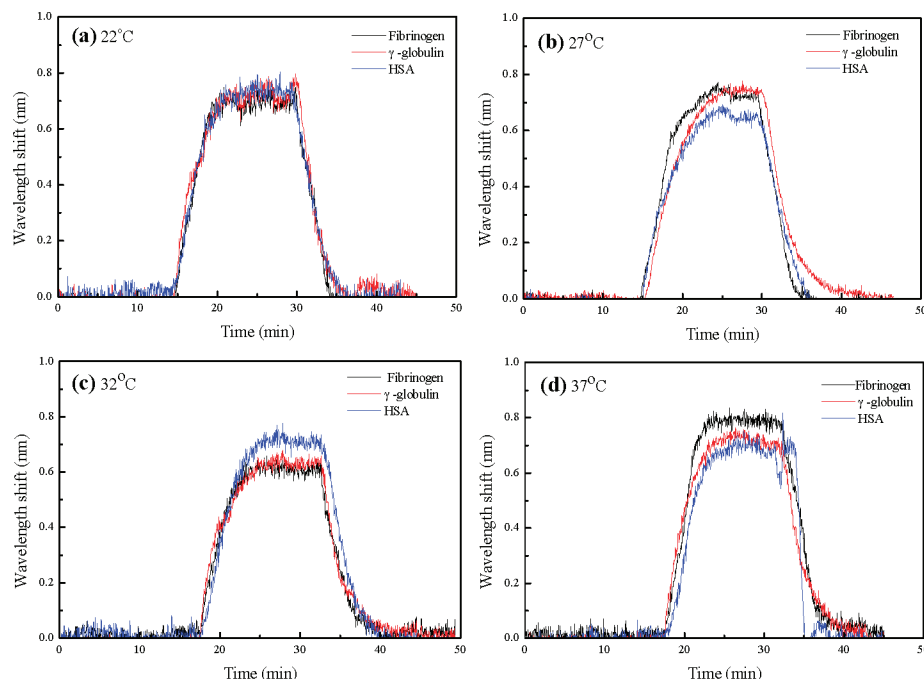
Figure 6. Adsorption of 1 mg/mL fibrinogen, γ-globulin, and HSA on polySBMA grafted surfaces as a function of solution temperature at (a) 22, (b) 27, (c) 32, and (d) 37 °C from SPR measurements.

interface.[28,29] In general, it is commonly observed that the acid-induced denaturation of proteins leads to a conformation change, which is due in part to the enhancement of electrostatic repulsion from the regions with more positive charge at lower pH. These denatured structures of the proteins with the exposure of positively and negatively charged regions to the solution might plausibly promote protein adsorption by electrostatic interaction to the polySBMA surface. In this analysis, it is important to note that the net charge of the protein becomes less positive at higher pH, so it will tend to be less attracted electrostatically to the polySBMA surface as the pH increases.

Thermal Stability of PolySBMA to Protein Adsorption The thermal stability of nonbiofouling surface is a crucial factor in retaining its biocompatibility in a human body environment. From the thermodynamic point of view, the increase of temperature usually enhances the exposure of hydrophobic patches of the protein in buffer medium and therefore promotes protein adsorption to the material surface by hydrophobic interaction. Some works showed that PEG-modified surfaces would lose their protein repulsive properties above 35 °C since the poor solubility of PEG changes.[23,26] It was also found in this work. Although the solubility of PEG can be regulated by the molecular weight, this is still a critical limitation for the PEG system in human body uses. For further understanding the protein repulsive properties of polySBMA associated with the change of medium temperature, Figure 6 shows the adsorption of various proteins on polySBMA with an increase in temperature from 22 to 37 °C in PBS buffer (0.15 M and pH 7.4), in which the ionic strength is controlled to be similar to that in a human biological system. The protein adsorption of human fibrinogen, γ-globulin, and HSA is almost resisted by polySBMA surfaces, as indicated by increased temperature cycling. To the best of our knowledge, this is the first example to show that polySBMA represents stable nonbiofouling brushes from 22 to 37 °C in protein solution.
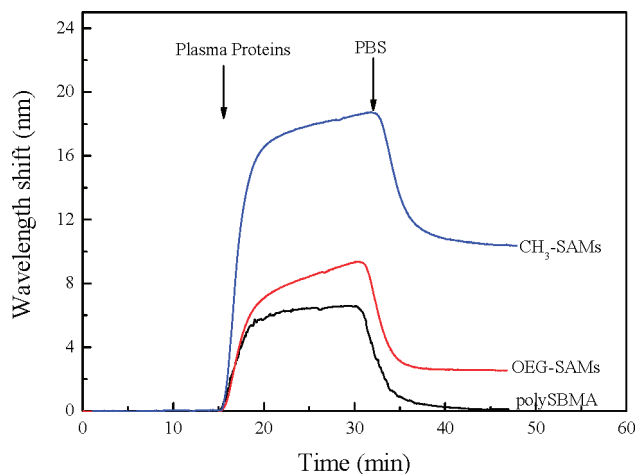
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Figure 7. Adsorption of plasma proteins on CH$_3$-SAMs, OEG-SAMs, and polySBMA grafted surfaces at 37 °C from human blood plasma. A wavelength shift of approximately 1 nm in the SPR response is equivalent to 15 ng/cm^2 of adsorbed proteins.

Plasma Protein Adsorption and Blood Platelet Adhesion. The initial stage of blood platelet adhesion onto a surface is a key determinant in the subsequent formation of thrombosis and embolism. Horbett et al. showed that the adhesion and activation of platelets from the blood stream might be correlated with the adsorption of proteins on surfaces.[5,6,14] This hypothesis was also tested in this study. Real-time adsorption of human plasma proteins onto polySBMA was monitored using SPR at 37 °C. CH$_3$-SAMs and OEG-SAMs were used as references. A diluted solution containing plasma proteins from platelet-poor plasma with 20% v/v PBS was used in this measurement to reduce the effects of plasma viscosity on the laminar flow channel. SPR results for the plasma protein adsorption are reported in Figure 7. We observed significant decreases in the adsorption of plasma proteins on OEG-SAMs as compared to CH$_3$-SAMs at 37 °C. Adsorption amount of plasma proteins on the hydrophobic surface
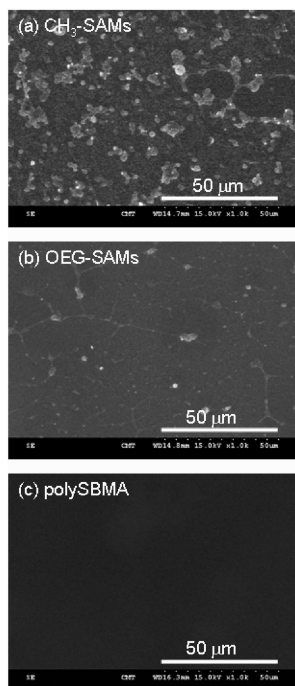
Figure 8. SEM photographs of platelets adhered onto the surface of (a) CH₃-SAMs, (b) OEG-SAMs, and (c) polySBMA surface. All images with magnification of 1000X.

(CH₃-SAM) is 75% higher than that on OEG-SAMs. However, there is a significant difference between OEG-SAMs and the polySBMA surface to resist plasma protein adsorption. Similar to reductions in the aforementioned single protein adsorption, the polySBMA grafted surface also greatly resists plasma protein adsorption. It is shown in Figure 7 that the adsorbed amounts of plasma proteins are 38.25ng/cm² on OEG-SAMs and 1.65ng/cm² on the polySBMA surface from diluted plasma solution at 37 °C. This indicates that polySBMA is a more effective alternative to OEG-SAMs for preventing plasma protein adsorption from human blood at 37 °C.

The platelet adhesion test has already become a recognized technique to estimate the blood compatibility of a prepared material surface. Thus, our work also employed this test to evaluate the blood compatibility of the polySBMA grafted surface. Figure 8 shows SEM photographs of platelets that adhered to the surfaces by contact of the prepared substrates with platelet-rich plasma solution for 60 min at 37 °C in vitro. The SEM results with image magnification of 1000X shows that the platelet adherance was remarkably suppressed on OEG-SAMs as compared with the hydrophobic surface (CH₃-SAM). It is clearly observed that the platelets have pseudopods and/or have spread on CH₃-SAMs, which indicates the activation of the platelet. However, there is still a small amount of slightly activated platelets on OEG-SAMs. As shown in Figure 8, the excellent performance of polySBMA in no obvious adhesion of blood platelet is due to its ability to highly resist nonspecific protein adsorption from blood plasma. It was shown that no platelets adhered to the polySBMA grafted surface as compared with OEG-SAMs. This might be attributed from the reduced plasma protein adsorption levels below 10 ng/cm² on polySBMA, while the adsorbed amount of plasma proteins is 38.25 ng/cm² on OEG-SAMs. The results confirmed previous statements that even a small amount of proteins on a surface can lead to the adhesion and activation of platelets from the blood stream. The possible reason why the polySBMA grafted surface more effectively resisted platelet adhesion and activation might be the much longer chains and higher densities of nonbiofouling groups of SBMA compared with OEG, similar to the previous reports from Ma et al. that both film thickness and polymer surface density contribute to the protein resistance.[30] Therefore, this result also suggests that the effectiveness of a nonbiofouling surface is likely due to not only the nature of the nonbiofouling group but also the thickness of the nonbiofouling films.

From the results of both protein adsorption and platelet adhesion in vitro, it is concluded that the well-packed polySBMA grafted surface performs stable and excellent blood compatibility at human body temperature. In the next stage of nonbiofouling material development, zwitterionic polysulfobetaine provides a significant impact and opportunity in searching for alternative stable nonbiofouling materials other than PEG.

Conclusions

In this work, we have combined SPR spectroscopy and surface polymer brushes of polySBMA to study the nonbiofouling stability of a zwitterionic polysulfobetaine grafted surface. We have found that polySBMA brushes possess excellent nonbiofouling stability in three cyclic protein adsorption tests and in a wide range of ionic strengths from 0.1 to 1.0 M, pH values from 7.4 to 11, and temperature cycling from 22 to 37 °C in PBS buffer. However, the increase in the amount of protein that adsorbed to the polySBMA surface at pH values below 5.0 might be attributed to the strong electrostatic interaction between the denatured proteins and the polySBMA brushes. Furthermore, results also show that there is a remarkably reduction of the adsorption of plasma proteins and the adhesion of platelets on the polySBMA grafted surface from human blood plasma. Results suggest that zwitterionic polySBMA is an effective and stable nonbiofouling material to provide a surface for uses in human blood and biomedical implants.

(30) Ma, H. W.; Hyun, J. H.; Stiller, P.; Chilkoti, A. *Adv. Mater.* **2004**, *16*, 338.