ACS Macro Letters

Letter

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Light-Triggered Switching of Reversible and Alterable Biofunctionality via β-Cyclodextrin/Azobenzene-Based Host–Guest Interaction

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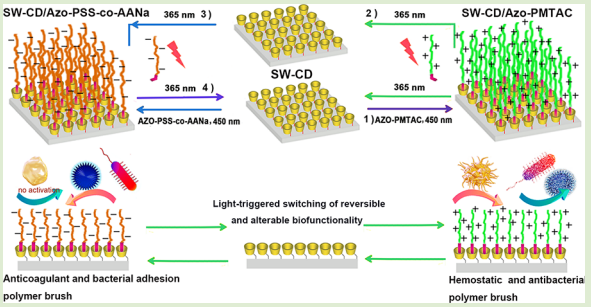
Supporting Information

ABSTRACT: Most of the recent reports focused on using cyclodextrin/azobenzene/polymer for reversible immobilization of biomolecules, the reversible photoswitching of biofunctions via universal and low-cost strategy, were barely investigated. Herein, we report light-triggered switching of reversible and alterable biofunctionality on silicon interface via β-cyclodextrin/azobenzene based host–guest interaction. Biofunctional azobenzene-grafted polymers were synthesized and assembled onto β-cyclodextrin anchored interfaces to form "smart" monolayers of light-triggered switchable brushes. The photoresponsive interfaces exhibit reversible and alterable biofunctionality switching from antibacterial/hemostatic to bioadhesion/anticoagulant upon ultraviolet and visible (UV–vis) light cycles.



The ability to stimuli-regulation of interactions between functional polymers provides great opportunity to develop novel materials with alterable functionality and cycling usage for reversible biosensors, bioadhesion, and many other biomedical fields.[1−3] To improve the performance of the material surface and endow the surface with reusability, self-assembled monolayers (SAMs) that respond to external stimuli, such as light,[4,5] pH,[6] and redox,[7] have been extensively studied, especially the smart surfaces that can recognize specific molecules, which have become increasingly important to design versatile biointerfaces.[8,9] Among the molecular recognition based host–guest interactions, the cyclodextrin (CD)/azobenzene (Azo) interaction is the most widely studied for the construction of reversible assembly of switching biofunctions.[10] CD with its hydrophobic cavity has been known to form inclusion complexes with a large number of complementary Azo derivatives via host–guest interaction. It is well-known that Azo can reversibly switch between *trans*- and *cis*-isomers upon UV–vis irradiation and the photoisomerization of Azo moiety dominates the inclusion and exclusion between CD and Azo due to the stability of the host–guest complexes.[11−13] Thus, the *trans*-Azo can be fully recognized by CD via hydrophobic and van der Waals interactions, while the *cis*-Azo cannot be due to the unmatched host–guest pairs. The processes of the inclusion and exclusion between CD and Azo are completely reversible under alternate irradiation with UV and vis light.

In addition, light is a fascinating stimulus since it can be remotely operated with high spatial and temporal precision easily. Therefore, the unique photoresponsive host–guest interaction has been widely used to design light-sensitive assembly systems, especially in the bioactive interface.[14] Based on the photoresponsive Azo and CD/Azo interaction, many typical bioactive systems had been founded. Jiang group designed Azo-RGD peptide grafted SAMs for the patterned growth of cells and ultrasensitive cell sensors.[15] Gong et al.[8] had reported a RGD grafted photoresponsive smart surface for reversible cell adhesion via the CD/Azo host–guest interaction. Though, the peptides grafted interfaces had been proved to be essentially useful for efficient regulation of biological responses, the methods involved with complex synthetic processes and the high-cost peptides might further restrict their applications in biomedical fields. More universal and low cost method by using synthetic functional polymers for the regulation of interface bioactivity has not been extensively explored.[16] While, most of recent reports focused on using CD/Azo/polymer for reversible immobilization of biomolecules, for instance, the cytochrome[17] and protein fouling resistance.[18] The photoswitching of biofunction was barely investigated. Thus, a universal and low-cost photoresponsive strategy should be explored to develop reversible and alterable biofunction switching via the CD/Azo/polymer based host–guest interaction, especially for some essential properties of biointerface, such as the thrombotic responses and the regulation of bioadhesion.

Received: September 8, 2014
Accepted: October 15, 2014
Published: October 16, 2014

Herein, we designed a CD/Azo/polymer based photoresponsive biofunctional system on the silicon interface (Figure 1A,B), which could switch its surface properties from

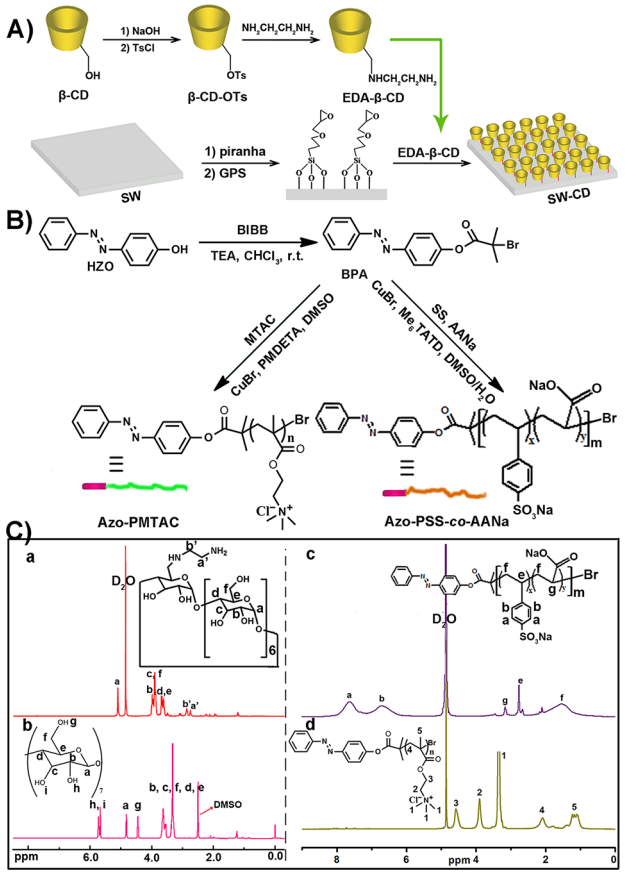


Figure 1. (A) Chemical routes for preparation of SW-CD; (B) Synthesis of BPA, Azo-PMTAC, and Azo-PSS-*co*-AANa; (C) ^{1}H NMR spectra for β-CD (in DMSO-d_6), EDA-β-CD (in D_2O), Azo-PMTAC (in D_2O), and Azo-PSS-*co*-AANa (in D_2O); ^{1}H NMR spectrum of β-CD-OTs in DMSO-d_6 (Figure S1).

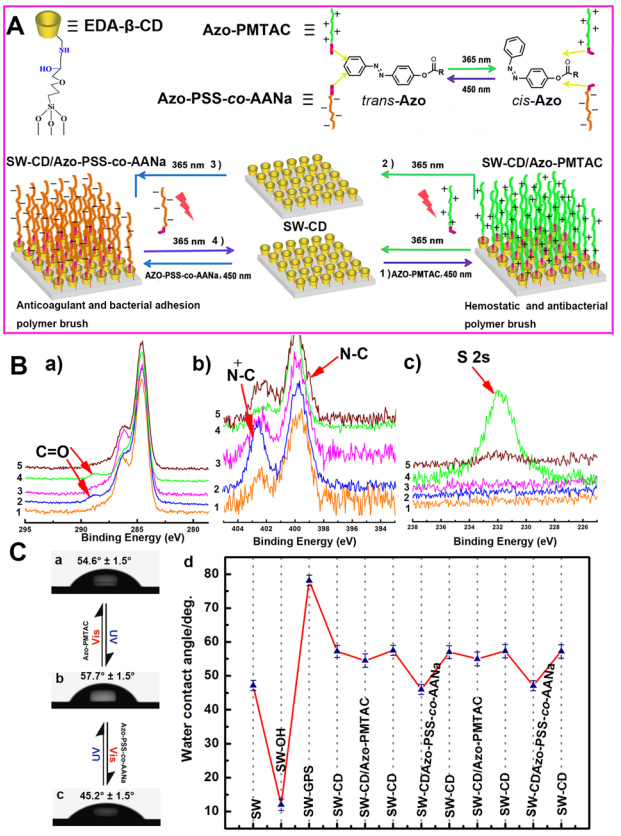


Figure 2. (A) Schematic depiction of light-triggered alternate and reversible assembly of Azo-PMTAC and Azo-PSS-*co*-AANa onto SW-CD; (B) XPS spectra for the substrates within one whole function switching cycle, C 1s spectra (B-a), N 1s spectra (B-b), and S 2s spectra (B-c): (1) SW-CD, (2) SW-CD/Azo-PMTAC, (3) Azo-PMTAC detached SW-CD, (4) SW-CD/Azo-PSS-*co*-AANa, (5) Azo-PSS-*co*-AANa detached SW-CD; (C) Images of water drops on three deferent kinds of surfaces: (a) SW-CD/Azo-PMTAC, (b) SW-CD, and (c) SW-CD/Azo-PSS-*co*-AANa; (d) reversible wettability transition of photoresponsive monolayer with UV−vis irradiation and alternate assembly of Azo-PMTAC and Azo-PSS-*co*-AANa.

antibacterial/hemostatic to bioadhesion/anticoagulant upon UV−vis cycles via alternate assembly of Azo-poly([2-(methacryloyloxy) ethyl] trimethylammonium chloride) (Azo-PMTAC) and Azo-poly(sodium 4-vinylbenzenesulfonate-*co*-sodium acrylate) (Azo-PSS-*co*-AANa; see Figure 2A). The positively charged Azo-PMTAC (antibacterial/hemostatic) and negatively charged Azo-PSS-*co*-AANa (the sulfonic acid groups and carboxyl acid groups in Azo-PSS-*co*-AANa could mimic the chemical structure and functionalities of heparin, such as anticoagulant, antithombotic, and promoting bioadhesion[19,20]) were applied as the model biofunctional Azo-grafted polymers for alterably assembling with the β-CD anchored interface to form monolayers of polymer brushes.

Silicon wafers (SW) were first treated scrupulously with a freshly prepared piranha solution and then coupled with 3-glycidoxypropyl trimethoxysilane (GPS). EDA-β-CDs were then grafted onto the silicon wafer surface to obtain SW-CD (Figure 1A). Azo-containing polymers (Azo-PMTAC and Azo-PSS-*co*-AANa) were synthesized by atom transfer radical polymerization (ATRP) from 2-bromoisobutyryl bromide 2-(4-phenylazophenyl) ethyl ester (BPA; Figure 1B, Supporting Information), and the corresponding chemical structures were confirmed by using ^{1}H NMR spectra (Figures 1C and S2).

Furthermore, as shown in Figure 2A, the Azo-PMTAC was assembled onto the SW-CD surface via the inclusion complexation between β-CD and Azo moiety to obtain the sample named SW-CD/Azo-PMTAC, and thus, the surface could be used for antibacterial/hemostatic tests. After being irradiated with UV light for 2 h, the azobenzene changed its configuration from trans to cis, resulting in the detachment of Azo-PMTAC from the SW-CD surface. To confirm the alterable biofunction, Azo-PSS-*co*-AANa was then attached onto the Azo-PMTAC detached SW-CD surface and named SW-CD/Azo-PSS-*co*-AANa, which might have excellent bioadhesion/anticoagulant ability since the PSS-*co*-AANa is a heparin-mimicking polymer.[21] And then four UV−vis cycles were carried out to verify the reversible and reusable abilities of the prepared surfaces.

X-ray photoelectron spectroscopy (XPS) was used to confirm the photoswitching of polymer brushes. As shown in Figure S4, the XPS peaks of silicon (binding energy: 152.8 eV (Si 2s), 104.1 eV (Si 2p)), oxygen (binding energy: 532.9 eV), and carbon (binding energy: 288.9 eV) were clearly detected for the SAMs. The intensity of the nitrogen for the SW-CD/Azo-PMTAC obviously increased compared to those for the SW-CD and SW-CD/Azo-PMTAC-UV. (Figure 2B-b). Sim-

dx.doi.org/10.1021/mz500568k | ACS Macro Lett. 2014, 3, 1130−1133

ilarly, the peak of sulfur for the SW-CD/Azo-PSS-*co*-AANa could be clearly observed, after being irradiated with UV light for 2 h in ultrapure water, the intensity was dramatically decreased and the peak became inconspicuous (Figure 2B-c). Meanwhile, in Figure 2B-a, the peaks at 288.8 eV were ascribed to C═O, which gave further evidence that the Azo-PMTAC and Azo-PSS-*co*-AANa were successfully attached onto the surface of SW-CD.

To study the photoswitchable property of the as-prepared SAMs, first, water contact angle (WCA) measurements were carried out to observe the transitions in surface wettability with UV−vis irradiation and alternating assembly of the Azo-PMTAC and Azo-PSS-*co*-AANa, as shown in Figure 2C.[22] The WCA for the SW-CD was 57.7 ± 1.5°; after assembling with the Azo-PMTAC, the WCA decreased to 54.6 ± 1.5°. After being irradiated with UV for 2 h in ultrapure water, Azo-PMTAC was detached from the substrate, and the WCA for the Azo-PMTAC detached surface decreased to the level of SW-CD. Then, Azo-PSS-*co*-AANa was used to assemble onto the Azo-PMTAC detached surface, and the WCA further decreased to 45.2 ± 1.5°. The reason should be the random copolymerization of SS and AANa, resulting in the asymmetric charge repulsion between the polymer brushes compared to that for SW-PMTAC, which might reduce the effect of the hydrophobic bromide groups at the outermost layer. Thus, the surface of the SW-CD/Azo-PSS-*co*-AANa was much more hydrophilic than the SW-CD/Azo-PMTAC.

Then, four UV−vis cycles were carried out to verify the reversible transitions in surface wettability with alternating assembly of the Azo-PMTAC and Azo-PSS-*co*-AANa (Figure 2C-d). It should be noted that the SW-CD substrate can be assembled with Azo-PMTAC and Azo-PSS-*co*-AANa alternately and repeatedly. The changes of WCAs are reversible for many UV−vis cycles. These results strongly indicated that the surfaces of the SW-CD/Azo-polymers were photoresponsive surfaces.

To further confirm that the as-prepared SAMs can be switched from antibacterial/hemostatic to bioadhesion/anticoagulant by alternate assembly of Azo-PMTAC and Azo-PSS-*co*-AANa; bacteria adhesion experiment was employed to evaluate the antibacterial and bioadhesion properties of the SAMs, followed by the evaluations of the hemostatic and anticoagulant photoswitchable properties via the measurements of platelet adhesion and activate partial thromboplastin time (APTT) and thrombin time (TT).

First, *Escherichia coli* (*E. coli*) and *Staphlococcus aureus* (*S. aureus*) were seeded onto the samples within two function switching cycles (C1 and C2; details were described in Supporting Information), and then the results were evaluated via a scanning electron microscope (SEM); as shown in Figure 3, few bacteria could adhere onto the surface of SW-CD/Azo-PMTAC, while after switching to Azo-PSS-*co*-AANa, the adhesions of both *E. coli* and *S. aureus* increased dramatically. In cycle 2, although the adhered bacteria on the Azo-PMTAC slightly increased, it still maintained a very low level compared to those for the SW-CD and Azo-PSS-*co*-AANa. Thus, confirming that the as-prepared SAMs can be photoswitched from antibacterial to promote bioadhesion by alterable assembly of Azo-PMTAC and Azo-PSS-*co*-AANa.

Platelets adhesion is a vital event during thrombus formation. The SEM images in Figure 4 show the results of platelet adhesion. For the SW-CD/Azo-PMTAC (Figure 4a), a large amount of platelets aggregated and accumulated on the SAM,

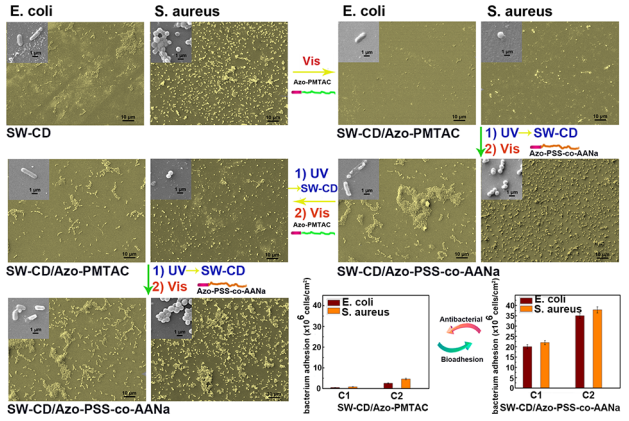


Figure 3. SEM images for *E. coli* and *S. aureus* cultured on SW-CD, SW-CD/Azo-PMTAC, and SW-CD/PSS-*co*-AANa within two whole cycles of functional switching; the histograms in the lower right show the antibacterial and bioadhesion transition, and the adhesion amounts were calculated from six SEM images. Note: due to the aggregation of bacteria, these data only present the approximate values.

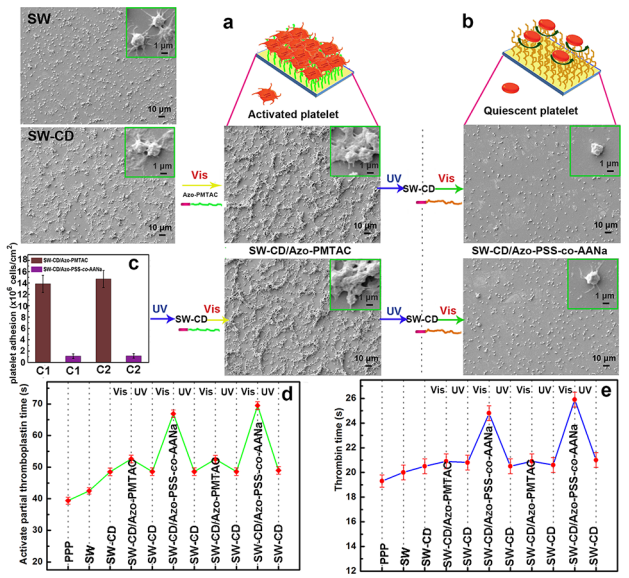


Figure 4. SEM images for the platelets adhering onto SW, SW-CD, SW-CD/Azo-PMTAC, and SW-CD/Azo-PSS-*co*-AANa; (a) presents the activated platelets on SW-CD/Azo-PMTAC surface and (b) presents the antiplatelet adhesion and the platelets remaining in quiescent after exposing with SW-CD/Azo-PSS-*co*-AANa. (c) The platelet adhesion amounts were calculated from six SEM images; note: due to the aggregation, these data only present the approximate values. (d, e) APTT and TT assays of the PPP, SW, and SAMs, values were expressed as means ± SD, n = 3.

and the platelets presented irregular shapes and an abundant amount of pseudopodium, while the adhered amount on the SW-CD/Azo-PSS-*co*-AANa was very low (Figure 4b) and both the spreading and the pseudopodium formation of the platelets were obviously suppressed, indicating lowered platelet activation and improved antithrombotic by the heparin-mimicking SAM of the Azo-PSS-*co*-AANa.

APTT and TT measurements were further performed to evaluate the hemostatic and anticoagulant activities of the SAMs, since the APTT and TT were widely used for the clinical examination of the abnormality of blood plasma and for the primary check of the activity of anticoagulative regents.

dx.doi.org/10.1021/mz500568k | *ACS Macro Lett.* 2014, 3, 1130−1133

Figure 4d,e shows the clotting times for the platelet poor plasma (PPP), pristine silicon wafer, and the samples for four UV−vis cycles. APTT for the SW-CD was approximately 46 s, while it slightly increased for the Azo-PMTAC assembled SAM to approximately 54 s, which indicated no obvious anticlotting activity. After switching to heparin-mimcking polymer, as for the Azo-PSS-*co*-AANa, assembled surface, the APTT prominently increased to approximately 70 s, indicating prolonged clotting time. For the TT results, the same changing tendency was observed. To verify the photoswitchable and reversible properties of these SAMs, four UV−vis cycles were performed. The results clearly indicated that the as-prepared SAMs showed excellent photoswitching reversibility. Combined with the data of the platelet adhesion and activation, the results indicated that the SAM of Azo-PSS-*co*-AANa was anticoagulant, while hemostatic for the SAM of Azo-PMTAC.

In summary, we have demonstrated a photoresponsive surface with reversible and alterable biofunctionality, which can switch its function from antibacterial/hemostatic to bioadhesion/anticoagulant upon UV−vis cycles via the alternate assembly of Azo-PMTAC and Azo-PSS-*co*-AANa onto the β-CD terminated silicon interface. The photoresponsibility, reversibility, and bioactivity switching for the system were demonstrated. This strategy is efficient, universal, and low-cost, which is expected to own great potential for light-triggered regulation of many different types of functional polymers reversibly, thus, to advance the design of bioactive, versatile, and "smart" biomaterials in the near future.

■ ASSOCIATED CONTENT

Supporting Information

Materials, synthesis steps, and characterization for CD and Azo grafted polymers and interfaces, antibacterial tests, and anticoagulant evaluation. 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.

■ ACKNOWLEDGMENTS

We acknowledge that this work was financially sponsored by the National Natural Science Foundation of China (Nos. 51173119, 51225303, and 51433007). We should also thank our laboratory members for their generous help and gratefully acknowledge the help of Ms. H. Wang of the Analytical and Testing Center at Sichuan University for the SEM.

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