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Development of Biocompatible Interpenetrating Polymer Networks Containing a Sulfobetaine-Based Polymer and a Segmented Polyurethane for Protein Resistance

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Interpenetrating polymer networks (IPNs) were prepared by the modification of a segmented polyurethane (SPU) with a cross-linked sulfobetaine methacrylate (SBMA) polymer. The IPN films that were prepared can effectively resist nonspecific protein adsorption when the distribution of SBMA units within the SPU film is well controlled, and they retain high mechanical strengths inherent from the base SPU films. Furthermore, the zwitterionic and biomimetic nature of sulfobetaine and the ease of SBMA preparation make SBMA-based materials very attractive for a wide range of applications. It is challenging to control the diffusion of highly polar SBMA into the hydrophobic network of SPU. In this study, various parameters governing the formation of IPNs containing SBMA were studied. The chemical composition depth profile of the IPN films was determined by confocal Raman microscopy. The morphology and thickness of these IPN films were examined by atomic force microscopy and scanning electron microscopy. The amount of adsorbed proteins on the IPN films was determined by an enzyme-linked immunosorbent assay. Results show that the amount of adsorbed proteins on the IPN films depends on the incubation conditions, including solvent polarity, incubation time, SBMA monomer ratio, and incubation concentration. It appears that the IPN films prepared in a mixed solvent of higher polarity with long incubation time lead to very low protein adsorption. This study not only introduces a new IPN system containing SBMA, but also provides a fundamental understanding of various parameters governing the formation of IPNs.

Introduction

Surface resistance to nonspecific protein adsorption is important for many applications, such as implanted biomaterials, biomedical diagnostics, drug delivery, and coatings for ship hulls.^{1–8} For antithrombogenic implants, three types of materials, including heparin,^{9–10} poly(ethylene glycol) (PEG),^{11–12} and phospholipids,^{13–15} have been employed for surface modification. Biomaterial surfaces modified with heparin molecules by a simple dipping method have become a major coating technique commercially to prevent the thrombin activation of existing materials. However, this approach lacks long-term stability for implantation. PEG-based materials are commonly used to effectively resist nonspecific protein adsorption. However, PEG is a polyether that autoxidizes relatively rapidly, especially in the presence of oxygen and transition metal ions found in most biochemically relevant solutions.¹⁶ On the outer surface of a cell, zwitterionic lipid phosphorylcholine (PC) is a major component and is known to be non-thrombogenic.¹³ Methacryloyloxyethyl phosphorylcholine (MPC)-based copolymers have become one of the major synthetic biocompatible materials.¹⁷

Segmented polyurethane (SPU) is one of the widely used biomaterials, especially in cardiovascular devices, due to its excellent mechanical properties. A series of studies have been

reported to improve its biocompatibility with MPC-based polymers via surface grafting, polymer blending, or interpenetrating polymer networks (IPNs).^{5,18–21} Recently, we demonstrated that the surfaces covered with zwitterionic poly(sulfobetaine methacrylate) [poly(SBMA)] highly resist nonspecific protein adsorption when its surface packing is well controlled.^{7–8, 22–25} In these studies, poly(SBMA) is grafted from a surface via the atom transfer radical polymerization (ATRP) method²⁵ or poly(SBMA) is grafted onto a surface via the physical adsorption of a diblock copolymer containing poly(SBMA).⁸ Results show that these surfaces have 0.3 and 3 ng/cm² of fibrinogen adsorption for these two approaches, respectively. The structure of sulfobetaine is similar to that of taurine betaine, which plays an important role in numerous physiological functions.²⁶ In addition, as compared to MPC, SBMA is easier to synthesize and handle. Thus, the zwitterionic and biomimetic nature of sulfobetaine and ease in SBMA preparation make SBMA-based materials very attractive for a wide range of applications.

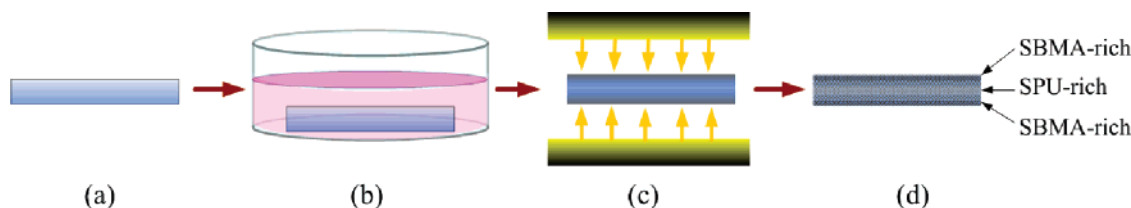
In this work, IPN films containing poly(SBMA) that effectively resist nonspecific protein adsorption and have high mechanical strengths were prepared by the modification of a segmented SPU with a cross-linked SBMA polymer. The previous work lacks a systematic study on the interpenetration of highly polar components into hydrophobic polymer networks.²⁷ It is challenging to control the diffusion of a polar component (MPC or SBMA) into the hydrophobic network of SPU. SBMA-based IPNs can be even more challenging since SBMA is soluble only in highly polar solvents. Although MPC-based IPNs have been studied before, the parameters governing the formation of IPNs are still not well understood. In this work,

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Scheme 1. Illustration of the Preparation Process of an IPN Film^a

^a (a) SPU film prepared from solvent evaporation in DMAc at 20 °C; (b) SPU film incubated in a methanol solution containing SBMA monomer, HEMA monomer, GDGDA cross-linker, and photoinitiators at 20 °C; (c) photopolymerization with visible light; and (d) IPNs of SPU/poly(SBMA).

the effects of incubation conditions, including solvent polarity, incubation time, SBMA monomer ratio, and incubation concentration, on the protein-resistant properties of the IPN films containing SBMA were studied. Results are compared with those of SBMA and 2-hydroxyethyl methacrylate (HEMA) hydrogels. This study not only introduces a new IPN system containing SBMA, but also provides a fundamental understanding of various parameters governing the formation of IPNs in general.

Materials and Methods

Chemicals. [2-(Methacryloyloxy)ethyl]dimethyl(3-sulfopropyl)ammonium hydroxide (sulfobetaine methacrylate, SBMA), 2-ethylhexyl methacrylate (EHMA), and glycerol 1,3-diglycerolate diacrylate (GDGDA) were purchased from Aldrich and used as received. SPU (Tecoflex 60) was obtained from Thermedics and purified by reprecipitation. The SPU is based on an aromatic polyetherurethaneurea with a soft segment of polytetramethyleneoxide and a hard segment of diphenylmethane diisocyanate and mixed diamines.²¹ Camphorquinone and ethyl 4-(*N,N*-dimethylamino) benzoate were also purchased from Aldrich. Phosphate buffer saline (PBS) was purchased from Sigma.

Preparation and Characterization of IPN Films Containing SPU and Poly(SBMA). As shown in Scheme 1, the SPU film of 100 μm thickness was prepared via the solvent evaporation method. A SPU solution was first prepared from 5.0 wt % SPU powder dissolved in dimethylacetamide (DMAc). After the solution was cast onto the glass slide, the slide was heated to 35 °C to dry the film. After the bulk of the solvent evaporated overnight, the SPU film was placed into a water bath at 60 °C for 24 h to remove traced DMAc and was then dried in a vacuum oven for 3 days. The SPU film was then immersed in an incubation solution containing SBMA monomer, EHMA monomer, GDGDA crosslinker, and photoinitiators for 24 h at 20 °C. Solvent polarity was varied by using a mixed solvent containing water, methanol, ethanol, or isopropanol with decreasing polarity in the incubation solution.

The total concentration of the incubation solution (or incubation concentration) was adjusted from 0.1 to 3.0 mol/L. The SBMA monomer ratio (mol %) is defined as the mole of SBMA monomer divided by the total moles of SBMA and EHMA monomers in the incubation solution. In this work, SBMA monomer ratio was adjusted between 0 and 100 mol % to optimize the preparation conditions for the IPNs while GDGDA was fixed at 1.0×10^{-2} mol/L for the case of incubation concentration at 1.0 mol/L (1 mol % incubation concentration). To eliminate side reactions, photoinitiators [i.e., camphorquinone and ethyl 4-(*N,N*-dimethylamino) benzoate] at 1.0×10^{-2} mol/L were added into the incubation solution in the presence of nitrogen in the dark. For photopolymerization, the SPU film placed between two mica sheets was irradiated with visible light ($\lambda = 400\text{--}500$ nm). After irradiation for 120 min at 20 °C, mica sheets were removed from the IPN films in water, unreacted monomers were extracted by soaking in ethanol, methanol, and water alternatively for several times, and the IPN film was dried in a vacuum oven. The chemical composition depth profile of the IPN film was characterized using a Raman microspectrometer, which combines a Renishaw in Via Raman spectroscopy and an inverted Leica DMIRBE microscope. The 785 nm laser was used

as an excitation source and was focused through a 40 \times objective to a ~ 1 μm light spot on the sample surface. Scattered light from the sample surface was collected through the same objective. Raleigh scattering light was cut off by a holographic notch filter. Raman light was passed through an entrance slit with a 65 μm opening and a 1200 l/mm diffraction grating and measured by a CCD camera. For the distribution depth profile of SBMA units within the SPU film, Raman spectra were acquired from the focal planes at the surface of the film and into the film with a 20 μm increment. The morphology and thickness of these IPN films were examined by atomic force microscopy (AFM) and scanning electron microscopy (SEM; FEI Sirion). All AFM images were acquired with a Digital Instruments (DI) multimode nanoscope IV (Santa Barbara, CA). The instrument is equipped with an E scanner (DI) and operated in air. For tapping-mode AFM, a commercial Si cantilever (TESP tip) of ~ 300 kHz resonant frequency from DI was used. The relative humidity was less than 40%.

Evaluation of Protein Adsorption Using the Enzyme-Linked Immunosorbent Assay (ELISA). The adsorption of human fibrinogen (Fg, purchased from Aldrich) onto the IPN films was evaluated using ELISA according to the standard protocol as described briefly below. First, the IPN films with 12 mm² of surface area were placed in individual wells of a 24-well tissue culture plate, and each well was incubated with 500 μL of PBS at room temperature. Then, the IPN films were soaked in 500 μL of 1 mg/mL Fg in PBS solution. After 90 min of incubation at 37 °C, the films were rinsed 5 times with 500 μL of PBS and then incubated in bovine serum albumin (BSA, purchased from Aldrich) for 90 min at 37 °C to block the areas unoccupied by Fg. The IPN films were rinsed with PBS 5 times again, transferred to a new plate, and incubated in a 500 μL PBS solution containing 5.5 $\mu\text{g/mL}$ horseradish peroxidase (HRP) conjugated anti-Fg (USbiological) for 30 min at 37 °C. The samples were rinsed 5 times with 500 μL of PBS and transferred into clean wells, followed by the addition of 500 μL of 0.1 M citrate-phosphate buffer (pH 5.0) containing 1 mg/mL chromogen of *o*-phenylenediamine dihydrochloride (OPD) and 0.03% hydrogen peroxide. After incubation for 20 min at 37 °C, the enzyme-induced color reaction was stopped by adding 500 μL of 1 M H₂SO₄ to the solution in each well, and finally the absorbance of light at 490 nm was determined by a microplate reader. Protein adsorption on the IPN samples was normalized with respect to that on the polystyrene (PS) plate as a reference. It should be noted that the amount of adsorbed proteins obtained could be higher than the actual amount due to the presence of multiple binding sites for Fg on the polyclonal anti-human Fg that was used.

Results and Discussion

In this work, IPNs were prepared by the modification of a segmented SPU with a cross-linked SBMA polymer. SPU was used as the matrix component to reinforce the mechanical strength of the IPN films while poly(SBMA) was used to reduce the nonspecific protein adsorption of the IPN films.^{8,25} Protein adsorption on IPN films was evaluated by ELISA. The objective of this work is to achieve very low protein adsorption on IPN films via controlling IPN preparation conditions, including solvent polarity, incubation time, SBMA monomer ratio, and

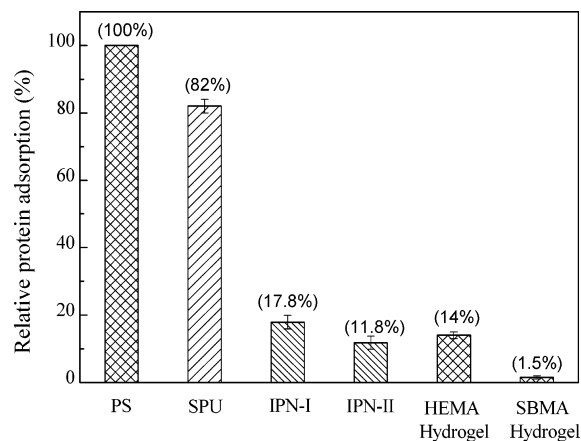


Figure 1. Relative human fibrinogen adsorption on various material surfaces determined from ELISA with PS as a reference. PS denotes polystyrene film; SPU, (unmodified) segmented polyurethane film; IPN-I, the IPN film prepared by incubating a SPU film in a methanol solution containing the SBMA monomer ratio of 70 mol %, the incubation concentration of 1.0 mol/L for 24 h at 20 °C; IPN-II, the IPN film prepared by incubating a SPU film in a solution containing the SBMA monomer ratio of 70 mol %, the incubation concentration of 2.0 mol/L and a mixed solvent of 95 vol % methanol and 5 vol % water for 24 h at 20 °C; HEMA, hydrogel pure 2-hydroxyethyl methacrylate hydrogel; and SBMA, hydrogel pure sulfobetaine methacrylate hydrogel. As a reference, the amount of adsorbed fibrinogen to PS is 336 ng/cm² determined from radiolabeled experiments.²⁸

incubation concentration. The process for IPN preparation can be generally divided into two stages. The first stage is associated with shorter incubation times. In this stage, the amount of poly-(SBMA) diffusing into the SPU matrix from the incubation solution is mainly controlled by the degree of SPU swelling. The second stage is associated with longer incubation times. In this stage, the amount of poly(SBMA) within a SPU matrix is determined by SBMA solubility within the SPU film. Thus, it is expected that solvent polarity plays a very important role in IPN preparation, and there is a tradeoff in solvent polarity. Incubation in less polar solvent will cause higher SPU swelling (or higher SBMA diffusion into the matrix or lower protein adsorption) initially, but will have lower SBMA solubility within the SPU film (or less SBMA-rich domains within the SPU matrix or higher protein adsorption) after long-time incubation. The incubation solution should be capable of both swelling hydrophobic SPU and dissolving hydrophilic ploy(SBMA) and an appropriate solvent polarity is needed to achieve the best performance of the IPN films.

Evaluation of Protein Adsorption on IPNs Prepared.

Protein adsorption on each IPN film prepared was evaluated by ELISA using PS as a reference. Relative protein adsorption for various samples with respect to that on PS is shown in Figure 1. As a reference, the amount of adsorbed fibrinogen on PS is ~336 ng/cm² from a fibrinogen solution of 0.03 mg/mL determined from radiolabeled experiments.²⁸ It can be seen from Figure 1 that protein adsorption on the IPN films are significantly reduced as compared with that on PS or the unmodified SPU film. The amount of adsorbed human Fg on the unmodified SPU film is 82% of that on PS. Protein adsorption on the IPN films is similar or even lower than on poly(HEMA) hydrogel while the IPN films have much better mechanical properties than poly(HEMA). The IPN film with the lowest protein adsorption was achieved by incubating a SPU film (Tecoflex 60) in a solution containing 95 vol % methanol and 5 vol % water for 24 h at 20 °C while the incubation solution contained the SBMA monomer ratio of 70 mol % and an incubation concentration of 2.0 mol/L. Poly(SBMA) hydrogel was also used

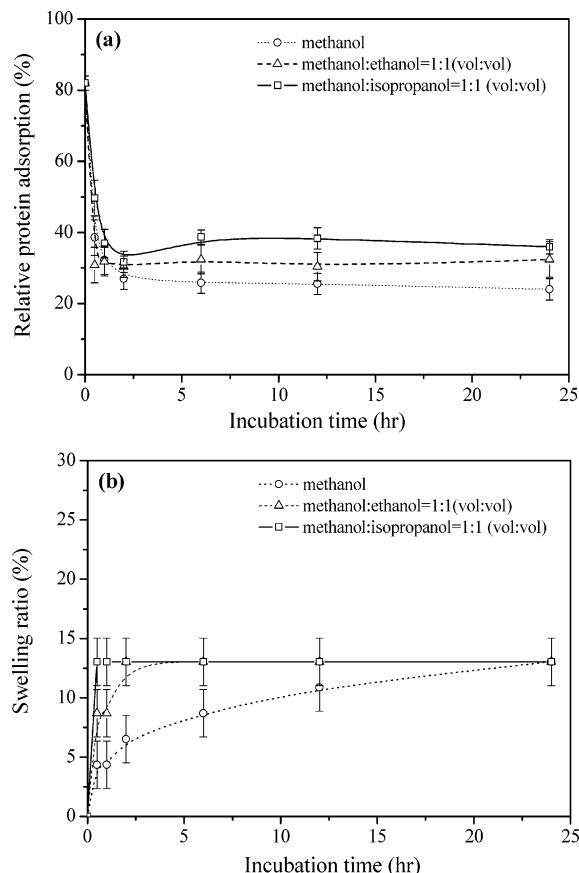


Figure 2. (a) Relative protein adsorption on IPNs versus incubation time for three solvents used—methanol (O), mixed ethanol/methanol of 1/1 volume ratio (Δ), and mixed isopropanol/methanol of 1/1 volume ratio (□) with the incubation concentration of 0.5 mol/L and the SBMA monomer ratio of 70 mol % at 20 °C; (b) the corresponding swelling ratio of the SPU film.

for comparison. It can be seen that relative protein adsorption on poly(SBMA) is only 1.5% of that on PS, indicating that poly-(SBMA) can highly resist nonspecific protein adsorption. These results show that IPNs containing poly(SBMA) and SPU are one of the excellent approaches for achieving low protein adsorption while maintaining mechanical strength.

Solvent Polarity. The resistance of IPNs to nonspecific protein adsorption strongly depends on the polarity of the solvent used. The IPN samples ability to resist nonspecific protein adsorption is determined by a balance between the degree of SPU swelling and the solubility of SBMA within the SPU film. For long incubation times, the resistance of IPNs to nonspecific protein adsorption is mainly determined by SBMA solubility within the SPU film. More polar solvent is preferred to prepare IPN samples with lower protein adsorption. After the SPU film is swelled over long incubation times by a more polar solvent containing highly polar SBMA, more SBMA can penetrate into the SPU film and form SBMA-rich domains within the SPU matrix. For short incubation times, reduction in protein adsorption on IPNs is mainly determined by the degree of SPU swelling. A less polar solvent will swell the SPU films more and allow more SBMA to diffuse into the film, leading to a reduction in protein adsorption initially. In this work, the effects of solvent polarity on the reduction in protein adsorption on prepared IPN films were studied mainly using three types of incubation solutions in the order of decreasing polarity: methanol > ethanol/methanol > isopropanol/methanol.

As shown in Figure 2a, less protein adsorption was observed on the IPN film, which was incubated in a more polar solvent

(i.e., methanol) for 24 h than those prepared from both less polar mixed solvents (i.e., ethanol/methanol and isopropanol/methanol). It is believed that more SBMA units can be partitioned into the SPU film in a more polar solvent environment during a long incubation period since SBMA monomers dissolve better in the polar solvent (methanol), resulting in the formation of SBMA-rich domains with the SPU matrix to provide better resistance to protein adsorption. In other words, the equilibrium between the swelled SPU film and the incubation solution was reached with a long enough incubation time, resulting in higher SBMA monomer partition within the SPU film. Thus, a more polar environment is preferred to achieve the formation of SBMA-rich domains within the SPU film to reduce protein adsorption over a long incubation time. This also explains why the IPN film (IPN-II) prepared by the addition of a stronger polar solvent (i.e., 5 vol % water) can further reduce nonspecific protein adsorption as shown in Figure 1. IPN-II is better than IPN-I prepared in pure methanol and HEMA hydrogels. It should be pointed out that pure water is not a good solvent for the IPN preparation since pure water cannot swell the SPU film. The solvent containing 95 vol % methanol and 5 vol % water appears to be a good compromise between SPU swelling and SBMA solubility.

Figure 2b shows SPU swelling vs incubation time. Results show that less polar solvents (e.g., ethanol/methanol or isopropanol/methanol) swell the SPU film more quickly initially. The swelling ratio (%) during IPN preparation is defined as the difference in diameter between the prepared IPN film and the unmodified SPU film divided by the diameter of the unmodified SPU film. It can be seen that the SPU film was quickly swelled to its maximum after 2 h when it was soaked in an ethanol/methanol (or isopropanol/methanol) solution. A similar degree of swelling was achieved in the methanol solution, but after 24 h. The swelling behavior can be correlated with that of the reduction of protein adsorption. As shown in Figure 2a, the reduction of protein adsorption is quicker for the SPU film soaked in ethanol/methanol (or isopropanol/methanol) solution than in methanol solution in the first 2 h. However, it should be pointed out that the reduction in protein adsorption on the IPN sample prepared in the ethanol/methanol (or isopropanol/methanol) solution is not as significant as that prepared in the methanol solution after 24 h. Even though a less polar solvent (e.g., isopropanol) can swell the SPU film and penetrate into the SPU matrix much faster, SBMA does not dissolve well in isopropanol-rich domains inside the SPU film. This clearly indicates that not only the degree of swelling is important for more SBMA to penetrate into the SPU film but also the polarity of the solvent inside the SPU film plays an important role for SBMA to dissolve within the SPU matrix. A more polar solvent can eventually promote SBMA penetration into the SPU matrix after long incubation times, but it has much slower kinetics for the swelling of the SPU films. Thus, appropriate solvents with intermediate polarities are desirable as incubation solutions for the IPN preparation to balance between the kinetics and thermodynamics of the IPN process.

Concentration of an Incubation Solution. The total concentration of an incubation solution can also affect the dispersion of SBMA units within the SPU film. In this work, it is varied between 0.1 and 3.0 mol/L, and protein adsorption on various IPN films prepared under different incubation concentrations was evaluated accordingly. As shown in Figure 3, there appeared an effective reduction in protein adsorption around 1.0 mol/L. For the lower concentrations of the incubation solution (<0.5 mol/L), the higher protein adsorption of the IPN film prepared

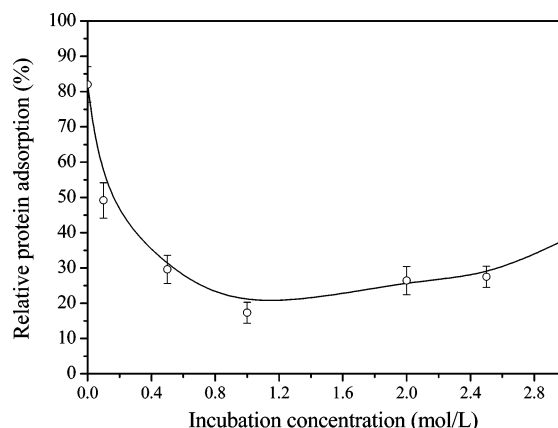


Figure 3. Relative protein adsorption versus incubation concentration in a methanol solution with the SBMA monomer ratio of 70 mol % for 24 h at 20 °C.

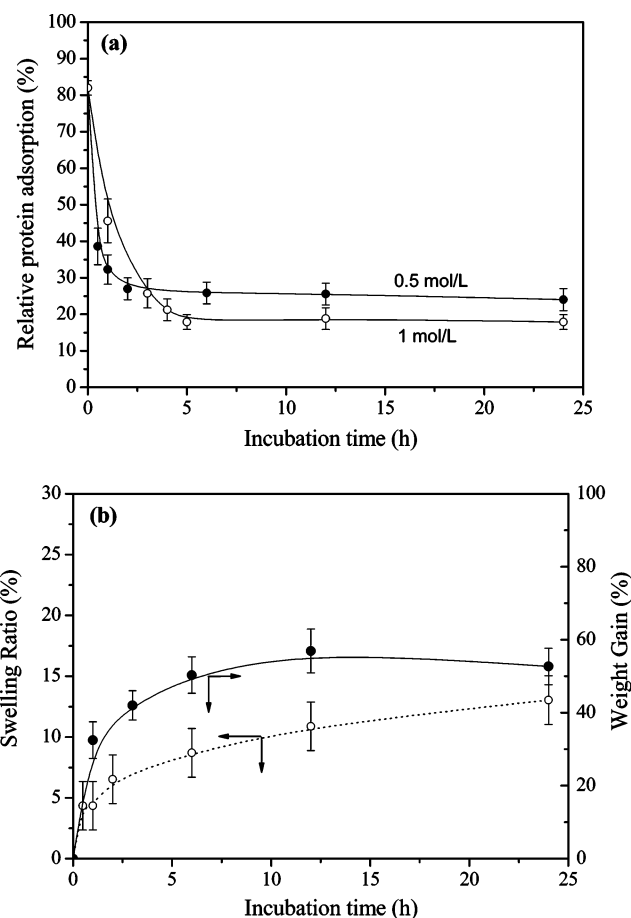


Figure 4. (a) Relative protein adsorption versus incubation time in a methanol solution with the SBMA monomer ratio of 70 mol % at 20 °C and the incubation concentration of 0.5 mol/L or 1.0 mol/L; (b) the corresponding swelling ratio and weight gain of the dry IPN film.

was observed due to the lack of SBMA-rich domains formed within the IPN film. For the IPN film incubated in highly concentrated solutions (>2.0 mol/L), the resistance of the IPN film to protein adsorption was not improved further due to the change in solvent behavior for a highly concentrated incubation solution containing highly charged SBMA. As shown in Figure 4a, the IPN films prepared from an incubation solution with a total concentration of 1.0 mol/L reduced protein adsorption more slowly than those prepared from the 0.5 mol/L solution over the first 2 h since less solvent molecules in the case of the 1.0 mol/L solution are available to swell the SPU film and promote

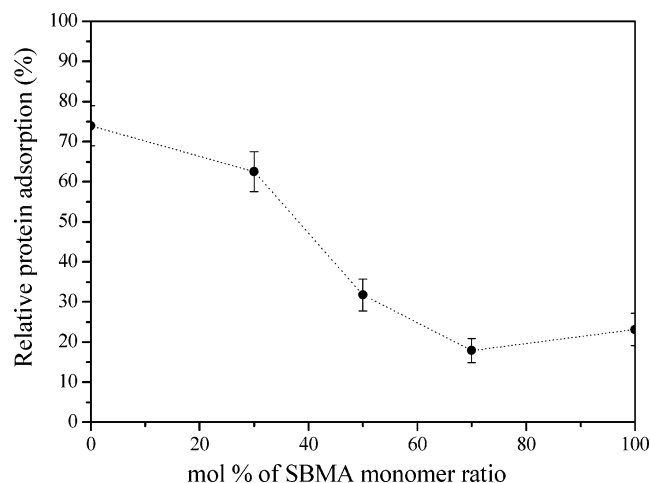


Figure 5. Relative protein adsorption versus different SBMA monomer ratios (mol %) with the incubation concentration of 1 mol/L for 24 h at 20 °C.

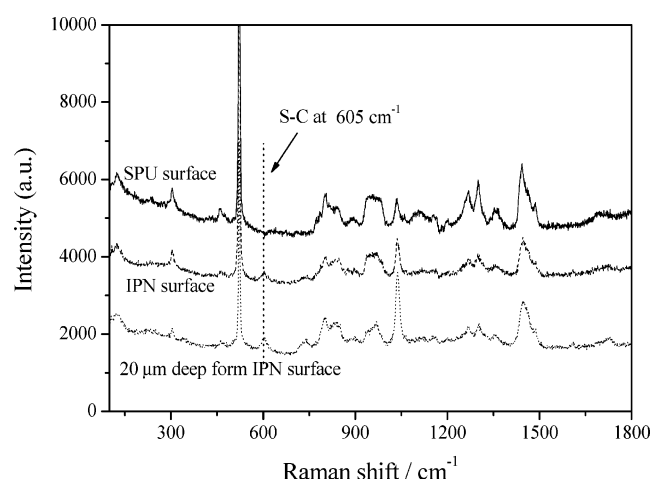


Figure 6. Raman spectra of the IPN-I film taken with a focal plane at the surface and 20 μm deep from the surface, respectively. Raman spectrum from the surface of an unmodified SPU film is also shown for comparison.

SBMA penetration into the SPU film. However, after incubation for 24 h, more effective reduction in protein adsorption was observed for the 1.0 mol/L solution. It is interesting to compare the variation of swelling ratio and weight gain with the variation of protein adsorption during IPN preparation. The weight gain (%) for the IPN preparation is defined as the difference in dry weight between the IPN film prepared and the unmodified SPU film divided by the dry weight of the unmodified SPU film. It can be seen from Figure 4b that there is a correlation among the swelling ratio, weight gain, and protein adsorption of the IPN film prepared. Results clearly show that the increase in swelling ratio and weight gain in Figure 4b corresponds to lower protein adsorption in Figure 4a as incubation time increases. Results also indicate that the monomer components indeed diffuse into the SPU film and form SBMA-rich domains within the IPN film.

Monomer Ratio of SBMA. Figure 5 shows the effects of different SBMA monomer ratios in an incubation solution on protein adsorption on the IPN films with the incubation time of 24 h at 20 °C and an incubation concentration of 1 mol/L. Relative protein adsorption decreased with increasing SBMA monomer ratio in the incubation solution. The maximum reduction of protein adsorption occurred when the molar ratio of SBMA to EHMA was 7:3. The results show that the

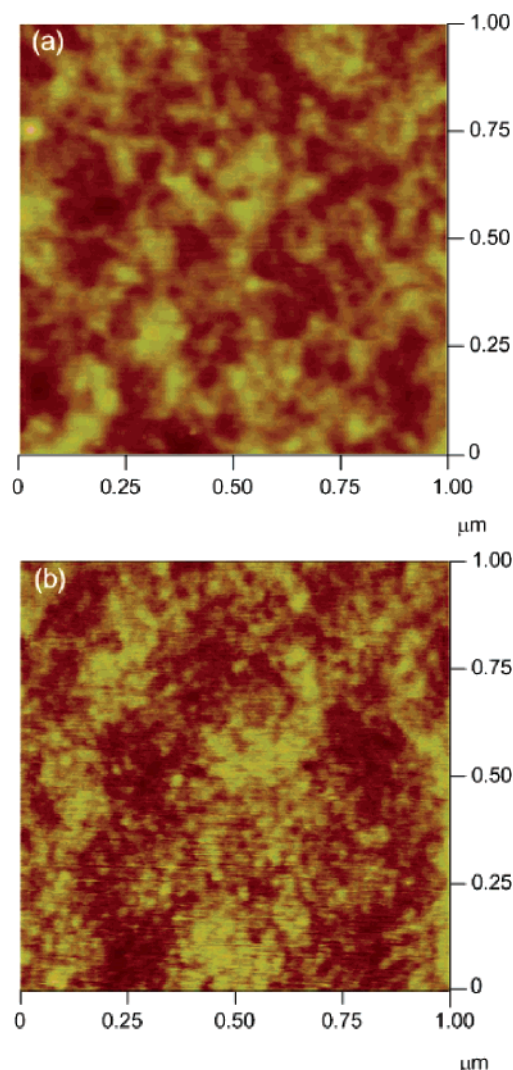


Figure 7. Tapping-mode AFM topographic images of (a) SPU and (b) IPN-I. The dimensions of the images are 1.00 μm \times 1.00 μm with a Z scale of 5 nm.

incubation solution with higher SBMA monomer ratio leads to more poly(SBMA)-rich domains within the IPN film for protein resistance. The results also show that some EHMA units in the solution can enhance the affinity between SPU and poly(SBMA). Thus, the well-controlled molar ratio of SBMA to EHMA is important for the dispersion of SBMA-rich domains within the SPU film and the formation of an excellent IPN structure with very low protein adsorption. Similar results have been reported for SPU/MPC by Ishihara and co-workers.^{20–21} In addition, they performed the detailed studies on the mechanical properties of SPU/MPC IPN films measured by tensile testing with repeated stress loading. Their results showed a Young's modulus of 18.6 MPa and a tensile strength of 31.0 MPa for their IPN films treated with MPC which was not significantly different from those of the unmodified SPU film. Thus, it is expected that the mechanical properties of IPNs containing poly(SBMA)/SPU should be similar to those of the unmodified SPU film.

Evidence for IPN Formation. Confocal Raman spectroscopy was used to characterize the IPN films. A typical spectrum is shown in Figure 6. The S—C chemical bonding from poly(SBMA) was observed at the Raman shift of 605 cm^{-1} , which appeared at both 0 and 20 μm depths from the top of the IPN film while no such a shift was observed for the unmodified SPU film. This indicates that poly(SBMA) indeed penetrated

into the SPU film. The Renishaw Raman microscope used in this work has a similar confocal configuration where the slits replace the pinholes in conventional confocal microscopes. From the cross-sectional SEM images of the IPN-I film (not shown), a homogeneous layer with a 120 μm thickness was observed. Figure 7 shows tapping-mode AFM topographic images for both a SPU (Figure 7a) and an IPN-I (Figure 7b) film. The AFM images show that the film roughness of the IPN-I is less than the unmodified SPU film. The major domains on the surface of the IPN-I are about 10–30 nm while those on the SPU have a larger width of 30–50 nm. The corresponding AFM phase images of Figure 7a,b (not shown) are relatively uniform. Thus, this indicates that the SBMA-rich domains are uniformly distributed over the SPU surface, which lower protein adsorption and also imply that the formation of the polymer networks are well interpenetrating without phase segregation.²⁹ As shown previously, reduction in nonspecific protein adsorption exhibited a time-dependent behavior (Figures 2a and 4a) and there is a correlation between the variation of swell ratio and weight gain (Figures 2b and 4b) and the variation of protein adsorption. For small SBMA molecules at 1 M concentration, SBMA physical adsorption should occur rather quickly. These results along with those from Raman indicate that SBMA does not simply adsorb onto the surface of the SPU films, but rather it penetrates into the SPU matrix. This observation agrees with what was observed previously for the IPNs containing poly(MPC)/SPU.

Conclusions

In this work, IPNs were prepared by the modification of a segmented SPU with a cross-linked SBMA polymer. The effects of solvent polarity, incubation time, SBMA monomer ratio, and incubation concentration on nonspecific protein adsorption on the IPN films were studied. The results show that appropriate solvent polarity and long incubation time are two key parameters for IPN preparation to achieve very low protein adsorption while there are optimal SBMA monomer ratios and incubation concentrations. The incubation solvent should be capable of both swelling hydrophobic SPU and dissolving hydrophilic poly-(SBMA) within the SPU film. The IPN film with the lowest protein adsorption was achieved by incubating a SPU film in a solution containing 95 vol % methanol and 5 vol % water for 24 h at 20 °C while the solution contained SBMA:EHMA = 7:3 (molar ratio) and the total concentration of the incubation solution was 2.0 mol/L. The process for IPN preparation can be divided into two stages. In the initial stage (short incubation times), the amount of poly(SBMA) diffusing into the SPU matrix from the incubation solution is mainly controlled by the degree of SPU swelling. In the later stage (long incubation times), the amount of poly(SBMA) within the SPU matrix is determined by the SBMA solubility within the IPN film. The results show that the IPN samples incubated in more polar solvent will reduce nonspecific protein adsorption more slowly initially, but they have higher SBMA partition within the SPU film after equilibrium is reached between the SPU film and the incubation solution. Results also show that there is a correlation among the swelling ratio, weight gain, and protein adsorption of the IPN film. Furthermore, results from confocal Raman microscopy, SEM, and AFM show that poly(SBMA) indeed

penetrates into the SPU film without phase segregation. Thus, IPNs are an effective approach to prepare a film that not only effectively resists nonspecific protein adsorption but also has excellent mechanical properties.

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