Surface-Initiated, Atom Transfer Radical Polymerization of Oligo(ethylene glycol) Methyl Ether Methacrylate and Subsequent Click Chemistry for Bioconjugation

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Introduction

The uncontrolled, nonspecific adsorption and adhesion of biological materials (e.g., proteins and cells) onto synthetic surfaces causes a complex cascade of undesired events[1] and bioincompatibility problems[2] in the applications of biomaterials. A number of studies on materials, such as polysaccharides,[3] dextran,[4] cellulose dialysis membranes,[5] and poly(ethylene glycol) (PEG),[6] have pursued the elimination/minimization of the unwanted, nonspecific adhesion of biological entities, but the verification of complete rejection to any protein adsorption remains a challenge. PEG shows good biocompatibility, low toxicity, non-immunogenicity, and high water solubility,[7,8] and in this respect PEG has extensively been investigated as a material with significant inertness to cell and protein adhesion.[9−15] The grafting of PEG-based polymers has been achieved by either the "grafting-onto"[16−18] or the "grafting-from" method.[19,20] Langer et al. constructed the non-biofouling, PEG-based polymeric surfaces by grafting a PEG-containing, SiO_2-reactive polymer onto surfaces.[16] While successful in minimizing the nonspecific adsorption of biological materials, the strictly controlled attachment of biologically active molecules (e.g., DNAs, biotin, and peptides), which would have many technologically important applications, such as sensors and microarrays, has not been demonstrated in this approach. The ''grafting-from" approach, surface-initiated polymerization (SIP), is an alternative to the "grafting-onto" approach. Because the polymer chain is generally grown from surface-anchored polymerization initiators, the lateral and vertical control of the polymer films, such as grafting density, thickness, and chemical composition, could be tuned depending upon the applications of interest. In addition, whereas the "grafting-onto" approach, in principle, suffers from steric hindrance, which limits the grafting density, SIP would yield highly dense polymer brushes.[10] Surface-initiated, atom transfer radical polymerization (SI-ATRP) has been applied to the construction of PEG-based, non-biofouling surfaces.[21−23] For example, Chilkoti et al. polymerized oligo(ethylene glycol) methyl ether methacrylate (OEGMA) from gold and silicon oxide surfaces and reported non-biofouling effect of the resulting *p*OEGMA films against proteins and cells.[11−13] Recently, poly(oligo(ethylene glycol)methacrylate), presenting hydroxyl groups at the polymer chains, was grown from surfaces, and the hydroxyl groups were utilized for the attachment of proteins onto the surfaces through multistep, biochemical processes.[24] It is prerequisite for wider applications to form surfaces that present a functionalizable group with a non-biofouling background, because the surfaces could act as a platform of the technological applications, such as microarrays and cellular micropatterns. In other words, the surfaces should possess dual properties, non-biofouling property and functionalizability. However, research on the polymeric surfaces has been devoted mainly to the generation of non-biofouling surfaces. In this paper, we extended our reported reaction,[25] Sharpless "click" chemistry, to the PEG-based polymeric films for the introduction of various functional groups onto the surfaces.

Recently, Cu(I)-catalyzed, highly specific, and efficient formation of 1,4-disubstituted 1,2,3-triazoles through 1,3-dipolar cycloaddition of azides and terminal alkynes (Sharpless "click" chemistry)[26] has intensively been utilized for modifying the surfaces of solid metals and cells by us and others, because the reaction gives very high yields and is stereospecific, and proceeds under mild reaction conditions.[27,28] The click chemistry also has been used for functionalizing polymers in solution.[29,30] For example, Matyjaszewski et al. reported the modification of side-chain functional groups of poly(3-azidopropyl methacrylate)[29] and polystyrene linear polymer[30] by the click chemistry. In this paper, we demonstrate that the click chemistry could be applied to polymeric nanobrushes presenting azide groups at the terminal. Specifically, we functionalized the non-biofouling *p*OEGMA film by the click chemistry to achieve maximized biospecific interactions between surface-attached ligands and target molecules with PEG as an inert background.

Experimental Section

Materials. Absolute ethanol (EtOH, HPLC grade, Merck), absolute methanol (MeOH, HPLC grade, Merck), acetone (HPLC grade, Merck), dichloromethane (HPLC grade, Merck), isopropyl alcohol (HPLC grade, Merck), bovine serum albumin (BSA, Sigma), fibrinogen (plasminogen-free, Sigma), ribonuclease A (RNase A, Sigma), lysozyme (Sigma), streptavidin (SA, Pierce), streptavidin−peroxidase conjugate (Sigma), 3,3′,5,5′-tetramethylbenzidine (TMB, Sigma), phosphate-buffered saline (PBS, Sigma), propargyl benzoate (98%, Aldrich), 4-pentynoic acid (95%, Aldrich), 5-hexyn-1-ol (96%, Aldrich), 1-hexyne (97%, Aldrich), copper(I) bromide (99.999%, Aldrich), 2,2′-dipyridyl (99+%, Aldrich), copper(II) sulfate pentahydrate (98+%, Aldrich), sodium azide (99.5%, Fluka), 2,3,5,6-tetrafluorophenol (98%, Aldrich), (+)-biotinyl-3,6,9-trioxaundecanediamine (biotin-PEO-LC-amine, Pierce), *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC, Aldrich), sodium L-ascorbate (99+%, Aldrich), and *N*,*N*-dimethylformamide (DMF, 99.8+%, Aldrich) were used as received. Poly(ethylene glycol) methyl ether methacrylate (OEGMA, Aldrich) was passed through a column of activated, basic aluminum oxide to remove inhibitors. The polymerization initiator, $[BrC(CH_3)_2COO(CH_2)_{11}S]_2$, was prepared according to the literature.[31]

Synthesis of Biotin-PEO-LC-*N*-Pentynoate. (*a*) *Synthesis of Pentynoic Acid Tetrafluorophenyl Ester.* 4-Pentynoic acid (0.50 g, 5.09 mmol) and 2,3,5,6-tetrafluorophenol (1.26 g, 7.60 mmol) were dissolved in 3 mL of anhydrous DMF at 80 °C under nitrogen. After the mixture was cooled to room temperature, EDC (1.95 g, 10.19 mmol) was added

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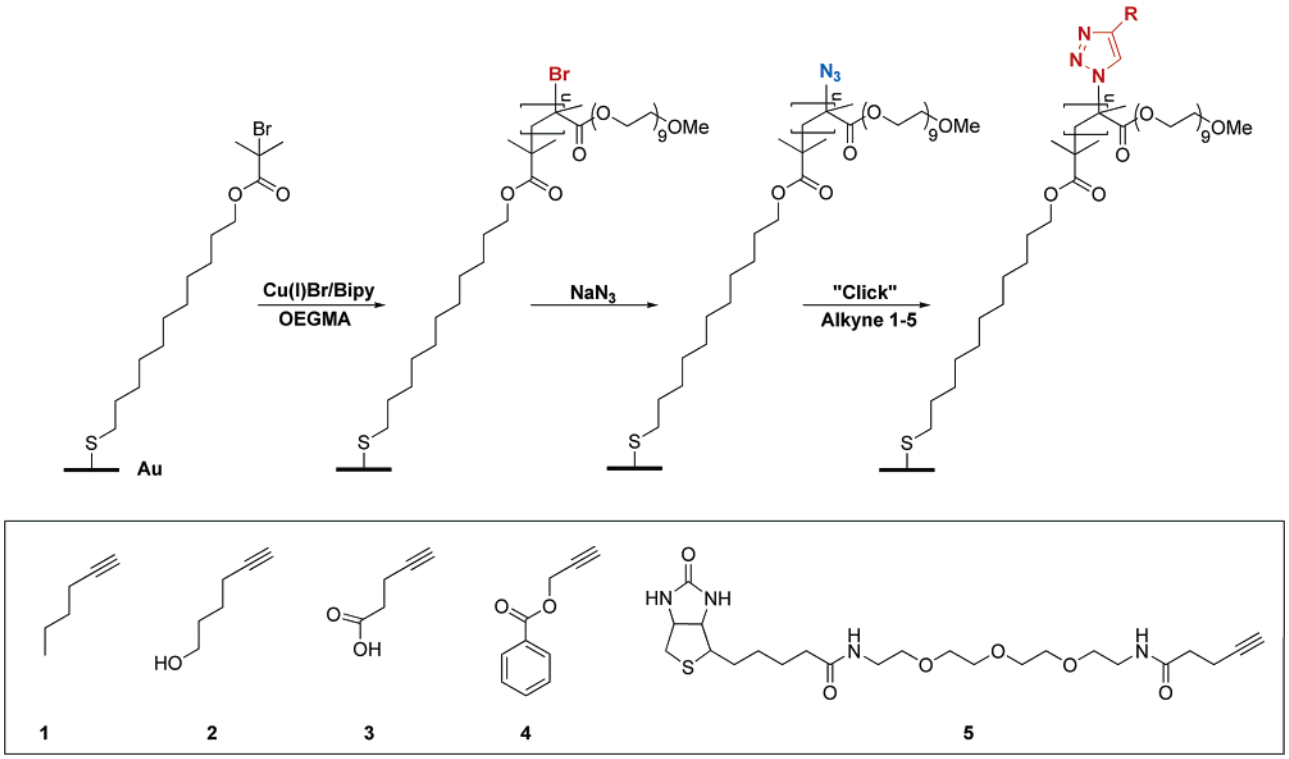

Figure 1. Schematic description of the procedure. Five acetylene group-containing compounds: 1-hexyne (**1**), 5-hexyn-1-ol (**2**), 4-pentanoic acid (**3**), propargyl benzoate (**4**), and biotin-PEO-LC-*N*-pentynoate (**5**).

to the reaction mixture, and the reaction was heated at 80 °C for 4 h. The solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography on silica gel (hexane/ethyl acetate = 5:1) to give pentynoic acid tetrafluorophenyl ester (1.05 g, 84%). ^{1}H NMR (400 MHz, CD_3OD): δ 2.34 (t, 1H, J = 2.63 Hz), 2.59−2.63 (m, 2H), 2.95 (t, 2H, J = 7.0 Hz), 7.38−7.44 (m, 1H).[32]

(*b) Synthesis of Biotin-PEO-LC-N-pentynoate.* Biotin-PEO-LC-amine (45 mg, 0.05 mmol), freshly distilled triethylamine (TEA) (0.30 mL, 0.10 mmol), and pentynoic acid tetrafluorophenyl ester (0.50 mg, 0.10 mmol) were dissolved in 3 mL of anhydrous DMF. The reaction was then stirred for 6 h at room temperature. The solvent was removed under reduced pressure, and the crude product was purified by flash column chromatography on silica gel (methylene chloride/methanol = 9:1) to give biotin-PEO-LC-*N*-pentynoate (49 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39−1.43 (2H, m), 1.64−1.68 (4H, m), 2.00 (1H, t, J = 2.58 Hz), 2.20 (2H, t, J = 7.46 Hz), 2.39 (2H, m), 2.47−2.51 (2H, m), 2.72 (1H, d, J = 12.76 Hz), 2.84−2.89 (1H, m), 3.09−3.12 (1H, m), 3.40−3.45 (4H, m), 3.52−3.60 (12H, m), 4.27−4.30 (1H, m), 4.46−4.50 (1H, m), 5.67 (1H, br, s), 6.64 (2H, br, s), 6.74 (1H, br, s).[33]

Preparation of SAMs. Gold substrates were prepared by thermal evaporation of 5 nm of titanium and 100 nm of gold onto silicon wafers. The SAM was prepared by immersing a gold substrate (1.2 × 1.5 cm^2) in a 1 mM ethanolic solution of $[BrC(CH_3)_2COO(CH_2)_{11}S]_2$ for 12 h at room temperature. After the formation of the SAM presenting a polymerization initiator, the surface was thoroughly rinsed with ethanol several times and then dried in a stream of argon.

Surface-Initiated, Atom Transfer Radical Polymerization of OEGMA. To a Schlenk tube containing deionized water (degassed, 2 mL) and methanol (degassed, 8 mL) were added Cu(I)Br (143 mg, 1.0 mmol) and 2,2′-dipyridyl (312 mg, 2.0 mmol). OEGMA (0.44 mL, 1 mmol) was then added, and the resulting dark red solution was bubbled with argon for 10 min. SI-ATRP was initiated by transferring the mixture to a degassed Schlenk tube that contained the gold substrate presenting an ATRP initiator, and then the mixture was stirred for 1 h under argon at room temperature. The polymerization was terminated by exposing the reaction to air and pouring water into the Schlenk tube. The termination step caused the reaction solution to turn blue, indicating the oxidation of Cu(I) to Cu(II). The *p*OEGMA-coated substrate was thoroughly washed with deionized water and methanol to remove any physisorbed polymers, and then dried in a stream of argon.

Preparation of Azide-Terminated *p*OEGMA. The nucleophilic substitution reaction of the terminal bromide group of *p*OEGMA with NaN_3 was performed to introduce azide groups onto surfaces for the click chemistry. The *p*OEGMA-coated substrate was immersed in a DMF solution (10 mL) of NaN_3 (65 mg, 1 mmol) for 1 h at room temperature. The substrate was washed with deionized water and DMF, and then dried in a stream of argon.

1,3-Dipolar Cycloaddition of Azide-Terminated *p*OEGMA with Acetylene Group-Containing Compounds (Click Chemistry). The 1,3-dipolar cycloaddition reaction, click chemistry, was performed by placing the gold substrate coated with N_3-terminated *p*OEGMA in the ethanol (or isopropyl alcohol)/deionized water solution (15 mL, 1:2, v/v) of sodium L-ascorbate (0.2 mmol) and copper(II) sulfate pentahydrate (0.04 mmol), and adding an acetylene group-containing compound, such as 1-hexyne (**1**, 56 μL), 5-hexyn-1-ol (**2**, 84 μL), 4-pentynoic acid (**3**, 49 mg), propargyl benzoate (**4**, 72 μL), or biotin-PEO-LC-*N*-pentynoate (**5**, 10 mg). Isopropyl alcohol was used as a cosolvent for **1** and **4**. The reaction mixture was stirred for 12 h at room temperature. After the reaction, the substrate was thoroughly washed with deionized water, acetone, ethanol, and dichloromethane, and then dried in a stream of argon.

Surface Plasmon Resonance (SPR) Study. SPR measurements were performed with a Biacore instrument (model: Biacore X). The ATRP and biotinylation were performed on a gold substrate (0.8 × 1.0 cm^2) (D.I. Biotech Ltd., Korea) that had been prepared for the SPR study by a sequential deposition of titanium (1.5 nm) and gold (39 nm) onto a glass cover slip (0.2 nm, No. 2 Corning, reflective index = 1.52). Non-biofouling effect of the biotinylated surface was tested with model proteins, such as BSA, fibrinogen, lysozyme, and RNase A, in a PBS solution (pH 7.4). The concentration of the protein solutions was set to be 1 mg/mL. The concentration of the SA (target protein) solution was set to be 0.1 mg/mL. The biotinylated surface was incubated in the buffer solution for 1 day and then washed with DI water and PBS. The surface was then glued onto a Biacore cassette.

Special care was taken to prevent some 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 a solution of sodium dodesyl sulfate (SDS) (BIAdesorb solution 1). SPR experiments were conducted with a constant flow of the solution (20 μL/min) over the surfaces. The binding of the model (1 mg/mL) and the target proteins (0.1 mg/mL) onto the biotinylated surface was carried out by one injection of 100 μL of a protein solution. After the elution of the protein solution for 5 min, the surface was washed with a constant flow rate of PBS buffer (20 μL/min). Protein binding resulted in a shift in the resonance angle that was reported in resonance units (RU; 10 000 RU = 1.0°).[34]

Protein Adsorption Experiments. Non-biofouling effect of the surfaces was tested with a PBS solution (1 mg/mL) of BSA (66 kDa, molecular biology reagent), fibrinogen (340 kDa, fraction I from human plasma), lysozyme (14.3 kDa, grade III from chicken egg white), or RNase A (13.7 kDa, type III-A from bovine pancreas) as a model protein at each step of the reaction processes. For SA (52 kDa, from streptomyces avidinii), the concentration of the PBS solution was set to be 0.1 mg/mL. After the substrate was immersed in the protein solution for 2 h at room temperature, the substrate was washed with distilled water and dried in a stream of argon. The change in thickness was measured by ellipsometry.

Enzyme-Linked Immunosorbent Assay (ELISA). Three substrates were used for ELISA: Br-presenting, N_3-presenting, and biotin-presenting surfaces. Before the ELISA experiments, the color of all three substrates was the same (gold). The substrates were preincubated in a PBS solution (pH 7.4) containing 0.1% Tween 20 for 16 h at 37 °C. The substrates were immersed in 3% BSA solution for 1 h and then soaked subsequently in a PBS solution of streptavidin-peroxidase conjugate (0.5 μg/mL) for 1 h at room temperature. The substrates placed in a 20-mL vial were shaken three times for 45 min and then washed three times with 0.1% Tween 20. Finally, the substrates were incubated in 3,3′,5,5′-tetramethylbenzidine (TMB) for 1 h at room temperature. The color change was optically observed, and their images were taken by a digital camera.

Polarized Infrared External Reflectance Spectroscopy (PIERS). PIERS spectra were obtained in a single reflection mode using a dry Ar-purged Thermo Nicolet Nexus FT-IR spectrophotometer equipped with the smart SAGA (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 (MCT) 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.

Ellipsometry. Ellipsometric measurements were performed by using a Gaertner Scientific ellipsometer (model: L116s) 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 10 or more locations on a bare gold substrate. The thickness was determined from ellipsometric measurements at 3−5 different 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.

X-ray Photoelectron Spectroscopy (XPS). The XPS study was performed with a VG-Scientific ESCALAB 250 spectrometer (UK) with monochromatized Al Kα X-ray source. Emitted photoelectrons were detected by a multichannel detector at a takeoff 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 3 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 4f7/2 core level peak at 84 eV as a reference.

Contact Angle Measurement. A Phoenix 300 apparatus (Surface Electro Optics Co. Ltd., Korea) equipped with a video camera was used to measure the static contact angle on water drops of ~3 μL in volume. Reported values represented the averages of at least four independent measurements.

Results and Discussion

Formation and Characterization of SAMs. The SAMs of the ATRP initiator-containing disulfide, $[BrC(CH_3)_2COO(CH_2)_{11}S]_2$, were formed by immersing a gold substrate in 1 mM ethanolic solution of the disulfide for 12 h at room temperature. The resulting SAMs were characterized by PIERS, contact angle goniometry, ellipsometry, and XPS. In the IR spectrum, we observed characteristic peaks of the SAMs at 2981 and 2853 cm^{-1} (symmetric methyl C−H stretching), 2929 cm^{-1} (asymmetric methyl C−H stretching), 1734 cm^{-1} (C═O stretching), 1466 cm^{-1} (methylene scissoring), 1284 cm^{-1} (methylene wagging and methylene twisting-rocking), and 1171 cm^{-1} (C−O−C stretching) (Figure 2a).[35−37] The thickness of the bromide-terminated SAMs was measured to be 17 ± 2 Å by ellipsometry, and the water static contact angle was 60 ± 2°. The XPS study also confirmed the presence of the SAMs on gold surfaces: the oxygen signal was observed at 531 eV (O 1s) and the carbon signal at 284.6 eV (C 1s), in addition to the gold signals.

Surface-Initiated, ATRP of OEGMA. The EG-containing polymer thin film was grown from the surface presenting dimethyl bromoester groups by surface-initiated, ATRP (SI-ATRP) of OEGMA as a monomer. After 1 h of polymerization in an oxygen-free environment, the resulting surface was characterized by PIERS and contact angle goniometry. The IR spectrum (Figure 2b) showed characteristic peaks at 1730 cm^{-1} (C═O stretching), 1452 cm^{-1} (−CH_2− scissoring), and 1149 cm^{-1} (C−O−C stretching), which were shifted as compared to those in the IR spectrum of the SAMs (Figure 2a). The contact angle measurement also confirmed the presence of EG chains at the surface. The static contact angle was changed from 60 ± 2° to 40 ± 2° after the polymerization, indicative of relatively hydrophilic nature of the surface.

Preparation of *p*OEGMA Surfaces Presenting Azide Groups: Reaction with Sodium Azide. To introduce azide groups onto the surface for subsequent click chemistry, the bromide-presenting *p*OEGMA surface was reacted with sodium azide for 1 h at room temperature. The IR spectrum indicates the facile transformation of bromide groups to azide groups: an intense absorption peak was observed at 2063 cm^{-1} (asymmetric N_3 stretching mode) (Figure 2c). In addition, the XPS spectra showed that the Br 3p peak at 182 eV disappeared and the N 1s peak at 399 eV appeared.

1,3-Dipolar Cycloaddition of Azide-Terminated *p*OEGMA with Acetylenyl Group-Containing Compounds (Click Chemistry). The reaction between terminal alkynes and azides is generally quite slow in the absence of a catalyst because alkynes are poor 1,3-dipole acceptors. It was reported by Sharpless that copper(I) dramatically accelerated the cycloaddition, and the reaction was highly regioselective.[27] Since the report, the reaction, Sharpless "click" chemistry, has intensively been utilized for functionalizing various surfaces, such as SAMs[37−40] and cells.[41] We and others have previously reported the use of the click chemistry for the post-modifications of SAMs, and herein we extended the approach to polymeric thin films. We tested several acetylene group-containing compounds to show the feasibility of the click chemistry on polymeric thin films. The compounds possessed methyl (**1**), hydroxyl (**2**), carboxylic acid (**3**), or benzoate group (**4**) at the other end. In addition, a

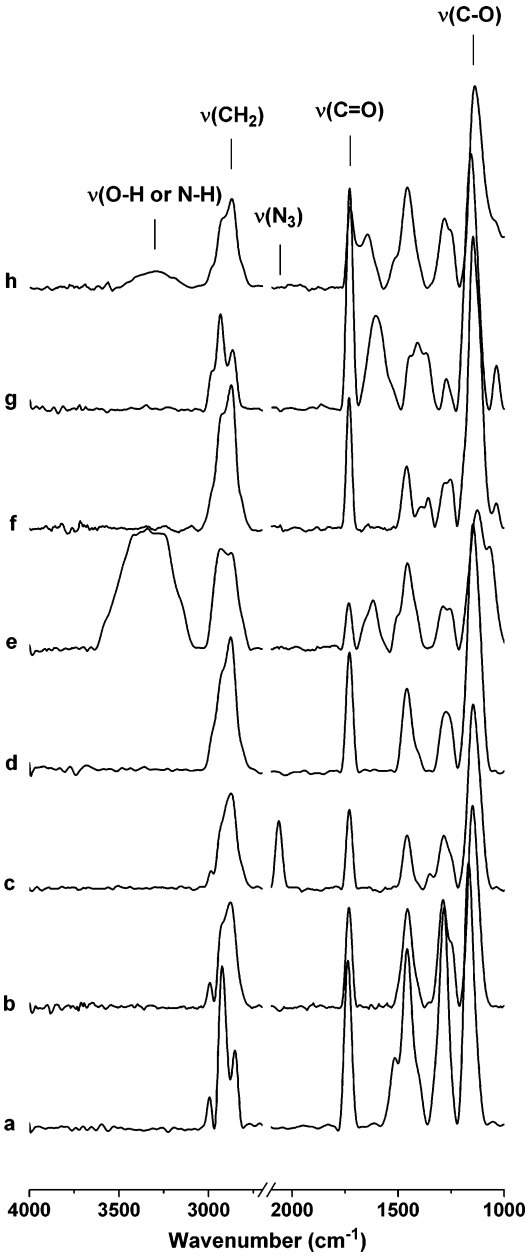

Figure 2. PIERS spectra of the substrates presenting (a) SAMs, (b) Br-presenting *p*OEGMA film, and (c) N_3-presenting *p*OEGMA film. PIERS spectra of substrates after the click chemistry with (d) **1**, (e) **2**, (f) **3**, (g) **4**, and (h) **5**.

biotin-containing compound (**5**) was synthesized and attached onto the surface as a model of biologically active compounds (Figure 1). Click chemistry was performed by placing a gold substrate coated with azide-terminated *p*OEGMA in the ethanol (or isopropyl alcohol)/deionized water solution (1:2, v/v) of sodium L-ascorbate and copper(II) sulfate pentahydrate, and adding an acetylene group-containing compound. The reactions were followed by PIERS (Figure 2d−h). Figure 2d−h shows the PIERS spectra of the methyl, the hydroxyl, the carboxyl, the phenyl, and the biotin moieties substituted at 4-site of triazoles, respectively. In all of the spectra, the asymmetric N_3 stretching peak at 2063 cm^{-1} disappeared, which indicates the successful coupling between the surface-anchored azide group and the acetylene group of the compound. Figure 2e (the PIERS spectrum of the substrate coupled with 5-hexyn-1-ol, **2**) shows ν(O−H) bands at 3338 and 1620 cm^{-1}.[42] The broadband at 1700−1750 cm^{-1} in Figure 2f accounts for ν(C═O) of the carboxylic group,[43] and the peak at 1600 cm^{-1} in Figure 2g was ascribed to the vibration mode of the benzene ring.[25] Figure 2h shows new peaks at 3297 (N−H stretching mode) and 1643 (amide I mode of C═O of biotin) and 1515 cm^{-1} (amide II mode of N−C═O of biotin).[44,45] Taken together, the PIERS spectra confirmed the successful introduction of various functional groups onto azide-terminated polymeric films by the click chemistry.

Table 1. Ellipsometric Mean Thickness before and after Protein Adsorption Experiments[a]

		thickness change after the treatment of proteins (Å)				
steps	thickness (Å)	BSA	fibrinogen	lysozyme	RNase A	SA
I	17	16	21	15	15	13
II	43	3	5	2	2	2
III	42	2	5	2	4	1
IV	41 (64)	3 (1)	4 (4)	3 (4)	4 (3)	1 (−20)

[a] Steps I, II, III, and IV indicate the formation of SAMs, SI-ATRP, the conversion of the bromide to the azide group, and the click chemistry, respectively. The values in parenthesis at step IV show the thickness changes for the biotin-presenting film. The concentration of SA, 0.1 mg/mL; the concentration of the other proteins, 1 mg/mL; adsorption time, 2 h.

Non-biofouling Effect. Ellipsometry was used to determine the nonspecific adsorption of proteins onto modified surfaces. The modified surfaces at each step were tested with the target protein (SA; concentration: 0.1 mg/mL in PBS, pH 7.4) and the model proteins (BSA, fibrinogen, lysozyme, and RNase A; concentration: 1 mg/mL in PBS, pH 7.4). After the formation of SAMs terminating in the polymerization initiator (step I in Table 1), apparent changes in thickness were observed probably because of weak, long-range hydrophobic interactions between the surface and proteins. The thickness increase was above 13 Å, and fibrinogen (a sticky protein) gave a 21 Å increase. In contrast, the thickness increase was greatly diminished after SI-ATRP, and the subsequent surface reactions (azide formation and the click chemistry) did not deteriorate the non-biofouling property of the surface. The thickness increase of no more than 5 Å was observed from steps II to IV (Table 1). The results indicate that the inert character of PEG was maintained regardless of organic functionalities present at the terminal of the PEG-based polymeric films, and the click chemistry would be a good candidate for the functionalization of the PEG-based, polymeric surface. Of interest, the biotin-presenting surface showed the thickness decrease (−20 Å) upon the exposure to streptavidin, while the surface did negligible thickness increase (<4 Å) with other proteins, such as BSA, fibrinogen, lysozyme, and RNase A. We believe that the specific interaction between biotin and SA made the polymeric layer more condensed, and consequently the thickness decreased. However, the detailed mechanism remained to be seen.

Biospecific Interactions between a Biotin-Presenting Surface and Streptavidin. The SPR technique is an optical method for measuring the refractive index of very thin layers of material adsorbed on a metal. The extent of binding between the solution-phase interactant and the immobilized interactant is easily observed and quantified by monitoring this reflectivity change.[34] One of the advantages of the SPR technique is its high sensitivity without any fluorescent or other labeling of the interactants. We used SPR to investigate the binding of SA onto the biotin-presenting surface. Figure 3 shows SPR sensorgrams of the association and dissociation phases for SA and other proteins. The difference in the steady-state SPR signals (ΔRU, defined

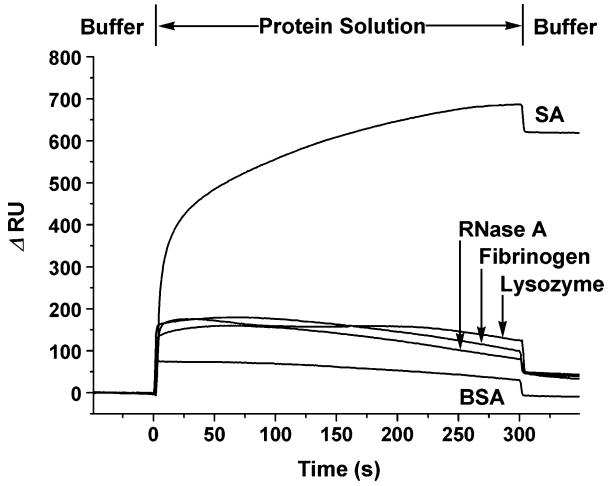

Figure 3. Surface plasmon resonance (SPR) sensorgrams.

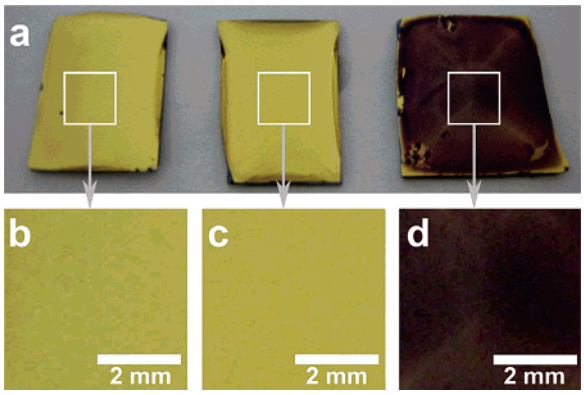

Figure 4. (a) Optical micrograph of substrates presenting Br, N_3, and biotin groups after ELISA (from left to right). (b−d) Optical micrographs of the selected areas from (a).

as ΔRU = RU (10 min after washing with buffer) − RU (steady-state signal prior to protein injection)) is proportional to the mass of adsorbed protein (1 ΔRU = 1 pg/mm^2).[34] The relative resonance unit (ΔRU) was measured to be 618 RU for SA. In the cases of lysozyme, fibrinogen, RNase A, and BSA, the values of ΔRU were measured to be 40, 37, 30, and 0 RU, respectively, which was consistent with the ellipsometric measurements. Assuming the average molecular weight of 52 kDa (1 Da ≈ 1.66 × 10^{-27} kg) for SA and the total area of 1 cm^2 of SPR chip, the value of 618 RU corresponded to ∼1.7 × 10^8 SA molecules/mm^2, or an average distance of ∼6 × 10^{-6} nm between adsorbed protein molecules.[13] The results mentioned above showed that biotin was successfully introduced onto the PEG-based polymeric surface, and SA could biospecifically recognize and bind to biotin at the surface.

Enzyme-linked immunosorbent assay (ELISA) was also performed for confirming the introduction and recognition of biotin. Figure 4 shows optical micrographs of three different surfaces after ELISA: Br-presenting, N_3-presenting, and biotin-presenting polymeric surfaces. The three samples were treated with TMB, and the color change was determined. Figure 4d showed the color change from yellow to deep blue on the biotin-presenting polymeric surface. No color change was observed with the Br-presenting and N_3-presenting polymeric surfaces (Figure 4b and c).

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 active functional groups with non-biofouling property of the supporting materials. In this report, we chose "click" chemistry as a potential candidate of coupling reactions between polymeric surfaces and incoming molecules of interest, and presented azide groups at the terminal of the non-biofouling polymeric film of *p*OEGMA. The "click" chemistry proceeded efficiently at the polymeric surfaces with various acetylenes bearing various functional groups, such as methyl, hydroxyl, carboxylic acid, and ester groups. As a model for bioconjugation, biotin was immobilized onto the *p*OEGMA film via click chemistry, and the biospecific recognition of streptavidin was demonstrated. The method described herein would be utilized for effectively attaching biologically active molecules onto non-biofouling, polymeric platforms because of the simplicity and efficiency of click chemistry, and be extended to the generation of microarrays with an aid of microfabrication techniques.

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References and Notes

(1) Collier, T. O.; Thomas, C. H.; Anderson, J. M.; Healy, K. E. *J. Biomed. Mater. Res.* **2000**, *49*, 141.
(2) Service, R. F. *Science* **1995**, *270*, 230.
(3) McArthur, S. L.; Mclean, K. M.; Kingshott, P.; St John, H. A. W.; Chatelier, R. C.; Griesser, H. J. *Colloids Surf., B: Biointerface* **2000**, *17*, 37.
(4) Österberg, E.; Bergström, K.; Holmberg, K.; Schuman, T. P.; Riggs, J. A.; Burns, N. L.; Van Alstine, J. M.; Harris, J. M. *J. Biomed. Mater. Res.* **1995**, *29*, 741.
(5) Kazuhiko, I.; Hideki, M.; Toshikazu, K.; Nobuo, N. *J. Biomed. Mater. Res.* **1995**, *29*, 181.
(6) Brink, C.; Österberg, E.; Holmberg, K.; Tiberg, F. *Colloids Surf.* **1992**, *66*, 149.
(7) Alcantar, N. A.; Aydil, E. S.; Israelachvili, J. N. *J. Biomed. Mater. Res.* **2000**, *51*, 343.
(8) Harris, J. M. *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*; Plenum Press: New York, 1992.
(9) Mougin, K.; Lawrence, M. B.; Fernandez, E. J.; Hillier, A. C. *Langmuir* **2004**, *20*, 4302.
(10) Edmondson, S.; Osborne, V. L.; Huck, W. T. S. *Chem. Soc. Rev.* **2004**, *33*, 14.
(11) Ma, H.; Li, D.; Sheng, X.; Zhao, B.; Chilkoti, A. *Langmuir* **2006**, *22*, 3751.
(12) Ma, H.; Hyun, J.; Stiller, P.; Chilkoti, A. *Adv. Mater.* **2004**, *16*, 338.
(13) Ma, H.; Wells, M.; Beebe, T. P., Jr.; Chilkoti, A. *Adv. Funct. Mater.* **2006**, *16*, 640.
(14) Michel, R.; Pasche, S.; Textor, M.; Castner, D. G. *Langmuir* **2005**, *21*, 12327.
(15) Snellings, G. M. B. F.; Vansteenkiste, S. O. S. I.; Corneillie, S. O.; Davies, M. C.; Schacht, E. H. *Adv. Mater.* **2000**, *12*, 1959.
(16) (a) Jon, S.; Seong, J.; Khademhosseini, A.; Tran, T.-N. T.; Laibinis, P. E.; Langer, R. *Langmuir* **2003**, *19*, 9989. (b) Khademhosseini, A.; Jon, S.; Suh, K. Y.; Tran, T.-N. T.; Eng, G.; Yeh, J.; Seong, J.; Langer, R. *Adv. Mater.* **2003**, *15*, 1995.
(17) (a) Koguma, I.; Sugita, K.; Saito, K.; Sugo, T. *Biotechnol. Prog.* **2000**, *16*, 456. (b) Sun, F.; Castner, D. G.; Mao, G.; Wang, W.; McKeown, P.; Grainger, D. W. *J. Am. Chem. Soc.* **1996**, *118*, 1856.
(18) Sawall, D. D.; Villahermosa, R. M.; Lipeles, R. A.; Hopkins, A. R. *Chem. Mater.* **2004**, *16*, 1606.
(19) Kim, D. J.; Kang, S. M.; Kong, B. K.; Kim, W.-J.; Paik, H.-j.; Choi, H.; Choi, I. S. *Macromol. Chem. Phys.* **2005**, *206*, 1941.
(20) Yoon, K. R.; Chi, Y. S.; Lee, K. B.; Lee, J. K.; Kim, D. J.; Koh, Y. J.; Joo, S. W.; Yun, W. S.; Choi, I. S. *J. Mater. Chem.* **2003**, *13*, 2910.
(21) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921.
(22) Kim, J. B.; Bruening, M. L.; Baker, G. L. *J. Am. Chem. Soc.* **2000**, *122*, 7616.

(23) Huang, X.; Wirth, M. J. *Anal. Chem.* **1997**, *69*, 4577.
(24) Tugulu, S.; Arnold, A.; Sielaff, I.; Johnsson, K.; Klok, H.-A. *Biomacromolecules* **2005**, *6*, 1602.
(25) Lee, J. K.; Chi, Y. S.; Choi, I. S. *Langmuir* **2004**, *20*, 3844.
(26) Feldman, A. K.; Colasson, B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 3897.
(27) (a) Rostovtsev, V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. (b) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.
(28) Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radič, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 1053.
(29) Sumerlin, B. S.; Tsarevsky, N. V.; Louche, G.; Lee, R. Y.; Matyjaszewski, K. *Macromolecules* **2005**, *38*, 7540.
(30) Gao, H.; Louche, G.; Sumerlin, B. S.; Jahed, N.; Golas, P.; Matyjaszewski, K. *Macromolecules* **2005**, *38*, 8979.
(31) Shah, R. R.; Merreceyes, D.; Husemann, M.; Rees, I.; Abbott, N. L.; Hawker, C. J.; Hedrick, J. L. *Macromolecules* **2000**, *33*, 597.
(32) Wilbur, D. S.; Chyan, M.-K.; Hamlin, D. K.; Kegley, B. B.; Risler, R.; Pathare, P. M.; Quinn, J.; Vessella, R. L.; Foulon, C.; Zalutsky, M.; Wedge, T. J.; Hawthorne, M. F. *Bioconjugate Chem.* **2004**, *15*, 203.
(33) Punna, S.; Kaltgrad, E.; Finn, M. G. *Bioconjugate Chem.* **2005**, *16*, 1536.
(34) Liedberg, B.; Lundström, I.; Stenberg, E. *Sens. Actuators, B* **1993**, *11*, 63.
(35) Kim, D. J.; Lee, K.-B.; Chi, Y. S.; Kim, W.-J.; Paik, H.-j.; Choi, I. S. *Langmuir* **2004**, *20*, 7904.
(36) Mostetler, M. J.; Stokes, J. J.; Murray, R. W. *Langmuir* **1996**, *12*, 3604.
(37) Jones, D. M.; Brown, A. A.; Huck, W. T. S. *Langmuir* **2002**, *18*, 1265.
(38) Lummerstorfer, T.; Hoffmann, H. *J. Phys. Chem. B* **2004**, *108*, 3963.
(39) (a) Collman, J. P.; Devaraj, N. K.; Chidsey, C. E. D. *Langmuir* **2004**, *20*, 1051. (b) Devaraj, N. K.; Miller, G. P.; Ebina, W.; Kakaradov, B.; Collman, J. P.; Kool, E. T.; Chidsey, C. E. D. *J. Am. Chem. Soc.* **2005**, *127*, 8600. (c) Devaraj, N. K.; Dinolfo, P. H.; Chidsey, C. E. D.; Collman, J. P. *J. Am. Chem. Soc.* **2006**, *128*, 1794. (d) Collman, J. P.; Devaraj, N. K.; Eberspacher, T. P. A.; Chidsey, C. E. D. *Langmuir* **2006**, *22*, 2457.
(40) Zhang, Y.; Luo, S.; Tang, Y.; Yu, L.; Hou, K.-Y.; Cheng, J.-P.; Zeng, X.; Wang, P. G. *Anal. Chem.* **2006**, *78*, 2001.
(41) Link, A. J.; Vink, M. K. S.; Tirrell, D. A. *J. Am. Chem. Soc.* **2004**, *126*, 10598.
(42) Beneš, L.; Melánová, K.; Zima, V.; Trchová, M.; Uhlířová, E.; Matějka, P. *Eur. J. Inorg. Chem.* **2001**, 713.
(43) Arnold, R.; Azzam, W.; Terfort, A.; Woll, C. *Langmuir* **2002**, *18*, 3980.
(44) Yam, C.-M.; Pradier, C.-M.; Salmain, M.; Marcus, P.; Jaouen, G. *J. Colloid Interface Sci.* **2001**, *235*, 183.
(45) Yam, C.-M.; Pradier, C.-M.; Salmain, M.; Fischer-Durand, N.; Jaouen, G. *J. Colloid Interface Sci.* **2002**, *245*, 204.

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