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A Facile Method of Using Sulfobetaine-Containing Copolymers for Biofouling Resistance

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ABSTRACT: Antifouling materials are desirable for many biomedical applications. In this work, the poly(sulfobetaine methacrylate-*co*-butyl methacrylate) (PSB) copolymers were investigated for their antifouling properties. The copolymers were synthesized via a simple free-radical polymerization with feed ratio of the zwitterionic sulfobetaine methacrylate (SBMA) varying from 0 to 20 mol %. The polymer composition was verified by nuclear magnetic resonance. The enzyme-linked immunosorbent assay and surface plasmon resonance were used to evaluate protein adsorption on a series of PSB copolymers from the single protein solution of fibrinogen, undiluted human blood serum, and undiluted human blood plasma. Results show that the protein adsorption amount decreased with the increasing content of SBMA in the copolymers. The adsorption levels achieved by PSB containing 20 mol % SBMA (PSB20) were only 4, 17, and 15 ng/cm^2 from fibrinogen, serum, and plasma, respectively, which represented 99%, 90%, and 90% reduction compared with the adsorption amounts on poly(butyl methacrylate) with no SBMA. The PSB20 film also completely inhibited endothelial cell attachment. Fouling resistance of PSB polymers can be well correlated with their receding water contact angles, which represent the polymer surface compositions in aqueous environment. The excellent antifouling abilities of PSB copolymers, combined with the facial synthesis method, commercial availability of all monomers, and low cost, render them highly promising for wide practical applications. The polymers can be applied versatilely as both solvent-cast films and surface coatings. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40789.

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INTRODUCTION

Surface properties of biomaterials play a vital role in determining interfacial phenomena such as protein and cell adsorption on the surfaces.[1–3] Materials in certain biological applications need to avoid unfavorable protein adsorption and cell attachment on the surfaces. For example, surfaces absorbing very low levels of fibrinogen (Fg) may still lead to platelet adhesion, thrombus formation, or tissue reaction.[4,5] Therefore, there is a great demand to develop anti-biofouling materials for various biomedical applications.

One well-known antifouling material is the hydrophilic poly(ethylene glycol) (PEG).[6–8] Because PEG gets degraded under oxidative conditions or in the presence of transition metal ions, which limits its long-term or *in vivo* applications, alternative antifouling materials are developed.[9,10]

Recently, zwitterionic polymers such as those based on sulfobetaine (SB)[11,12] or 2-methacryloyloxyethyl phosphorylcholine (MPC)[13,14] have drawn the most attention because of their ultralow biofouling properties. The reported SB-based antifouling polymers mainly include homopolymer poly(sulfobetaine methacrylate) [poly(SBMA)][11,15–17] and diblock copolymers, with the zwitterionic poly(SBMA) block and a second block (biodegradable, hydrophobic, or ionic) for multifunctionality.[18–21] The SB-based antifouling polymers have almost been exclusively synthesized via atom transfer radical polymerization (ATRP),[11,15,16,18,20,21] which is ideal for fundamental studies because it can provide precise control of polymer properties. However, ATRP needs relatively harsh reaction conditions (e.g., transition metal catalyst, no oxygen) and complex procedure.[22,23] A simple and versatile synthesis method is thus more desirable for wide practical applications, provided that the synthesized polymers still retain good antifouling ability.

On the other hand, MPC-based polymers have been proven to decrease protein adsorption effectively. Ishihara and co-workers[24–27]developed the poly(MPC-*co*-butyl methacrylate) (PMB) random copolymers that can be coated easily on various substrates. Although the hydrophobic moiety (i.e., butyl methacrylate, or BMA) is incorporated into the copolymers, the studies showed that the surface-coated PMB could strongly resist

adsorption of proteins from buffer or complex media (such as serum and plasma) and prevent cell attachment.[24–26] The techniques for studying protein adsorption in these works included enzyme-linked immunoassay, radioimmunoassay, ultraviolet (UV) absorbance, or MicroBCA protein assay, some of which can provide only the relative protein adsorption percentage whereas others are less sensitive.

Both SBMA and MPC monomers are currently commercially available, whereas SBMA is at a much lower cost (~25 times cheaper). Therefore, in this work, we intended to develop the SBMA-based poly(SBMA-*co*-BMA) (PSB) random copolymers at low cost with a facile synthesis method and investigate their antifouling properties. The PSB random copolymers, with different SBMA contents, were synthesized via a very simple free-radical polymerization approach. Protein adsorption from the single protein solution of Fg, undiluted human blood serum, and undiluted human blood plasma, and cell attachment on the PSB surfaces were thoroughly studied. The protein adsorption amount on PSB surfaces were quantified by a surface plasmon resonance (SPR) biosensor. Compared with other methods to evaluate protein resistance, SPR is the most widely used quantitative and *in situ* approach, with the reported detection limit of less than 0.3 ng/cm^2.[2] For the biological applications, material surfaces often need to contact complex media, which contain many kinds of proteins at high concentrations, rather than single proteins. Thus, it is important to evaluate the resistance of an antifouling material to the adsorption from complex media.

MATERIALS AND METHODS

Materials

N-(3-sulfopropyl)-*N*-(methacryloxyethyl)-*N*,*N*-dimethylammonium betaine (SBMA), BMA, azobisisobutyronitrile, 1-dodecanethiol, hydrogen peroxide, *o*-phenylenediamine, fluorescein diacetate, tetrahydrofuran (THF, 99%), acetone (99.5%), methanol (99.8%), and ether (99%) were all obtained from Sigma-Aldrich (Milwaukee, WI). Phosphate-buffered saline (PBS, pH 7.4, 10 m*M*, 138 m*M* NaCl, 2.7 m*M* KCl) and phosphate-citrate buffer (pH 5.0) were purchased from Sigma-Aldrich. Water used in the experiments, filtered by a Millipore system, had a minimum resistivity of 18.0 MΩ cm.

Human plasma Fg was purchased from EMD Biosciences (San Diego, CA). Bovine serum albumin (BSA) was purchased from Sigma-Aldrich. Horseradish peroxidase (HRP)-conjugated polyclonal goat anti-human Fg was obtained from US Biological (Swampscott, MA). Pooled human blood serum and plasma (with citrate-phosphate-dextrose anticoagulant) were purchased from BioChemed Services (Winchester, VA). Bovine aortic endothelial cells were provided by Prof. Shaoyi Jiang at the University of Washington. Dulbecco's modified Eagle medium was purchased from Thermo Scientific (Waltham, MA). Other cell culture reagents were acquired from Invitrogen (Grand Island, NY).

Synthesis of PSB

The PSB copolymers were synthesized by free-radical polymerization. Methanol (200 mL) was first purged with N_2 for 1 h. SBMA (0.04 mol) and BMA (0.16 mol) monomers were then dissolved in it, followed by the addition of 40 mg free-radical initiator azobisisobutyronitrile and 200 μL 1-dodecanethiol. The reaction mixture was purged for 1 h and heated to 60°C. The polymerization was allowed to proceed for 24 h. Solvent was then removed. The copolymer was precipitated in diethyl ether and washed with deionized water, and finally recrystallized into powder. The copolymers with different compositions were prepared similarly, using varying SBMA/BMA molar ratios. Molecular weight of polyBMA was determined by gel permeation chromatography in THF eluent with a refractive index detector, using a calibration curve of polystyrene (PS) standards.

Analysis of Polymer Composition

Composition of the PSB polymers was determined by ^{1}H 500-MHz Fourier transform nuclear magnetic resonance (NMR) spectroscopy in deuterated chloroform. The peak at 3.9 ppm is associated with two protons adjacent to the oxygen atom in the BMA pendant group, whereas the peak at 3.3 ppm arises from six protons in two methyl groups attached to the quaternary amine in the SBMA pendant group. Relative areas of two peaks were used to determine the molar percentage of each repeat unit (i.e., SBMA or BMA) in the copolymers.

Preparation of Polymer Films

The PSB copolymers with different SBMA fractions (PSB0, PSB5, PSB10, and PSB20) were dissolved at 5 wt % in the mixed solvent of THF and methanol (1 : 1). The polymer solutions were then poured into Teflon dishes. Solvent was allowed to evaporate at room temperature to form thin PSB films.

Protein Adsorption by Enzyme-Linked Immunosorbent Assay

A standard enzyme-linked immunosorbent assay (ELISA) was used to evaluate protein adsorption on PSB copolymer films, using Fg as the model protein.[17,19,25] The polymer films were first equilibrated in PBS overnight. Two milliliters of Fg solution (1 mg/mL in PBS) was added onto each film and incubated at 37°C for 90 min. Then, the surfaces were rinsed five times with PBS and incubated with BSA solution (1 mg/mL in PBS) for 90 min at 37°C to block the surface sites unoccupied by Fg molecules to inhibit the nonspecific adsorption of antibody molecules later. The films were washed with PBS five times again, and incubated with 2 mL HRP-conjugated anti-Fg solution (10 μg/mL in PBS) for 30 min at 37°C. The substrates were then rinsed with PBS, followed by the addition of 2 mL of 0.1*M* phosphate-citrate buffer (pH 5.0) containing 1 mg/mL *o*-phenylenediamine chromogen and 0.03% hydrogen peroxide. After incubation for 20 min at 37°C, the enzyme-induced color reaction was stopped by adding 2 mL of 1*M* H_2SO_4 to each solution. Finally, light absorbance of the solutions at 490 nm was determined by a microplate reader. The light absorbance from tests on PS Petri dish surfaces was set to 100% for calculating the relative protein adsorption values.

Modification and Characterization of SPR Chips

Resistance of PSB copolymers (PSB0, PSB5, PSB10, and PSB20) to protein adsorption was also thoroughly evaluated by an SPR biosensor. SPR chips were prepared by depositing 2-nm chromium and 48-nm gold (surface plasmon-active) onto glass substrates by e-beam evaporation under vacuum. Before coating the polymer films, SPR chips were cleaned by acetone, ethanol,

and water sequentially, treated under UV/ozone for 20 min, washed again with water and ethanol, and air dried. PSB polymers with different SBMA fractions were dissolved in 1 : 1 THF/methanol to make 1 wt % solutions. The clean SPR chips were then immersed into polymer solutions and incubated overnight at room temperature. Chips were rinsed with water and dried before use.

Film thickness of the polymer layer was evaluated by an α-SE ellipsometer (J. A. Woollam Co., Lincoln, NE) equipped with a 632.8-nm He–Ne laser at incidence angles of 65°–75°. A refractive index of 1.45 was assigned to the polymer layer.

Static, advancing, and receding contact angles of water on the PSB-coated surfaces were measured by sessile drop technique using a goniometer (model 100-00; Rame-Hart Inc., Mountain Lakes, NJ) under ambient conditions. Multiple readings from randomly chosen spots on the samples were taken and averaged. One-touch video capture was used to record the drop shapes, and ImageJ was used to measure the contact angles.

Protein Adsorption by SPR

Protein adsorption on PSB films was evaluated using a four-channel SPR sensor (Plasmon-IV, Institute of Photonics and Electronics, Academy of Sciences, Czech Republic), which measures change in the resonant wavelength at a fixed light incident angle.

The PSB-coated SPR chip was attached to the base of a prism. Optical contact between two surfaces was realized using a refractive index matching oil (Cargille). A pre-adsorptive baseline was first established by flowing the PBS buffer over the chip surface through the sensor for 10 min. Fg solution (1 mg/mL in PBS), 100% human blood serum, or 100% human blood plasma was then flowed through different channels for 10 min, and buffer was then flowed for 10 min to remove the unbound protein molecules and to re-establish the postadsorptive baseline. Flow rate was 0.05 mL/min throughout the experiments. The bare SPR chip without any polymer coating was used as control for comparison. Protein adsorption was finally quantified by measuring wavelength change between the preadsorptive and postadsorptive baselines and converting it to the amount of the adsorbed protein. A 1-nm SPR wavelength shift at 750 nm corresponds to a surface coverage of 15 ng/cm^2.[28]

Cell Attachment

Bovine aortic endothelial cells were maintained in continuous growth in a humidified incubator with 5% CO_2 at 37°C. The culture medium was Dulbecco's modified Eagle medium with 10% fetal bovine serum, 1% sodium pyruvate, 1% nonessential amino acids, and 2% penicillin–streptomycin. Cells were passaged once a week and used only before passage 15.

Polymer films were peeled off from the Teflon plate, cut into 2 × 2 cm^2 pieces, equilibrated in sterile PBS overnight, and sterilized by exposure to UV light for 30 min before cell seeding. To prepare the cell suspension, cells were detached from the flask wall by trypsin/ethylenediaminetetraacetic acid (0.05%/0.53 m*M*), washed with PBS, and dispersed in culture medium to reach a concentration of 10^5 cells/mL. Four milliliters of cell suspension was then added onto each sample and incubated for

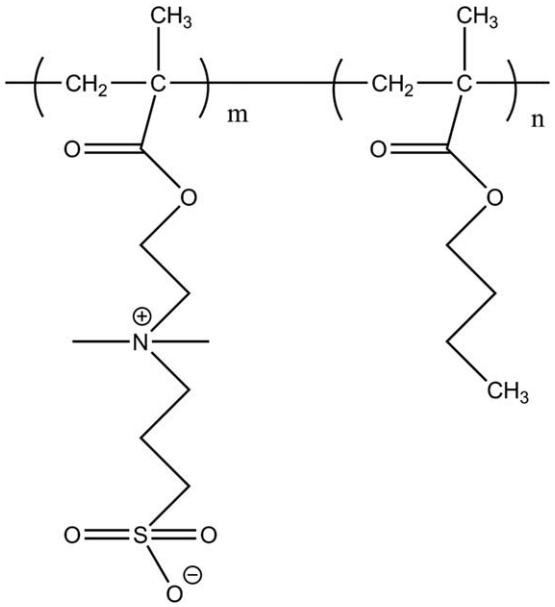

Figure 1. Chemical structure of PSB.

7 days, with the culture medium refreshed on the fourth day. After the incubation, polymer films were transferred into a new six-well plate containing 4 mL sterile PBS in each well. Fluorescein diacetate solution (10 mg/mL in acetone, 20 μL) was then added to each well and incubated for 5 min under opaque conditions. Culture surfaces were finally imaged using a fluorescence microscope (Olympus IX70) at ×10.

RESULTS AND DISCUSSION

Synthesis of PSB copolymers (Figure 1) was carried out by simple free-radical polymerization at 60°C. The feed molar ratio of the zwitterionic SBMA in copolymers ranges from 0% to 20%. Molecular weight of the homopolymer polyBMA was determined by gel permeation chromatography, which shows M_n of 46,000, M_w of 56,000, and polydispersity of 1.22. Chemical composition of the copolymers was verified by ^{1}H NMR. Figure 2 shows the NMR spectra of PSB0 and PSB20 at a range of 0.5–5 ppm. Comparing with the PSB0 spectrum, a peak at 3.3 ppm appeared in the spectrum of PSB20 owing to the incorporation of SBMA, whereas the peak at 3.9 ppm representative of BMA decreased. Relative areas of the two peaks were used to determine the molar percentage of SBMA and BMA in each polymer. The polymer compositions determined by NMR are presented in Table I. We also observed very small peaks at 5.5 and 6.1 ppm (data not shown), which can be attributed to the alkenyl protons from the residual unreacted monomers. Because of the high viscosity of polymers during synthesis, the unreacted monomers could be encapsulated in the polymer samples. Therefore, it was difficult to completely remove the unreacted monomers from the polymers by precipitation in diethyl ether once. Nevertheless, based on the peak areas, the amount of residual monomers was very small compared with the amount of polymers; and it should not affect our other results. The

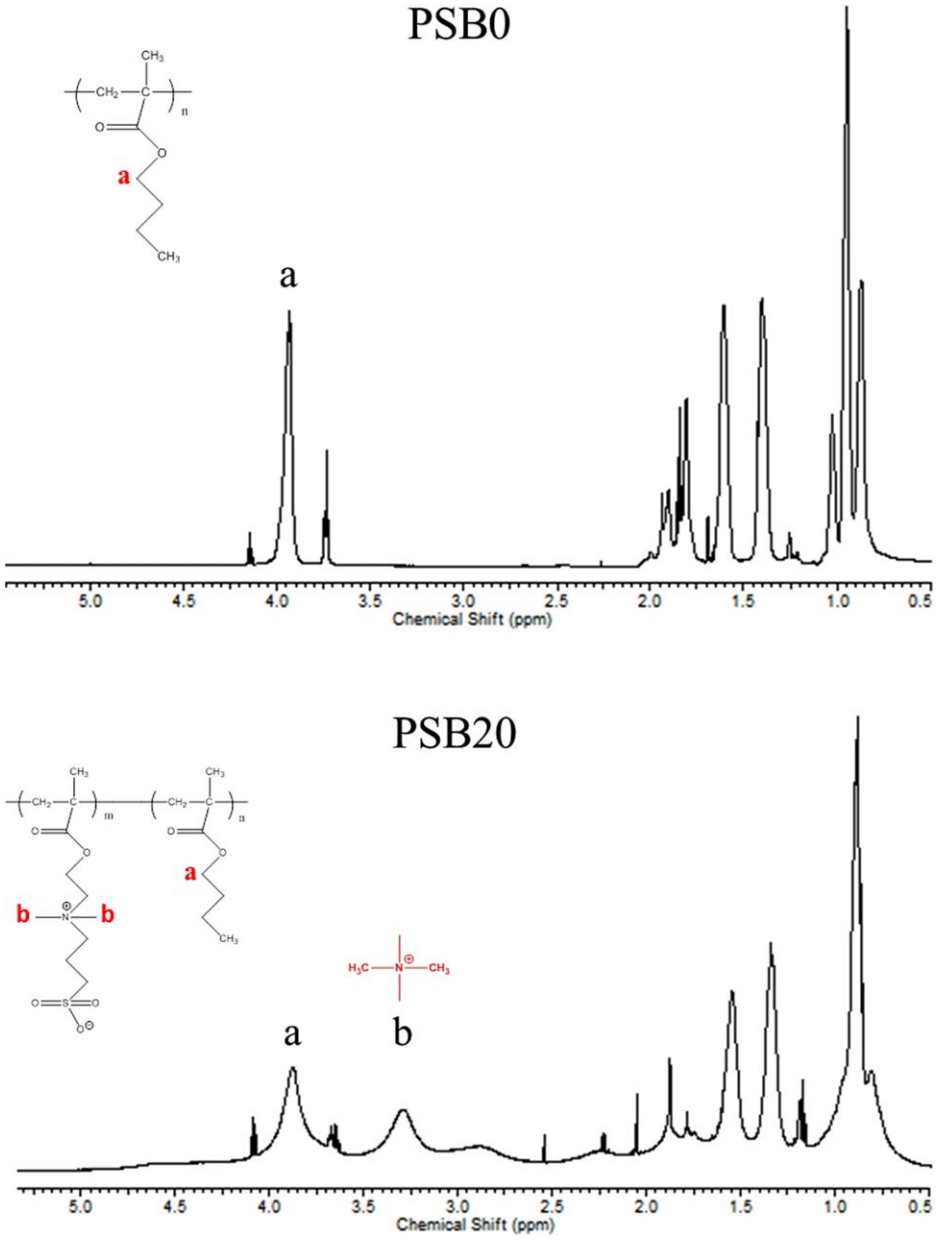

Figure 2. ^{1}H NMR spectra of PSB0 and PSB20. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

unreacted monomers can be completely removed after re-precipitation in diethyl ether two more times.

Fg is an important blood plasma protein involved in platelet adhesion, blood clot formation, and inflammatory response, and has been widely used to evaluate antifouling properties of many materials.[4] Therefore Fg was chosen to be a model protein in this work to study protein adsorption on different PSB films by ELISA. It is shown in Figure 3 that comparable amount of Fg adhered to the PS positive control and PSB0 (i.e., poly-BMA without SBMA) surfaces. The amount of Fg adsorbed on

Table I. Polymer Composition and Corresponding Static Water Contact Angle

Polymer abbreviation	Monomer feed ratio (mol % SBMA/BMA)	Polymer composition (mol % SBMA/BMA)	Contact angle (degree)
PSB0	0/100	0/100 ± 0/0	95 ± 4
PSB5	5/95	4/96 ± 0/0	79 ± 3
PSB10	10/90	10/90 ± 1/1	68 ± 3
PSB20	20/80	23/77 ± 3/3	56 ± 5

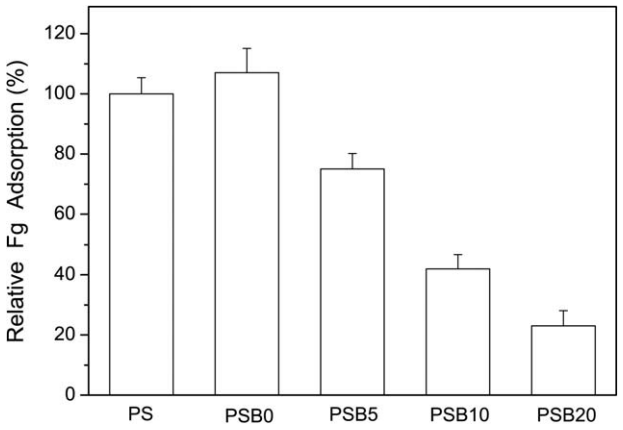

Figure 3. Relative Fg adsorption on PSB films with different SBMA fractions (PSB0, PSB5, PSB10, and PSB20) as determined by ELISA. The optical density at 490 nm from tests on PS surfaces was set to 100% for calculating the relative protein adsorption values. Error bars represent the standard deviation of the mean.

the surfaces decreased with the increasing SBMA fraction of the copolymers. If setting the Fg adsorption (OD_{490nm}) on the PS surface as 100%, Fg adsorption on the PSB20 surfaces was only about 23%. Results indicate that protein adsorption can be effectively suppressed by incorporating antifouling SBMA moieties into the copolymers.

The amount of protein adsorbed on PSB copolymers with different SBMA fractions was more accurately quantified by SPR. For the SPR measurements, PSB copolymers were first coated onto the SPR chips (gold-coated glass slides). Thickness of the coated films of PSB0, PSB5, PSB10, and PSB20 was found similar (35–45 nm, measured by ellipsometry). Protein adsorption on the PSB-coated and bare gold surfaces was then tested. Figure 4(a) shows the amount of the absorbed Fg. A great amount of Fg adhered on the gold surfaces and PSB films with low SBMA contents (PSB0 and PSB5), with adsorption of 349 ± 26, 330 ± 32, and 273 ± 29 ng/cm^2, respectively, indicating that with low SBMA contents the copolymers have limited protein resistance because of insufficient surface hydration. As the SBMA content of PSB copolymers increased, protein adsorption was much more effectively suppressed. The Fg adsorption on PSB10 and PSB20 films was only 26 ± 5 and 4 ± 2 ng/cm^2, respectively. Compared with PSB0, PSB20 (with ~20 mol % of SBMA) led to 99% reduction of Fg adsorption. Surfaces with Fg adsorption below 5 ng/cm^2 have been referred to as ultralow fouling surfaces[2,5]; the PSB20 surface is thus ultralow fouling.

Comparison between Figures 3 and 4(a) indicates that the relative Fg adsorption measured on the same set of polymer surfaces using ELISA is mostly higher than those measured using SPR. This may be attributed to two possible reasons: nonlinear dependence of optical density (OD) on the amount of adsorbed Fg in ELISA, and possible entrapment of proteins inside the thicker ELISA samples. ELISA is a labeled technique. In ELISA, the adsorbed proteins on a surface were first detected by antibodies that are labeled with enzyme (i.e., HRP); the enzyme was then reacted with its substrate to develop a colourful solution; OD of the solution was finally measured to reflect the

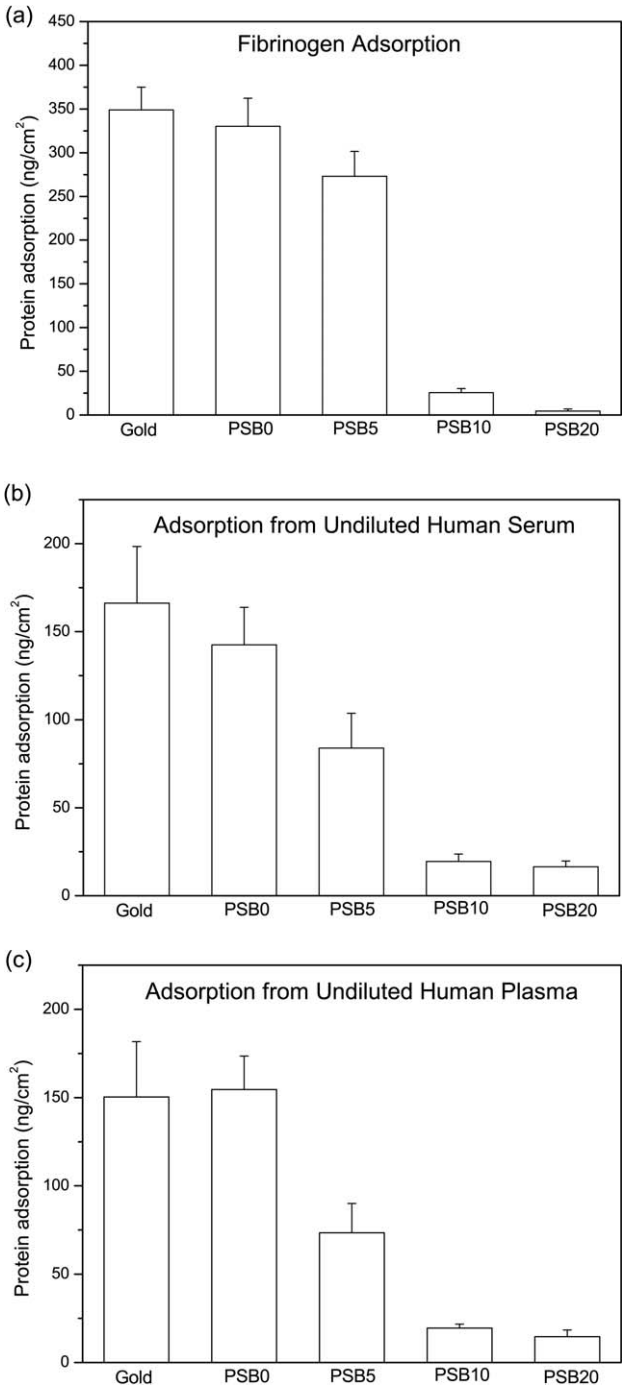

Figure 4. Adsorption of (a) 1 mg/mL Fg solution, (b) undiluted human serum, and (c) undiluted human plasma on PSB polymer films with different SBMA fractions (PSB0, PSB5, PSB10, and PSB20) and gold control surfaces, as measured by SPR. Error bars represent the standard deviation of the mean.

quantity of the initially adsorbed proteins. Note that the measured OD value is not necessarily in linear correlation with the amount of the adsorbed proteins. For the purpose of quantifying the amount of proteins adsorbed on a surface, ELISA only provides an OD value rather than an absolute adsorption mass density. On the other hand, SPR is a nonlabeled and very sensitive technique, providing direct measurement of how much

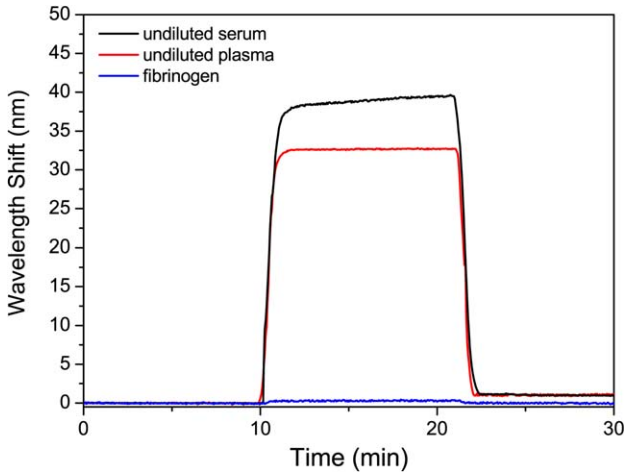

Figure 5. Typical SPR sensorgrams showing very low adsorption from Fg, undiluted human serum, and undiluted human plasma on PSB20 films. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

proteins adsorb on a surface (e.g., in ng/cm^2). Therefore, SPR results are more quantitative and accurate. Second, polymer films used for our ELISA test, prepared by solvent cast, were much thicker than the polymer coatings on SPR chips (30–45 nm). Some proteins may be trapped inside the ELISA samples rather than on sample surfaces, leading to higher OD.

Our results are consistent with others' work. For example, a random copolymer of SBMA, hexyl methacrylate, and methyl methacrylate (15/70/15) showed excellent resistance to protein adsorption using fluorescence-labeled Fg.[29] Ishihara and co-workers[24,25]reported random copolymers of zwitterionic MPC and BMA, showing that the increase in MPC molar fraction significantly reduced the adsorption amount of Fg, BSA, and fibronectin.

Surfaces of biomedical materials often need to contact complex media. Therefore, it is of great importance to determine whether a new material intended for antifouling applications can suppress nonspecific adsorption from complex media. Notice that compared with the single proteins, materials' resistance to complex media is much more challenging, owing to the high protein concentration and the existence of many kinds of proteins in complex media.

Figure 4(b,c) shows the resistance of PSB copolymers with different SBMA fractions to adsorptions from the undiluted human blood serum and plasma. The adsorption amount decreases significantly with the increasing SBMA content in the copolymers, similar to the trend observed for the Fg resistance. The lowest adsorption levels achieved are 17 ± 3 and 15 ± 4 ng/cm^2 from serum and plasma, respectively, on PSB20 films, which represent 90% reduction of adsorption, compared with the PSB0 films with no SBMA. Figure 5 provides typical SPR

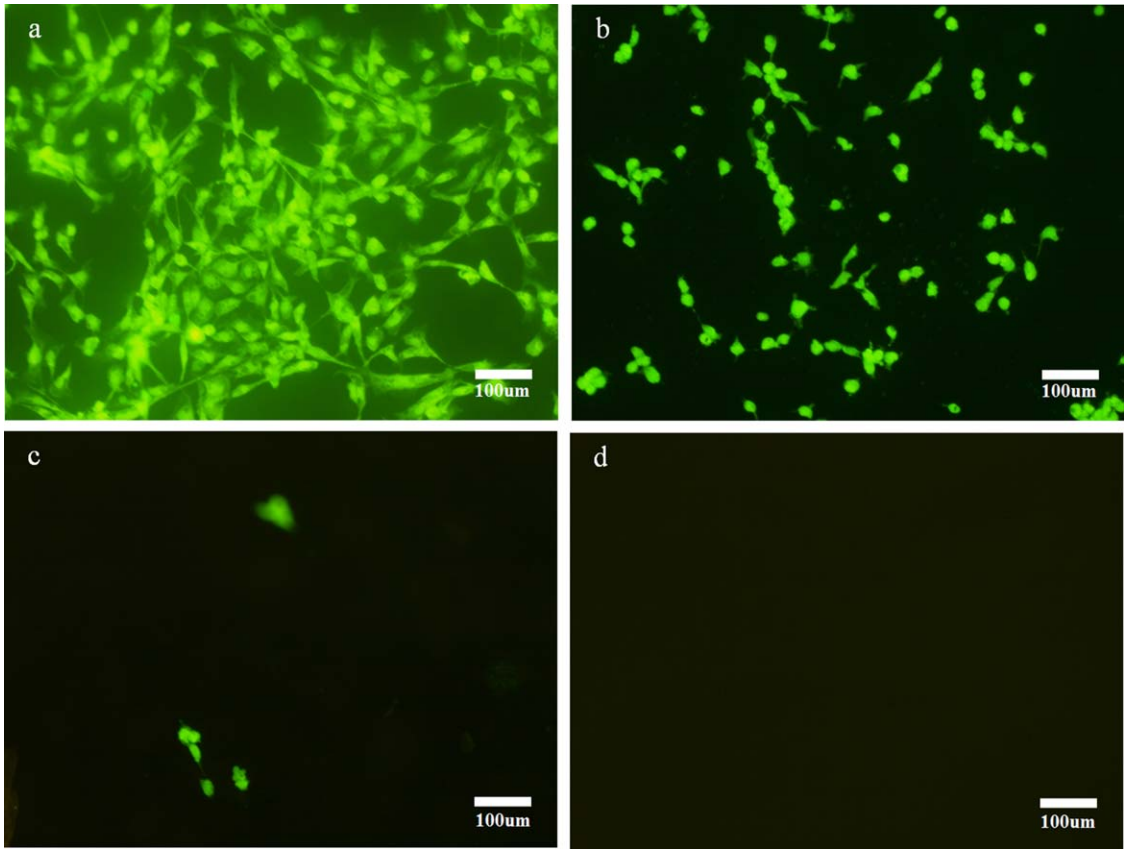

Figure 6. Fluorescence microscopic images of bovine aortic endothelial cells attached after 1-week culturing on (a) PSB0, (b) PSB5, (c) PSB10, and (d) PSB20. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

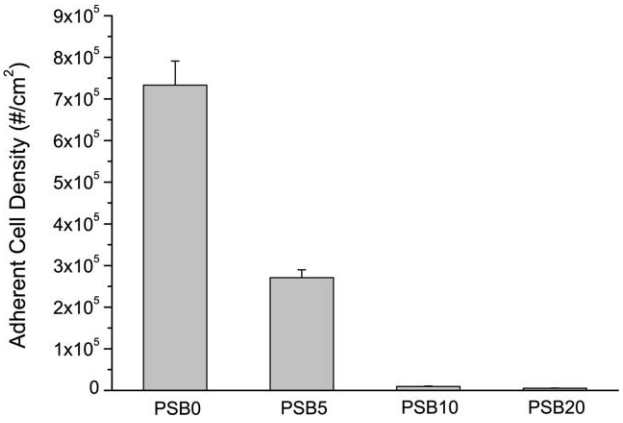

Figure 7. Density of bovine aortic endothelial cells adhered on PSB surfaces.

curves for PSB20 films, showing very low adsorption from Fg, serum, and plasma. Notice that the sharp increase in wavelength at ~10th min for the serum and plasma curves was caused by the change in refractive index when the solution was switched from PBS buffer to complex media. The curves dropped back to the baseline level at ~21st min when the serum or plasma solution was changed back to the PBS buffer. The nonspecific adsorption on surfaces was only related to the difference between the postadsorptive and preadsorptive baselines. Our results indicate that the PSB copolymer with 20 mol % SBMA can not only resist single protein adsorption, but also effectively prevent nonspecific adsorption from complex media.

Ishihara et al.[26] reported that PMB, with 30 mol % MPC, reduced the relative amounts of individual proteins (e.g., Fg, albumin, IgG, and fibronectin) from human plasma by 40%–50% compared with polyBMA (i.e., PSB0) or glass, as determined by a radioimmunoassay. Because of different sample preparation procedures and protein adsorption measurement methods used, it is hard to directly compare the antifouling efficiency of various polymers. Nevertheless, our data clearly show that the PSB, with 20 mol % SBMA, can reduce the total protein adsorption from serum or plasma by 90%, down to a level of <20 ng/cm^2.

It has been reported that the minimal adsorption on poly(SBMA) brushes, grafted on gold by ATRP, were 6.0 and 6.1 ng/cm^2 from serum and plasma, respectively.[16] Higher adsorption on the PSB copolymers can be mainly attributed to the lower density of the zwitterionic antifouling SB groups in copolymers, as compared with the highly packed polymer brushes via ATRP. Nevertheless, PSB20 still represents an excellent antifouling material in resisting nonspecific adsorption from plasma and serum, with levels comparable with the conventional PEG- or oligo(ethylene glycol)-based materials.[30,31]

The PSB copolymer films containing various SBMA contents were next incubated with endothelial cells in culture medium supplemented with 10% fetal bovine serum. Because the SBMA moiety suppressed protein adsorption effectively, we expected that the copolymers with high SBMA fractions could also prevent cell attachment. Figure 6 shows the fluorescence images of cells on the PSB films after 1 week incubation, and Figure 7 provides the quantitative data of the adherent cell density (i.e., number per area). A large number of cells adhered to the PSB0 surface. With the incorporation of 5 mol % SBMA moiety, the number of cells on the PSB5 surface already decreased markedly. Only a few cells were observed on PSB10, and almost no cells attached on PSB20. The cell adhesion results convincingly corroborate the protein adsorption data. Attachment of anchorage-dependent cells on a surface is caused by the interaction of cell membrane integrins and adhesive proteins on the surface. Because the PSB10 and PSB20 surfaces prevent protein adsorption effectively, they also resist cell attachment.

Surface hydration is generally considered to be the key factor for antifouling properties.[2] To better understand the antifouling mechanisms of the PSB polymers, contact angles of water on different polymer films were determined. Table I shows the static water contact angle of each PSB polymer. It is obvious that the water contact angles on PSB surfaces decrease with the increasing SBMA fraction. Notice that even for the PSB20 surfaces, the water contact angle is still relatively high (~56°). The contact angle was measured in air; so the hydrophobic BMA chains tend to stay outward in the air environment, leading to high contact angle. If in an aqueous environment, the hydrophilic SBMA pendant groups are expected to reorient outward and generate desirable antifouling properties. Under-water surface reconstruction of polymers with both hydrophobic and hydrophilic domains (regardless of random copolymer, block copolymers, or homopolymers) has been previously demonstrated by near-edge X-ray absorption fine structure and sum frequency generation spectroscopy, which provide strong evidences on the migration of hydrophobic domains away from the surface after immersion in water and the tendency of hydrophilic domains present at the polymer–water interface.[32,33]

Surface reconstruction of PSB copolymers after immersion in water is inferred from dynamic water contact angle measurements. As shown in Table II, both the advancing contact angle (θ_{adv}) and the receding contact angle (θ_{rec}) of water decrease with the increasing SBMA ratio. The difference in θ_{adv} and θ_{rec} (i.e., hysteresis) show an increasing trend with the SBMA

Table II. Advancing and Receding Contact Angles of Water on PSB Surfaces

Polymer abbreviation	Advancing angle, θ_{adv} (degree)	Receding angle, θ_{rec} (degree)	Hysteresis, $\theta_{adv} - \theta_{rec}$ (degree)
PSB0	105 ± 4	79 ± 5	26
PSB5	89 ± 2	44 ± 4	40
PSB10	79 ± 5	28 ± 5	51
PSB20	62 ± 5	10 ± 3	52

fraction in copolymers, with the largest hysteresis for PSB20. Contact angle hysteresis is caused by factors such as surface roughness, surface reconstruction of the polymer after liquid contact, and chemical heterogeneity of the top layer.[34] The surface roughness of different PSB films should be similar. Therefore, the observed large hysteresis for PSB10 and PSB20 is most likely due to higher degree of under-water surface rearrangement and chemical heterogeneity (a combination of polar SBMA and nonpolar BMA in copolymers).

PSB10 and PSB20 also show very low receding water contact angles of 28° and 10°, respectively. For the surfaces of copolymers consisting of both polar and nonpolar groups, the advancing water contact angle is determined primarily by the nonpolar component, whereas the receding water contact angle is sensitive to the polar groups at the surface.[34] As described above, PSB10 and PSB20 show excellent resistance to protein adsorption and cell attachment. Therefore, the antifouling properties of the PSB polymers show strong correlation with their receding water contact angles, which are representative of the polymer surface compositions in aqueous environment. Our results are consistent with the dynamic contact angle behavior of the PMB copolymers[35] and a previous report showing that it is the receding water contact angle, not the advancing or static water contact angle, representing the polar groups in the near-surface region of the coating.[34]

CONCLUSIONS

Random copolymers of zwitterionic SBMA and hydrophobic BMA, with different SBMA/BMA molar ratios, were synthesized by a very simple free-radical polymerization method. Although the hydrophobic moiety is incorporated, the PSB copolymers with more than 10 mol % SBMA retained excellent antifouling properties. Both ELISA and SPR show that the protein adsorption decreased with the increasing content of SBMA in the copolymers. The PSB20, with 20 mol % SBMA, not only exhibited robust resistance to single protein adsorption (<5 ng/cm^2 Fg) but also prevented adsorption from undiluted human blood serum and plasma. PSB20 film also completely resisted endothelial cell adhesion. Considering their excellent antifouling properties, along with the extremely simple synthesis method and low cost, PSB copolymers hold great potential for biomedical applications, in the form of both solvent-cast film and surface coating.

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