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Library of Antifouling Surfaces Derived From Natural Amino Acids by Click Reaction

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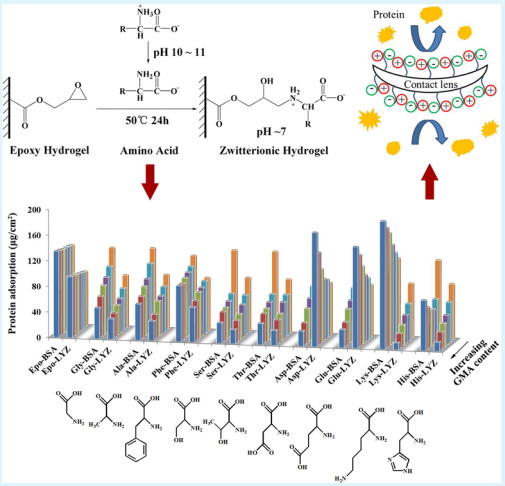
ABSTRACT: Biofouling is of great concern in numerous applications ranging from ophthalmological implants to catheters, and from bioseparation to biosensors. In this report, a general and facile strategy to combat surface fouling is developed by grafting of amino acids onto polymer substrates to form zwitterionic structure through amino groups induced epoxy ring opening click reaction. First of all, a library of poly(2-hydroxyethyl methacrylate-*co*-glycidyl methacrylate) hydrogels with zwitterionic surfaces were prepared, resulting in the formation of pairs of carboxyl anions and protonated secondary amino cations. The analysis of attenuated total reflectance Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy confirmed the successful immobilization of amino acids on the hydrogel surfaces. After that, the contact angle and equilibrium water content of the modified hydrogels showed that the hydrogels exhibited improved hydrophilicity compared with the parent hydrogel. Furthermore, the protein deposition was evaluated by bicinchoninic acid assay using bovine serum albumin (BSA) and lysozyme as models. The results indicated that the performance of the hydrogels was determined by the nature of incorporated amino acid: the hydrogels incorporated with neutral amino acids had nonspecific antiadsorption capability to both BSA and lysozyme; the hydrogels incorporated with charged amino acids showed antiadsorption behaviors against protein with same charge and enhanced adsorption to the protein with opposite charge; the optimal antiadsorption performance was observed on the hydrogels incorporated with polar amino acids with a hydroxyl residual. The improvement of antiprotein fouling of the neutral amino acids grafted hydrogels can be ascribed to the formation of zwitterionic surfaces. Finally, a couple of soft contact lenses grafted with amino acids were fabricated having improved antifouling property and hydrophilicity. The result demonstrated the success of amino acids based zwitterionic antifouling strategy in ophthalmology. This strategy is also applicable to substrates including filtration membranes, microspheres and nanofibers as well. It is a versatile method for amino acids grafting onto polymer substrates to construct zwitterionic surfaces and achieve antifouling properties.



KEYWORDS: *amino acids, zwitterionic, antifouling surfaces, hydrophilicity, click reaction*

1. INTRODUCTION

Biofouling is a critical issue affecting biomedical implants and devices,[1,2] biosensors,[3] food packaging,[4] water treatment[5,6] and marine equipment.[7] Nonspecific protein fouling on biological implants and devices provides a conditioning layer for microbial colonization and subsequent biofilm formation.[8,9] It may result in harmful side effects, such as thrombosis and infections.[10] Biofouling on porous filtration membranes reduces water permeability and may also induce microbial contamination in bioseparation.[11] It is a major challenge for commercial and naval shipping as well. The biofouling slimes that form on ship hulls are reported to raise fuel consumption by as much as 30% in some cases.[12] Therefore, there is a constant need to develop versatile, convenient and cost-effective antifouling strategies in healthcare and many industries.

One strategic approach to combat surface fouling is to prevent biofoulants from attaching.[13] Surface functionalization with antifouling structure is a convenient way for imparting adhesion resistance.[14] Four principles of constructing protein resistant surfaces are summarized in the literature:[15] (1) contain polar functional groups, (2) incorporate hydrogen bond accepting groups, (3) do not contain hydrogen bond donating groups and (4) have no net charge. Poly(ethylene glycol) (PEG) is the common moiety to endow surface with protein adhesion resistance because of the formation of hydrated layer.[16] Other hydrophilic polymers can also be grafted to give surface antifouling features by surface initiated

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polymerization.[17] Recently, introducing zwitterions onto material surface becomes an attractive method to prepare antiprotein fouling surface.[18−21] G. M. Whitesides and coworkers are the frontrunners in this area and have published several works on this subject.[15,22,23] E. F. Murphy et al.[24] assumed the mechanism for zwitterion antiprotein fouling is that zwitterion is highly polarized and can combine with a large amount of water molecules, thus forming a hydration layer near the material surface that can prevent protein from fouling. However, reagents that can be used to introduce zwitterion onto material surface are usually limited to phosphorylcholine[25,26] and betaine.[27−29]

Natural amino acids exist predominantly as zwitterions at neutral pH in aqueous solutions because of the formation of protonated primary amino group ($-NH_3^+$) and deprotonated carboxyl group ($-COO^-$), and the biocompatible nature of amino acids promises them as a potential candidate for synthesizing zwitterionic biomaterials. However, only a few amino acids were applied as zwitterions for the development of antifouling materials. Q. Liu et al.[30] synthesized a serine based zwitterionic poly(serine methacrylate) (PSerMA) that can strongly suppress biofouling of bovine serum albumin (BSA), full serum and full plasma. Q. Shi et al.[31] reported lysine grafted polyacrylonitrile (PAN) membrane via a carbodiimide chemistry which suppressed both BSA and lysozyme adsorption. J. Shen et al.[32] grafted lysine onto polydopamine coated poly(ethylene terephthalate) sheets via Michael addition reaction, showing improved resistance to nonspecific protein adsorption and platelet adhesion. A. L. Zydney et al.[33] reported the fouling behavior of a lysine zwitterionic ultrafiltration membrane in comparison with charged and neutral membranes generated by covalent attachment of different end group ligands to a base cellulose membrane using the same activation chemistry. The results exhibited that the extent of fouling was strongly affected by electrostatic and hydrophobic interactions between protein and membrane, and the zwitterionic membranes had very low degree of protein fouling over a wide range of conditions. J. E. Rosen et al.[34] grafted cysteine onto nanoparticles using −SH to open epoxy group, resulting in cysteine zwitterionic surface with antibiofouling property. L. Zou et al.[35] reported cysteine modified aromatic polyamide reverse osmosis membrane through bonding the thiol group of the cysteine to the allyl functionalized membrane, which displayed lower BSA and dodecyltrimethylammonium bromide (DTAB) adsorption. So far, these researches are all limited to the concept, that simple amino acids must have active side groups including −OH (serine), −SH (cysteine) and $-NH_2$ (lysine) bonded with polymer chains, which means pairs of protonated primary amino groups ($-NH_3^+$) and deprotonated carboxyl groups ($-COO^-$) must been maintained after grafting as bare amino acids possess.

To prepare antifouling materials using the library of amino acids including those having no active side group and having active side groups (the quantity of the former is much more than that of the latter), a general and facile strategy is developed by a combination of amino acids and epoxy chemistry: zwitterionic structure was obtained by click reaction−grafting amino acids onto polymer chains via ring opening reaction between primary amino groups of amino acid and epoxy groups of polymer chains in weakly alkaline aqueous solution followed by protonation of secondary amino groups and deprotonation of carboxyl groups in the vicinity of pH 7. It means that protonated secondary amino cations (PSA, $-NH_2^+-$) and deprotonated carboxyl anions (DPC, $-COO^-$) are paired to form the PSA-DPC zwitterionic structure. To confirm the concept, zwitterionic poly(2-hydroxyethyl methacrylate-*co*-glycidyl methacrylate) (poly(HEMA-*co*-GMA)) hydrogels were prepared by grafting different types of amino acids. Their hydrophilic properties and antiprotein adsorption performance were investigated further in detail. Antiprotein adsorption properties were tested by using BSA and lysozyme as target proteins, and adsorption amounts were determined by a Bradford Protein Assay Kit method. Moreover, a couple of amino acids grafted contact lenses were fabricated and *in vivo* wearing evaluation was performed in a rabbit model. Other types of substrates including micropsheres, nanofibers and filtration membranes were also tested to impart zwitterionic surfaces in order to extend the applications of the concept.

2. EXPERIMENTAL SECTION

2.1. Materials. 2-Hydroxyethyl methacrylate (HEMA) from Acros Co. (Beijing, China) and glycidyl methacrylate (GMA) from JingChun Chemical Co. (Shanghai, China) were used as received. Free radical photoinitiator, Darocur1173, obtained from Ciba Co. (Switzerland) and ethylene glycol dimethacrylate (EGDMA) obtained from TCI Co. (Japan) were used as received. L-α-Amino acids, including glycine, alanine, phenylalanine, serine, threonine, aspartic acid, glutamic acid, lysine and histidine, were all supplied from JingChun Chemical Co. (Shanghai, China). BCA Protein Assay Reagent Kit K3000 was purchased from Shanghai Biocolor BioScience & Technology Co. (Shanghai, China). All other chemical reagents were commercially analytical grade and used without further purification.

2.2. Methods. *2.2.1. Preparation.* HEMA monomer (100%, 97%, 94%, 91%, 88% and 85% w/w based on the total weight 100% of HEMA and GMA), GMA monomer (0%, 3%, 6%, 9%, 12% and 15%), cross-linker EGDMA (0.3%) and photoinitiator Darocur 1173 (0.3%) were mixed as stock solutions. The mixture was introduced between two glass plates (length 7.5 cm × width 2.5 cm) and cured under a UV lamp (Spectroline SB-100P/F, 20 cm away from the plate, 365 nm) for 1 h. Poly(HEMA-*co*-GMA) membranes were obtained with a fairly consistent thickness of 0.25 mm controlled by a Teflon gasket. The obtained poly(HEMA-*co*-GMA) membranes were extracted by ethanol/water mixture (v/v, 1/3) at 60 °C for 6 h.

Amino acids were dissolved in an alkaline solution of 25 vol % ethanol aqueous solution with the concentration of 1.0 mol/L and the pH value of the mixture was regulated to 10−11 by adding NaOH. Then the amino acid solutions were allowed to swell the pristine poly(HEMA-*co*-GMA) hydrogels until the equilibrium had been reached (approximately 24 h) in vials. The vials containing the hydrogels and the remaining amino acid solutions were placed in a shaker at 50 °C for 24 h. Subsequently, the hydrogels were removed from the amino acid solution. The grafted zwitterionic hydrogels were obtained after extraction using distilled water.

2.2.2. Surface Characterization. The surface chemistry of the grafted hydrogels at dry state was characterized by attenuated total reflectance Fourier transform infrared spectroscopy (FTIR/ATR, Nicolet Magna-IR 550 Spectrometer Series II, Thermo Nicolet, Corporation Madison, WI) and X-ray photoelectron spectroscopy (XPS, AXIS ULTRA DLD, KRATOS Corporation, Japan). The FTIR spectra with wavenumber ranging from 4000 to 500 cm^{-1} were collected by cumulating 32 scans at a resolution of 4 cm^{-1}. XPS analysis was conducted on a Shimadzu ESCA 750 spectrometer using Mg Kα as the radiation source (the takeoff angle of the photoelectron was set to 45°).

2.2.3. Incorporation Efficiency. The incorporation efficiency was calculated as follows[36]

$$\text{incorporation efficiency}\ (\%) = (W_{zp} - W_{cp})/W_{th} \times 100\% \qquad (1)$$

where W_{zp} and W_{cp} are the weights of grafted zwitterionic hydrogel and pristine hydrogel at dry state, respectively. W_{th} is the weight gain

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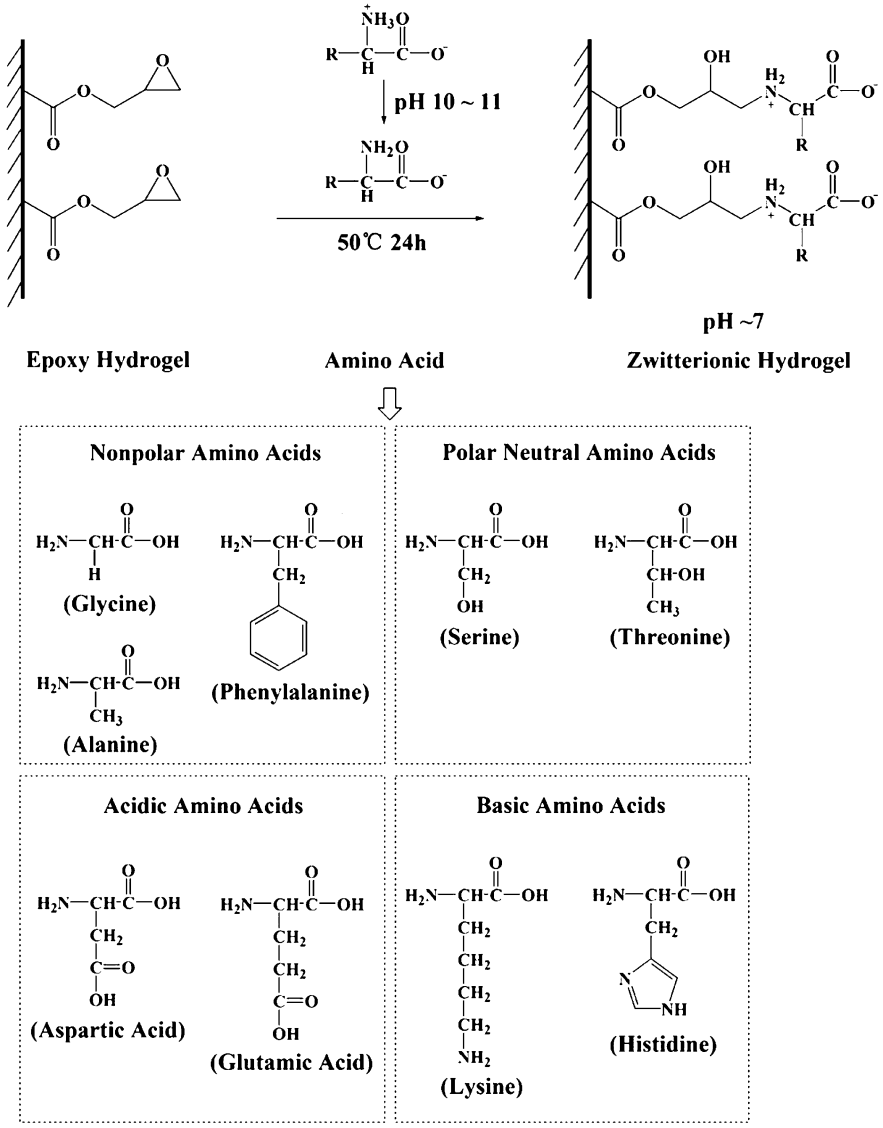
Scheme 1. Reaction Mechanism of Amino Acids Induced Epoxy Ring Opening Reaction for Constructing Zwitterionic Structures



of amino acid assuming all epoxide groups of pristine HEMA/GMA hydrogel are opened by amino groups of amino acids, which equals to the molecular weight of amino acid times GMA moles in the composition of pristine hydrogel. The result reported was an average value of at least three measurements.

2.2.4. Contact Angle. The water contact angle of zwitterionic hydrogels was measured at ambient humidity and temperature by the sessile drop method, using JC2000C1 goniometer of Zhongchen Digital Technical Co. (Shanghai, China). The result reported here was an average value of at least three measurements.

2.2.5. Equilibrium Water Content. The equilibrium water content was calculated as follows

$$\text{equilibrium water content } (\%) = \frac{W_s - W_d}{W_s} \times 100\% \quad (2)$$

where W_s and W_d were the weights of zwitterionic hydrogels at hydrated state and dry state, respectively. The result reported was an average value of at least three measurements.

2.2.6. Protein Adsorption. Protein adsorption resistance evaluation was conducted: amino acid grafted membranes were kept in phosphate buffered saline (PBS) (pH 7.4) for 12 h followed by immersing in 5 mg/mL BSA or lysozyme in PBS for 12 h at 37 °C and then rinsed with 500 mL of fresh PBS twice by a stirring method (300 rpm for 5 min). The adsorbed protein was detached in 1 wt % sodium dodecyl sulfate (SDS) on a shaker at 37 °C for 12 h and the concentration of protein in the SDS solution was determined by the bicinchoninic acid assay method. From the concentration of protein, the amount of protein adsorbed on the surface was calculated.

2.2.7. Preparation and Characterization of Contact Lenses. Contact lenses with the composition HEMA/GMA (91/9, wt/wt) and EGDMA 0.3% (based on the total weight HEMA and GMA) were prepared by UV in the presence of free radical initiated polymerization using polypropylene mold (supplied by Hydron Contact Lenses, China). Grafting of amino acids (Ser, Asp and His) were carried out using the same procedure as that described in section 2.2.1. Finally, the contact lenses were kept in normal saline at room temperature and steam sterilized at 121 °C for 40 min. The as-prepared contact lenses grafted with amino acids have the dimensions of about 14.5 mm diameter and 0.07 mm thickness. The water contact angle of the contact lenses was measured at ambient humidity and temperature by the sessile drop method following the same procedure as that described in section 2.2.4.

The cytotoxicity of the hydrogel contact lenses was evaluated by an extraction test according to the ISO 10993-5 standard.[37] The samples were steam sterilized at 121 °C for 40 min, then immersed in DMEM (10% FBS) at an extraction ratio of 1 cm^2/mL and incubated at 37 °C

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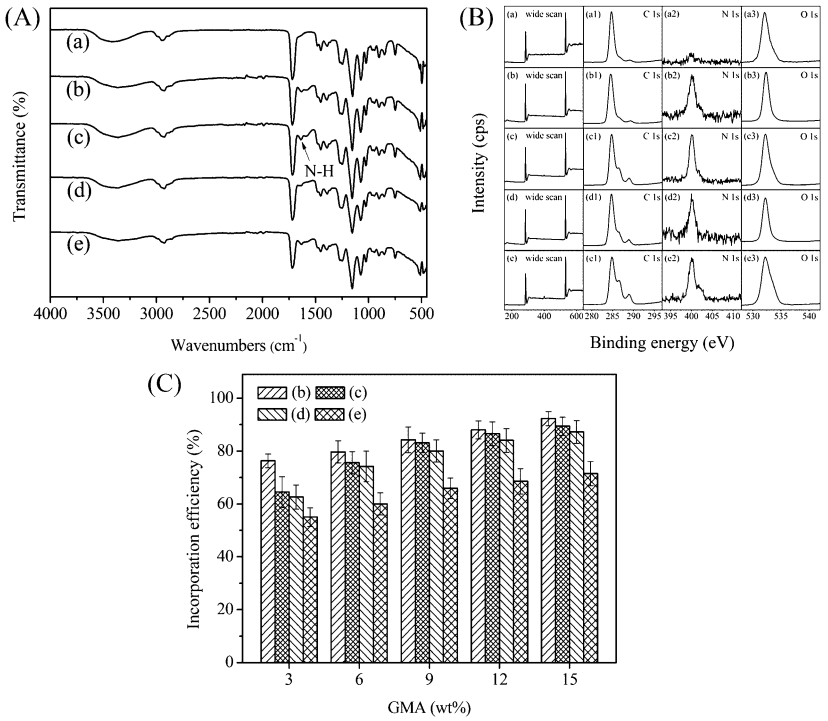

Figure 1. FTIR/ATR (A), wide-scan XPS spectra (B) (with C 1s, N 1s and O 1s core-level spectra coded in the same pattern) and incorporation efficiency (C) of (a) poly(HEMA-*co*-GMA) gel (GMA was 15% based on total weight of HEMA and GMA) and zwitterionic gels grafted with (b) glycine, (c) serine, (d) aspartic acid and (e) lysine.

for 24 h. The extract DMEM solutions were collected for MTT assay. L929 cells were seeded on 96-well plates at a density of 5000 cells/well and cultured in 100 μL DMEM (10% FBS) for 24 h in a humidified incubator (37 °C, 5% CO_2). Then the medium was discarded, 100 μL of the extract solution and 100 μL of DMEM (10% FBS) was added to the sample group. As a blank group, 200 μL of DMEM (10% FBS) was used. Both the sample and blank group were incubated at 37 °C in a 5% CO_2 humidified incubator. On the first, third and fifth day, 20 μL of MTT solution (5 mg/mL) was added into each well and the cells were further incubated for 4 h to allow formation of formazan crystals. Subsequently, the supernatant was removed carefully and 150 μL of dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. The ultraviolet absorbance was measured at 490 nm using the Bio-Rad Model 680 microplate reader (Philadelphia, PA). The cell relative growth rate (RGR) of hydrogel lens extracts was calculated as a percentage of the sample group to the blank group. All data was obtained from an average of six samples.

In vivo wearing evaluation of the contact lenses is performed in rabbit models (weight 2−2.5 kg) supplied by the Animal Center of Southeast University, China (License No. SYXK 2010-0004). The corneas were carefully observed with a slit lamp corneal microscope after dyeing with fluor-strip (Tianjin Jingming New Technological Development). The contact lenses were carefully placed on the cornea of rabbits without anesthetization. After the rabbit eyes wore the contact lenses for 8 h, the contact lenses were collected and rinsed with 100 mL of fresh PBS twice by stirring method (300 rpm for 5 min). The adsorbed protein was measured by the same procedure as that described in section 2.2.6. During the experiment, the rabbits were individually housed in a light controlled room at 20 °C and relative humidity of 50%. Water and food were not restricted. The local Ethics Committees for Animal Experimentation approved all the experiments.

3. RESULTS AND DISCUSSION

3.1. Preparation. The rationale for designing the zwitterionic surfaces was to create a protonated secondary amino cation (PSA) by employing a method of reacting epoxide group with primary amine-click reaction followed by protonation, a deprotonated carboxyl anion (DPC) with variation in the nature of the amino acids. It can be illustrated as shown in Scheme 1. In aqueous solution over appropriate pH ranges, neutral amino acids can exist as zwitterions with no overall electrical charge. After hydroxide ions are added, the pH shifts to a higher value, and the $-NH_3^+$ groups convert to $-NH_2$ groups by removing the hydrogen ions. The nitrogens of the $-NH_2$ groups in the amino acids attack the epoxide groups on the surface of substrates to form secondary amino groups −NH− by ring opening reaction hanging on the polymer network. After that, by adding hydrogen ions, the pH shifts to a lower value approaching 7, and secondary amino groups (−NH−) become protonated secondary amino cations (PSA) as $-NH_2^+-$. As the net charge of amino acids grafted surface is zero in the appropriate pH range of about 7, the amino acids based zwitterionic surface was obtained because of the formation of PSA and DPC. This kind of zwitterion is noted as PSA-DPC.

First, we demonstrate the concept by grafting amino acids on hydrogels. Poly(HEMA-*co*-GMA) hydrogels as substrates were prepared via photoinitiated free radical cross-linking polymerization with different GMA content. After that, a series of different L-α-amino acids were grafted onto poly(HEMA-*co*-GMA) hydrogels. When neutral L-α-amino acids were used, PSA-DPC zwitterionic hydrogels were obtained. When the acidic L-α-amino acids are grafted, the resultant zwitterionic hydrogels with excess carboxyl groups are polar and negatively charged at neutral pH. When the basic L-α-amino acids are grafted, the resultant zwitterionic hydrogels with excess amino groups are polar and positively charged at neutral pH. The progress was monitored by detecting the change of the surface composition and incorporation efficiency as follows.

3.2. Surface Characterization. The surface chemistry of poly(HEMA-*co*-GMA) gel and amino acid grafted zwitterionic

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gels were investigated with ATR-FTIR and XPS analyses. As shown in the FTIR spectra (Figure 1A), take serine zwitterionic gel for an example, compared with the original poly(HEMA-*co*-GMA) gel, the intensity of the epoxy peak (900 cm^{-1}) was lower and the new absorption signal at 1630 cm^{-1} was ascribed to the N−H bending vibration. In addition, XPS analysis was also performed to determine the chemical compositions of gel surfaces. As shown in Figure1B, for all samples, two major emission peaks were observed at 285 eV for C 1s and 533 eV for O 1s. Compared with Figure 1B(a), a new emission peak at 402 eV for N 1s appeared on the surface of zwitterionic gel (from b to e), and amino acids were the only source of nitrogen (N). The results confirm that the amino acids were successfully grafted onto the poly(HEMA-*co*-GMA) hydrogel surfaces. Moreover, the atomic percentage on the zwitterionic gel surfaces is summarized in Table 1. According to the data, the nitrogen (N 1s) content of poly(HEMA-*co*-GMA) gels grafted with three amino acids including glycine, serine and aspartic acid (1.06%, 1.13% and 1.19%, respectively) were remarkable higher than that of the lysine based zwitterionic gel (0.68%), indicating that the grafting of the three amino acids was more effective compared with lysine.

Table 1. Surface Element Composition (at. %) of Poly(HEMA-*co*-GMA) Gels (GMA 15%) before and after Grafting with Amino Acids Determined by XPS

gels	C 1s	N 1s	O 1s
poly(HEMA-*co*-GMA) gel	70.08	0	29.92
poly(HEMA-*co*-GMA) gel−glycine	72.08	1.06	26.86
poly(HEMA-*co*-GMA) gel−serine	72.20	1.13	26.67
poly(HEMA-*co*-GMA) gel−aspartic acid	72.31	1.19	26.50
poly(HEMA-*co*-GMA) gel−lysine	70.35	0.68	28.97

Incorporation efficiency of zwitterionic gels was monitored by a gravimetry method (Figure 1C). The results revealed that the incorporation efficiency of zwitterionic gels increased along with increasing GMA content in pristine gel component, confirming that amino acids were successfully incorporated into the hydrogels. The experiment values were smaller than theory values, which seems to be inevitable as the reaction efficiency was restricted by the steric hindrance effect between reaction groups and diffusion of amino acids into hydrogels. We noticed that the incorporation efficiency is in the range of 55%−90%. Apparently, most epoxy groups are opened by grafting of amino groups. Thus, we assumed that the amino acids were not only grafted onto hydrogel surface but also incorporated into hydrogel interior parts. Besides, the incorporation efficiency of zwitterionic gels grafted with three amino acids including glycine, serine and aspartic acid were significantly higher than that of lysine based zwitterionic gel, as indicated in Figure 1C.

The incorporation efficiency should be affected by the swelling of gel in ethanol aqueous solution containing different amino acids, whereas the reaction activity of amino acid with epoxy groups also played a role in the grafting. Although the different hydrophilicity of amino acid had an effect on the swelling of hydrogel, the solvent especially ethanol had the most significant influence. After trials, 25 vol % ethanol aqueous solution was chosen as the solvent, inducing gel swelling to a proper extent during grafting (with the amino acid concentration of 1.0 mol/L). It was found that most of epoxy groups were opened by grafting of amino groups with high incorporation efficiency (55% ∼ 90%). In other words, the solvent played a leading role in swelling and mainly determined the degree of reaction. Meanwhile, the difference of the reaction activity of amino acid with epoxy groups and the change in swelling due to the different hydrophilicity of the amino acid ligands contributed to the change of the incorporation efficiency. However, the click reaction of primary amine groups of amino acid with epoxy groups occurred easily at room temperature as observed in this research, which may have similar activity to classic primary amine−epoxy ring opening. Therefore, the difference of the incorporation efficiency may be mainly ascribed to the change in swelling due to the different hydrophilicity of the amino acid ligands.

3.3. Hydrophilicity. Incorporation of all selected amino acid species led to hydrophilicity improvement of hydrogel surfaces, and contact angles decrease from 80 to near 15° as revealed in Figure 2A, which is relevant to nature of certain amino acids. Zwitterionic hydrogels based on polar and charged amino acids showed greater decrease of contact angle, whereas those grafted with hydrophobic amino acids showed lesser decrease. The most prominent decreases were observed in samples grafted with charged amino acids. This result corresponded to the concept that ionic interaction between ions and water molecules are stronger than hydrogen-bond, so that an ionic surface is more hydrophilic than a neutral surface. However, the lysine modified surface showed the least improvement in hydrophilicity, which may be ascribed to the lower grafting being consistent with the surface nitrogen content (Table 1) and the incorporation efficiency (Figure 1C). In addition, equilibrium water contents of zwitterionic

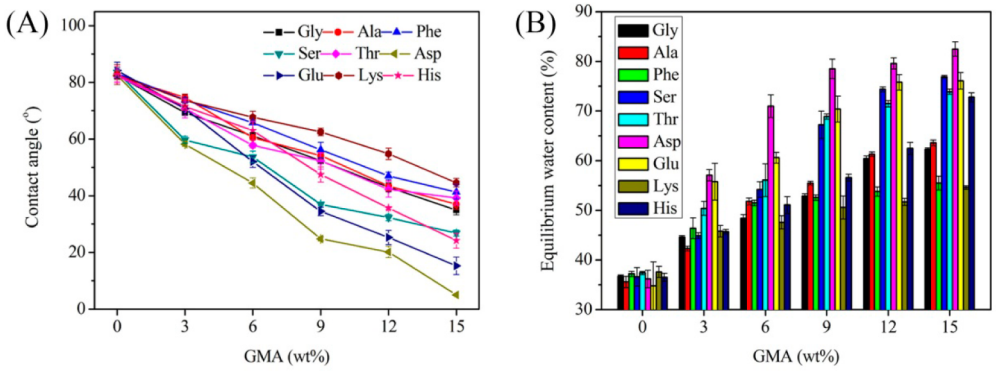

Figure 2. Dependence of water contact angle (A) and equilibrium water content (B) of zwitterionic hydrogels grafted with amino acids on GMA content.

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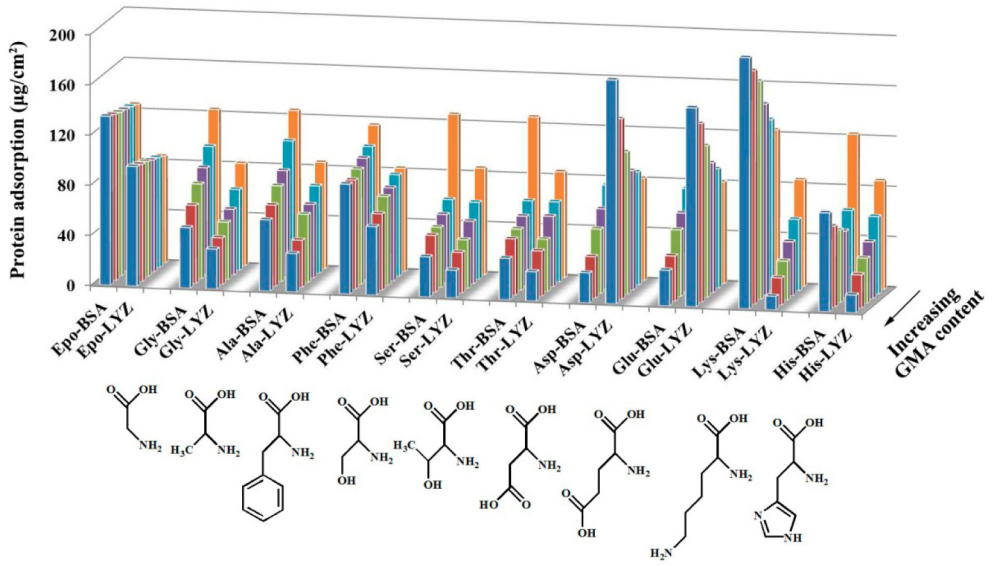

Figure 3. Protein adsorption onto zwitterionic surfaces of hydrogels grafted with different amino acids, pristine hydrogel surfaces and epoxide hydrogel surfaces (noted as Epo-BSA and Epo-LYZ).

hydrogels are given in Figure 2B. All nine zwitterionic hydrogels showed increasing water content, with the most significant increase approaching near 200% of pristine poly(HEMA-*co*-GMA) gels water content value. The equilibrium water contents of glycine, serine and aspartic acid grafted hydrogels were higher than that of hydrogel grafted with lysine. It may be attributed to their higher incorporation efficiency in comparison with lysine grafted gel (Figure 1C).

The enormous improvement in the material bulk properties coordinated with the fact that amino acid was incorporated onto the hydrogel surface and into hydrogel bulk as well, which was verified by the weight increase of gel before and after amino acid incorporation. This may be caused by a synergy effect of the nature of the copolymer and the incorporation of amino acids. The majority of copolymer matrix was HEMA, which can highly swell in a mixture of water and ethanol. When the hydrogel surface was grafted with amino acids, it becomes more hydrophilic and swells further. Because the degree of cross-linking is relatively low, the copolymer network can expand to allow amino acids to diffuse into the internal part and react with interior epoxy groups, which in turn causes more swell. As a result, amino acids were not only grafted onto gel surface but also incorporated into the interior part. Hydrogel grafted with diverse amino acids showed different improvement in water content and the regular pattern is similar to that with contact angle. The hydrogels grafted with more polarized amino acids performed much better wettability than those grafted with amino acids with alkyl or aromatic residuals.

3.4. Protein Adsorption. Protein adsorption is one of the important phenomena for evaluating the antifouling properties of materials for application in bioengineering field. Zwitterionic hydrogels are divided into four groups for better illustration according to the amino acids grafted: (1) nonpolar amino acids, (2) polar neutral amino acids, (3) acidic amino acids and (4) basic amino acids. Two proteins with different charge states are used as target protein, namely BSA with negative charges and lysozyme (LYZ) with positive charges.

The amino acid grafted hydrogel surface is highly hydrated due to the zwitterionic structure of amino acid. In neutral solution, the secondary amino group −NH− residual protonated (PSA) and carboxyl group −COOH residual deprotonated (DPC) carry a positive charge and a negative charge respectively, resulting in the formation of a PSA-DPC zwitterion. Water is strongly bound to these charged sites due to ionic solvation and form a dense hydration layer. This layer of water molecules can prohibit protein molecules from approaching toward hydrogel surface and suppress protein adhesion, as indicated in Figure 3.

Nonpolar amino acids possess a nonpolar R group, such as hydrogen atom, hydrocarbon chain, or aromatic side chain. The antiprotein adsorption properties of poly(HEMA-*co*-GMA) hydrogels grafted with three hydrophobic amino acids (glycine, L-alanine and L-phenylalanine) were investigated, as revealed in Figure 3. The results showed that glycine and alanine incorporated in hydrogels suppressed the adsorption of both BSA and lysozyme, and the antiadsorption performance was enhanced with ever increasing grafting amount. When incorporation efficiency approached nearly 90%, the adsorption amount was reduced to approximate 40%, and glycine based zwitterionic hydrogels showed slightly better performance than alanine grafted hydrogel. However, phenylalanine based zwitterionic hydrogels seemed to be less resistive to protein adsorption due to two competitive effects: On one hand, the zwitterionic structure of incorporated phenylalanine improved hydrogel hydrophilicity, which was verified by the contact angle and water content data. On the other hand, the grafting may induce protein to foul onto hydrogel through the hydrophobic effect between phenylpropyl group and hydrophobic residuals in the protein molecules. The synergy of the two effects resulted in a weak resistance to protein adsorption.

Polar neutral amino acids have a neutral but polar R group. L-Serine (Ser) is the simplest and most representative polar neutral amino acid that can be thought as a version of L-alanine (Ala) with a hydroxyl group attached. The antiadsorption performance of serine based and threonine based zwitterionic hydrogels against BSA and LYZ is also given in Figure 3. Significant reduction to both protein adsorptions was observed even under low grafting degree. This may be a result of synergy of zwitterion interfacial hydration and strong hydrophilicity of hydroxyl group in serine and threonine residual. These two

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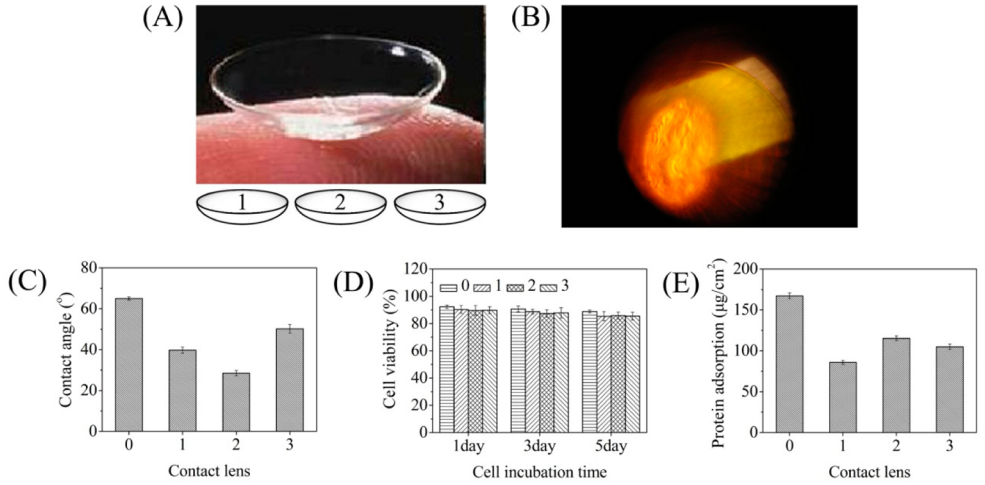

Figure 4. (A) Photo of amino acid grafted soft contact lens; (B) photo of the rabbit eye wearing amino acid grafted soft contact lens observed by the slit lamp corneal microscope; contact angle (C), cytotoxicity (D) and protein adsorption (E) of soft contact lenses (0 was conventional polyHEMA contact lens; 1, 2 and 3 were soft contact lenses grafted with Ser, Asp and His, respectively).

factors improved material surface wettability and enhance the hydration layer between hydrogel and protein, thus strongly resisting protein fouling.

L-Aspartic acid (Asp) and L-glutamic acid (Glu) have an R group with an extra carboxyl group, negatively charged at physiological pH. The anti-BSA fouling performances of Asp and Glu grafted hydrogels are shown in Figure 3. When BSA was used as the target protein, significant resistivity was observed for both amino acids grafted hydrogels. The antiadsorption performance was as good as that of a polar neutral amino acid incorporated hydrogel. However, when the target protein was changed to lysozyme, the hydrogel seemed to shift to protein attractive and an increase of adsorption amount along with increasing grafting degree was observed. Given both Asp and Glu grafted hydrogel surfaces were negatively charged, electrostatic interaction between hydrogel surface and protein should be considered. Because the hydrogels have net negative charges, and BSA is negatively charged, the electrostatic interaction between the hydrogels and BSA is repulsion, protein adsorption is reduced. Lysozyme has net positive charges and the electrostatic interaction between material and protein is attraction, thus enhancing the adsorption of lysozyme.

Two different basic amino acids, L-histidine (His) and L-lysine (Lys), were used to incorporate into epoxide hydrogel and the antiprotein adsorption properties of resultant zwitterionic hydrogels are also given in Figure 3. Hydrogels incorporated with His showed increasing resistivity to lysozyme along with increasing grafting amount; but with regard to BSA, the adsorption amount first decreased then increased with increasing grafting amount. As the case in negatively charged amino acids, the antifouling performance is the result of competitive effect of zwitterion repelling and electrostatic interaction. The difference is that in Asp and Glu the extra carboxyl group is strongly ionized, but in His the imidazole group is a relatively weak polar group. At low grafting degrees, the zwitterion repellant effect played the major role and exhibited protein resistance characteristics. At high grafting degrees hydrogel surface ionic strength further enhanced, and the electrostatic interaction between protein and hydrogel surface became stronger and increased protein fouling. Hydrogels incorporated with Lys showed an opposite regular pattern. BSA is negatively charged at pH 7 and thus more likely to absorb to the positively charged amine groups on the Lys modified hydrogel surface. The high level of adsorption to the basic membrane is again consistent with a significant electrostatic contribution arising from the attractive interactions between the negatively charged BSA and the positively charged membrane, as indicated in the literature.[33]

3.5. Applications. To evaluate the potential application of our concept, a couple of zwitterionic hydrogel soft contact lenses grafted with Ser, Asp and His as examples were fabricated (Figure 4A) using polypropylene mold. All the grafted contact lenses have better hydrophilicity than that of conventional polyHEMA contact lens, as revealed in Figure 4C, and the Asp grafted soft contact lens had lowest contact angle of 28.5°. MTT assay was used to evaluate the cytotoxicity of the hydrogel contact lenses. As revealed in Figure 4D, the RGRs of all the amino acid grafted zwitterionic hydrogels were above 85%. The results indicated that the amino acid grafted contact lenses were noncytotoxic.

In vivo wearing evaluation was performed using a rabbit model checked by slit lamp corneal microscope (Figure 4B) and the protein adsorption on the surface of the contact lens was measured by bicinchoninic acid assay (Figure 4E) after 8 h of wearing. It was found that all the amino acid grafted contact lenses had much lower protein adsorption in comparison to conventional polyHEMA contact lens. Meanwhile, the soft contact lens incorporated with Ser has the lowest protein deposition. Apparently, the amino acid grafted zwitterionic hydrogel contact lenses show effective antifouling property which can be attributed to the formation of PSA-DPC pairs of protonated secondary ammonium cations and deprotonated carboxyl anions. Therefore, common canonical amino acids grafting by epoxy ring opening reaction may have applications in combating biofouling by introducing zwitterions of PSA-DPC pairs.

4. CONCLUSIONS

In summary, we have presented for the first time a simple and efficient strategy for constructing antifouling surfaces by click reaction—grafting of the library of natural amino acids onto polymer substrates. As an example, the zwitterionic poly(HEMA-*co*-GMA) hydrogels were found to exhibit improved

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hydrophilicity after grafting with amino acids by ring opening reaction of epoxy groups. The antiprotein adsorption performance of the grafted zwitterionic hydrogels was depended on the nature of incorporated amino acid. The zwitterionic hydrogels incorporated with neutral amino acids had nonspecific antiadsorption capability to both BSA and lysozyme because of the formation of protonated secondary amino groups ($-NH_2^+-$) and deprotonated carboxyl groups ($-COO^-$) zwitterionic pairs by primary amino−epoxy click reaction. The optimal antiadsorption performance was observed on the zwitterionic hydrogels incorporated with polar amino acids with a hydroxyl residual. The application trial of amino acids based hydrogel contact lenses has demonstrated the success of amino acids based PSA-DPC zwitterionic antifouling strategy in ophthalmology. This strategy is also applicable to substrates including hydrogels, microspheres, nanofibers and filtration membranes as well, and the results will be published in the near future. Moreover, the advantages of the present strategy include abundance of raw materials (libraries of epoxy grafted substrates and amino acids), easy fabrication (one step reaction) and good biocompatibility.

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Notes

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ABBREVIATIONS

BSA, bull serum albumin
DMSO, dimethyl sulfoxide
DPC, deprotonated carboxyl anion
EGDMA, ethylene glycol dimethacrylate
FTIR/ATR, attenuated total reflectance Fourier transform infrared spectroscopy
GMA, glycidyl methacrylate
HEMA, 2-hydroxyethyl methacrylate
LYZ, lysozyme
PBS, phosphate buffered saline
PSA, protonated secondary amino cation
SDS, sodium dodecyl sulfate
RGR, relative growth rate
XPS, X-ray photoelectron spectroscopy
Ala, L-alanine
Asp, L-aspartic acid
Glu, L-glutamic acid
Gly, glycine
His, L-histidine
Lys, L-lysine
Phe, L-phenylalanine
Ser, L-serine
Thr, L-threonine

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