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A Cu-free clickable surface with controllable surface density

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Electronic Supplementary Material (ESI) available: Materials, experimental procedures, and additional results.

Abstract

A Cu-free clickable polymer was synthesized and used to dip-coat plastic substrates to prepare Cu-free clickable surfaces. The surface density of dibenzocyclooctyne moieties was quantified using an azide-conjugated fluorophore and a conventional fluorometer, indicating that the clickable moieties displayed on a dip-coated surface were accessible from solutes in water. The present approach also succeeded in the control of the surface density of the clickable moieties. Azide-conjugated oligo DNA was immobilized on the surface using a Cu-free click reaction.

Introduction

Rapid progress in nanotechnology and microscale engineering has increased the use of surfaces and interfaces as confined fields for chemical and biological reactions. Chemically engineered surfaces are particularly important in catalysts, separation techniques, biosensors, microfluidic devices, adhesion, and electronic devices, among others. As engineered surfaces are often used by non-chemists, there is a high demand for a simple, robust, and reproducible methods for functionalizing material surfaces. Self-assembled monolayers prepared from thiol compounds are useful and reliable, but suitable substrate surfaces for their immobilization, such as gold, are limited. Although a wide variety of plastics are used in commodity and industrial products, methodology for their surface functionalization is limited. Some existing methods require special apparatus, harsh conditions, or specialist skill. Coating with a polymer solution is among the most facile methods for functionalizing the surface of a plastic substrate. However, the surface and bulk of a solid polymeric material have significantly different properties [1-3]. The surface segregation of a polymer is key for preparing a molecularly-engineered surface through coating [4-9].

Among various chemical methods available for surface functionalization, click chemistry is a family of powerful and efficient reactions used to synthesize complicated and functional materials under various conditions [10]. The Huisgen 1,3-dipolar cycloaddition of azides and alkynes has become the most widely used click reaction since Sharpless *et al.* and Meldal *et al.* developed the Cu-catalyzed azide–alkyne cycloaddition, in which Cu(I) catalyzes the rapid quantitative reaction of azides and alkynes at ambient temperature in an aqueous solution [11-13]. To avoid the toxicity of Cu, Bertozzi *et al.* developed a Cu-free click reaction (strain-promoted azide–alkyne cycloaddition) using cycloalkynes [14]. This reaction proceeds rapidly with high yield and high selectivity under mild conditions in organic and aqueous solutions. The most attractive advantage of this reaction is its orthogonality to the chemistry used in aqueous solution. This reaction is highly reliable, bio-orthogonal, and often applied to label biological materials, such as proteins, lipids,

oligonucleotides, and cells [15-17].

Since these pioneering works on click chemistry, clickable surfaces have attracted research attention as a platform for immobilizing functional substances for electrodes, analytical devices, and biomaterials [18-28]. However, in previous reports, Au-S, silane coupling, special surface pretreatment (e.g. ozone treatment) and chemical vapor deposition chemistry have been employed for preparing clickable surfaces on substrates, but have limited suitable substrate materials (such as gold and silicon) or require specific apparatus. As plastic substrates are inexpensive and widely used in analytical, biological and medical fields, a simple method to prepare a Cu-free clickable surface on a plastic surface is highly promising. Quantifying and controlling the surface density of reactive sites on the functionalized surface are also important [24, 29-32]. There is a practical demand for the reliable quantification and control of the surface density of clickable groups.

Dip-coating, in addition to brush- and spray-coating, is a simple and easy method for functionalizing surfaces and is applicable to both flat and curved surfaces without requiring special apparatus. Therefore, dip-coating has attracted attention as a practical and versatile method for functionalizing surfaces of a three-dimensional object. To date, our group has reported the introduction of functional groups on a plastic surface by dip-coating using the surface segregation of functional polymers [7, 8, 33]. In the present study, we proposed preparing a Cu-free clickable surface on a plastic surface by simply dip-coating with a functional polymer containing dibenzocyclooctyne moieties. We also succeeded in quantifying the dibenzocyclooctyne moieties present on the clickable surface and controlling the surface density of the clickable functional groups.

Materials and methods

Materials and methods are in the Electronic Supplementary Material.

Results and discussion

To synthesize a Cu-free clickable polymer (termed PMHD, Fig. 1), methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA) were first copolymerized through free radical polymerization in ethyl acetate to obtain a backbone polymer (termed PMH). The ^1H NMR spectrum of PMH showed a monomer ratio (m:n) of 9:1. Size-exclusion chromatography analysis showed that the number-average and weight-average molecular weights of PMH were 41,000 and 85,000, respectively. Dibenzocyclooctyne acid (DBCO acid), which contains a Cu-free clickable functional group, was conjugated to the hydroxy groups of PMH to afford PMHD. ^1H NMR measurements showed that 83% of hydroxy groups in PMH were conjugated with DBCO acid in PMHD.

PMHD was dissolved in a mixture of DMF (1.8 mL) and NaOH aqueous solution (0.2 mL, 1 M) and stirred for 20 h at 25 °C to investigate ester bond hydrolysis in the DBCO-conjugated side chain of PMHD. ^1H NMR measurement of the resultant polymer showed that, after NaOH treatment, chemical shifts derived from the aromatic rings of DBCO moieties (at 7.27–7.70 ppm) and from cyclooctyne rings (at 3.65–3.72 and 5.13 ppm) had disappeared (Fig. S1). These results showed that an ester bond between the DBCO moiety and polymer backbone was cleaved by aqueous NaOH to liberate a DBCO moiety.

Polymers synthesized for dip-coating were dissolved in ethyl acetate to give a concentration of 1 wt%. Nylon 6, polyethylene terephthalate (PET), and poly(methyl methacrylate) (PMMA) substrates were dip-coated by immersing in polymer solution, withdrawing slowly, and drying in a vacuum chamber. Dip-coating with PMHD did not significantly change the appearance of the nylon and PET substrates (Fig. S2). Tape-peeling tests (using scotch-tape) showed that the coated polymer layers did not detach, indicating stable adhesion of the polymer layers onto the substrates. The weight increase of the PET substrates after dip-coating indicated that the PMH and PMHD layers on PET were about 120–140 nm thick, assuming that the density of these polymers was identical to that of PMMA (1.18 g cm⁻³) [33].

To quantify reactive DBCO moieties displayed on the dip-coated surface, we synthesized an

azide-conjugated fluorescent compound (Fig. 2a and S3). The dip-coated surface was immersed in an aqueous solution containing the azide-conjugated fluorescent compound to allow Cu-free click reactions with the surface DBCO moieties. After washing, a fluorophore was liberated from the surface using 0.1 M NaOH aqueous solution (Fig. 2a). The liberated fluorophore in solution was quantified using a conventional fluorophotometer to provide quantitative information on DBCO moieties on the dip-coated surface. DBCO moieties quantified in this way are considered “accessible and reactive to solutes in an aqueous solution” [34]. Bare and PMH-coated substrates (nylon, PET, and PMMA) afforded no liberated fluorophore, suggesting that no DBCO moieties were present on their surfaces. In contrast, all substrates coated with PMHD showed relatively large amounts of fluorophore, indicating that equivalent amounts of reactive DBCO moieties were present on their surfaces. For example, a PMHD-coated PMMA substrate afforded 63 pmol cm⁻² of fluorophore (Fig. 2b). These results showed that DBCO moieties were present on the outermost surface and that Cu-free clickable surfaces were prepared on plastic substrates by simple polymer dip-coating. In previous reports, we demonstrated that hydrophobic moieties promoted the surface segregation of functional groups in a coating polymer [7, 8, 33]. The hydrophobicity of the DBCO moieties also contributes to their surface segregation in the present study.

X-ray photoelectron spectroscopy (XPS) measurements showed that bare and PMH-coated substrates did not have an N1s peak (Fig. S4a and S4b). In contrast, the PMHD-coated PMMA substrate showed an N1s peak derived from the nitrogen of the DBCO moiety in PMHD (Fig. S4c). These results suggested that DBCO moieties were introduced on the surface by dip-coating with PMHD, which agreed with the results of DBCO moiety quantification described above.

The morphology of the dip-coated surfaces was observed using atomic force microscopy (AFM, Fig. S5). The root-mean-square (RMS) surface roughness of bare PMMA and PMH- and PMHD-coated substrates was 1.1, 2.0, and 2.3 nm, respectively. The AFM images and the surface roughness indicated that dip-coated surfaces were flat compared with those of the bare substrates.

Field-emission scanning electron microscopy (FE-SEM) observations elucidated the surface morphology of a large surface area ($40\ \mu\text{m} \times 30\ \mu\text{m}$). Fig. S6 shows neither rolling nor holes on bare and dip-coated substrate surfaces, which also indicated that dip-coating did not change the surface morphology of the substrate.

We next attempted to control the amount of DBCO moieties present on the dip-coated surface. PMHD and PMH were mixed together in dip-coating solutions in various weight ratios. As the PMHD ratio increased, the density of DBCO moieties on the surface increased (Fig. 3), meaning that the surface density of DBCO moieties can be controlled by simply varying the PMHD content in the polymer mixture.

We finally conducted DNA immobilization on the surface of a PMHD-coated PMMA substrate. An aqueous solution containing azide-conjugated DNA oligonucleotide (N_3 -DNA, 19-mer, Table S1) was loaded onto a dip-coated surface to immobilize N_3 -DNA on the surface through a Cu-free click reaction. An aqueous solution of a complementary DNA strand with fluorescein isothiocyanate (FITC) at the 5' end (FITC-DNA, Table S1) was loaded onto the surface to allow DNA hybridization on the surface. The hybridized FITC-DNA was liberated by heating the solution and the solution fluorescence was measured (Fig. S7). Although FITC-DNA was not detected for bare and PMH-coated PMMA substrates, considerable amounts of FITC-DNA were detected on the PMHD-coated surfaces (Fig. 4). For example, when the N_3 -DNA-immobilized surface was prepared using $4\ \mu\text{M}$ N_3 -DNA, $5.4\ \text{pmol cm}^{-2}$ of FITC-DNA was observed, representing the surface density of surface immobilized N_3 -DNA. These results indicated that N_3 -DNA was successfully immobilized on the PMHD-coated surface using Cu-free click reaction. The surface density of immobilized DNA was comparable with that of previous reports [7, 35]. As the concentration of N_3 -DNA increased in the Cu-free click reaction, the surface density of the immobilized N_3 -DNA also increased. Sola et al. also reported that the concentration of DBCO-DNA affected the yield of a Cu-free click reaction [35]. These results demonstrated that the clickable surface prepared by simple dip-coating of PMHD can

be used for biomolecule immobilization.

Conclusions

We have designed and synthesized a Cu-free clickable polymer (PMHD). The surfaces of plastic substrates were easily functionalized through facile dip-coating with PMHD to afford Cu-free clickable functional groups on the outermost surface. We also synthesized an azide-conjugated fluorophore to quantitate a reactive Cu-free clickable functional group (DBCO) on a dip-coated substrate surface. The surface density of DBCO moieties was successfully quantified as 63 pmol cm⁻², when a PMMA substrate was dip-coated with 1 wt% PMHD solution. The surface density of DBCO can be controlled by varying the weight ratio of PMHD in the PMHD/PMH mixed dip-coating solution. Investigating oligo DNA immobilization showed that the PMHD-coated surface immobilized 5.4 pmol cm⁻² oligo DNA via a Cu-free click reaction. The present study is the first report of simple dip-coating with a Cu-free clickable polymer solution producing a clickable surface and control of the surface density of the clickable functional group. The present method is simple, versatile, and will further increase the potential of using click chemistry for solid surface engineering.

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Compliance with ethical standards

The authors declare no competing financial interest.

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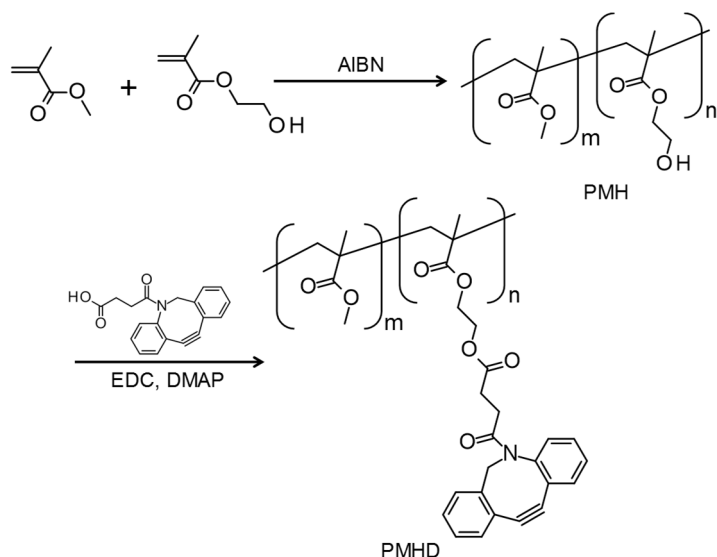


Fig. 1. Synthesis of a Cu-free clickable polymer (PMHD).

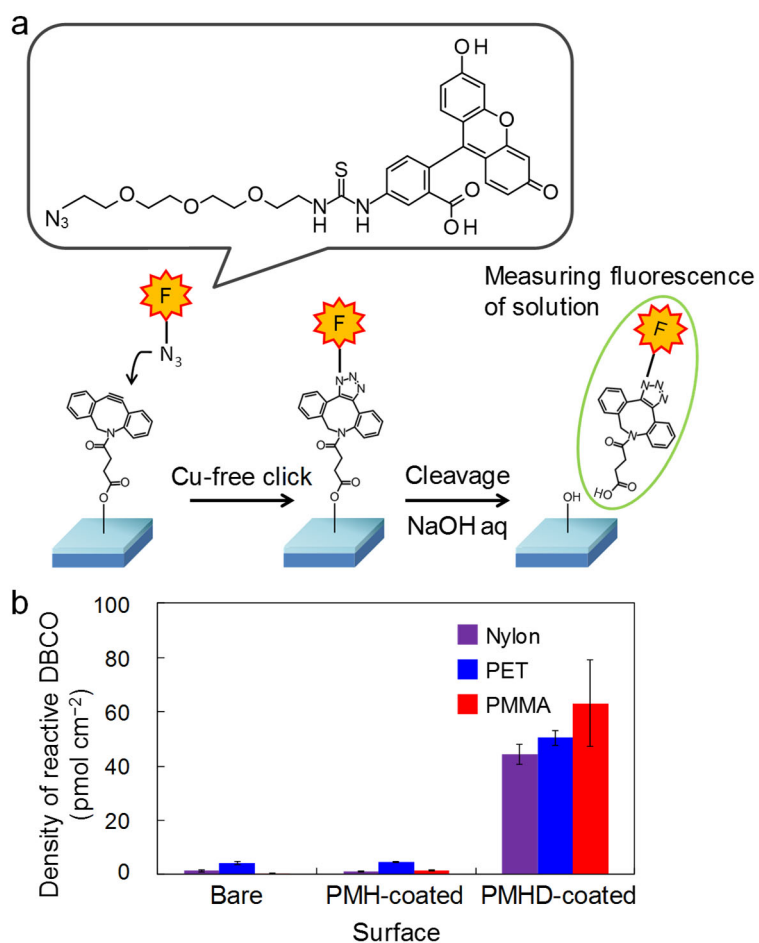


Fig. 2. (a) Schematic representation of density quantification for surface DBCO moieties. (b) Density of DBCO moieties on substrate surfaces. PMHD concentration for dip-coating was 1 wt%.

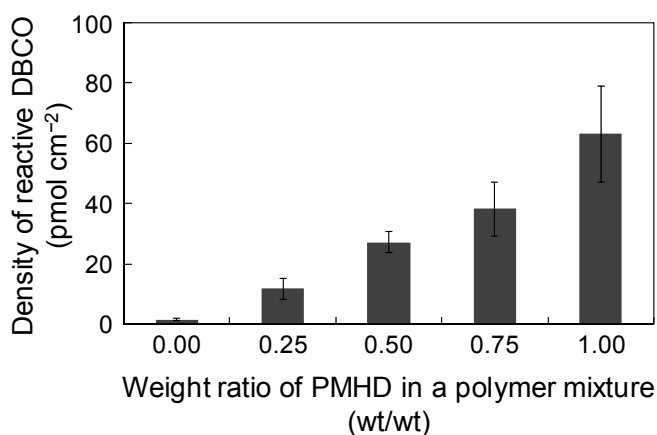


Fig. 3. Density of DBCO moieties on a surface prepared with various weight ratios of PMHD and PMH. A PMMA substrate was used and the sum of PMHD and PMH concentrations for dip-coating was 1 wt%.

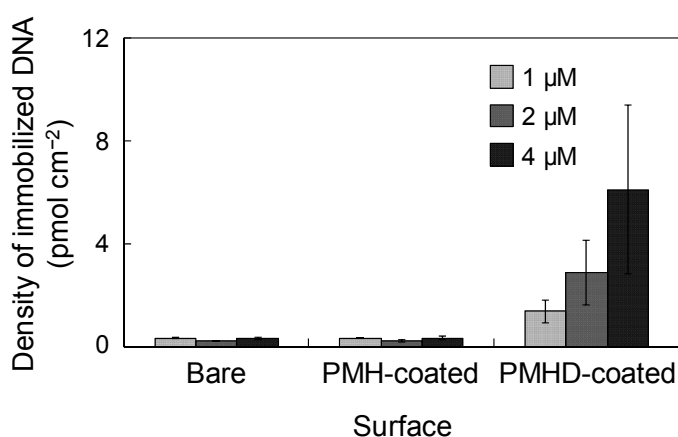


Fig. 4. Density of surface-immobilized oligo DNA prepared using various concentrations of N₃-DNA. A PMMA substrate was used and the PMHD concentration for dip-coating was 1 wt%.