Selective immobilization of biomolecules onto an activated polymeric adlayer

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The authors report a facile method for the selective immobilization of biomolecules onto a gold surface that was preactivated by a polymeric adlayer. The polymeric adlayer was designed to perform triple functions: high resistance to nonspecific protein adsorption, efficient surface anchoring, and subsequent covalent attachment of biomolecules. For this purpose, a random copolymer, poly(PEGMA-*r*-NAS), was synthesized by radical polymerization of poly(ethylene glycol) methyl ether methacrylate (PEGMA) and *N*-acryloxysuccinimide (NAS). In the first step, the polymeric adlayer was formed onto amine-terminated self-assembled monolayers (SAMs) on gold through covalent bond formation between reactive *N*-hydroxysuccinimide (NHS) ester of the copolymer and the amine of the SAMs. In the second step, amine-bearing biotin as a model biomolecule was covalently attached onto the polymeric adlayer that still contained unreacted NHS esters. The degrees of the binding sensitivity for a target protein and the nonspecific binding for four model proteins on the biotinylated polymeric adlayer were examined by surface plasmon resonance spectroscopy. Finally, the specific immobilization of rhodamin (TRITC)-conjugated streptavidin on the biotinylated polymeric adlayer was achieved by a simple microcontact printing technique, resulting in well-defined patterns of the protein. © *2007 American Vacuum Society.* [DOI: 10.1116/1.2801974]

I. INTRODUCTION

Microarrays of various biological probe molecules (DNA,[1] proteins,[2] oligosaccharides,[3] cells,[4] etc.) have recently served as miniaturized tools, such as biochips and biosensors.[5] The ability to simultaneously identify a number of analytes in the limited quantity of a biological sample would aid in determining biomarkers for early disease detection as well as understanding the underlying molecular mechanisms of biological processes and diseases.[6] Biochips and biosensors mostly use biological molecules that are immobilized onto solid surfaces in the proximity of a transducer to detect interactions or reactions with analytes. Therefore, one of the key steps in the construction of microarrays is the immobilization of biomolecules onto solid surfaces. Up to date, a variety of surface modification methods, including self-assembled monolayers (SAMs),[7–10] poly(*L*-lysine) coating,[11,12] nitrocellulose adlayers,[13,14] have been utilized to immobilize biomolecules. In particular, SAMs have been widely used and considered as a reliable strategy to construct microarrays on gold surfaces. On the other hand, another key consideration in the biochip fabrication is how to minimize nonspecific adsorption/adhesion of biomolecules in order to obtain high signal-to-noise ratio (low background signal). It is, therefore, required to impart the antibiofouling property to the SAMs. Typically, poly(ethylene glycol) (PEG) has been introduced onto the SAMs to reduce the nonspecific adsorption.

Generally, there are two ways to the introduction of PEG-containing, polymeric adlayers on substrates: physisorption[15,16] and covalent attachment.[17–22] In particular, the covalent attachment for irreversible grafting can be accomplished by either "grafting-onto"[17–19] or "grafting-from" approach.[20–23] The grafting-from approach, surface-initiated polymerization, is a reliable method to achieve tunable and robust polymer brushes and to control their grafting density

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by varying polymerization time[21,22] and monomer concentrations.[23] The polymerization process, however, sometimes requires sophisticated processes, such as a vacuum technique, and additional reagents, such as catalysts and ligands. Alternatively, the grafting-onto approach has also been used for the formation of ultrathin films due to the experimental simplicity and ease. For example, Hillier *et al.* constructed cell-resisting surfaces with ultrathin PEG-coating layers of about or less than 2 nm.[19] Langer *et al.* also reported that poly(TMSMA-*r*-PEGMA), composed of a surface-reactive part (trimethoxysilane) and a functional part (PEG), could spontaneously form polymeric SAMs on Si/SiO_2 wafers, and the resulting surfaces showed highly protein- and cell-resistant property.[24] Despites the excellent antibiofouling property, the previous polymeric adlayers were lack of the ability to immobilize biomolecules onto the surfaces. In this work, we designed a new random copolymer capable of forming a polymeric adlayer on a gold surface by grafting-onto approach, which possessed additional dual functions, the immobilization ability of biomolecules and the antibiofouling property. The new random copolymer, denoted as poly(PEGMA-*r*-NAS), was synthesized by radical polymerization of poly(ethylene glycol) methyl ether methacrylate (PEGMA) and *N*-acryloxysuccinimide (NAS). The successful immobilization of biomolecules through the polymer adlayer on the amine-terminated SAMs and the construction of model protein patterns were demonstrated.

II. EXPERIMENTAL SECTION

A. Materials

PEGMA (average $M_n = \sim 475$), NAS, and 2,2′-azobisisobutyronitrile (AIBN) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Absolute ethanol (HPLC grade, Merck), anhydrous dichloromethane (HPLC grade, Merck), triethylamine (99.5%, Aldrich), 2-(2-aminoethoxy)ethanol (EG_2-NH_2) (98%, Aldrich), acetic acid (99%, Junsei), (+)-biotinyl-3,6,9-trioxaundecanediamine (biotin-NH_2) (Pierce), bovine serum albumin (BSA) (Sigma), fibrinogen (Sigma), ribonuclease A (RNase A) (Sigma), lysozyme (Sigma), streptavidin (SA) (Pierce), and phosphate buffered saline (PBS) (Sigma) were used as received. 11-amino-1-undecanethiol $[HS(CH_2)_{11}NH_2]$ was purchased from Dojindo Molecular Technologies, Inc.

B. Synthesis of poly(PEGMA-*r*-NAS)

Prior to polymerization, neat PEGMA was flowed through the inhibitor-removal basic alumina column (Aldrich Chemical Co.). PEGMA (2.38 g, 5 mmol), NAS (0.85 g, 5 mmol), and AIBN (17 mg, 0.01 equiv) were placed in a vial and dissolved in tetrahydrofuran (anhydrous, inhibitor free, 99.9%, 10 mL). The mixture was degassed for 20 min using an Ar gas stream, and the vial was sealed with a Teflon-lined screw cap. The polymerization reaction was carried out at 70 °C for 24 h. After evaporation of solvent under vacuum, the copolymer was obtained as a viscous liquid. We obtained the polymer with molecular weight (M_n) of ~17 633 and polydispersity index of 2.15. ^{1}H NMR (300.40 MHz, $CDCl_3$): δ 4.15 (br, 2H, CO_2-CH_2 of PEGMA), 3.68–3.55 (br, 30H, -OCH_2CH_2O of PEGMA), 3.41 (s, 3H, -OCH_3 of PEGMA), 2.82 (s, 4H, CO-CH_2CH_2-CO of NAS), 2.20–1.63 (br, 5H), and 1.23 (br, 3H).

C. Preparation of NH_2-terminated SAMs and polymeric adlayers

Gold substrates were prepared by thermal evaporation of 5 nm of titanium and 100 nm of gold onto silicon wafers. The amine-terminated self-assembled monolayers (NH_2-SAMs) were formed by overnight soaking of an UV ozone-cleaned, gold-coated substrate in a 1 mM ethanolic solution of $[HS(CH_2)_{11}NH_2]$ with 3%(v/v) $N(CH_2CH_3)_3$. The substrate was then rinsed sequentially with ethanol, ethanolic solution of CH_3COOH 10%(v/v), and ethanol, followed by drying in a stream of Ar.[25] The gold substrate presenting the NH_2-SAMs was immersed in the 1 wt % solution of poly(PEGMA-*r*-NAS) in anhydrous dichloromethane for 2 h at room temperature. The substrate was rinsed with dichloromethane and ethanol, followed by drying in a stream of Ar.

D. Covalent attachment of biotin-NH_2 onto poly(PEGMA-*r*-NAS) adlayers

A substrate coated with the poly(PEGMA-*r*-NAS) adlayer was immersed in absolute ethanol solution of amine-terminated biotin ligand (biotin-NH_2, 1 mg/mL) for 2 h at ambient temperature. The resulting substrate was washed with ethanol, followed by purging with a stream of Ar. The remaining NHS ester groups of the poly(PEGMA-*r*-NAS) adlayer were deactivated (passivated) by immersing the substrate in the ethanolic solution of 2-(2-aminoethoxy)ethanol (1 mg/mL) for 1 h at ambient temperature. After blocking, the substrate was rinsed with ethanol and distilled water, followed by purging with a stream of Ar.

E. Ellipsometric and surface plasmon resonance studies

Nonspecific binding of proteins was studied by measuring the thickness of the biotinylated polymeric adlayer before and after incubation in a PBS solution of BSA, fibrinogen, lysozyme, or RNase A (1 mg/mL) as model proteins. The concentration of SA (target protein) was set to be 0.1 mg/mL. After immersing the biotinylated substrate in each protein solution for 2 h at ambient temperature, the substrate was washed with distilled water and dried in a stream of Ar. The change in the thickness was measured by ellipsometry.

Surface plasmon resonance (SPR) measurements were performed with a Biacore instrument (model: Biacore X, Kalmar Högskola, Sweden). The biotinylation were performed on a gold substrate (0.8×1.0 cm^2) (D.I. Biotech Ltd., Korea). The surface was then glued onto a Biacore cassette. Special care was taken to prevent noise (e.g., air spark) due to accumulation of air bubbles or contamination. Prior to each set of experiments, the channels of the SPR

instrument were cleaned with solutions of sodium dodesyl sulfate (BIAdesorb solution 1) and 50 mM glycine-NaOH (BIAdesorb solution 2). The degree of nonspecific protein adsorption onto the biotin-attached polymeric adlayer was monitored with model proteins, including BSA, fibrinogen, lysozyme, and RNase A in a PBS solution (*p*H 7.4). The concentration of each protein was set to be 1 mg/mL, whereas that of streptavidin (SA; target protein) was 0.1 mg/mL. SPR experiments were conducted with a constant flow of the solution (20 μL/min) over the surfaces. The binding of the model and the target proteins onto the biotin-attached polymeric adlayer was carried out by a single injection of 100 μL of each protein solution. After elution of each protein solution for 5 min, the surface was washed with a constant flow rate of PBS buffer (20 μL/min). Protein binding caused a shift in the resonance angle that was reported in resonance units (RU; 10 000 RU=10°).[23]

F. Microcontact printing of biotin-NH_2

A PDMS stamp was prepared according to the literature method using Sylgard 184 silicone elastomer (Dow Corning, Midland, MI).[26] Before its use, the PDMS stamp was oxidized by an oxygen plasma cleaner (medium setting, Harrick PDC-002, USA) for 1 min. After inking, biotin-NH_2 (10 mM in ethanol) was printed by contacting the PDMS stamp onto the poly(PEGMA-*r*-NAS) adlayer for 60 s. The sample was then immersed immediately in a solution of EG_2-NH_2 (1 mg/mL, 0.1 M sodium bicarbonate) for 30 min and rinsed with ethanol and distilled water. After the pattern generation of biotin, the sample was immersed in a solution of TRITC-conjugated streptavidin (0.1 mg/mL) in PBS (*p*H 7.4) containing 0.1%(w/v) BSA and 0.02%(v/v) Tween 20 at ambient temperature. After 60 min, the sample was removed and washed several times with PBS and distilled water. Fluorescence images were acquired on an LMS 510 laser scanning confocal microscope (Carl Zeiss, Germany).

G. Measurements

1. Polarized infrared external reflectance spectroscopy

Polarized infrared external reflectance spectroscopy (PIERS) spectra were obtained in a single reflection mode using a dry N_2-purged Thermo Nicolet Nexus Fourier-transform infrared (FTIR) spectrophotometer equipped with the smart smart apertured grazing angle accessory. The *p*-polarized light was incident at 80° relative to the surface normal of the substrate and a narrow band mercury-cadmium-telluride detector cooled with liquid nitrogen was used to detect the reflected light. The IR spectra of functionalized gold surfaces at each reaction step were collected over the range 900–4000 cm^{-1}.We averaged 16 000 scans to yield the spectra at a resolution of 4 cm^{-1} and all spectra were reported in the absorption mode relative to a clean gold surface.

2. Ellipsometry

Ellipsometric measurements were performed by using a Gaertner Scientific ellipsometer (model: L116s, Chicago, IL) equipped with a He-Ne laser (λ=6328 Å) at an angle of incidence of 70°. The constants of gold substrates were derived from ellipsometric measurements conducted at ten or more locations on a bare gold substrate. The thickness was determined from ellipsometric measurements at different 3–5 spots (separated by at least 0.5 cm), using the recorded substrate constants and assuming that the refractive index of the film was 1.46 and the film was completely transparent to the laser beam.

3. X-ray photoelectron spectroscopy

The x-ray photoelectron spectroscopy (XPS) study was performed with a VG-Scientific ESCALAB 250 spectrometer (UK) with monochromatized Al $K\alpha$ x-ray source. Emitted photoelectrons were detected by a multichannel detector at a take-off angle of 90° relative to the surface. During the measurements, the base pressure was $10^{-9}-10^{-10}$ Torr. Survey spectra were obtained at a resolution of 1 eV from three scans and high-resolution spectra were acquired at a resolution of 0.05 eV from 5 to 20 scans. All binding energies were determined with the Au 4*f*7/2 core level peak at 84 eV as a reference.

III. RESULTS AND DISCUSSION

A. Design and synthesis of a PEG-containing polymer

We designed and synthesized a new PEG-containing polymer capable of forming a polymeric adlayer on amine-presenting surfaces by grafting-onto approach. For this purpose, a random copolymer, poly(PEGMA-*r*-NAS), was synthesized by radical polymerization of PEGMA and NAS. ^{1}H NMR spectrum revealed that the actual composition of monomers in the resulting copolymer (1:1.08) turned out to be almost identical to the initial feeding ratio (1:1). Poly(PEGMA-*r*-NAS) was composed of a PEG part for antibiofouling function and a NHS ester part for the immobilization of biomolecules as well as the formation of the polymeric adlayer onto amine-terminated SAMs (NH_2-SAMs) on gold. In other words, the NAS moiety was designed to play the dual functions in our scheme. Overall processes including the formation of the polymer adlayer on NH_2-SAMs and the subsequent attachment of biomolecules are illustrated in Fig. 1.

B. Formation and characterization of NH_2-SAMs

The NH_2-SAMs were formed by immersing a gold substrate in 1 mM ethanolic solution of $HS(CH_2)_{11}NH_2$ with 3%(v/v) $N(CH_2CH_3)_3$ for 12 h at room temperature. The resulting SAMs were characterized by FTIR spectroscopy, ellipsometry, and XPS. In the IR spectrum, we observed characteristic peaks at 2926 [$\upsilon_{as}(CH_2)$], 2853 [$\upsilon_s(CH_2)$], 1452 (CH_2 scissoring), and 1285 and 1245 cm^{-1} (CH_2 wagging and twisting-rocking) [Fig. 2(a)]. The mean thickness of

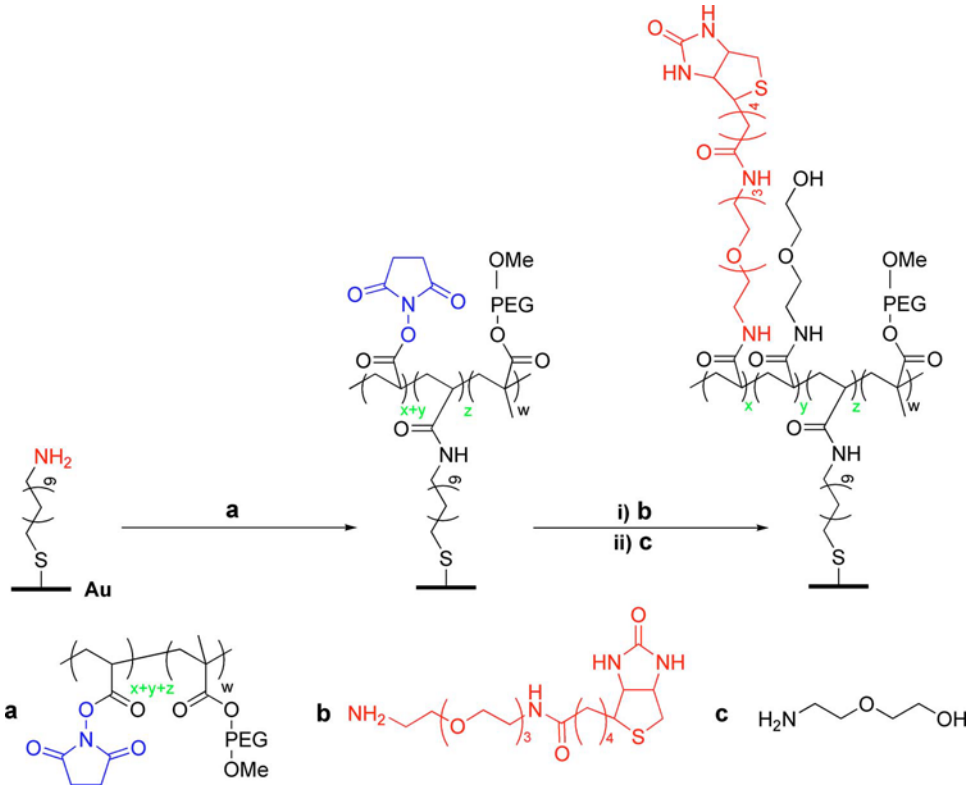

FIG. 1. Schematic of the overall procedure for the preparation of biotinylated polymeric adlayers: chemical structure of (a) poly(PEGMA-*r*-NAS) ($x+y+z:w=1:1$), (b) (+)-biotinyl-3,6,9-trioxaundecanediamine, and (c) 2-(2-aminoethoxy)-ethanol.

the NH_2-SAMs was measured to be 14 Å by ellipsometry (Table I). The XPS study also confirmed the presence of elements in the SAM on gold substrates. We observed nitrogen and sulfur peaks at 400[N(1*s*)] and 162 eV [S(2*p*)] from the NH_2-SAM, in addition to the gold peak at 84 eV. We also observed O(1*s*) peak at 531 eV, indicative of the possible

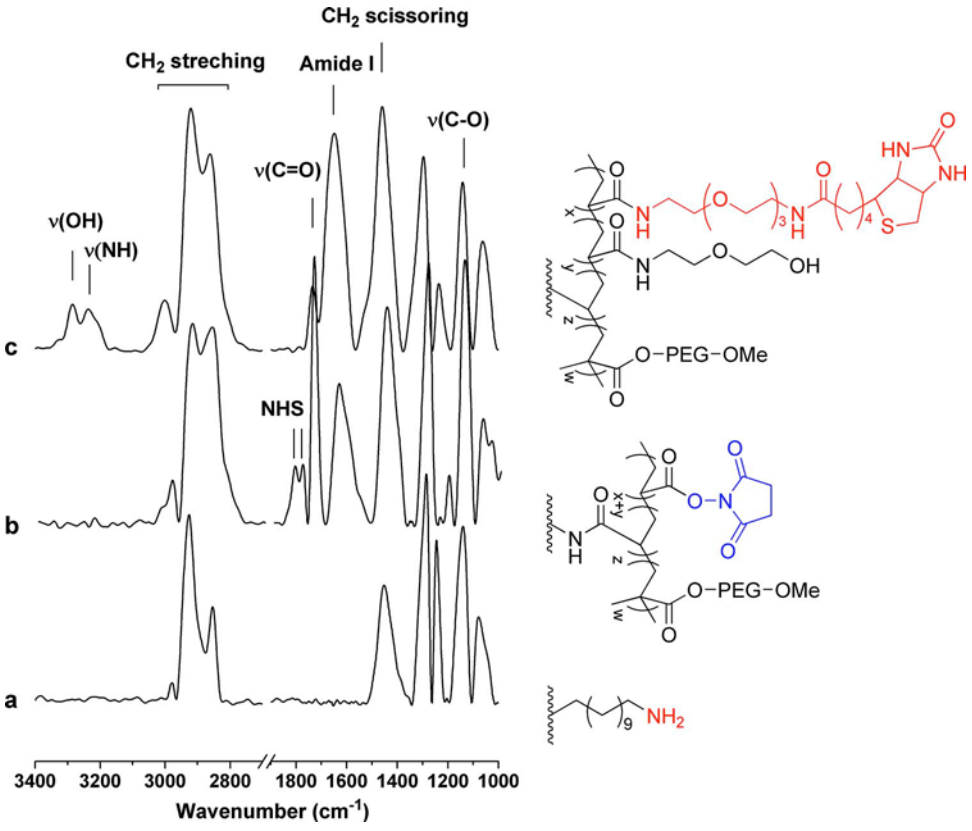

FIG. 2. PIERS spectra of (a) NH_2-SAMs, (b) poly(PEGMA-*r* -NAS) adlayers, and (c) biotinylated poly(PEGMA-*r* -NAS) adlayers.

TABLE I. Ellipsometric mean thickness and thickness change before and after protein adsorption. (The concentration of SA: 0.1 mg/mL; the other proteins: 1 mg/mL; incubation time: 2 h.)

Surfaces	Thickness (Å)	Thickness change after the treatment of proteins (Å)				
		BSA	Fibrinogen	Lysozyme	RNase A	SA
NH_2-SAM	14	18	54	13	7	7
Poly(PEGMA-*r*-NAS) adlayer	23	10	28	11	5	5
Biotinylated poly(PEGMA-*r*-NAS) adlayer	23	3	8	4	4	27

presence of oxidized sulfur species (SO_3) (Ref. 25) [Fig. 3(a)]. The experimental ratios of average atomic concentrations ($N/C=0.087$, $S/C=0.094$) were close to those expected from the stoichiometric ratios (0.091) (Table II).

C. Formation of poly(PEGMA-*r*-NAS) adlayers and subsequent immobilization of biotin

The NAS moiety in the polymers was used for the formation of adlayers and subsequent bioconjugation. First, the formation of the poly(PEGMA-*r*-NAS) adlayer via covalent amide bonding with the NH_2-SAM was characterized by FTIR spectroscopy and XPS. In the IR spectrum, we observed characteristic peaks of the grafted copolymer at 1739 (C=O stretching of -CO_2-PEG or -CO_2-succiimide ester), 1815 (C=O stretching of the ester bond), 1785 (symmetric C=O stretching of NHS), 1641 (C=O stretching of amide I in the succiimide ring), and 1206 and 1071 cm^{-1} (C-O stretching) [Fig. 2(b)].[8] The high-resolution XPS spectrum of the C(1*s*) photoemission envelope also confirmed the adlayer formation. The high-resolution C(1*s*) peak fitted with four unique carbon moieties: -CH_2- (284.6 eV), -C-O-C- (286.2 eV), -C(=O)- (287.1 eV, ester or amide linkage), and O-CH_3 (288.5 eV) [Fig. 3(b)].[27] The high-resolution N(1*s*) peak was deconvoluted into two components: peaks appeared at 399.9 and 401.7 eV correspond to the amide linkage (between amine terminated SAMs and the polymer) and the amide of succiamide ring, respectively. These characteristic XPS peaks of the poly(PEGMA-*r*-NAS) adlayer indicated that the surface-anchoring occurred by the formation of amide linkage. The difference in the binding energies of two nitrogen peaks was 1.8 eV, which was consistent with the previously obtained values [Fig. 3(c)].[28,29]

Amine-bearing biotin (biotin-NH_2) was used as a model compound to investigate the postfunctionalizability of the poly(PEGMA-*r*-NAS) adlayer. The amide bond could be formed between biotin-NH_2 and the reactive NHS ester groups that still remained in the copolymer after the formation of the adlayer. In the IR spectrum of the biotinylated surface, we observed a peak at 1648 (amide I) and peaks around 3174–3352 cm^{-1} [ν(N-H) of biotin and ν(O-H) of 2-(2-aminoethoxy)ethanol] [Fig. 2(c)].[30] In addition, a weak band was observed at 1532 cm^{-1} (amide II) as a shoulder. Taken together, these characterization data confirmed the successful formation of the poly(PEGMA-*r*-NAS) adlayer and the subsequent bioconjugation with biotin.

D. Protein adsorption experiment: Ellipsometric and SPR studies

After validation of the adlayer formation and the subsequent bioconjugation, ellipsometric and SPR studies were employed to assess the degree of nonspecific adsorption of various proteins (streptavidin, BSA, fibrinogen, lysozyme, and RNase A) onto the poly(PEGMA-*r*-NAS)-modified surfaces. Thickness change before and after incubation of the model proteins is shown in Table I. In the case of substrates presenting the NH_2-SAMs, we observed distinct thickness increase for all the tested proteins. In particular, significant increase in the thickness was observed for fibrinogen (a sticky protein) by ~54 Å, indicative of high nonspecific adsorption. After the formation of the poly(PEGMA-*r*-NAS) adlayer onto the NH_2-SAMs, such thickness change decreased, presumably because of the presence of PEG in the adlayer. However, we still observed noticeable non-specific adsorption of proteins onto the adlayer. This nonspecific adsorption was thought to be caused by the remaining NHS ester groups, which would react with amines in the proteins. In contrast, when the remaining NHS esters of the adlayer was first reacted with biotin-NH_2 and subsequently with excess of 2-(2-aminoethoxy)ethanol for passivation, the thickness change was dramatically decreased to be less than 8 Å for all nontarget proteins. The data with fibrinogen would be a representative: the formation of the poly(PEGMA-*r*-NAS) adlayer diminished the nonspecific adsorption from 54 to 28 Å and the subsequent passivation further minimized it up to 8 Å in thickness change. In contrast, SA was specifically immobilized onto the biotinylated surface but not onto other two surfaces, resulting in ~27-Å-thickness increase. The observed adsorption thickness of SA was in agreement with the previous reports.[31,32] These results suggest that the poly(PEGMA-*r*-NAS) adlayer can be used as an active platform for the attachment of biomolecules with the minimization of nonspecific protein adsorption.

We also used SPR to further examine whether the biotinylated polymeric adlayer specifically interact with its target, SA. SPR would be a complimentary technique to ellipsometric measurements: it enables real-time monitoring of interactions with external analytes, while ellipsometry measures static adsorption of analytes onto a solid surface after washing. Figure 4 shows SPR sensorgrams of the association and dissociation phases for SA and other proteins. The difference in the steady-state SPR signals, defined as ΔRU, is propor-

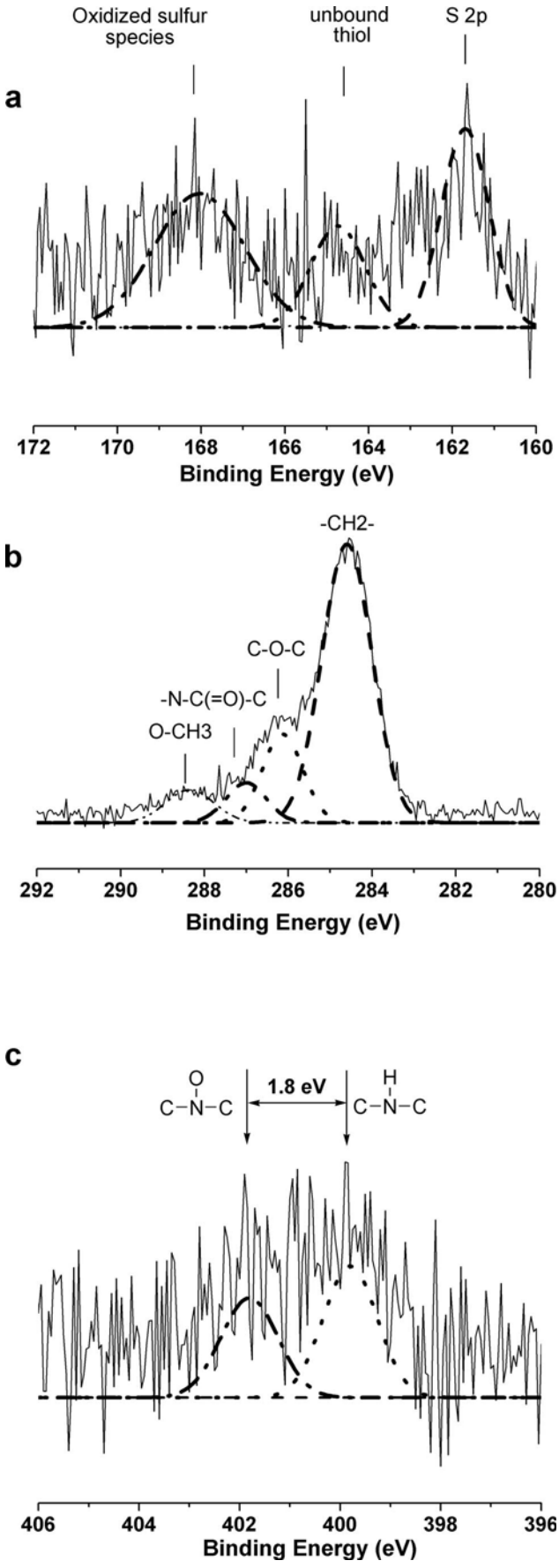

FIG. 3. Narrow XPS spectra of (a) sulfur peaks of NH_2-SAMs, (b) carbon, and (c) nitrogen peaks of poly(PEGMA-*r* -NAS) adlayers.

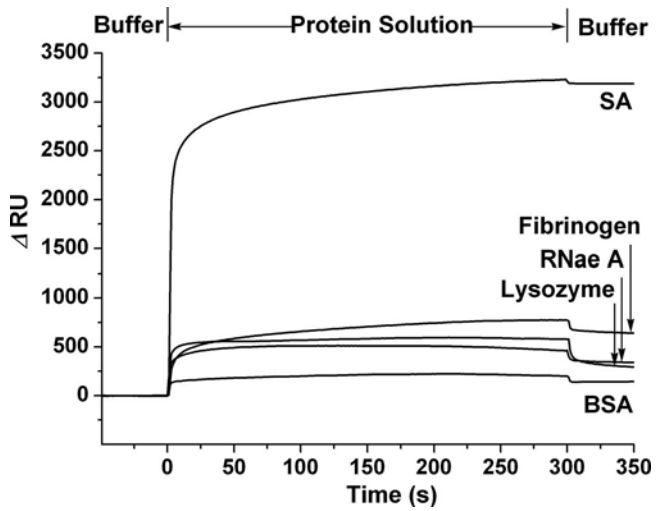

FIG. 4. SPR sensorgrams.

tional to the mass of adsorbed protein (1 ΔRU =1 pg/mm^2).[23] While the relative resonance unit (ΔRU) was measured to be 3190 RU for SA, other proteins showed the ΔRU values of less than ~637 RU, being consistent with the results of the ellipsometric measurements. Assuming the average molecular weight of 52 kDa (1 Da $\sim 1.66 \times 10^{-27}$ kg) for SA and the total area of 1 cm^2 per SPR chip, the value of 3190 RU corresponded to $\sim 8.8 \times 10^8$ SA molecules/mm^2 with an average distance of $\sim 3 \times 10^{-5}$ nm between bound SA molecules. Both ellipsometric and SPR characterizations confirmed that SA was specifically immobilized onto the biotinylated poly(PEGMA-*r*-NAS) adlayer.

E. Construction of protein patterns

Pattern generation of biotin on the poly(PEGMA-*r*-NAS) adlayer was achieved by microcontact printing (μCP) of biotin-NH_2 using a PDMS stamp that had micrometer-sized relief features (50-μm circles and lines). After patterning biotin-NH_2 by the amide bond formation, the surface was treated with EG_2-NH_2 to deactivate any remaining NHS esters. The resulting substrate was immersed in the solution of rhodamin (TRITC)-conjugated streptavidin. The patterned images of SA with different features were taken by fluores-

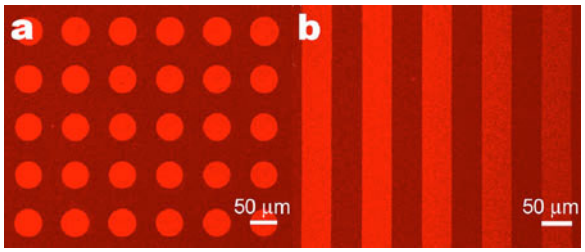

FIG. 5. Fluorescence microscopic images of TRITC-conjugated streptavidin bound to micropatterns of biotin on the poly(PEGMA-*r* -NAS) adlayer: (a) 50-μm-wide circles separated by 50 μm and (b) 50-μm-wide lines separated by 50 μm.

TABLE II. Average atomic concentrations based on XPS survey scans.

Substrates	Elements				
	Au 4*f*	C 1*s*	O 1*s*	S 2*p*	N 1*s*
NH_2-SAM	36.7	45.9	9.1	4.3	4.0
Poly(PEGMA-*r*-NAS) adlayer	20.0	54.6	16.7	4.7	4.0
Biotinylated poly(PEGMA-*r*-NAS) adlayer	20.8	54.4	12.8	2.5	9.5

cence microscopy (Fig. 5). Circle and line patterns, where TRITC-conjugated streptavidin was immobilized, were clearly observed with high contrast, suggesting that the poly(PEGMA-*r*-NAS) adlayer may be used in the fabrication of protein arrays. Although one pair of binding sites in SA was used for immobilization on surfaces, the other pair in the opposite side was still available. Therefore, various biomolecules, such as antibodies and DNAs bearing biotin moiety, could be attached for biochip or sensor applications.[33,34]

IV. CONCLUSIONS

Research in nanobiotechnology and biomedical sciences involves the manipulation of interfaces between manmade surfaces and biomolecules (and cells). The manipulation generally requires the construction of surfaces that present chemically reactive functional groups with antibiofouling property to minimize nonspecific adsorption of biomolecules. In this article, we constructed an activated polymeric adlayer onto amine-terminated SAMs on gold by using a newly designed polymer. We utilized a design concept of the polymers required for the grafting-onto approach. For the facile grafting of polymers and subsequent functionalization while minimizing nonspecific adsorption of biomolecules, the polymers should contain three functional moieties, such as "surface-reactive," "antibiofouling," and "functionalizable" ones. As a proof-of-demonstration, we utilized the NHS ester group as both surface-reactive and functionalizable moieties and used amine-terminated SAMs as a model surface. The work clearly confirmed that the design concept worked for biospecific and selective immobilization of biomolecules. The synthesis of polymers containing the three functional moieties for direct surface modification of bare oxide-based substrates is being investigated to generalize the method described in this work.

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