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Charge Tunable Zwitterionic Polyampholyte Layers Formed in Cyclic Olefin Copolymer Microchannels through Photochemical Graft Polymerization

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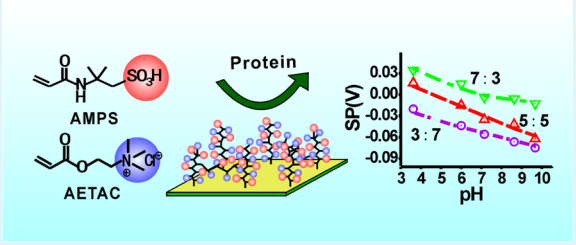
ABSTRACT: Zwitterionic layers immobilized on various surfaces exhibit ideal biocompatibility and antifouling capability, but direct immobilization of zwitterionic molecules provides limited choice of surface charges. In this paper, the formation of charge tunable zwitterionic polyampholyte layers onto the surface of microfluidic channels of cyclic olefin copolymer by photochemical graft polymerization of mixed acrylic monomers, [2-(acryloyloxy) ethyl] trimethyl ammonium chloride and 2-acrylamido-2-methyl-1-propanesulfonic, under UV illumination was reported. With this method, surface charge of the resulting modification layers could be tailored through the initial monomer ratio and reaction conditions. The incorporation of both monomers into the grafted layers was confirmed by X-ray photoelectron spectroscopy (XPS) and attenuated total reflection Fourier transform infrared (ATR-FTIR). The results indicate that the modified layers are hydrophilic with contact angles of 33.0−44.3°, and the isoelectric points of the modified layers can be tuned from <3 to >9 simply by adjusting the monomer ratios. Elimination of the nonspecific adsorption of proteins on the zwitterionic layers thus formed was proved by fluorescent microscopy and streaming potential measurement. The uniformity of the modified layers was verified through a comparison of electrophoresis inside the modified and native microchannels. A whole blood coagulation time measurement was performed to show its applicability.



KEYWORDS: *surface charge, tunable, zwitterionic, streaming potential, protein, nonspecific adsorption*

INTRODUCTION

Nonspecific adsorption of proteins and other biomolecules might cause adverse effects in many applications.[1] Despite overwhelming efforts on the elimination of these effects, there are limited protocols that are universally applicable to various surfaces presumably due to the complexity of biomacromolecules. Exploring novel surface modification methods is still necessary. Among the substances that have been used for this purpose, natural origin zwitterionic compounds such as betaine[2] and phospholipid[3,4] have been proved to have very good antiadsorption performance, which inspires many studies on zwitterionic surfaces for the elimination of nonspecific adsorption. Whitesides' group has systematically studied the surfaces of self-assembled monolayers (SAMs) on gold with charged groups, and they found the SAMs fabricated from a 1:1 mixture of positive and negative charged groups could effectively eliminate the adsoption of fibrinogen and lysozyme.[5] Chang et al. modified gold by surface-initiated atom transfer radical polymerization of sulfobetaine methacrylate (SBMA) to form polymer brushes that showed remarkable reduction of protein adsorption from blood plasma.[6] Zwitterionic modification has also been used in reverse osmosis[7] as well as nanoparticle capping to minimize nonspecific cell uptake[8] and nonspecific protein adsorption.[9]

The methods used to introduce zwitterionic layers are diverse. To name a few, Kuo et al. immobilized a copolymer of zwitterionic SBMA and acrylic acid onto tissue culture polystyrene, polyurethane, and polydimethylsiloxane (PDMS) with layer-by-layer polyelectrolyte films through electrostatic interaction, and the result suggested its potential to be used in blood contacting biomedical devices.[10] Keefe et al. modified the surface of PDMS by the super hydrophilic zwitterionic poly(carboxybetaine methacylate) (pCBMA), which effectively suppressed protein adsorption from complex media.[11] Zhao et al. have successfully introduced poly(SBMA) onto the polypropylene (PP) surface through UV-induced graft polymerization of hydroxyethyl methacrylate and then surface-initiated atom transfer radical polymerization of SBMA after coupling with 2-bromopropionyl bromide, and the modified PP showed significantly reduced BSA fouling.[12]

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All these works proved that zwitterionic surfaces were promising to eliminate the nonspecific protein adsorption or fouling, but zwitterionic compounds like sulfobetaine normally possess both positively and negatively charged groups in the same molecules; the ratios of these groups are fixed. Due to the huge variation of the surface properties and their applications, direct immobilization of the zwitterionic molecules is somehow cumbersome, and the resulting surfaces may biased far from their isoelectric points (pIs) and other characteristics of the free molecules. To facilitate the formation of antifouling surfaces, Jiang's group constructed zwitterionic hydrogel and monolayers through mixed positively and negatively charged compounds through techniques such as two-component atom transfer radical polymerization.[13,14] Nonfouling polymer brushes were also prepared using surface-initiated two-component atom transfer radical polymerization by the same group.[15] The resulting surfaces exhibited high resistance to nonspecific adsorption of proteins; however, the procedures to make the surfaces are complicated and hard to be implemented in materials without active groups, such as cyclic olefin copolymer (COC).

Lab-on-a-chip (LOC) devices are considered to be a novel miniaturized platform for biological applications. Polymers have been widely used in the fabrication of these devices,[16−18] while the notorious nonspecific adsorption of biomacromolecules by pristine polymer surfaces may give rise to some serious detrimental consequences, such as poor separation efficiency and analyte loss. Surface modification is therefore indispensable for achieving better analytical performance. Protocols of surface modification can be roughly classified as permanent surface modification and dynamical coating.[19,20] Permanent modification introduces functional groups onto the surface through chemical bonding or very strong physical adsorption, which increases the surface hydrophilicity and governs the electroosmotic flow.[21,22] It has been broadly applied in electrophoretic separations of proteins and DNA fragments.[23] Dynamic coating can be realized through addition of chemicals that are prone to change the surface properties into the solutions. The required surfaces are obtained through the dynamic interaction between additives and the surfaces.[24,25] Dynamical coating is simple to apply; however, its chemical or mechanical stability is not as good as permanent modification in the long term, so permanent modification is preferred in many real applications.[26,27]

Photoinitiated graft polymerization has been successfully used for permanent surface modification.[28] For example, Hu introduced dimethylacrylamide (DMA) and poly(ethylene glycol)monomethoxyl acrylate (PEG) onto the PDMS surface through graft polymerization for electrophoretic separation of the peptide sequences.[29] COC has been proved as an ideal substrate of LOC devices[30] and is suitable to be modified with photochemical graft polymerization too.[31−33] A broad range of monomers, with acidic, basic, and zwitterionic groups, have been introduced into COC microchips to get hydrophilic surfaces, for the purposes including electroosmosis modulation, physical adsorption elimination, and online sample processing inside channels.[34−40] We have attempted to tune the surface charge and hydrophilicity of COC microchannels with photochemical graft polymerization of acrylic monomers. The applicability of these modified microchannels for microchip electrophoresis was demonstrated.[41] Six monomers with different functional groups were used separately because surface charge was the main concern, but these surfaces possessing single charged groups might not be competent in elimination of nonspecific adsorption of proteins.

In the present work, photochemical graft polymerization of two monomers, one with cationic groups and the other with anionic groups, is performed to form zwitterionic surfaces onto COC microfluidic chips. Through mixing of two monomers in different ratios, surfaces with various pIs were obtained simply by UV irradiation of the chips for a short period of time. The monomers used in the work, [2-(acryloyloxy) ethyl] trimethyl ammonium chloride (AETAC) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), are easy to get and handle. The most significant merit of the present work relies on its facile formation of zwitterionic polyampholyte layers directly onto the inner surface of COC microchannels and simple pI control through the initial reactant ratio. The effects of the experimental conditions on hydrophilicity, surface charge, and capability of antiadsorption were evaluated by contact angle, streaming potential, and observation of adsorption of FITC-labeled BSA under a fluorescence microscope. The effectiveness of reduction of nonspecific protein adsorption of the zwitterionic polyampholyte layers was confirmed with a blood coagulation time measurement in modified COC microchannels.

EXPERIMENTAL SECTION

Materials. Benzophenone (BP, >99%), ethanol, Na_2HPO_4, and NaH_2PO_4 were purchased from the Tianjin Guangfu Fine chemical Research Institute (Tianjin, China). Fluorescein isothiocyanate (FITC), 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS, 99%), [2-(acryloyloxy) ethyl]-trimethyl ammonium chloride (AETAC, 80% aqueous solution), bovine serum albumin (BSA), fibrinogen (FBG), and lysozyme (LYZ) were obtained from Sigma-Aldrich. Sodium tetraborate was from the Xi'an Chemical Reagent Factory (Xi'an, China). Phosphoric acid, sulfuric acid, and sodium hydroxide (analytical grade) were purchased from Tianjin Hongyan Chemical Reagent Factory (Tianjin, China). Glycine, glutamic acid, arginine, and leucine (analytical grade) were obtained from Shanghai Zhongqin Chemical Reagent Factory (Shanghai, China). Distilled water was used in all experiments.

Fabrication of COC Plates and Microfluidic Chips. COC plates were thermoformed from resin granules at a temperature of 180 °C into square plates (1250 × 1500 mm, 1 mm thick). The plates were cut into the desired sizes, and each small plate was annealed at 50 °C for 120 min to improve the surface smoothness and release the internal stress that might come from the thermoforming process. The annealed plates were used for the modification and other experiments.

Microfluidic chips were made by the same procedure described previously[42] with a little variation of temperature. Briefly, a blank plate of COC (15 mm × 55 mm) was sandwiched between two microscope slides with copper wire (80 or 50 μm diameter) stretched on one of the slides. The assembly was then fixed with six binder clips and heated in an oven at 140 °C for 25 min. After cooling, the copper wires were etched away in nitric acid for about 15 min. The microchannels used for blood coagulation time measurement were formed in the same way using stainless steel rods of 0.9 mm diameter instead of copper wires, and the rods were pulled away after the assembly was cooled to room temperature. Holes (3 mm in diameter) were drilled at the appropriate location on the channels which act as access holes. After cleaning in ethanol, the plate with microchannels was bonded with another blank COC plate of the same size thermally at 122 °C for 10 min. Small pieces of plastic tube (8 mm long and 6 mm in diameter) were glued at the access holes as the solution reservoirs. Microchannels of the microchips used for streaming potential and blood coagulation measurement were parallel and for electrophoresis were arranged to a cross configuration.

Surface Photochemical Modification. Two 250 W high-pressure mercury lamp bulbs placed side-by-side (5 cm apart) were

used as an UV light resource in the photochemical modifications to provide uniform and strong irradiation. The irradiation intensity was controlled by the distance between the COC plate and the bulbs and calibrated by an ultraviolet irradiation meter (UV-A, Photoelectric Instrument Factory of Beijing Normal University, Beijing, China). Modification solution contains 10% of a monomer mixture (AETAC/AMPS) and 0.5% BP as the photoinitiator. For the COC plate modification, the modification solution was applied on the plate, and the thickness of the solution film was controlled by a pair of spacers placed between the COC plate and a quartz plate. UV radiation (7.6 mW/cm^2) was allowed to reach the monomer solution and initiate the graft polymerization reaction for the desired time (30−300 s). For microchannel modification, the graft polymerization solution was filled into the channel, and the microchip was put under UV (5.0 mW/cm^2 for chip) for a given time (Figure 1). After the reaction, the channel

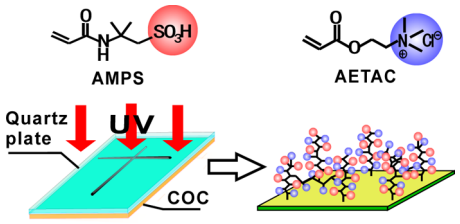

Figure 1. Structure of two monomers and schematic illustration of COC microchip modification.

was rinsed with ethanol to remove free polymer and unreacted monomers. At least three COC plates or microchips were modified for each modification condition to ensure the reliability of the surface characterization or application.

Surface Characterization. The hydrophilicity of the COC surface was evaluated by the contact angle measurement as described earlier.[41] X-ray photoelectron spectroscopy was achieved using an ESCALAB 210 Spectrometer (VG, UK) equipped with a twin anode (MgKa/AlKa) source as the excitation radiation at 300 W. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) spectra were recorded by a Nicolet iS10 FTIR spectrometer. An atomic force microscope (AFM, Agilent SPM 5500) in tapping mode was used to evaluate the thickness of modified polyampholyte layers. The surface charge of the microchannels was characterized by streaming potential measurement as described previously.[43]

Adsorption of Protein on a COC Microfluidic Chip. BSA, FBG, and LYZ with concentrations of 500 μg/mL in PBS, pH 7.4, were used in the adsorption experiment. Generally, both unmodified and modified COC microchannels and plates were exposed to FITC-labeled BSA (labeling procedure as described by Verzola et al.[44]) for 20 min, after proper rinsing with blank PBS, and streaming potential was measured. Fluorescence images were taken with excitation by an ultrabright blue LED (filtered with a 420−490 nm band-pass filter, Shenyang HB Optical Technology Co., Ltd., Shenyang, China) after a long-pass filter with a cutoff wavelength of 520 nm.

Microchip Electrophoresis. The high voltage for the electrophoresis was supplied by a high voltage module (DW-P602, Dongwen High-Voltage Power Supply, Tianjin, China), which was controlled by a program written in Labview (National Instruments) together with a three-way solenoid valve and data acquisition of the laser-induced flourescence (LIF) signal. Sample injection detail was described previously.[45,46] The electropherograms were recorded with a LIF detector similar to that described previously,[47] but an APD (AD500-8-TO52S2, Silicon sensor, Germany)[48] was used instead of PMT.

Whole Blood Coagulation Time Measurement. The fresh blood was collected from the orbital sinus of rats immediately before the measurement. The blood was filled into one reservoir of the microchip as soon as possible and timing was started. The microchip was then tilted gently back and forth to let blood flow through the channel from one end to the other. The time required for the blood flow to stop was taken as the coagulation time. The microchannel was checked under a stereo microscope (NOVEL NTB-4B, Ningbo, China) to make sure the stop of flow was caused by blood coagulation. The effective length of the microchannel was 5.20 cm. The velocity of the blood flow into the channel was calculated through the time it took to travel a certain distance.

RESULTS AND DISSCUSSION

Hydrophilicity. Zwitterionic groups can be introduced into solid surfaces through physical or chemical anchoring. However, pI, which is a critical parameter of the zwitterionic substances, of the obtained surface is presumably unchangeable, the same as that of the anchored molecules if they are directly anchored. Although there are plenty of zwitterionic molecules available, tailoring of the pI is cumbersome because different molecules may need different immobilization protocols. To develop a universal way to form a zwitterionic surface is therefore important. Fréchet et al. have proved that butyl acrylate and AMPS could photochemically copolymerize on the surface of COC,[33] which implies a possibility to use two monomers with different charges, i.e., one with cationic and the other with anionic groups to build zwitterionic layers on COC surfaces.

According to this speculation, two monomers of AETAC, with a tertiary amine group, and AMPS with a sulfonic group were used to check its applicability. Since both of the monomers are hydrophilic while COC is hydrophobic, contact angle was used as an indicator of the surface reaction. With BP as a photoinitiator, photochemical graft polymerization of the monomer mixture gave rise to apparent contact angle changes (Figure 2a and b). For all monomer ratios studied (AETAC/

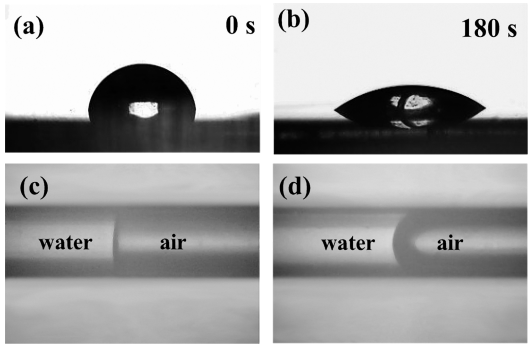

Figure 2. Comparison of the hydrophilicity of COC before and after the photochemical modification with mixed monomers. (a) Unmodified COC plate; (b) modified COC plate with AETAC/AMPS = 5:5 for 180 s; (c) the profile of the air−water interface in an unmodified COC microchannel; (d) the profile of the air−water interface in a COC microchannel modified with AETAC/AMPS = 5:5 for 180 s.

AMPS = 9:1, 7:3, 5:5, 3:7, 1:9), contact angles decreased with the UV illuminating time, as shown in Figure 3. The lowest contact angle was obtained for AETAC/AMPS = 5:5, most probably due to the complete association of both kinds of ionic groups. With a further increase of illumination time, contact angles might rise back after about 300 s, which is consistent with that we observed for single monomers.[41] The unexpected effect of prolonged illumination time on contact angles might be a consequence of increased cross-linking of the polymer chains, which blocked some of the hydrophilic groups buried inside the polymer layers. The UV-caused breakage of linkage

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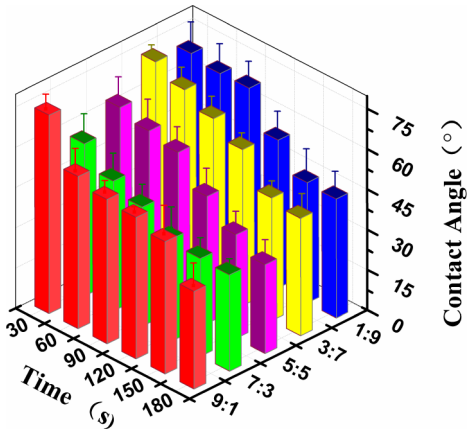

Figure 3. Contact angles obtained with AETAC/AMPS in different monomer ratios along with illumination time. Experimental condition: the monomer concentration was 5%, the photoinitiator concentration was 0.5%, the irradiation times were from 30 to 180 s, and temperature of reaction was 50 °C.

of hydrophilic groups from the polymer backbone could be another reason.

The photochemical graft polymerization of the mixed monomers could also be performed inside sealed COC microchannels by simple illumination of the microchannels that filled with monomer solutions. As shown in Figure 2c and d, the profiles of water−air interfaces in a native COC microchannel and one modified with mixed monomers evidently reflect the change of the hydrophilicity of the surface. These results proved that the COC surface could be successfully modified by the monomer mixture.

Composition. X-ray photoelectron spectroscopy (XPS) and attenuated total reflection Fourier transform infrared (ATR-FTIR) were used to verify the incorporation of both groups into the polymerized layer. Figure 4 shows the XPS survey

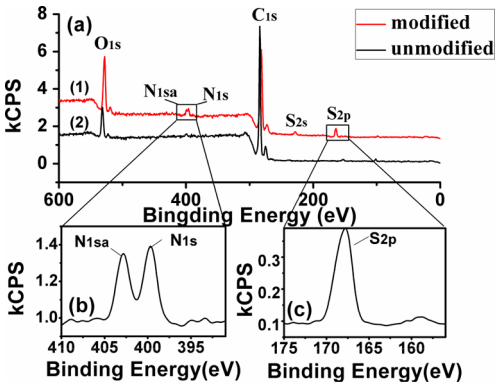

Figure 4. (a) XPS survey spectra of modified COC(1) and native COC(2), (b) magnified peaks of N 1s and N 1sa of modified COC with AETAC/AMPS = 5:5, and (c) magnified characteristic peak of S 2p of modified COC with AETAC/AMPS = 5:5.

spectra of the native and modified COC. For the native COC, no N or S peak was observed, while N 1s at 402.83 eV and S 2p at 167.78 eV were evidently observed for modified COC [Figure 4a(1)]. Contents of N and S atoms evaluated from XPS were 2.6% for N 1sa, 3.0% for N 1s, and 2.6% for S 2p. These values proved that both monomers were introduced into the copolymer at roughly the same ratio of the monomer mixture (5:5). The presence of amine and sulfonic groups on the modified surface was also confirmed by ATR-FTIR. As illustrated in Figure 5, only C−H vibration can be seen at

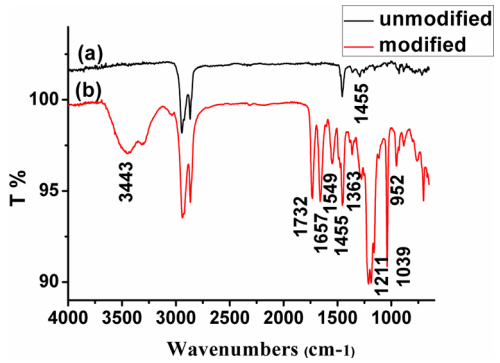

Figure 5. ATR-FTIR of (a) unmodified COC and (b) modified COC with AETAC/AMPS = 5:5.

1455 cm^{-1} for the unmodified COC surface (Figure 5a). For the COC plates modified with AETAC and AMPS, the N−H stretching vibration was observed at 3443 cm^{-1}; the C−O stretching vibration was observed at 1039 cm^{-1}; and the peak at 1211 cm^{-1} should come from the sulfonic group (Figure 5b). The bands at 1657, 1549, and 1363 cm^{-1} can be attributed to the amide carbonyl stretching vibration of AMPS. The carbonyl stretching vibration and bending vibration of AETAC units should be at 1732 and 952 cm^{-1}. All this evidence proved that zwitterionic monomers were successfully grafted polymerized onto the COC surface.

An increase of the thickness of the photochemically grafted polymer layer with UV illumination time has been observed previously.[49] Under the selected condition, the thickness of the modified layer measured with AFM was 45 ± 5 nm, which is near the optimum depth of zwitterionic layers for the elimination of nonspecific protein adsorption.[6]

Surface Charge. To verify the surface charge status, streaming potentials across the microchannels were measured from pH 3.6 to 9.7. For convenient comparison of the results, the conductivity of all measuring solution was maintained at 248 ± 2 $\mu S \cdot cm^{-1}$. As shown in Figure 6a, modification with a binary monomer mixture in different ratios gave different surface charge. When AETAC/AMPS = 3:7, negative streaming potentials were obtained for all measured pHs. When AETAC/AMPS = 5:5, streaming potentials of modified channels were positive at pH lower than 5, which indicated that the surface carried positive charge. At pH > 5, the surface charge turned to negative. For AETAC/AMPS = 7:3, the channels exhibited positive streaming potential when pH < 7 in phosphate buffers: that is, it carries more positive charge, exactly as expected. The pIs for these three layers are <3, 5, and 7, respectively. As a comparison, the surface modified with pure AETAC (AETAC/AMPS = 10:0) carried positive charge at all measured pHs, and that modified with AMPS (AETAC/AMPS = 0:10) carried negative charge in the same range of pHs. These results clearly demonstrated that the surface property can be easily tailored through the adjustment of the monomer ratios. Compared with the traditional way for introducing zwitterionic moieties, the surface properties obtained with the proposed method can be easily tuned by monomer composition.

The effect of the illumination time on the streaming potential of modified channels is shown in Figure 6b. Only moderate changes in streaming potentials were observed when the

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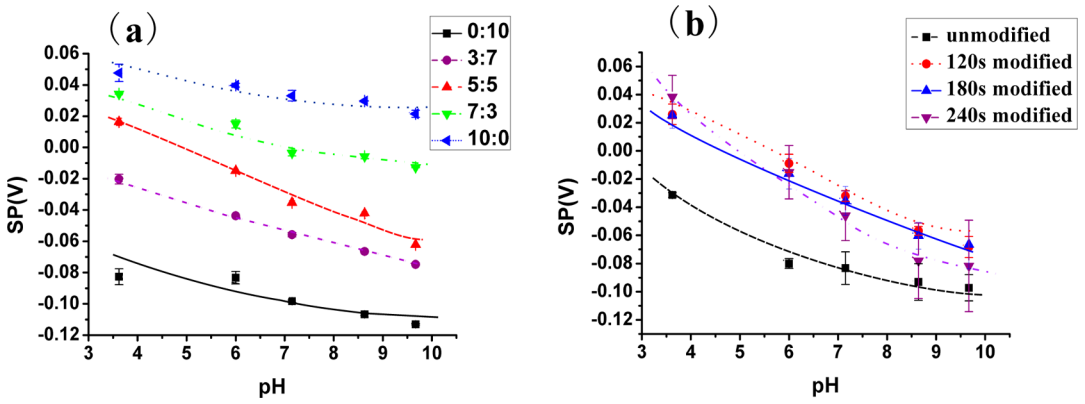

Figure 6. Streaming potentials of (a) COC microchannels modified with the mixture of AETAC and AMPS at various ratios and (b) modified COC microchannels at various pHs for different UV illumination time. Experimental conditions: the monomer concentration was 5%, the photoinitiator concentration was 0.5%, the temperature of reaction was 50 °C, and the irradiation intensity of UV illumination was 5.0 mW/cm^2.

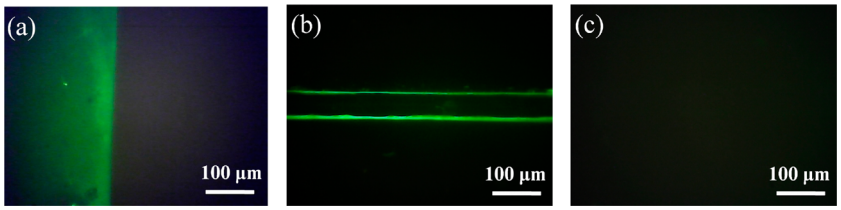

Figure 7. Fluorescent images of (a) the partially modified COC plate (left half unmodified by blocking the light), (b) a native COC microchannel, and (c) a modified COC microchannel after treatment of FITC-BSA.

illumination time increased from 120 to 240 s. This result implied that the surface charges were mainly dependent on the modified polymer layer after 120 s. Also, when illumination time increased to 240 s, the measured streaming potential became unstable, as indicated by a significant increase of the standard deviation for multiple measurements. The irregularity of streaming potential after long time illumination was related to the increased cross-linking and possible decomposition polymerized layers as mentioned previously. The accumulation of defects along with the growth of the modified layers and the elasticity of thick hydrogel-like polymer might be responsible for the unstable streaming potential too.

Protein Adsorption. To confirm the resistance of nonspecific adsorption to proteins on the photochemically modified zwitterionic surfaces, half of a COC plate was modified with a mixture of AMPS and AETAC of 5:5, while the other half was left unmodified by covering this area with aluminum foil during the UV irradiation. After a thorough rinsing, the plate was immersed in a solution containing 2 mmol/L of FITC-labeled BSA for 20 min and then rinsed with the PBS. The fluorescence of the plate was checked on a florescence microscope. As showed in Figure 7a, the unmodified area exhibited strong fluorescence, while the area modified with a binary monomer mixture was dark. Similar experiments performed in microchannels gave the same results. Flushing unmodified microchannels with FITC-BSA rendered strong fluorescence at the channel wall (Figure 7b), while modified channels showed virtually no fluorescence (Figure 7c) after the same flushing.

Because of the presence of labeling groups, the property of a protein may be changed to some extent, and the adsorption behavior obtained with fluorescently labeled protein may deviate largely from the native one. Therefore, adsorption of native proteins on modified and unmodified channels was systematically compared by streaming potential measurement. Streaming potential measurement is a label-free technique, and the comparison is based on the change of surface charge before and after proteins attached to the microchannel surfaces. According to literature results,[1] the adsorption of a protein is highly dependent on the protein property and other conditions such as pH. Therefore, three proteins, BSA, FBG, and LYZ, which have different pIs and molecule weights, were employed as model compounds. Streaming potentials were performed at two extreme pHs for most biological applications, 3.6 and 9.1, to prove the effectiveness of the photochemically modified zwitterionic layers. Figure 8 shows the percentage of streaming

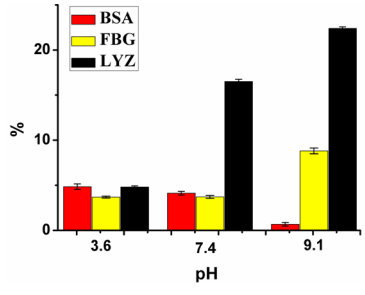

Figure 8. Relative streaming potential change of modified COC microchannels after flushing with proteins at three different pHs, 3.6 (phosphate buffer), 7.4 (phosphate buffer), and 9.1 (borax buffer).

potential changes before and after flushing of proteins in modified microchannels. For clarity, streaming potential change caused in unmodified microchannels was taken as 100% for each protein and pH condition, and the data in the figure represent the relative streaming potential change with regard to that of unmodified. At pH 3.6, all proteins caused a rather small change (<4.8%) in streaming potential, indicating effective

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elimination of the adsorption of these proteins. While at pH 9.1, although effective elimination of adsorption of BSA was observed, the streaming potential change caused by LYZ was still quite large (22.4%). Streaming potential change due to the flushing of FBG was about 8.8%. At the physiological pH, 7.4, the situation was between these two extremes, and the streaming potential change caused by BSA (4.1%) was smaller than that at pH 3.6 but larger than that at pH 9.1. For FBG, the value was almost the same as that at pH 3.6, while for LYZ, apparent streaming potential change was found (16.5%). These results could be explained by the surface charge interaction. The pIs of BSA, FBG, and LYZ are 4.7, 5.6, and 11.1, respectively. At pH 3.6, they all carry positive charge, and the modified surface carries positive charge too. So, adsorptions of these proteins were inhibited by the charge repulsion. At pH 9.1, the situation is quite different because the modified surface is negative, which expels molecules with negative charges like BSA and FBG, and flushing with these two proteins gave rise to 0.67% and 8.8% of change in streaming potential of modified channels. At pH 7.4, surface charge was negative too; however, its density was lower, so only LYZ caused evidence streaming potential change. It should be mentioned that adsorption of FBG, which is an important protein in adhesion and aggregation of platelets, was greatly inhibited (only 3.7% of streaming potential change) at this pH.[6]

For LYZ, significant streaming potential change was obtained, most probably because of the positive charge of LYZ at this pH. If the microchannel was modified with AETAC/AMPS ratios of 7:3 and 9:1, the surface carries less negative charges than that obtained with AETAC/AMPS ratio of 5:5, and the streaming potential changes were 18.3% and 11.0%, respectively, just as expected. Previous research demonstrated that zwitterionic polymers can take up large quantities of free water molecules and form a stable hydration layer to prevent protein adsorption.[50] The results obtained here implied that even for zwitterionic layers surface charge was still a factor that affected the adsorption of proteins. Tailoring the surface property of zwitterionic layers is necessary for efficient elimination of nonspecific adsorption.

Uniformity. Surface uniformity of the modified microchannels was confirmed with microchip electrophoresis because the separation efficiency of free solution electrophoresis was highly affected by the microchannel surface status. If the surface charge is not uniform along the microchannel, uneven electroosmosis at different locations of the microchannel might induce complicated flow pattern and cause severe dispersion of analyte bands. Unexpected analyte adsorption onto the microchannel due to the poor microchannel surface quality might cause similar consequences. As shown in Figure 9a, broad peaks were obtained for FITC-labeled amino acids inside unmodified COC microchannels. On the contrary, with microchannels modified with a mixture of AETAC and AMPS (5:5), highly efficient separation of these amino acids was achieved (Figure 9b). This result indicated that the photochemically grafted polymerization of zwitterionic layers was uniform.

Application. To prove the applicability of the prepared zwitterionic polyampholyte layers, measurement of whole blood coagulation time was attempted. Blood coagulation time is important for the therapies of thrombotic diseases and for caring for patients with bleeding tendencies. Because of the complexity of the whole blood, measuring whole blood coagulation time should be a very suitable way to confirm the

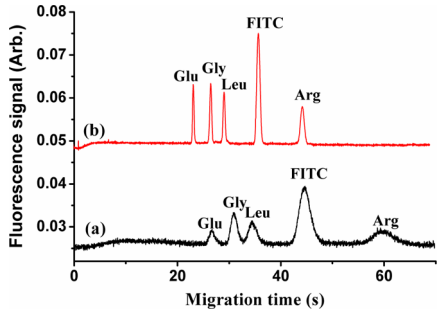

Figure 9. Electropherogram of the separation of FITC-labeled amino acid, (a) using unmodified COC microchannels and (b) using modified COC microchannels. Separation conditions: running buffer was 10 mmol/L of borax, pH 9.2, the separation length was 3.0 cm, the separation voltage was 2000 V, and the concentrations of amino acids were 1 μmol/L.

capability of antibiofouling property of a material. For easier and meaningful comparison of the results, microchannels with inner diameter of 0.9 mm were adopted to match the normal capillary. The results indicated that the freshly collected blood could flow through the channel easily, and the initial flow rate was 0.48 cm/s. Coagulation happened at 9.0 ± 0.3 min, which was consistent with that obtained with a traditional capillary method and within the normal range of 8.5−15 min.[51] As a comparison, in unmodified microchannels of the same size, the blood coagulated in less than 2 min because of its strong interaction with blood components. The flow of blood in the unmodified channel slowed down evidently after it entered the channel and coagulated when it traveled about 1.9 cm. These results demonstrate the applicability of the prepared zwitterionic polyampholyte layer in bioassays. It should be more valuable to use smaller microchannels with such kinds of zwitterionic layers in the measurement of more specific parameters such as prothrombin time and activated coagulation time. In addition, the results also indicate that the modified zwitterionic layers can effectively prevent extra blood coagulation caused by foreign objects, which is of great significance for medical implants and in vivo sensors.[6,52]

CONCLUSIONS

The results reported here proved that zwitterionic layers can be easily formed onto the COC microchannel surface with photochemical graft polymerization of mixed monomers containing different charge groups. The most important advantage of the proposed protocol is its flexibility of adjustment of the composition of the modified layers and their surface charge status. Surface properties such as hydrophilicity, pI, and antifouling capability could all be experimentally tuned. The photochemical approach also makes this method applicable to irregular surfaces, as long as proper measures are taken to ensure the light illumination. The modification area can be accurately patterned through photomasks, and inner surfaces of UV transparent materials can be modified too. Compared with previously reported ways for obtaining zwitterionic layers, which were normally through immobilization of zwitterionic molecules using various coupling routes, this method is simple, fast, and easy to be performed in large scale. It is, in some extent, a universal way to form modification layers carrying groups with different charges or other characteristics. Although only two monomers were adopted in the present work to demonstrate the feasibility,

the method can be readily extended for other combinations of photochemically polymerizable molecules to get various surfaces. Due to the indispensable role of zwitterionic surfaces in biological process and wide acceptance of the photochemical process in industry, the method should have application potential in many biology or medicine related areas.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Tangpasuthadol, V.; Pongchaisirikul, N.; Hoven, V. P. *Carbohydr. Res.* **2003**, *338*, 937−942.
(2) Chevalier, Y.; Storet, Y.; Pourchet, S.; Le Perchec, P. *Langmuir* **1991**, *7*, 848−853.
(3) Santonicola, M. G.; Memesa, M.; Meszyńska, A.; Ma, Y. J.; Vancso, G. J. *Soft Matter* **2012**, *8*, 1556−1562.
(4) Kaznessis, Y. N.; Kim, S.; Larson, R. G. *Biophys. J.* **2002**, *82*, 1731−1742.
(5) Holmlin, R. E.; Chen, X. X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 2841−2850.
(6) Yang, W.; Chen, S. F.; Cheng, G.; Vaisocherova, H.; Xue, H.; Li, W.; Zhang, J. L.; Jiang, S. Y. *Langmuir* **2008**, *24*, 9211−9214.
(7) Azari, S.; Zou, L. *J. Membr. Sci.* **2012**, *401*−*402*, 68−75.
(8) Liu, X. S.; Jin, Q.; Ji, Y.; Ji, J. *J. Mater. Chem.* **2012**, *22*, 1916−1927.
(9) Rouhana, L. L.; Jaber, J. A.; Schlenoff, J. B. *Langmuir* **2007**, *23*, 12799−12801.
(10) Kuo, W. H.; Wang, M. J.; Chien, H. W.; Wei, T. C.; Lee, C.; Tsai, W. B. *Biomacromolecules* **2011**, *12*, 4348−4356.
(11) Keefe, A. J.; Brault, N. D.; Jiang, S. Y. *Biomacromolecules* **2012**, *13*, 1683−1687.
(12) Zhao, Y. H.; Wee, K. H.; Bai, R. *J. Membr. Sci.* **2010**, *362*, 326−333.
(13) Chen, S. F.; Jiang, S. Y. *Adv. Mater.* **2008**, *20*, 335−338.
(14) Chen, S. F.; Yu, F. C.; Yu, Q. M.; He, Y.; Jiang, S. Y. *Langmuir* **2006**, *22*, 8186−8191.
(15) Bernards, M. T.; Cheng, G.; Zhang, Z.; Chen, S. F.; Jiang, S. Y. *Macromolecules* **2008**, *41*, 4216−4219.
(16) Fan, Z. H.; Harrison, D. J. *Anal. Chem.* **1994**, *66*, 177−184.
(17) Jacobson, S. C.; Moore, A. W.; Ramsey, J. M. *Anal. Chem.* **1995**, *67*, 2059−2063.
(18) Lamonte, R. R.; McNally, D. *Adv. Mater. Process.* **2001**, *159*, 33−36.
(19) Lai, S. Y.; Cao, X.; Lee, L. J. *Anal. Chem.* **2004**, *76*, 1175−1183.
(20) Dang, F. Q.; Zhang, L. H.; Hagiwara, H.; Mishina, Y.; Baba, Y. *Electrophoresis* **2003**, *24*, 714−721.
(21) Soper, S. A.; Ford, S. M.; Qi, S.; McCarley, R. L.; Kelly, K.; Murphy, M. C. *Anal. Chem.* **2000**, *72*, 642A−651A.
(22) Soper, S. A.; Henry, A. C.; Vaidya, B.; Galloway, M.; Wabuyele, M.; McCarley, R. L. *Anal. Chim. Acta* **2002**, *470*, 87−99.
(23) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. *Anal. Chem.* **1998**, *70*, 4974−4984.
(24) Wang, A. J.; Xu, J. J.; Chen, H. Y. *Anal. Chim. Acta* **2006**, *569*, 188−194.
(25) Zhang, J. Y.; Das, C.; Fan, Z. H. *Microfluid. Nanofluid.* **2008**, *5*, 327−335.
(26) Liu, J.; Lee, M. L. *Electrophoresis* **2006**, *27*, 3533−3546.
(27) Wong, I.; Ho, C. M. *Microfluid. Nanofluid.* **2009**, *7*, 291−306.
(28) Oster, G.; Shibata, O. *J. Polym. Sci.* **1957**, *26*, 233−234.
(29) Hu, S. W.; Ren, X. Q.; Bachman, M.; Sims, C. E.; Li, G. P.; Allbritton, N. *Anal. Chem.* **2002**, *74*, 4117−4123.
(30) Nunes, P. S.; Ohlsson, P. D.; Ordeig, O.; Kutter, J. P. *Microfluid. Nanofluid.* **2010**, *9*, 145−161.
(31) Lin, R.; Burns, M. A. *J. Micromech. Microeng.* **2005**, *15*, 2156−2162.
(32) Ro, K. W.; Liu, J.; Knapp, D. R. *J. Chromatogr., A* **2006**, *1111*, 40−47.
(33) Rohr, T.; Ogletree, D. F.; Svec, F.; Fréchet, J. M. J. *Adv. Funct. Mater.* **2003**, *13*, 264−270.
(34) Garnett, J. L.; Ng, L.-T.; Viengkhou, V.; Hennessy, I. W.; Zilic, E. F. *Radiat. Phys. Chem.* **2000**, *57*, 355−359.
(35) Deng, J. P.; Yang, W. T. *J. Polym. Sci., Polym. Chem.* **2001**, *39*, 3246−3249.
(36) Deng, J. P.; Yang, W. T.; Rånby, B. *Macromol. Rapid Commun.* **2001**, *22*, 535−538.
(37) Ma, H. M.; Davis, R. H.; Bowman, C. N. *Macromolecules* **2000**, *33*, 331−335.
(38) Irwan, G. S.; Kuroda, S.-I.; Kubota, H.; Kondo, T. *J. Appl. Polym. Sci.* **2002**, *83*, 2454−2461.
(39) Kubota, H.; Suka, I. G.; Kuroda, S.-I.; Kondo, T. *Eur. Polym. J.* **2001**, *37*, 1367−1372.
(40) Seo, J. H.; Shibayama, T.; Takai, M.; Ishihara, K. *Soft Matter* **2011**, *7*, 2968−2976.
(41) Du, G. F.; Cai, Q. L.; Zhao, L.; Wei, H.; Wang, J.; Wang, X. Y.; Guo, G. S.; Pu, Q. S. *Chem. Eng. J.* **2012**, *195*−*196*, 132−139.
(42) Wang, L. L.; Wu, J.; Wang, Q.; He, C. H.; Zhou, L.; Wang, J.; Pu, Q. S. *J. Agric. Food Chem.* **2012**, *60*, 1613−1618.
(43) Pu, Q. S.; Elazazy, M. S.; Alvarez, J. C. *Anal. Chem.* **2008**, *80*, 6532−6536.
(44) Verzola, B.; Gelfi, C.; Righetti, P. G. *J. Chromatogr., A* **2000**, *874*, 293−303.
(45) Pu, Q. S.; Luttge, R.; Gardeniers, H. J. G. E.; van den Berg, A. *Electrophoresis* **2003**, *24*, 162−171.
(46) Chen, H.; Fang, Q.; Yin, X. F.; Fang, Z. L. *Lab Chip* **2005**, *5*, 719−725.
(47) Wang, Q.; Zhang, Y.; Ding, H.; Wu, J.; Wang, L. L.; Zhou, L.; Pu, Q. S. *J. Chromatogr., A* **2011**, *1218*, 9422−9427.
(48) Wu, J.; Liu, X. H.; Wang, L. L.; Dong, L. J.; Pu, Q. S. *Analyst* **2012**, *137*, 519−525.
(49) Pu, Q. S.; Oyesanya, O.; Thompson, B.; Liu, S. T.; Alvarez, J. C. *Langmuir* **2007**, *23*, 1577−1583.
(50) Ishihara, K.; Nomura, H.; Mihara, T.; Kurita, K.; Iwasaki, Y.; Nakabayashi, N. *J. Biomed. Mater. Res.* **1998**, *39*, 323−330.
(51) Poon, C. Y. In *Remington: the science and practice of pharmacy*, 21st ed.; Troy, D. B., Ed.; Lippincott Williams & Wilkins: Philadelphia, 2006; p 572.
(52) Ladd, J.; Zhang, Z.; Chen, S. F; Hower, J. C.; Jiang, S. Y. *Biomacromolecules* **2008**, *9*, 1357−1361.