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Display of Amino Groups on Substrate Surfaces by Simple Dip-Coating of Methacrylate-Based Polymers and Its Application to DNA Immobilization

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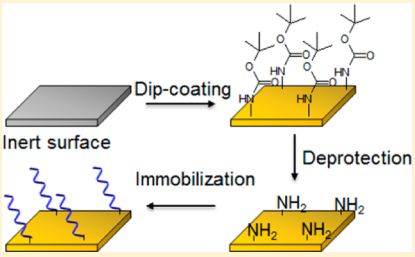
Supporting Information

ABSTRACT: The implementation of a reactive functional group onto a material surface is of great importance. Reactive functional groups (e.g., an amino group and a hydroxyl group) are usually hydrophilic, which makes it difficult to display them on a dry polymer surface. We here propose a novel method for displaying amino groups on the surfaces of polymeric substrates through dip-coating of a methacrylate-based copolymer. We synthesized copolymers composed of methyl methacrylate and 2-aminoethyl methacrylate with different protecting groups or ion-complexes on their amino groups, then dip-coated the copolymers onto a poly(methyl methacrylate) (PMMA) substrate. Evaluation using a cleavable fluorescent compound, which was synthesized in the present study to quantify a small amount (pmol/cm^2) of amino groups on a solid surface, revealed that the protection of amino groups affected their surface segregation in the copolymer coating. *p*-Toluenesulfonate ion-complex and *tert*-butoxycarbonyl (Boc) protection of amino groups were found to effectively display amino groups on the surface (more than 70 pmol/cm^2). The density of amino groups displayed on a surface can be easily controlled by mixing the copolymer and PMMA before dip-coating. Dip-coating of the copolymer with Boc protection on various polymeric substrates also successfully displayed amino groups on their surfaces. Finally, we demonstrated that the amino groups displayed can be utilized for the immobilization of a DNA oligonucleotide on a substrate surface.



1. INTRODUCTION

Chemical modification of bulk material surfaces is a rational approach to surface functionalization. Various methods have been developed to functionalize material surfaces, including plasma treatment,[1] self-assembled monolayer formation,[2] vapor deposition,[3,4] layer-by-layer assembly,[5] Langmuir−Blodgett deposition,[6,7] dip- and spin-coating, and specific peptides binding to material surfaces.[6] Dip-coating is a simple method for altering surface properties and functionalizing material surfaces. Dip-coating of a polymer solution can immobilize functional polymers onto an inert material surface and endow the surface with the functional properties of the coating polymers. One example of a successful chemical modification through polymer coating involves the use of perfluoroalkyl-containing polymers to achieve highly hydrophobic surfaces, because perfluoroalkyl groups tend to be segregated on a surface in contact with air.[8−10] From a practical standpoint, there is a strong demand for introducing reactive functional groups onto inert material surfaces and for controlling the density of reactive functional groups on surfaces.[11,12] Reactive functional groups, including hydroxy and amino groups, can act as reactive sites for subsequent reactions (immobilization of biomolecules and ligands or polymer grafting).[13−15] However, such reactive functional groups are usually hydrophilic, and it is difficult to display them on a dry polymer surface because they are likely to be buried within the bulk phase of the polymer matrix during the drying of a dip-coated solution, as hydrophobic moieties are preferentially segregated at the air/polymer interface to minimize its surface energy.[2] To display hydrophilic reactive groups on the surface, some studies have reported the adsorption of water-soluble amphiphilic polymers containing the reactive functional groups on a material surface in an aqueous solution.[16] The physical adsorption of hydrophilic polymers might, however, suffer from desorption of the polymer in an aqueous solution and limit the available surface groups because of limitations in adhesion between the hydrophilic polymer and the substrate surface. It is also difficult to control the density of functional groups on a coated surface.

In the present study, we propose a novel strategy to display amino groups on a surface through dip-coating of a methacrylate-based copolymer onto polymeric substrates. We conjectured that the protection of amino groups in the polymer would render the amino groups hydrophobic, leading to the display of the amino groups on the surface after dip-coating (Figure 1). We synthesized methacrylate-based copolymers containing amino groups in the side chains and modified the amino groups with different protecting groups. Quantification of the amino groups using a cleavable fluorescent compound developed in the present study revealed that *tert*-butoxycarbonyl (Boc) protection displayed amino groups in a dip-coated

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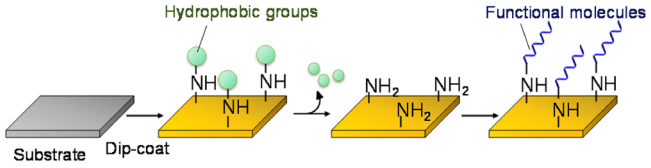


Figure 1. Illustration of surface modification by dip-coating of a copolymer with a protecting group.

copolymer and that the amino groups on the surface were accessible to a reactant in an aqueous solution.

2. RESULTS AND DISCUSSION

To Quantify Amino Groups on a Solid Surface. Prior to the investigation of dip-coating, we developed a novel method for quantifying amino groups on a solid surface. Although many methods for quantifying amino groups have been reported,[17−22] there are difficulties in quantifying small amounts (~pmol/cm^2) of amino groups on a solid surface. In previous studies, amino groups on surfaces were analyzed using XPS (X-ray photoelectron spectroscopy), ATR-FTIR (attenuated total reflectance-Fourier transform infrared spectroscopy), and a fluorescent imager (fluorescent laser scanner).[16,23−25] Direct spectrometric measurements (XPS and ATR-FTIR) can, however, detect amino groups on a surface and also those buried within certain depths (10 nm for XPS and a few micrometers for ATR-FTIR) from a surface, which are not accessible to reactants in bulk solution.[26,27] In our preliminary investigation, we attempted covalent modification of amino groups displayed on a surface using fluorescein isothiocyanate (FITC), but failed to reproducibly quantify the amino groups by analyzing the fluorescent images of the substrate surfaces.

Noel et al. reported the quantification of primary amine groups on a solid surface.[21] They employed an anionic dye that electrostatically binds to amino groups on the surface and is liberated by pH adjustment. We extended their strategy to a fluorescent dye for picomole-order detection and synthesized a cleavable fluorescent compound, which consisted of a reactive carboxy group, a disulfide bond, and a fluorescent moiety (Scheme 1). The cleavable fluorescent compound was designed to covalently bind to amino groups via amide bonds in the presence of a condensation agent (carbodiimide) and to liberate the fluorescent moiety into an aqueous solution under reducing conditions. This approach enables the quantification of amino groups in homogeneous solution using a conventional fluorescence spectrophotometer, rather than at the solid surface. HPLC analysis revealed that the disulfide bond in the compound was able to be cleaved in 2 mM dithiothreitol (DTT) aqueous solution. This method means that the amino groups detected on the surface can be accessible to a water-soluble reactant (cleavable fluorescent compound) in an aqueous solution.

Syntheses of Methacrylate-Based Copolymers Containing Amino Groups and Dip-Coating. We designed and synthesized random copolymers of methyl methacrylate (MMA, 90 mol %) and 2-aminoethylmethacrylate (AEMA, 10 mol %) via free radical polymerization (Figure 2).[28] The

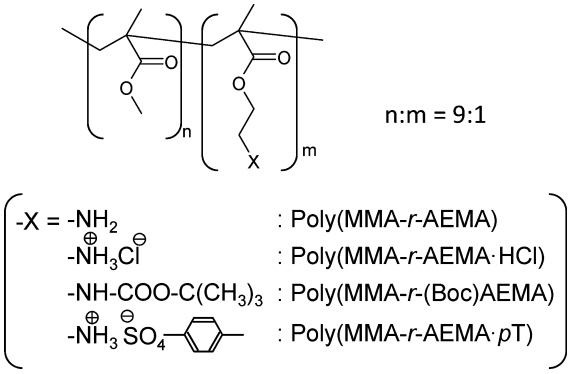
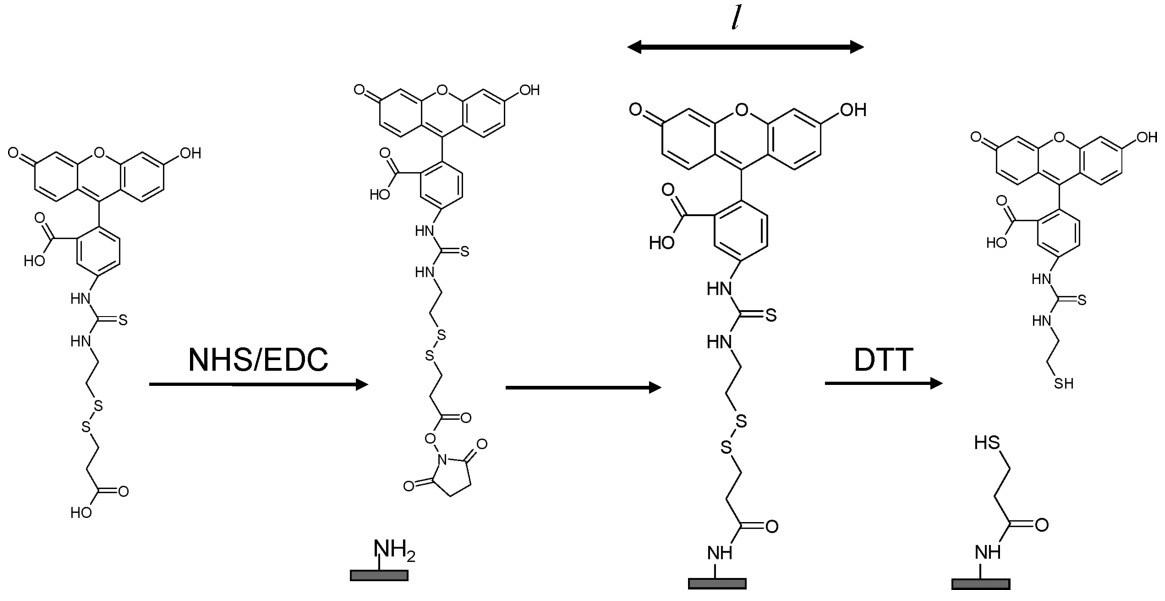


Figure 2. Chemical structures of synthesized copolymers.

polymerization was identified by ^{1}H NMR, FT-IR, and GPC. NMR chemical shifts at 3.6 ppm (s, 31H, $-CO_2-CH_2-$, $-CO_2-CH_2-CH_2-$, $-CO_2-CH_3$), 1.9−1.81 ppm (m, 17H, $C-CH_2-$), and 1.25−0.85 ppm (m, 34H, $C-CH_3$) indicate the MMA and AEMA moieties. The FTIR measurement shows

Scheme 1. Quantification of Amino Groups Using a Cleavable Fluorescent Compound



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the absorbance at 1729 and 1148 cm^{-1}, suggesting the C−O and C═O bonds in the copolymer. The monomer compositions in the copolymers were determined by the elemental analysis. These copolymers (poly(MMA-*r*-AEMA)) consisted of adhesive (MMA) and reactive groups (AEMA). The MMA groups were supposed to act as an adhesive matrix to some of polymeric substrates, and also as an insoluble moiety in water. The adhesion of the coated copolymer to the polymeric substrates was supposed to be van der Waals force and dipole−dipole interaction. In particular, because a methacrylate-base polymer is compatible with poly(methyl methacrylate) (PMMA), the dip-coating of the copolymers would produce the boundaryless coating on a PMMA substrate. AEMA groups provided amino groups as reactive points, which can immobilize functional molecules in a subsequent reaction. The amino groups in the copolymers were modified by counterions and a protecting group: ionic complexes with chloride ions and with *p*-toluenesulfonate, and *tert*-butoxycarbonyl groups. These protecting groups can be deprotected readily using appropriate aqueous solutions. Size-exclusion chromatography suggested that poly(MMA-*r*-AEMA) had an M_n of 130 × 10^3 g/mol and an M_w/M_n of 1.5. When the copolymers were dip-coated onto a PMMA substrate, the coated copolymers was not detached from a substrate in an aqueous buffer (to the naked eye) and were not soluble in water. Indeed, there was no weight change after immersing coated substrates in aqueous solutions and drying.

The dip-coating of these copolymers was carried out on both sides of PMMA substrates (1 cm × 1 cm). The dip-coated substrates apparently kept the intrinsic transparency. Weight measurements of the substrates before and after coating revealed that the thickness of the coated layer was 1.4 ± 0.5 μm on average. We confirmed that the deprotection of amino groups did not change the weight of a coated substrate. After deprotection, the cleavable fluorescent compound was conjugated with the amino groups on the surface using water-soluble carbodiimide, then liberated using DTT. The liberated fluorescent compound was then measured to quantify the amino groups displayed on surfaces. Figure 3 shows the effect of the protecting groups on the cleavable fluorescent compound immobilized on dip-coated surfaces. A bare substrate and the PMMA-coated substrate did not exhibit significant fluorescence, indicating the absence of amino groups on the surface. PMMA coating on a bare substrate was carried out to ensure that the coating process did not affect the present quantification. Small amounts of background fluorescence would be due to physical (nonspecific) adsorption of the cleavable fluorescent compound on the surfaces. Because a bare substrate and the PMMA coating have the same surface properties, it is reasonable that these surfaces exhibited similar signals during the quantification of amino groups. The dip-coating of copolymers without protecting groups (poly(MMA-*r*-AEMA)) and with hydrochloride (poly(MMA-*r*-AEMA·HCl)) exhibited fluorescence higher than that of bare and PMMA-coated substrates, indicating that these copolymers introduced amino groups onto the surfaces. The estimated amino groups on the surfaces were 15 ± 9 and 28 ± 10 pmol/cm^2 for poly(MMA-*r*-AEMA) and poly(MMA-*r*-AEMA·HCl), respectively. Copolymers with Boc protection (poly(MMA-*r*-(Boc)AEMA)) and with *p*-toluenesulfonate (poly(MMA-*r*-AEMA·*p*T)) showed fluorescence signals 5-fold higher than that of poly(MMA-*r*-AEMA). These results mean that the amounts of amino groups on the surface depended on the protection of amino groups, and also suggest that hydrophobic protecting groups enable amino groups to locate at the surface. The Boc protection and *p*-toluenesulfonate ion-complex produced amino groups of 73 ± 8 and 79 ± 38 pmol/cm^2 on coated surfaces. The lateral long axis (l in Scheme 1) of the fluorescent moiety was 1.26 nm in length according to a space-filling model calculation.[29] Assuming that the fluorescent moiety can rotate freely on the surface and occupy an area of 1.26 nm × 1.26 nm and that the coated surface was flat, the estimated maximal amount of the cleavable fluorescent compound immobilized is 100 pmol/cm^2. Although this value shows the limitation of the present quantification method, it is comparable to the values obtained in the present study.

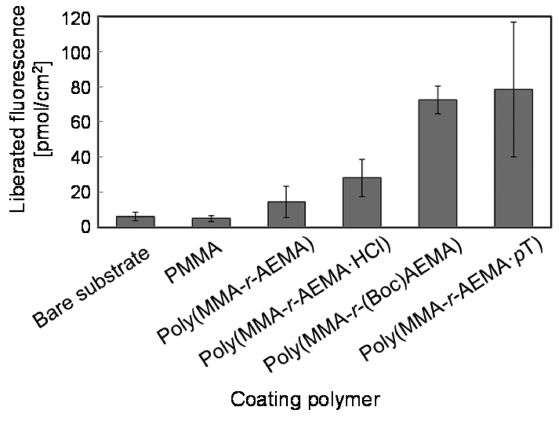

Figure 3. Effect of the protecting groups on the cleavable fluorescent compound immobilized on dip-coated surfaces. A bare substrate represents a PMMA substrate without any coating. PMMA-coating represents a PMMA substrate coated with PMMA in the same manner.

X-ray Photoelectron Spectroscopy (XPS) Measurements. The X-ray photoelectron spectroscopy (XPS) measurements were carried out for the surfaces coated with poly(MMA-*r*-AEMA), poly(MMA-*r*-AEMA·HCl), poly(MMA-*r*-(Boc)-AEMA), and poly(MMA-*r*-AEMA·*p*T) (Figure 4). A peak of N1s at 405 eV was hardly observed for the poly(MMA-*r*-AEMA)- and poly(MMA-*r*-AEMA·HCl)-coated surfaces, although a small amount of amino groups on the surface was detected using the cleavable fluorescent compound. These results would be explained as follows. Tanaka et al. reported the swelling of surfaces of PMMA induced by water, even though water is a nonsolvent for PMMA.[30] The swelling took place at a depth of more than 10 nm. In the present study, the amino groups of poly(MMA-*r*-AEMA) would be buried at a certain depth from a dry polymer surface because an XPS measurement was carried out for a dry sample in a high-vacuum chamber. Once the surface got wet with water, the methacrylate-based polymer swelled, and the amino groups came out to the surface. Indeed, the poly(MMA-*r*-AEMA) coating produced a very small amount of amino groups on a surface, which was only one-fifth or less than that of poly(MMA-*r*-AEMA·*p*T). On the other hand, the XPS measurement proved the presence of nitrogen (N1s at 405 eV) for the poly(MMA-*r*-(Boc)AEMA) and the poly(MMA-*r*-AEMA·*p*T)-coated surfaces, meaning the display of amino groups on surfaces by dip-coating of these copolymers. Sulfur (S2p at 173 eV) was also observed on the poly(MMA-*r*-AEMA·*p*T)-coated surface, which indicates that *p*-toluenesulfonate, which was supposed to be complexed with an amino group, was present on the coated surface. The N/C and O/C

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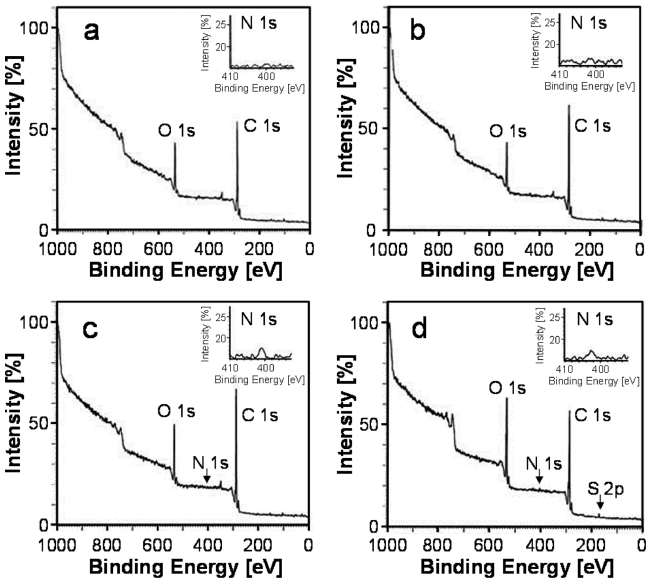

Figure 4. XPS measurements of the surfaces of dip-coated substrates. (a) Poly(MMA-*r*-AEMA)-, (b) poly(MMA-*r*-AEMA·HCl)-, (c) poly(MMA-*r*-(Boc)AEMA)-, and (d) poly(MMA-*r*-AEMA·*p*T)-coated surfaces. Insets are N1s core-level spectra.

ratios, derived from the XPS spectral area ratios of N1s and C1s core-level, were summarized in Table 1. The poly(MMA-*r*-(Boc)AEMA)- and poly(MMA-*r*-AEMA·*p*T)-coated surfaces gave relatively high N1s/C1s ratios (3.9% and 4.2%) as compared to poly(MMA-*r*-AEMA)- and poly(MMA-*r*-AEMA·HCl)-coated surfaces. The poly(MMA-*r*-AEMA·*p*T)-coated surface had a high O1s/C1s ratio (34%), which also supports the display of *p*-toluenesulfonate on a surface. These results agree with the quantification results using the cleavable fluorescent compound.

Table 1. Spectral Area Ratios of Nitrogen and Oxygen to Carbon Derived from XPS Measurements of Copolymers-Coated PMMA Substrates

	N1s/C1s [%]	O1s/C1s [%]
poly(MMA-*r*-AEMA)	1.0	24
poly(MMA-*r*-AEMA·HCl)	1.1	21
poly(MMA-*r*-(Boc)AEMA)	3.9	21
poly(MMA-*r*-AEMA·*p*T)	4.2	34

Although Boc protection and *p*-toluenesulfonate ion-complex displayed amino groups on the surface most effectively among the copolymers tested, *p*-toluenesulfonate protection provided variable results (large error bars). This may be because the stability of ionic complexes is relatively low as compared to covalent bonds under the present conditions.

The use of Boc protection for the display of amino groups has important features, as follows. First, Boc protection can be readily deprotected by acid in aqueous and organic solvents. Second, the Boc-deprotection reaction produces gaseous isobutene and carbon dioxide as byproducts, which can be separated from coated substrates rapidly and easily. Third, stable protection with a covalent bond can improve the stability of amino groups on coated surfaces. Amino groups are reactive and may be subject to unexpected reactions, including oxidation. Amino groups can thus be stored using Boc protection and the protecting group conveniently removed immediately prior to use.

Control of the Density of Amino Groups on a Surface. Control of the density of amino groups on the surface is an important issue for surface functionalization. Excessive amino group density on the surface results in nonspecific adsorption of biomolecules[31] and might lead to low conversion of amino groups due to restricted accessibility by functional molecules (e.g., ligands and biomacromolecules) when immobilizing them on the surface. Unreacted amino groups on the surface would induce undesired high cationic charges, which may cause electrostatic adsorption and denaturation of biomacromolecules. We therefore attempted to control the density of amino groups on the surface using poly(MMA-*r*-(Boc)AEMA). Poly(MMA-*r*-(Boc)AEMA) and PMMA were first mixed, and the mixture was then dip-coated onto PMMA substrates. The poly(MMA-*r*-(Boc)AEMA) contents at 25 and 50 wt % resulted in 27 and 46 pmol/cm^2 (Figure 5). The immobilized fluorescence increased proportionately with the content of poly(MMA-*r*-(Boc)AEMA), suggesting that the amino group density on the surface can be controlled by simply varying the content of poly(MMA-*r*-(Boc)AEMA) in the polymer mixture.

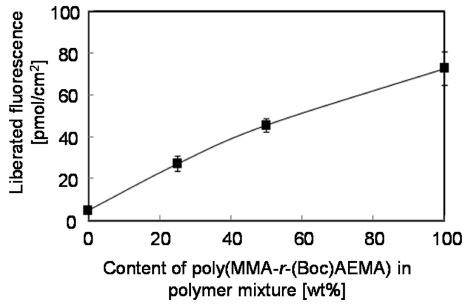

Figure 5. Effect of the poly(MMA-*r*-(Boc)AEMA) content in the polymer mixture on the cleavable fluorescent compound immobilized on dip-coated surfaces. Poly(MMA-*r*-(Boc)AEMA) was mixed with PMMA, and the mixture was dip-coated onto a PMMA substrate.

Dip-Coating on Different Kinds of Substrates. One of the attractive features of dip-coating is that it is applicable to different kinds of substrates. Poly(MMA-*r*-(Boc)AEMA) was dip-coated onto a variety of polymeric substrates, polyethylene terephthalate (PET), poly(vinyl chloride) (PVC), and nylon 6. The coated copolymer (or PMMA) was not detached from the substrates during deprotection and washing procedures. Bare substrates exhibited physical adsorption of the cleavable fluorescent compound, with the amount adsorbed depending on the particular substrate (Figure 6). However, dip-coating of PMMA onto the substrates suppressed physical adsorption, resulting in low levels of adsorption (less than 10 pmol/cm^2), similar to that of a PMMA substrate. Dip-coating of poly(MMA-*r*-(Boc)AEMA) onto the substrates increased the fluorescence immobilization to that of the dip-coated PMMA substrate. The estimated amino groups on these substrates were from 66 to 88 pmol/cm^2, which was comparable to that on a PMMA substrate (73 pmol/cm^2). These results indicate that the dip-coating method can display a similar amount of amino group on a variety of substrates.

DNA Immobilization on the Dip-Coated Substrates. One of the applications of the surfaces displaying reactive functional groups is the immobilization of functional molecules on the surface. In the last two decades, DNA or RNA

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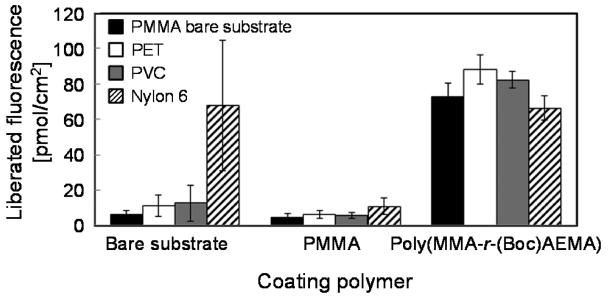

Figure 6. Immobilization of cleavable fluorescent compound on various kinds of substrates by copolymer dip-coating. PMMA, PET (polyethylene terephthalate), PVC (polyvinyl chloride), and nylon 6 substrates were used. For poly(MMA-*r*-(Boc)AEMA) coating, p values were >0.05. Between substrates coated with poly(MMA-*r*-(Boc)-AEMA) and bare substrates except nylon 6, p values were <0.05. All of the data were based on 7-fold experiments.

oligonucleotides have attracted a broad attention as functional molecules because of their specific hybridization to the complements and of the aptamer-binding ability. We carried out DNA immobilization on a copolymer-coated surface. A PMMA substrate, a PET film, a PVC film, and a nylon 6 film were first coated with poly(MMA-*r*-(Boc)AEMA). After deprotection using appropriate solutions, the amino groups on the surface were modified with a bifunctional cross-linker (*N*-succinimidyl 6-maleimidohexanoate), followed by conjugation of the cross-linker with a 5′-end thiolated DNA oligonucleotide (19-mer). A complementary DNA strand with FITC at the 5′-end (FITC-DNA) was loaded to allow DNA hybridization on the surface. The hybridized FITC-DNA was liberated by heating and measured. Figure 7 shows that

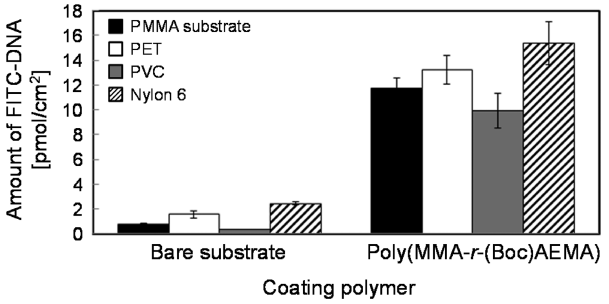

Figure 7. FITC-DNA hybridization on thiolated DNA (19-mer) immobilized on copolymer-coated substrates.

considerable amounts (10−15 pmol/cm^2) of FITC-DNA were liberated from all of the coated substrates, indicating successful immobilization of thiolated DNA on the coated surfaces. The amounts of hybridized DNA (FITC-DNA) in the present study were similar to those reported in the literature.[23,32,33] In the case of the PMMA substrate coated with the copolymer without any protection (poly(MMA-*r*-AEMA), there was only 2.3 pmol/cm^2 immobilized on a coated surface. The initial density of amino groups affected the DNA immobilization.

3. CONCLUSIONS

We synthesized copolymers with amino groups with different protecting groups to functionalize polymer surfaces by simple dip-coating. We also developed a cleavable fluorescent compound to quantify small amounts (~pmol/cm^2) of amino groups on solid surfaces. The present study successfully demonstrated that protection of amino groups affects the surface segregation of amino groups during polymer dip-coating and that Boc protection of amino groups effectively and reproducibly displays amino groups on the surfaces of a variety of polymer substrates. The density of amino groups displayed on a surface can be easily controlled by varying the content of the copolymer incorporating the amino groups. Investigation of DNA immobilization revealed that the dip-coated substrate can be used to immobilize functional molecules, including DNA, antibodies, enzymes, and other ligands. The dip-coating of the copolymers did not affect the visible-light transparency of the substrates, which means that the present methodology using dip-coating has a potential for the application in the optical sensors and biosensors. The present study suggests that functional groups with appropriate protection can control the surface segregation of the functional groups, and therefore that this method has great potential for surface functionalization of polymeric matrixes.

4. EXPERIMENTAL SECTION

Materials. Methyl methacrylate (MMA), 2-propanol, 2,2′-azobisisobutyronitrile (AIBN), triethylamine, *N*,*N*-dimethyl-4-aminopyridine (DMAP), *N*,*N*-dimethylformamide (DMF), potassium carbonate (K_2CO_3), chloroform, and *N*-hydroxysuccinimide (NHS) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2-Aminoethyl methacrylate hydrochloride (AEMA·HCl) and fluorescein isothiocyanate (FITC) were purchased from Sigma (St Louis, MO). Di-*tert*-butyl dicarbonate (Boc_2O) was purchased from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). *N*-Succinimidyl 6-maleimidohexanoate (EMCS, bifunctional cross-linker) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Dojindo Molecular Technologies Inc. (Kumamoto, Japan). 3-[(2-Aminoetyl)dithio]propionic acid was purchased from Toronto Research Chemicals (Toronto, Canada). MMA was distilled at reduced pressure prior to use, and other reagents were used without further purification. Thiolated and (FITC)-modified DNA oligonucleotides (19-mer) were purchased from Tsukuba Oligo Service Co. Ltd. (Tsukuba, Japan). DNA sequences used in the study were thiol-5′-TTAGTTCTCCAGCTATCTT-3′ and FITC-5′-AAGATAGCTGGAGAACTAA-3′ (the complementary strand).

A PMMA substrate (1 mm thick) was obtained from Nitto Jushi Kogyo Co., Ltd. (Tokyo, Japan). A PET film (0.1 mm thick) was obtained from Takafuji Chemical Industry. A PVC film (0.3 mm thick) was obtained from Hitachi Maxell, Ltd. (Tokyo, Japan). Nylon 6 was obtained from Toray Industries, Inc. (Tokyo, Japan) and melt-pressed to prepare nylon films (0.1 mm thick). These substrates were cleaned by a detergent solution and cut to 1 cm × 1 cm pieces prior to use.

Synthesis of Poly(MMA-r-AEMA·HCl). MMA (54.3 mmol), AEMA·HCl (6.04 mmol), and AIBN (0.071 mmol) were dissolved in 11.4 mL of a 2-propanol/water mixture (5:1 weight ratio) in a three-neck flask, followed by purging with nitrogen gas. Polymerization was performed under a nitrogen atmosphere at 60 °C overnight. The copolymer was precipitated by adding excess water, and the precipitate was then freeze-dried overnight. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 3.60 (s, 31H, $-CO_2-CH_2-$, $-CO_2-CH_2-CH_2-$, $-CO_2-CH_3$), 1.95−1.81 (m, 17H, $C-CH_2-$), 1.22−0.85 (m, 34H, $C-CH_3$). IR (ATR): 1148 (C−O), 1727 (C═O), and 2959 (CH_2) cm^{-1}.

Synthesis of Poly(MMA-r-(Boc)AEMA). Poly(MMA-*r*-AEMA·HCl) (2 g) was dissolved in 56 mL of chloroform. DMAP (0.050 mmol), triethylamine (12.6 mmol), and Boc_2O (7.55 mmol) were added to the chloroform solution. Reaction was performed at 4 °C overnight. The polymer was precipitated by adding excess water, and the precipitate was freeze-dried overnight. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 3.60 (s, 31H, $-CO_2-CH_2-$, $-CO_2-CH_2-CH_2-$, $-CO_2-CH_3$), 1.90−1.81 (m, 24H, $C-CH_2-$), 1.60−1.46 (m, 11H, O−

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$(CH_3)_3$), 1.27−0.85 (m, 38H, C−CH_3). IR (ATR): 1215 (C−O), 1735 (C═O), and 2972 (CH_2) cm^{-1}.

Synthesis of Poly(MMA-r-AEMA). Poly(MMA-*r*-AEMA·HCl) (0.8 g) was dissolved in 28 mL of chloroform containing K_2CO_3 (0.58 mmol). The polymer was precipitated by adding excess water, and the precipitate was freeze-dried overnight. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 3.60 (s, 31H, −CO_2−CH_2−, −CO_2−CH_2−CH_2−, −CO_2−CH_3), 1.90−1.81 (m, 22H, C−CH_2−), 1.25−0.85 (m, 30H, C−CH_3). IR (ATR): 1148 (C−O), 1729 (C═O), and 2953 (CH_2) cm^{-1}.

Synthesis of Poly(MMA-r-AEMA-p-toluenesulfonate) (Abbreviated as poly(MMA-r-AEMA·pT)). AEMA·HCl (6.04 mmol) was dissolved in 20 mL of toluene containing K_2CO_3 (18.1 mmol) at 90 °C. After the solution was filtered, *p*-toluenesulfonic acid (7.03 mmol), MMA (54.3 mmol), 9.2 mL of toluene, and AIBN (0.11 mmol) were added in a three-neck flask, followed by purging with nitrogen gas. Polymerization was performed under a nitrogen atmosphere at 90 °C for 24 h. The copolymer was precipitated by adding excess hexane, and the precipitate was vacuum-dried overnight. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.80 (d, 2H), 7.28 (d, 2H), 3.62 (s, 31H, −CO_2−CH_3, −CO_2−CH_2−CH_2−), 1.92−1.83 (m, 16H, C−CH_2−), 1.28−0.86 (m, 35H, C−CH_3). IR (ATR): 1150 (C−O), 1727 (C═O), and 2954 (CH_2) cm^{-1}.

Characterization of Copolymers. Copolymers synthesized were characterized by ^{1}H NMR (300 M Hz; in chloroform-*d*, Varian Gemini 300) and ATR-FTIR (Bruker Optics, ALPHA with Ge). Molecular weights of copolymers were determined by GPC (Jasco LC2000 plus) equipped with an 80 × 300 mm column × 2 (Showa Denko K. K.) and a RI detector (Jasco RI2031 plus) at 40 °C. Chloroform was used as an elution solvent, and PMMA molecular-weight standards were used for a standard curve.

Synthesis of Cleavable Fluorescent Compound. 3-[(2-Aminoetyl)dithio]propionic acid (0.55 mol) and FITC (0.50 mol) were dissolved in 40 mL of THF/water (1:1 volume ratio) mixture (containing 0.1 vol % triethylamine) and kept for 3 h at room temperature. After the solution was evaporated, the resultant solid was dissolved in 1 wt % Na_2CO_3 aqueous solution, and 1 M HCl aqueous solution was added to adjust the solution pH to 1−2. The precipitate obtained was centrifuged and washed with 1 mM HCl aqueous solution (10 mL) three times and freeze-dried overnight. The yield was 73%. The cleavable fluorescent compound was characterized by ^{1}H NMR and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF-MS) (Voyager, Applied Biosystems, Framingham, MA). ^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) = 6.68−6.57 (m, 9H, fluorescence), 2.99−2.90 (m, 4H, S−CH_2), 2.73−2.66 (t, 2H, CH_2−COOH), 1.21−1.16 (t, 2H, NH−CH_2). MALDI TOF/MS ($[M + Br]^+$ calcd for $C_{26}H_{22}N_2O_7S_3$, m/z = 570.7; found, 572.1.

The reduction of the cleavable fluorescent compound was evaluated by HPLC. The cleavable fluorescent compound was dissolved in a 1/15 M phosphate buffer solution (pH 8.0, 1 mL) containing 2 mM DTT and mixed at 40 °C for 1 h to reduce the disulfide bond. The reduced cleavable fluorescent compound was applied to an HPLC (Jasco LC2000 plus) equipped with an inertsil ODS-3 (10 × 250 mm) column (GL Science, Tokyo) and a UV detector. The elution program and a flow rate were shown in Table S1. The eluted compounds were observed by detecting absorbance at 280 nm.

Dip-Coating. Polymers synthesized were dissolved in chloroform/DMF (70/30 volume ratio) to give a polymer concentration of 50 g/L. Polymer substrates (PMMA plates, PET, PVC, and nylon 6 films) were immersed in the copolymer solutions (dip-coating) for a few seconds and dried in a vacuum chamber at room temperature overnight. Deprotection of Boc groups was carried out by immersing the substrates in 4 M HCl aqueous solution for 2 h at 25 °C. Deprotection of HCl and *p*-toluenesulfonate was carried out using a 10 mM Na_2CO_3 aqueous solution for 2 h at 25 °C.

The detachment of the coated layer was assessed by immersing the coated substrates (dip-coated after 2 day) in a 4 M HCl aqueous solution or a phosphate buffer for 2 h or longer at 25 or 40 °C. Neither detachment nor peel-off was observed for the copolymers coated on four kinds of substrates by the naked eye. We also confirmed no weight change after immersing and subsequent drying procedures.

Ten pieces of dip-coated PMMA substrates were weighed using a microbalance, and the thickness of a coated layer was calculated from the increment of the weight and the density of PMMA, because the copolymers were composed mainly of a MMA moiety.

Quantification of Amino Groups on Surfaces. The substrates were washed with water (5 mL) three times and immersed in a 1/15 M phosphate buffer solution (pH 8.0, 1 mL) containing 0.2 mM cleavable fluorescent compound, 0.2 mM NHS, and 1 mM EDC, followed by 2 h incubation. The substrates were washed with phosphate buffer (5 mL) three times and immersed in 5 mM NaOH aqueous solution (10 mL) at 40 °C for 1 h. The NaOH aqueous solution was replaced with fresh solution and shaken for 2 h at 40 °C. The repeated wash with a NaOH solution was to solubilize and wash out the unreacted but adsorbed fluorescent compound. Among several washing solutions tested (e.g., SDS, Tween 20, DMSO/water mixture solutions), a NaOH aqueous solution was appropriate in the present study. The substrates were finally rinsed with phosphate buffer (5 mL) three times and immersed in phosphate buffer containing 2 mM DTT at 40 °C for 1 h. The fluorescence intensity of the DTT aqueous solution was measured using a fluorescence spectrophotometer (FP-8200 fluorescence spectrometer, Jasco, Tokyo, Japan). Excitation and emission wavelengths were 494 and 518 nm. Excitation and emission band widths were 5 nm. Sensitivity of the apparatus was set at low. A standard curve of the fluorescent compound was shown in Figure S1. Typically the dip-coating and the quantification were carried out in triplicate unless otherwise stated.

X-ray Photoelectron Spectroscopy (XPS) Measurements. XPS analyses were performed with an ESCA-850 (Shimadzu) equipped with a Mg Kα source (powered at 30 mA and 8 kV) at a constant dwelling time of 200 ms. The pressure in the analysis chamber was about 10^{-15} Pa. The pass energy was set at 100, 2, and 4 eV for wide scan, and narrow scan of N and S, respectively. The spectra were calibrated in relation to the C1s binding energy (284.6 eV).

DNA Immobilization. Poly(MMA-*r*-(Boc)AEMA) was coated on a substrate as described above, followed by deprotection. Prior to DNA immobilization, 5′-thiolated DNA (19-mer, 84 nmol) was dissolved in 834 μL of 0.1 M DTT solution in 0.1 M phosphate buffer (pH 8.0, containing 1 M KCl) in a micro tube to allow reduction of the 5′-thiolated DNA for 30 min. The reduced 5′-thiolated DNA was purified by a NAP-5 column (GE-Healthcare, Piscataway, NJ) using 0.1 M phosphate buffer containing 1 M KCl, pH 8.0. Adequate dilution of the purified DNA with the same phosphate buffer gave 20 μM solutions of the reduced 5′-thiolated DNA. A bifunctional cross-linker solution (2 mM EMCS in 0.1 M phosphate buffer, pH 8.0) was applied as droplets on copolymer-coated substrates at 25 °C for 1 h. These surfaces were rinsed three times with 5 mL of water. Next, a solution of the reduced 5′-thiolated DNA (20 μM in 0.1 M phosphate buffer containing 1 M KCl, pH 8.0) was applied as droplets to the EMCS-modified surfaces. The substrates were incubated at 25 °C for 2 h. These surfaces were rinsed with a copious amount of phosphate buffer. A complementary DNA strand (1 μM, 200 μL), conjugated with FITC at its 5′-end, was loaded onto the DNA-immobilized surfaces at 25 °C for 1 h to allow DNA hybridization. After the substrates were rinsed with a phosphate buffer (5 mL) three times, substrates were dipped in a fresh phosphate buffer (1 mL) at 85 °C for 30 s to dissociate the DNA duplex. The DNA complement liberated in the supernatant was measured by a fluorescence spectrophotometer.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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