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Protein adsorption resistance of PVP-modified polyurethane film prepared by surface-initiated atom transfer radical polymerization

Huihui Yuan[a,1], Bin Qian[a,1], Wei Zhang[a], Minbo Lan[a,b,*]

[a] Shanghai Key Laboratory of Functional Materials Chemistry and Research Center of Analysis and Test, East China University of Science and Technology, Shanghai 200237, China
[b] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China

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ABSTRACT

An anti-fouling surface of polyurethane (PU) film grafted with Poly(*N*-vinylpyrrolidone) (PVP) was prepared through surface-initiated atom transfer radical polymerization (SI-ATRP). And the polymerization time was investigated to obtain PU films with PVP brushes of different lengths. The surface properties and protein adsorption of modified PU films were evaluated. The results showed that the hydrophilicity of PU–PVP films were improved with the increase of polymerization time, which was not positive correlation with the surface roughness due to the brush structure. Additionally, the protein resistance performance was promoted when prolonging the polymerization time. The best antifouling PU–PVP (6.0 h) film reduced the adsoption level of bovine serum albumin (BSA), lysozyme (LYS), and brovin serum fibrinogen (BFG) by 93.4%, 68.3%, 85.6%, respectively, compared to the unmodified PU film. The competitive adsorption of three proteins indicated that LYS preferentially adsorbed on the modified PU film, while BFG had the lowest adsorption selectivity. And the amount of BFG on PU–PVP (6.0 h) film reduced greatly to 0.08 μg/cm^2, which was almost one-tenth of its adsorption from the single-protein system. Presented results suggested that both hydrophilicity and surface roughness might be the important factors in all cases of protein adsorption, and the competitive or selective adsorption might be related to the size of the proteins, especially on the non-charged films.

1. Introduction

Polyurethane (PU) has been used extensively as biomaterials for low cost and excellent properties, such as abrasion resistance, toughness, tensile strength, and good biocompatibility compared to other polymers [1]. At present, PU has attracted increasing attention in the design of medical devices such as catheters and heart valves. However, the intrinsic hydrophobicity of PU limits its long-term application in vivo. Since after insertion of PU medical devices into the body, the bio-macromolecules will immediately diffuse to PU medical devices to form the conditioning film [2] which is a great risk for future bacteria adhesion [3]. Many of the biomolecules in conditional film are proteins [4]. Therefore, preventing protein adsorption could be a promising strategy to increase the service life of medical devices.

The modification is a pervasive way to change the physicochemical property while keep the intrinsic nature of biomaterials [5]. Some reports have demonstrated that the surface of PU device can be modified to obtain hydrophilicity [6], low roughness [7], and nanotopography [8], which normally show relatively low non-specific adsorption of proteins. To improve the hydrophilicity, several methods like coating [9], plasma treatment [10], UV grafting [11], and chemical grafting [12] have been employed for immobilizing some hydrophilic groups or charged functional groups onto PU. Among the chemical grafting, surface-initiated atom transfer radical polymerization (SI-ATRP) is commonly used to prepare polymer brushes grafting [13]. Introducing polymer brushes, a kind of nanotopography on the surface of devices, is beneficial for repelling protein adsorption due to their ability of swinging in solution at micro-scale.

On the other hand, poly(*N*-vinylpyrrolidone) (PVP) is attractive in the biomaterials due to its chain-typed, low toxicity, good solubility, chemical stability, and biocompatibility [14–16]. Over the past few years, PVP has been widely grafted onto substrate for biomaterials applications. Wu et al. [17] successfully carried out SI-ATRP of NVP on silicon surfaces. Simultaneously, the chain-length of PVP could be controlled by reaction time. Furthermore,

* Corresponding author at: Shanghai Key Laboratory of Functional Materials Chemistry and Research Center of Analysis and Test, East China University of Science and Technology, Shanghai 200237, China. Tel.: +86 21 64253574.
E-mail address: minbolan@ecust.edu.cn (M. Lan).
[1] Equal study contribution as first Authors.

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Robinson et al. [18] reported that PVP-coated silica reduced bovine serum albumin (BSA) adsorption by 75% compared to the unmodified material. Since PVP modified silica demonstrated a preferable surface against protein adsorption, we can assume that a more profound antifouling PU modified with PVP brushes might be achieved.

In this study, we aimed to prepare a protein adsorption resistance PU film modified with hydrophilic polymer PVP. A two-step SI-ATRP method was used to synthesize PU–PVP films. The water contact angle, chemical composition, ATR–FTIR spectra, and surface morphology of the modified PU films at different stages were investigated. In addition, three proteins, bovine serumalbumin (BSA), bovine serum fibrinogen (BFG), and lysozyme (LYS), with different molecular weight were introduced to evaluate the protein adsorption on PU films. Relationship between properties of PU films and their corresponding antifouling activity was also discussed.

2. Materials and methods

2.1. Materials

Polyurethane (pellethane 2363-80AE) was supplied by Lubrizol Corporation (USA). The 4,4'-diphenylmethane diisocyanate (MDI, 98%) and 2-Bromoisobutyryl bromide (BiBB, 98%) were obtained from Aladdin Chemistry Co., Ltd. (Shanghai, China) and used as received. *N*-vinylpyrrolidone (NVP, 99%, Aladdin) was purified by distillation under reduced pressure to remove the inhibitors before use. Copper(I) bromide (CuBr, 98.5%, Sinopharm, Shanghai, China) was purified by being stirred in glacial acetic acid overnight, washed with absolute methanol and dried under vacuum. Sodium hydroxide (NaOH, AR) and hydrochloric acid (HCl, 36.5%) were purchased from Lingfeng Chemical Reagent Co., Ltd (Shanghai, China). The cyclic ligand, 5,7,7,12,14,14-hexamethyl-1,4,8,11-tetra-azacyclotetradecane (Me_6TATD), was synthesized according to the method described by Hay et al. [19] α-cyano-4-hydroxycinnamic acid (CHCA) was from Sigma-Aldrich, St. Louis, MO, USA. BFG, BSA, and LYS were all obtained from Sigma-Aldrich (New Jersey, USA). Micro BCA Protein Assay Reagent Kit, ammonium persulfate (98%), glycine (99%), acrylamide (99.9%), N,N'-methylene diacrylamide (98%), sodium dodecyl sulfate (99%), tetramethylethylenediamine (99.9%), coomassie brilliant blue R250, isopropanol (99.5%), acetic acid (99.5%), protein marker (14.4 kDa–97 kDa), and loading buffer were purchased from Sangon Biotech (Shanghai) Co., Ltd.

2.2. Preparation of PVP-modified PU films

The procedure of PVP grafting on PU films was showed in Fig. 1:

2.2.1. Preparation of PU films

PU films were made by casting from a 5% (w/v) PU solution in DMF on glass Petri dishes and the solvent was slowly evaporated under vacuum at 65 °C for 48 h [20]. PU films, 6 mm in diameter, were then cut from the films, extracted by Soxhlet extraction in toluene for 24 h, and dried in a vacuum oven.

2.2.2. Initiator-immobilization on the PU films

To introduce isocyanate into the PU films [21], PU films reacted with 7.5% (w/v) MDI at the presence of 2.5% (w/v) TEA as a catalyst in anhydrous toluene for 2.5 h at 60 °C to obtain PU–NCO films.

Then the films were rinsed twice with toluene and immersed in 200 mL of a 2% (w/v) solution of 1,2-ethanediamine in toluene and reacted for 2 h at 60 °C [22]. Subsequently, the films were removed from solution and rinsed thrice with methanol and distilled water, and dried in vacuum.

The next reaction was under the anhydrous condition. The PU–NH_2 films were immersed in 25 mL of toluene containing 0.84 mL triethylamine (6 mmol). Then a mixture of 0.75 mL BiBB (6 mmol) in toluene was added dropwise into the above solution over a period of 1 h under ice-water bath [6]. The reaction was then carried out at 0 °C for 0.5 h and at room temperature for 24 h. The PU–Br films were obtained by washing thrice with toluene, methanol, and distilled water to remove the unreacted components.

2.2.3. SI-ATRP on theinitiator-functionalized films

A typical procedure for grafting PVP onto the initiator-functionalized PU–Br films was as follows [17]. CuBr (72 mg, 0.5 mmol), Me_6TATD (28.4 mg, 0.1 mmol), NVP (1.04 mL, 10 mmol), and 20 mL of the mixture of methanol (12.5 mL) and water (7.5 mL) were added separately to a glass flask. Wherein, the CuBr was used as catalysts and the Me_6TATD was used as ligand. Before polymerization, the mixture in flask was stirred for 30 min under a nitrogen atmosphere. After that the initiator-functionalized PU–Br films were placed into the glass flask. Polymerization was carried out at 60 °C for 1.5 h, 3.0 h, and 6.0 h under a nitrogen atmosphere, respectively, and was terminated by exposure to air. The PU–PVP films were obtained and washed successively with methanol and distilled water, dried in a vacuum oven and kept in a dryer.

2.3. Characterization of unmodified and modified PU films

Elemental analysis of films was determined by X-ray Photoelectron Spectroscopy (XPS, Thermo Fisher, Escalab 250Xi).

ATR–FTIR spectra of films were obtained from a Fourier Transform Infrared Spectrometer (FTIR, Thermo Nicolet 6700) with a resolution of 4 cm^{-1} in absorbance mode.

The topology of films was performed by Atom Force Microscope (AFM, Veeco, CSPM5500). The root-mean-square (RMS) surface roughness was calculated from the roughness profiles.

Water contact angle measurements of films were studied with a contact angle measuring system from Powereach, model JC 2000D, equipped with a video CCD-camera and JC 2000D software. Measurements were carried out using the sessile drop method: a total of 2 μL deionized water was dropped on the film at room temperature. Then images were taken every 4 s over 60 s. Three replicates were used to get a reliable value.

2.4. PVP grafting density and molecular weight on PU films

The grafting density (GD) is an important parameter to measure the amount of PVP grafted onto the PU films. The GD was calculated according to the following equation:

$$GD = \frac{W_t - W_0}{W_0}$$

where, W_t is the weight of PU–PVP film after t hours' grafting and W_0 is the weight of PU–Br film. The films were dried in a vaccum oven at 60 °C for 4 h and weighed by an electronic balance with the accuracy of 0.1 mg. Thirty-six PU–Br films were divided into three groups randomly, and then were grafted with PVP for 1.5 h, 3.0 h, and 6.0 h, respectively.

The molecular weight of PVP grafted on PU films was also determined to evaluate the relationship between polymerization time and the chain length of PVP. PU–PVP films were hydrolyzed in 300 μL of 1.5 mol/L NaOH for 2 h at 90 °C. Then hydrolyzed solution was adjusted to litmusless and analyzed by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry

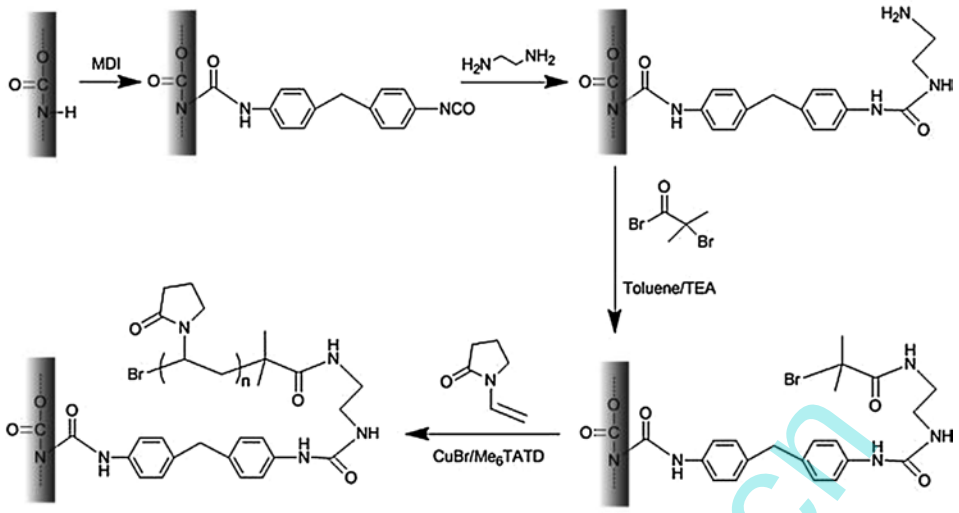

Fig. 1. Schematic illustration of the immobilization of the initiator and subsequent SI-ATRP of PVP on PU films.

(Maldi-TOF-MS, SCIEX, 4800plus) to get the molecular weigh of PVP [23]. CHCA was used as matrix.

2.5. Single protein adsorption

BFG, BSA, and LYS were used as model protein in this study. A phosphate-buffered saline (PBS, pH 7.4) was used to prepare a protein solution with a concentration of 1 mg/mL. The films were incubated at 37 °C for 24 h in phosphate-buffered saline (PBS, pH 7.4). After incubation, the films were immersed in protein-containing solution and shaken at 100 rpm at 37 °C for 1 h. After that, the films were gently rinsed with double distilled water to remove the non-adherent proteins. Then the films were put into 500 μL of 1% sodium dodecyl sulfate (SDS) and shaken for 2 h at 37 °C to desorb the adsorbed proteins on the surface. Subsequently, the SDS solution was mixed with 500 μL BCA working-reagent. After incubation for 1 h at 60 °C, the adsorption of the BFG (or BSA or LYS) was measured at 562 nm using an ultraviolet spectrophotometer.

2.6. Mixed proteins adsorption

A phosphate-buffered saline (PBS, pH 7.4) was used to prepare a protein solution of BSA, BFG, and LYS with a concentration of 1 mg/mL, respectively. According to the process in 2.4, the total protein adsorption quantity was obtained.

The solution from the same batch containing 1% SDS and proteins was dried using vacuum freeze drying for 24 h. The powder was dissolved in 16 μL PBS and 4 μL loading buffer. Then, the solution was boiled for 3 min and determined by SDS-PAGE method [24]. The images were taken by canon MF-Chemi B and analyzed by image J software to calculate the content of protein.

3. Results and discussion

To develop a hydrophilic surface on PU substrate with excellent protein resistance, PU films were firstly reacted with MDI to introduce isocyanate, secondly reacted with ethanediamine to obtain films with $-NH_2$ and then added BIBB to perform initiator-immobilization. Finally NVP was polymerized on the PU films through SI-ATRP. The PVP brushes of different length were sucessfully grafted on PU films after different polymerization time.

3.1. Surface characterization

3.1.1. XPS

Fig. 2 showed the changes of the chemical composition of the PU films at different stages of surface modification. A typical XPS survey spectrum was confirmed as PU films (Fig. 2a): the characteristic signals for carbon (C1s at 285 eV), oxygen (O1s at 533 eV), and nitrogen (N1s at 400 eV) were clearly observed [25]. The nitrogen peak of PU–NH_2 film was higher than those of PU films and PU–NCO films, because 1,2-ethanediamine is composed mainly of nitrogen. After introducing —Br onto the films, the appearance of weak signals assigned to bromine (Br3d at 69 eV, inset of Fig. 2d) indicated that the initiator had been tethered covalently to the PU–NH_2 films. Fig. 4f illustrated the high-resolution spectra of C1s which can be curve-fitted in three peaks centered at 284.83 eV (C—H), 286.18 eV (C—N), and 288.08 eV (C═O) [26].

3.1.2. ATR–FTIR

ATR–FTIR was further used to determine the tethering of PVP on the film surface. Fig. 3 presented the typical ATR–FTIR spectra of the origin PU films and modified PU films. The most significant difference between the origin PU and PU–NCO was the appearance of a relatively strong new band of —NCO stretching vibration at 2284 cm^{-1} [27] (Fig. 3b) on the PU–MDI films, which disappeared on the PU–NH_2 films (Fig. 3c). And on the PU–NH_2 film, the sharp peak amplified at 3307 cm^{-1} was the stretching vibration of amino group ($-NH_2$). Then the strongest peak at 1702 cm^{-1}(Fig. 3d) arising from the stretching vibration of carbonyl group (C═O) [28] in BiBB indicated that the initiator was successfully grafted onto the PU films. For the PU–PVP film (Fig. 3e), the peak at 1702 cm^{-1} splitting into two peaks because of the different chemical circumstance of C═O in BIBB and PVP, demonstrated that the PVP was also grafted onto the PU films by the SI-ATRP method.

3.1.3. Water contact angle

The water contact angle is a common and convenient way to assess the hydrophilic/hydrophobic properties of the prepared films. The changes of the water contact angles of the modified PU films at different stage were clearly given in Fig. 4. As the reaction progress, the water contact angle fluctuated with the hydrophily of the surface groups. After NVP was grafted onto the films, the contact angles of the surfaces were significantly decreased. Especially in Fig. 5, with prolongation of the polymerization time, the

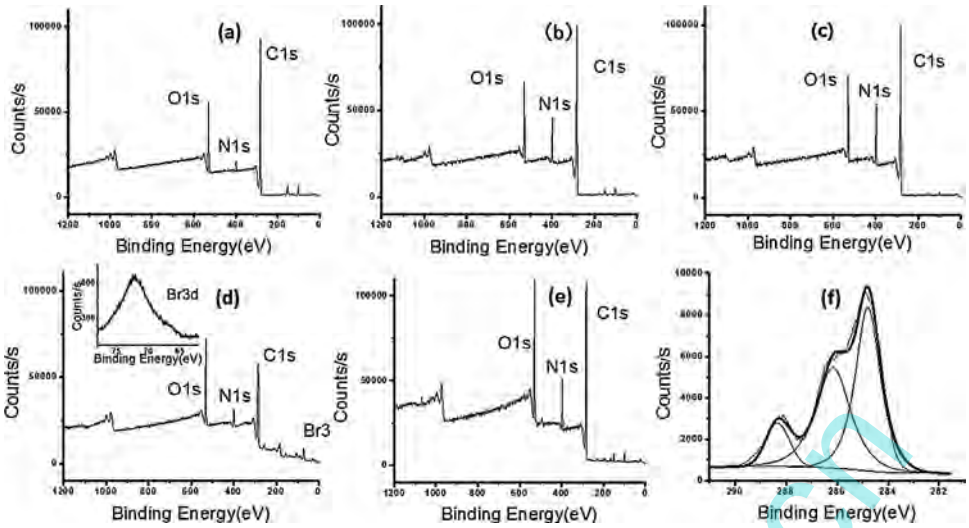

Fig. 2. XPS spectra of (a) PU, (b) PU–NCO, (c) PU–NH_2, (d) PU–Br inset: Br 3d, (e) PU–PVP, and (f) High-resolution C1s XPS spectra of PU–PVP (6.0 h).

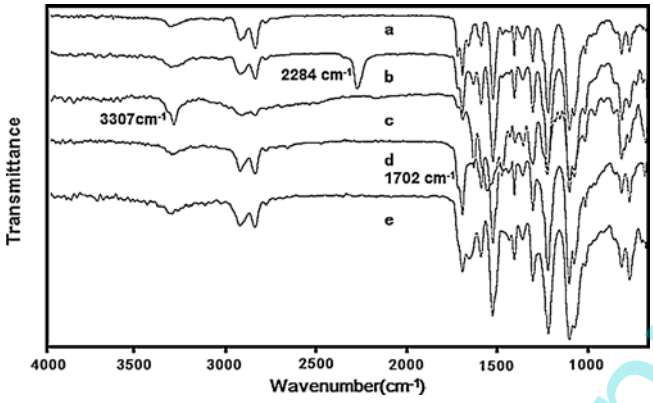

Fig. 3. ATR–FTIR spectra of (a) origin PU film, (b) film with —NCO group, (c) film with —NH_2 group, and (d) PVP-modified film (6.0 h).

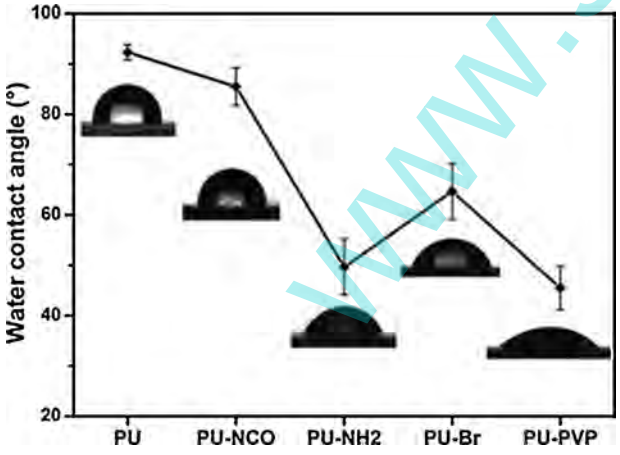

Fig. 4. Water contact angle of unmodified and modified PU films.

water contact angles decreased from 60° to 46°, indicating that the hydrophilic PVP was successfully grafted on the PU films.

3.1.4. AFM

The surface morphology of unmodified and modified PU films at dry state was examined by tapping mode AFM. The unmodified PU films had a rather uniform and smooth surface, with a RMS roughness value of about only 1.517 nm (Fig. 6a). After surface graft polymerization of NVP via SI-ATRP, the surface roughness of the PU–PVP surface increased to 48.445 nm (1.5 h), 79.379 nm (3.0 h), 29.120 nm (6.0 h), respectively. It was believed that the increasing roughness was related to the coverage and length of PVP chains on the film. When the polymerization time was prolonged, the PVP chains on films became longer and the polymerization proceeded at the first 1.5 or 3.0 h, the PVP brush might not well cover the surface in this case. So the roughness of PU–PVP (1.5 h) was smaller than that of PU–PVP (3.0 h) and larger than that of unmodified PU. After polymerization for 6.0 h, the grafted chain density and chain length increased, and the chain length also became uniform, which resulted in brush clusters smaller and neater. Hence, the roughness of PU–PVP (6.0 h) became relatively smaller than those of other two modified PU–PVP.

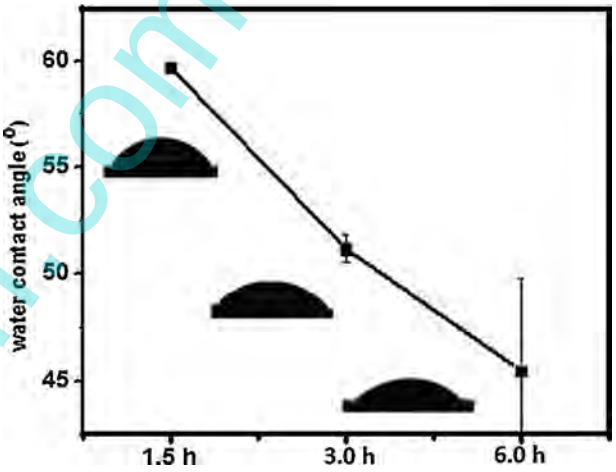

Fig. 5. Water contact angle of PU–PVP films with different polymerization time.

3.2. Grafting density and chain length of PVP

Grafting density of PVP was determined by the weight increase of PU–Br and PU–PVP. The GD increased with the prolongation of polymerization time. The order of GD on PU–PVP films was 123, 143, and 163 μg/cm^2 for PU–PVP films (1.5 h, 3.0 h, and 6.0 h), respectively. Fig. 7 showed the distribution of molecular weigh of PVP cleaved from the PU films measure by Maldi-TOFs. Similarly,

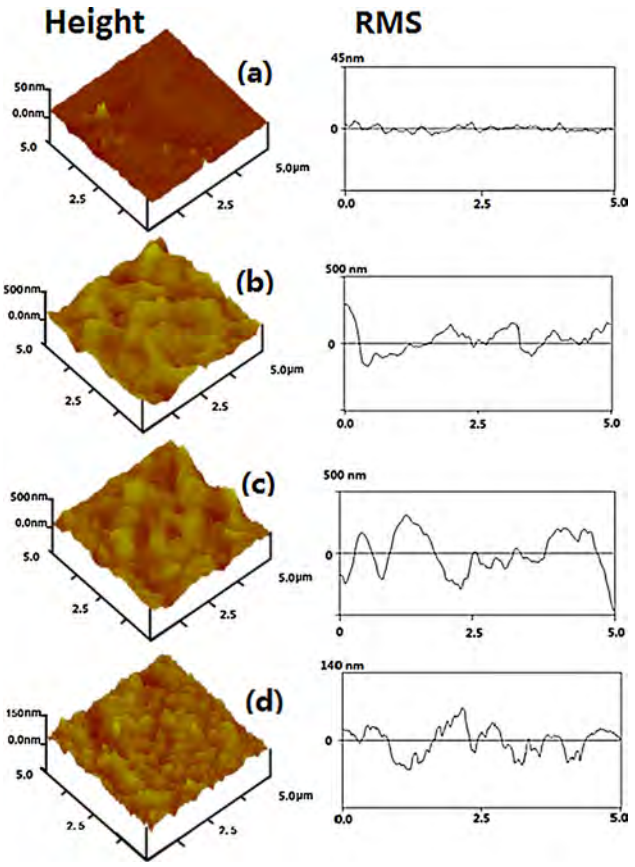

Fig. 6. AFM images of (a) PU, (b) PU–PVP (1.5 h), (c) PU–PVP (3.0 h), and (d) PU–PVP (6.0 h).

the shorter the polymerization time was, the smaller the molecular weigh of PVP was. The chain length of PVP was longest on PU–PVP (6.0 h) and shortest on PU–PVP (1.5 h).

3.3. Protein adsorption

Since the protein adsorption on biomaterials' surfaces is always considered as the first step of many undesired bio-reactions and bio-responses [29], the primary target for preparing biomaterials is to construct ultra-low fouling or non-fouling surfaces. In this study, the protein adsorption property of the films was tested by measuring the adsorption level of BFG, BSA, and LYS which had remarkably different sizes and charge characteristics. LYS (14.3 kDa, 45 Å × 30 Å × 30 Å) [30] has a net positive charge as opposed to the net negative charge of BFG (340 kDa,

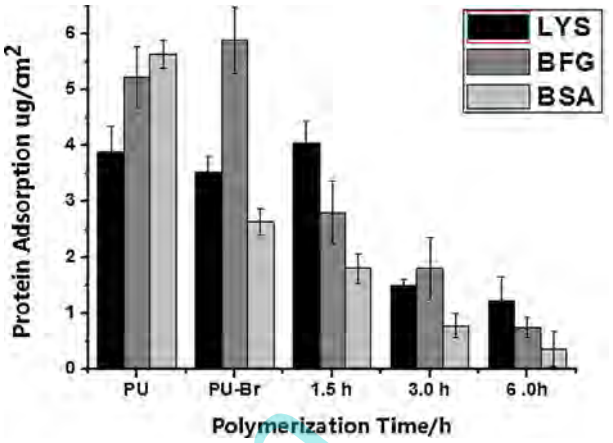

Fig. 8. Protein adsorption on PU–Br and PU–PVP films with different polymerization time. Data are means ± the standard error, $n = 3$.

450 Å × 90 Å × 90 Å), and BSA (66 kDa, 140 Å × 40 Å × 40 Å) [31] at physiologic pH.

The theoretical adsorption amounts on a surface in the end-on and side-on close-packed monolayer surface coverage were 2.26 and 0.24 μg/cm^2 for BFG, and 0.72 and 0.21 μg/cm^2 for BSA, respectively [32]. As shown in Fig. 8, the levels of BFG, BSA, and LYS adsorption on PU films were 5.23, 5.65, and 3.90 μg/cm^2, respectively. The adsorbed amounts of BFG and BSA on PU were greatly larger than the theoretical adsorption amounts, which indicated surface was adsorbed by multilayer protein. After step-by-step modification, the PU–Br films obtained had slight change in the protein adsorption except for BSA compared with the unmodified PU films. Though BSA adsorbed on PU–Br films was less than that on PU films, it was higher than that on PU–PVP films. With the increase of polymerization time, the protein adsorption of PU–PVP films decreased rapidly. After long polymerization time (6.0 h), the PU–PVP films adsorbed merely 0.75, 0.37, and 1.23 μg/cm^2 of BFG, BSA, and LYS, respectively. The reduction in the level of protein adsorption, 85.6% for BFG, 93.4% for BSA, and 68.3% for LYS, was achieved, compared to the unmodified PU films. Likewise, the amounts of BFG or BSA adsorbed on PU–PVP (6.0 h) suggested that these two proteins adsorbed on the surfaces of PU–PVP (6.0 h) were a mixture of end-on and side-on orientations in contrast with theoretical adsorption amounts, respectively [33]. It was obvious to find that PU–PVP (6.0 h) possessed the best protein repelling performance due to its highest hydrophilicity. The results indicated that the smaller the contact angle of PU–PVP film was, the less the protein was adsorbed. On the other hand, the surface roughness of films (caused by brush structure) and intrinsic property of protein had important effects on the protein adsorption. The order of antifouling property of PU–PVP was BSA > BFG > LYS. LYS demonstrated the

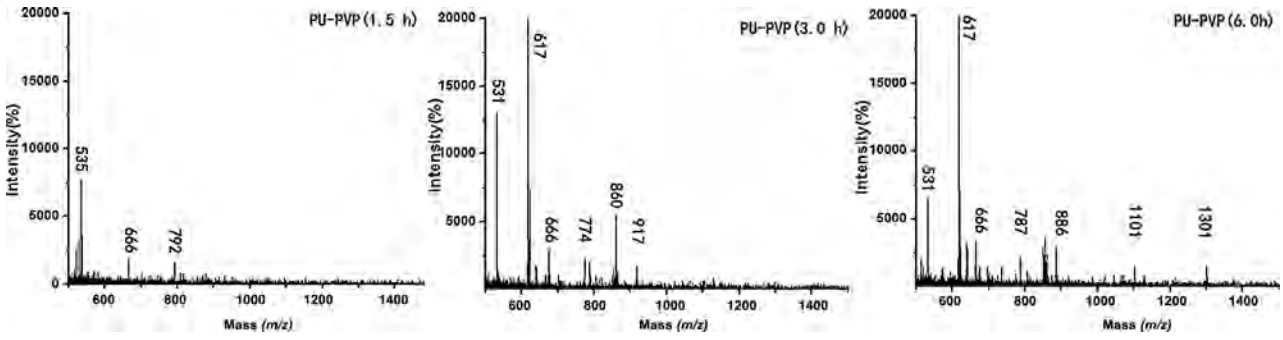

Fig. 7. Mass spectra of PVP from PU–PVP films.

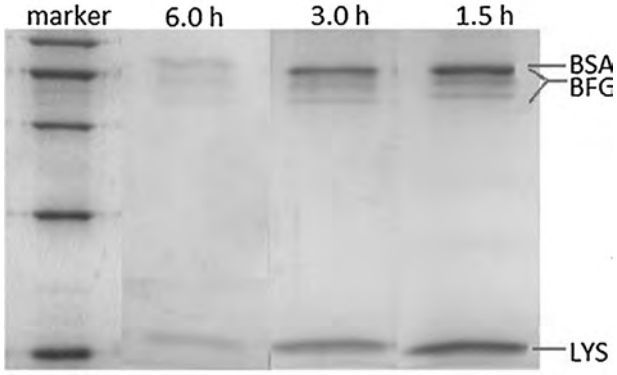

Fig. 9. SDS-PAGE of mixed proteins samples.

Table 1
The percentage of single protein in total absorbed proteins on different PU–PVP films.

	BSA%	LYS%	BFG%
PU–PVP (1.5 h)	28.72 ± 0.62	56.42 ± 0.75	14.86 ± 0.67
PU–PVP (3.0 h)	30.21 ± 1.28	55.32 ± 0.40	14.47 ± 1.10
PU–PVP (6.0 h)	25.00 ± 1.27	55.00 ± 0.57	20.00 ± 0.92

relatively strong adsorbability on the PU–PVP. Notably, the amount of LYS on PU–PVP (1.5 h) was almost the same as or a little bit higher than that on unmodified PU, although the hydrophilicity of PU–PVP (1.5 h) was favorable compared with the PU films. The possible reason for LYS adsorption might be as follows: (1) The cationic amino acids in LYS attracted the electron cloud on hydrogen atom and could easily form N—H—O hydrogen bond with PVP [34], which made the adsorption stable. (2) LYS could adsorb on top of, as well as be embedded within, the brush [35]. And it is considered to be a 'hard' protein which is unlikely to undergo alteration in its secondary structure during adsorption [36], resulting in hard desorption from the surface when the PVP brush swung. For BFG and BSA, their structures were rearranged to reach an energetically advantageous conformation, leading to the hydrophobic side chains entering the interior part of the proteins [33]. (3) The immobilized long chain PVP increased the surface roughness and did not well cover the surface which might allow LYS entrapment in its groove regions [37]. LYS entrapping in brushes and exchanging an equivalent volume of water leaded to an increase in entropy which was thermodynamically favorable [34]. For BFG and BSA with the relatively larger size and molecular weight than LYS, they were unfavorable to sink in the groove regions. Thus, the PVP brushes were compressed by proteins, making the entropy of the surface being higher, which was thermodynamically unfavourable and resulted in the resistance of protein adsorption [38]. In general, the PVP brushes showed good repelling protein performance.

In fact, the proteins were co-existed in physiological environment. Protein solution maintaining BSA, BGF, and LYS was prepared to investigate the competitive adsorption of three proteins on different PU–PVP films. The total amount of adsorbed proteins on films was 3.26 μg/cm^2 for PU, 2.96 μg/cm^2 for PU–PVP (1.5 h), 2.35 μg/cm^2 for PU–PVP (3.0 h), and 0.40 μg/cm^2 for PU–PVP (6.0 h), respectively. The PU–PVP (6.0 h) resisted protein adsorption significantly, with a reduction of 87.7% total protein adsorption compared to PU. The adsorbed proteins were separated and analyzed by SDS-PAGE (Fig. 9), among which the amount of every protein adsorption was calculated by Image J [39]. Table 1 showed that LYS preferentially adsorbed on the modified PU films, and it occupied over half of total amount of protein adsorption. Furthermore, compared to BSA, BFG had low selectivity in mixed protein solution, which was expected to be favorable for some biological environment [40]. The amount of adsorbed BFG on PU–PVP (6.0 h) reduced greatly to 0.08 μg/cm^2, which was almost one tenth of its adsorption from the single-protein system. Presented results suggested that the competitive or selective adsorption might be related to the size of the proteins especially on the non-charged films.

4. Conclusion

In this work, polyurethane films were successfully grafted with hydrophilic poly(*N*-vinylpyrrolidone) polymer by using SI-ATRP method. With the longer polymerization time, the PVP grafting density increased and the PVP chains on PU films became longer. Subsequently PU–PVP films had a better hydrophilic surface which was favorable to resist the protein adsorption. Although the grafted PVP brushes increased the surface roughness of modified PU films compared to the unmodified PU films, the nanotopography was beneficial for protein fouling resistance. Presented results indicated that both hydrophilicity and surface roughness might be the important factors in all cases of protein adsorption, and the competitive or selective adsorption might be related to the size of the proteins especially on the non-charged films. However, further studies are required to investigate the real samples like plasma and urine, and obtain a better understanding of protein adsorption in vivo.

Acknowledgments

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