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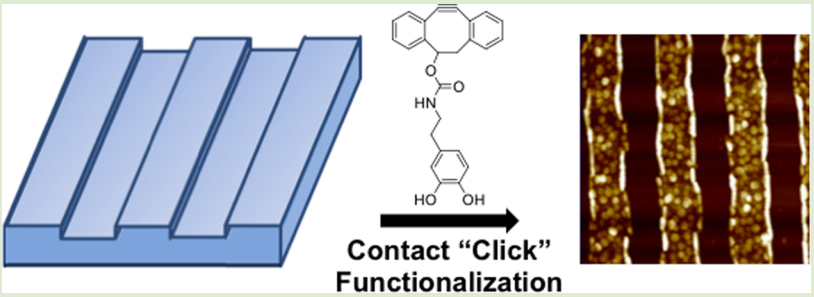
Dopamine-Based Copper-Free Click Kit for Efficient Surface Functionalization

Jukuan Zheng,[†,‡] Ying Chen,[§,‡] Alamgir Karim,[§] and Matthew L. Becker*[,†]

[†]Department of Polymer Science and [§]Department of Polymer Engineering, The University of Akron, Akron, Ohio 44325, United States

Supporting Information



ABSTRACT: Strain-promoted azide−alkyne cycloaddition reactions are combined with a dopamine functional species to generate a highly efficient method for surface modification. The resulting conjugate containing 4-dibenzocyclooctynol (DIBO) and dopamine results in a versatile surface labeling technology that can replicate patterns generated from photolithography and microcontact printing techniques.

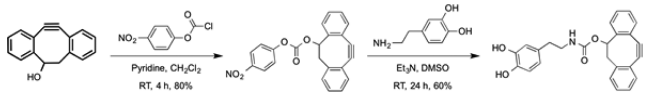
Since the discovery of self-polymerization and the surface-independent adhesion of poly(dopamine),[1] catechol groups or dopamine derivatives have been used widely for anchoring small molecules,[2] polymer chains,[3,4] and biomolecules[5] onto many different kinds of surfaces.[6−8] The outstanding adhesion of poly(dopamine) to various kinds of surfaces including copper,[9] steel,[10,11] glass,[3,12] organic polymers,[13] silicone oxide,[3] titanium oxide,[14,15] aluminum oxide,[16,17] and hydroxyapatite[18,19] has been used in a wide variety of surface modification and biomedical applications. Small molecules that contain catechol or dopamine have also been utilized as adhesive agents for surface functionalization, and surface-initiated polymerization was demonstrated using catechol-bearing initiators.[6,20] In addition there is an increasing interest in strain-promoted azide−alkyne cycloaddition (SPAAC) due to its highly efficient, orthogonal, and metal-free characteristics. Its use has been widely reported in bioimaging,[21−25] biomaterial fabrication,[26−31] surface modification,[32,33] and nanoparticle[34] and polymer[35−37] functionalization. Using a combination of copper-free click reaction and dopamine surface binding characteristics, we have developed a versatile and efficient method for derivatizing surfaces with azide-derivatized molecules.

In this work, dopamine and 4-dibenzocyclooctynol (DIBO) were combined to create a small molecule possessing both an anchoring motif and a reactive handle. The catechol group on the dopamine acts as the adhesive effectively tethering the conjugate to different surfaces, while the DIBO group enables efficient, orthogonal, and catalyst-free modification with any number of azide-derivatized molecules. We expect the combination of dopamine and DIBO to serve as a versatile and efficient surface modification technology for microfluidic and biomedical applications.

DIBO was synthesized according to methods described previously,[22] and the hydroxyl group was activated with *p*-nitrophenyl chloroformate. The activated DIBO derivative was then coupled to dopamine yielding the desired DIBO−dopamine conjugate in approximately 60% yield (Supporting Information (SI)). The desired conjugate was confirmed with ^{1}H NMR, ^{13}C NMR, and ESI, all of which are located in the SI. This DIBO−dopamine molecule is quite stable in an organic solvent such as THF without observation of the typical signature for oxidation (color change) for at least 1 week.

Polydimethylsiloxane (PDMS) has been utilized widely in microfluidic applications and the demonstration of surface fluorescence patterning.[38,39] In this work, PDMS was used to demonstrate the rapid and selective binding of the catechol species to the oxidized silanol groups following ozone treatment and show that the DIBO group was available for

Scheme 1. Synthesis of DIBO−Dopamine from Dopamine and *p*-Nitrophenyl Carbonate Activated DIBO



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further reaction with the fluorescent probes via SPAAC. The surface chemistry and energetics of PDMS can be varied to near silicon oxide-like structure using mild oxidative treatment with ultraviolet ozone (UVO, 185/254 nm) irradiation.[40] Flat PDMS sheets were treated with UVO for 15 min (accumulated radiation energy ~5.3 J/cm^2). The surfaces were immediately coated with trimethylchlorosilane (TMCS, Thermo Scientific) for 24 h using vapor deposition to prevent nonspecific adsorption. Contact angle results (SI) were obtained to confirm the vapor deposition of the TMCS.

A TEM grid was utilized as a photomask to generate patterned (UVO exposed) hydrophilic regions (Figure 1A).

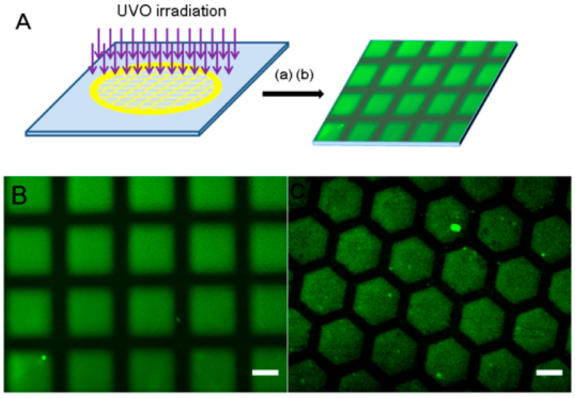

Figure 1. (A) Procedures for the fluorescently patterned PDMS surfaces using a photolithography mask and adhesion-assisted copper-free click reaction. (a) Immersion in a DIBO−dopamine solution ($CHCl_3$, 2 mg/mL) for 30 min. (b) Immersion in a Chemo 488 azide solution (4 μg/mL) for 5 min. (B), (C) Fluorescence pattern present on DIBO−dopamine-coated PDMS shows successful recognition of the oxidized regions. The respective fluorescence patterns were generated using different TEM grids as photomasks. The scale bars are 20 μm for both images B and C.

The irradiated elastomer was then immersed in DIBO−dopamine solution ($CHCl_3$, 2 mg/mL). After 30 min, the patterned PDMS was removed from solution, rinsed with chloroform, and dried with nitrogen. The elastomer was then immersed in a solution of Chemo 488 azide (4 μg/mL) for 5 min, washed with water, and dried with nitrogen. A control experiment was carried out with the same procedures without processing in a DIBO−dopamine solution.

Figure 1(B) and Figure 1(C) show the surface fluorescence patterns generated using square and hexagonal TEM grids. The control experiment without the dopamine−DIBO conjugate showed no fluorescence. Therefore, the patterned fluorescent regions confirm the selective derivatization of the azide-labeled molecule to the UVO-irradiated regions. This selectivity is due to the binding of dopamine to the silanol groups present in the oxidized regions that resulted from UVO treatment. The aromatic C 1s signals can be observed in the XPS results shown below.

TiO_2 and SiO_2 surfaces were used as PDMS surrogates to demonstrate the selective binding to the oxidized regions in the XPS experiment as PDMS cannot be used in high-vacuum XPS environments. However, the fluorescence pattern confirms the successful binding of DIBO−dopamine.

Due to the utility of PDMS in microcontact printing and microfluidic applications, additional surfaces were tested for their ability to efficiently transfer the conjugate. First, control experiments were used to demonstrate the ability of DIBO−dopamine to bind to TiO_2 and SiO_2 prior to the contact-printing application. TiO_2 and SiO_2 were immersed in 2 mg/mL of DIBO−dopamine solution in THF for 12 h, after that the substrates were taken out and washed with THF. Then they were dried overnight under vacuum. XPS measurement was utilized to confirm the adhesion of DIBO−dopamine to these two different surfaces. TiO_2 and SiO_2 substrates that were not treated with DIBO−dopamine were used as control experiments. As shown in Figure 2(A,B), we are able to conclude that

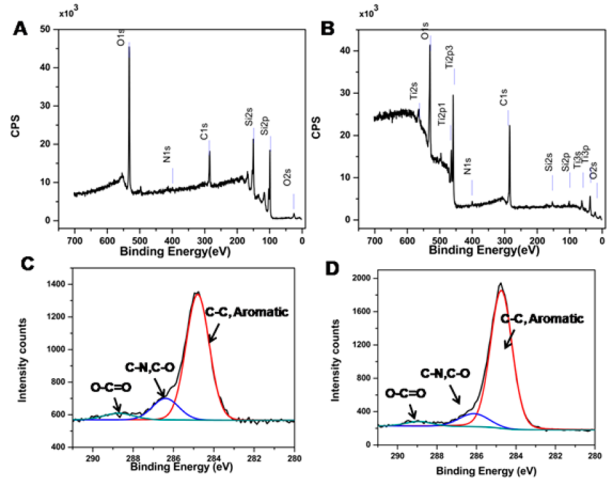

Figure 2. XPS results confirm the binding of DIBO-dopamine to TiO_2 (A) and SiO_2 (B), and the corresponding high resolution spectra (C,D) further resolve the signal corresponding to the DIBO-dopamine molecules.

the small molecule is anchored to the substrate as there is no signal from C 1s and N 1s present on the untreated substrate (SI). The DIBO−dopamine-treated surfaces have excitations corresponding to C 1s and N 1s. High-resolution XPS results clearly show the peaks corresponding to the C*−O−C═O, C*−N, and O═C*−N excitations, which further confirm the binding of DIBO−dopamine to these surfaces.

Then the patterned template transfer and surface modification was carried out using a nanogrooved PDMS elastomer. Following an annealing procedure (SI) the PDMS template was coated with DIBO−dopamine. It was brought into contact with several different surfaces including TiO_2, SiO_2, Al_2O_3, and Au. A 100 g weight was put on top of the template, and after 10 min, the PDMS was peeled off in the direction of the ridges. The modified surface was immersed in a click-on fluorescence dye solution (3-azido-7-hydroxycoumarin, 1 μg/mL) for 5 min. The substrates were then washed thoroughly with water (Figure 3). As shown in Figure 3, a blue fluorescence pattern was observed on all four types of surfaces (Figure 3 is SiO_2) confirming the successful pattern transfer. The fluorescence images of modified TiO_2, Al_2O_3, and Au surfaces are noted in the Supporting Information. The reactivity of the DIBO group is preserved under these conditions for copper-free click functionalization.

To further demonstrate the fidelity of the microcontact printing techniques, gold nanoparticle patterns were generated on silicon oxide surfaces modified with the patterned DIBO−dopamine via copper-free click reaction. The modified surface was immersed in a solution of azide-functionalized gold nanoparticles (50 nm, Nanocs) for 12 h. The surface was

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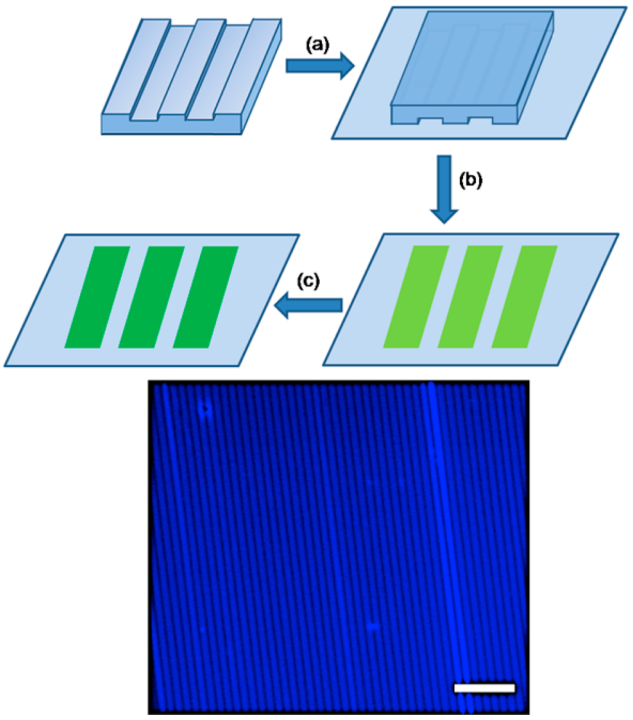

Figure 3. Schematic outlining the sequential procedure for contact printing modification of surfaces. (a) DIBO−dopamine coating on the patterned PDMS substrate. (b) Contact print transfers nanopattern to a secondary substrate. (c) Functionalization of the transferred pattern with dye or nanoparticle. Fluorescence image (bottom) shows the successful pattern transfer and subsequent copper-free click functionalization. The scale bar is 10 μm.

washed with water and dried under vacuum. AFM imaging measurements were carried out before and after gold nanoparticle functionalization. As shown in Figure 4(A), prior to the gold nanoparticle modification, the surface has grooved patterns of the small molecule with height around 5 nm. This means multilayers of the small molecule were transferred onto the surface via contact printing. After the gold nanoparticle modification, shown in Figure 4(B), a grooved pattern with gold nanoparticles located only inside the grooves was visualized. The height of the features increased from 5 to nearly 50 nm, which also confirms the functionalization of the gold nanoparticle. The gold nanoparticles could not be removed via sequential washing procedures showing the covalent nature of the triazole formation. While we have not fully explored the resolution limits for the pattern transfer technology or stability of the resulting inorganic patterns, we envision a number of molecular sensing and microfluidic applications. We believe that the nanoparticle and substrate could likely be used in any number of combinations of interest. However, the binding constant of the catechol species will likely depend on the nature of the d and f orbital spacing of the metal oxide substrates the pattern is transferred to.

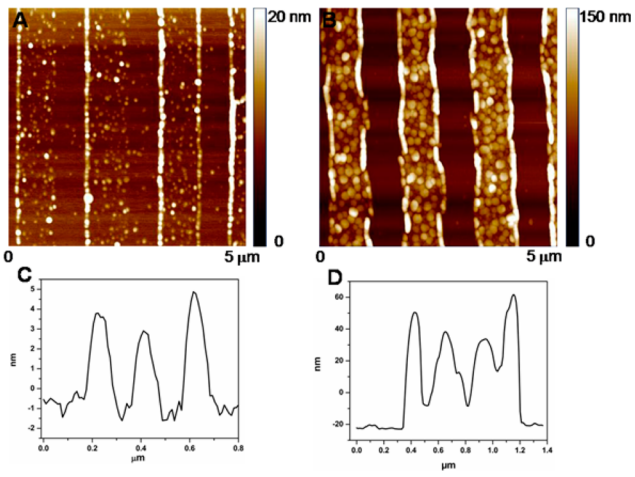

Figure 4. AFM image showing the successful pattern transfer of a small molecule and the following gold nanoparticle functionalization. A nanopatterned gold nanoparticle is generated.

In conclusion, we have developed a versatile surface modification method by combining the adhesive properties of a dopamine and the copper-free click reaction of the DIBO reactive handle. Micro or nanopatterns were generated on several different surfaces without using complicated lithography facilities. This method could be applied universally as a modification method for a number of different metal oxide surfaces. This highly versatile and efficient surface functionalization method will find widespread applications in biomedical and nanodevice fabrication.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The detailed synthetic procedures for the DIBO−dopamine conjugate are listed in addition to the materials and methods list. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: becker@uakron.edu. Phone: (330) 972-2834. The University of Akron, Department of Polymer Science, Goodyear Polymer Center 517, Akron, OH 44325-3909.

Author Contributions

‡These authors contributed equally. The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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