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COMMUNICATION TO THE EDITOR

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Photoresponsive "Smart Template" via Host−Guest Interaction for Reversible Cell Adhesion

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Supporting Information

INTRODUCTION

For tissue engineering applications, the interactions between cells and scaffold materials are of critical importance and thus have attracted intense research interest in recent years.[1] The cell behaviors are affected precisely by all sorts of physical and mechanical factors in physiological environments, in particular, cell−substrate interactions.[2−4] Most commonly, the cell adhesion and migration, the most important fundamental cellular behaviors, could be regulated by noncovalently interactions between integrins on cell membranes and some specific biomolecules incorporated on the polymer scaffolds, such as laminin, fibronectin, and peptides containing arginine-glycine-aspartate (RGD) sequence.[5−9] To improve the performance of the polymer scaffolds, various strategies to develop new biointerfaces used as mimics of the extracellular matrix (ECM) have been explored.[10−12] Among them, the approaches based on the self-assembled monolayers (SAMs) technology could introduce biospecific adhesion motifs on the material surfaces.[13−16] Mrksich et al. designed SAMs presenting the benzenesulfonamide moiety which can facilitate the specific recruitment of carbonic anhydrase-RGD to the surface and the subsequent specific cell adhesion.[17] SAMs that respond to external stimuli, such as light,[18−21] heat,[22] and voltage,[23,24] are currently the available class of "smart surfaces" in numerous research fields, not only in tissue engineering but also in the formation of cell microarrays, regenerative medicine, and drug discovery studies.[25] For example, Lutz et al. fabricated thermoresponsive oligo(ethylene glycol) SAMs which allowed efficient control over cell-adhesion within an applicable temperature range.[22] Yousaf et al. reported a electroactive quinine-terminated SAM that captured and subsequently released proteins and cells at different electrochemical potentials.[24] However, with few exceptions, most existing technologies have relied on irreversible control and cannot be regenerated for further use if the original structure on surface is altered.

In this Communication, we designed a novel "smart template" mainly consisting of α-cyclodextrin (α-CD)-terminated alkanesilane which allowed to assemble with azobenzene-glycine-arginine-glycine-aspartate-serine (azo-GRGDS) via host−guest interaction for controlling cell adhesion reversibly. This template allows forming inclusion complex with different ligands such as azobenzene, naphthalene, and stilbene via host−guest recognitions. Therefore, when peptide, poly(ethylene glycol), or other special molecules are modified on different ligands, the template will recognize different complex ligands, and these ligands can be displaced freely. By this way, this surface will possess multifunction. In this study, azobenzene was selected as a model ligand because of its perfect photoresponsive property. After being irradiated with UV light, azobenzene isomerizes from thermodynamically stable trans configuration to cis form and then reverses to trans form by heating or by irradiation with visible light.[26,27] This photoswitchable surface can be easily induced to change its conformation upon the UV irradiation at target site and regenerated to its initial state reversibly, and the irradiation could be controlled rapidly, cleanly, and remotely.

EXPERIMENTAL SECTION

Quartz was chosen as substrate as it is a common, inexpensive, and nontoxic material, and in addition, quartz also enables microscopic observation in view of optical properties to be used for biomedical studies conveniently.[28] The quartz substrates we used were treated scrupulously with "piranha" solution and dried under nitrogen previously.[29] As shown in Scheme 1, undecylenic acid, TSTU, and NH_2-α-CD were used to synthesize the α-CD-termined SAMs on the treated substrates, and the synthesis route is represented in Scheme S1. And then, the SAMs containing the inclusion complexes which consisted of α-CD and azo-GRGDS were formed (assembly step), and thus the surface could be used for the cell adhesion. After being irradiated with UV light, azobenzene changed its configuration from trans to cis, leading to the detachment of azo-GRGDS from the monolayer.

RESULTS AND DISCUSSION

In order to study the photoswitchable property of these SAMs, contact angle (CA) measurements were carried out to observe the transitions in surface wettability with UV irradiation.[30] CA measurements were performed first on the clean quartz substrate, and its CA was 35.4 ± 1.8°. After the substrate modified with the alkanesilane terminated with an *N*-hydroxysuccinimide (NHS) group, the CA significantly increased to 86.8 ± 2.4°. The CA of the SAMs containing α-CD was 45.3 ± 1.9°. The reason for the remarkable decrease should be the hydrophilic outer part of α-CD. When the inclusion complex formed, because of the existence of RGD peptide, the CA increased to 58.7 ± 1.5°. Then α-CD/azo-GRGDS SAMs was immersed into the deionized (DI) water and its CA restored to 45° when the surface was exposed to UV light for 30 min, which responded to the

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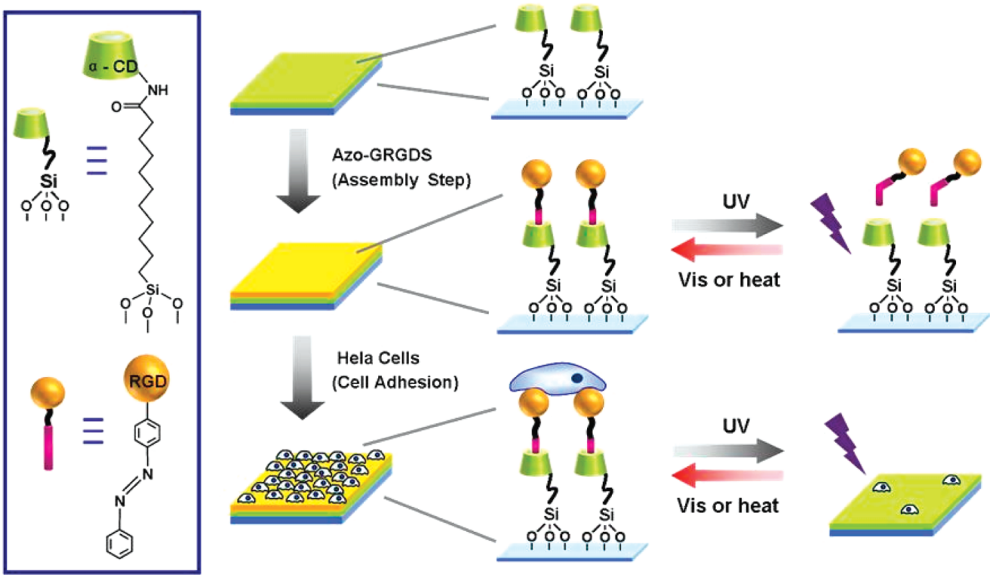
Scheme 1. Strategy for Cell Adhesion on SAMs by Using Host−Guest Assembly of α-Cyclodextrin and Azobenzene-GRGDS[a]



[a] The green monolayer on SiO_2 substrate represents silane terminated with α-CD. α-CD and azobenzene can form inclusion complex via host−guest recognition (assembly step), and Hela cells are cultured on the substrate. When *trans*-azobenzene is transformed to *cis*-azobenzene upon UV irradiation at 365 nm, azo-GRGDS and cells are detached from the substrate. This process is reversible.

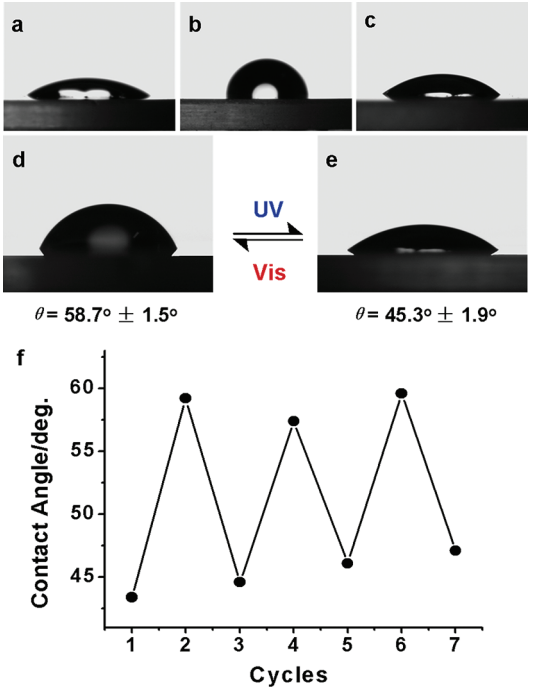


Figure 1. Images of water drops on silicon dioxide substrate and four different kinds of surfaces: (a) clean silicon dioxide substrate, (b) the SAM containing silane terminated with NHS groups, (c) the SAM containing α-CD, (d) the SAM containing α-CD/azo-GRGDS, (e) α-CD/azo-GRGDS SAMs after UV irradiation, and (f) reversible wettability transition of photoreponsive monolayer with UV and vis irradiations.

photocontrollable molecular motion of azobenzene in SAMs. It should be noted that this SAM can assemble with azo-GRGDS repeatedly. Figure 1 shows the photographs of water drop profile

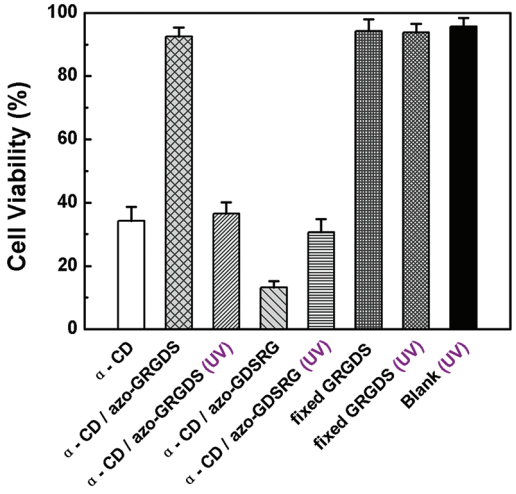


Figure 2. Viabilities of Hela cells on different substrates.

on these surfaces, and the changes of CA are reversible for many circles at different wavelengths of light. These results clearly indicated that the surfaces modified with α-CD/azo-GRGDS SAMs were photoresponsive surfaces.

On the other hand, the X-ray photoelectron spectroscopy (XPS) was used to analyze the α-CD SAMs and α-CD/azo-GRGDS SAMs surfaces before and after UV irradiation. The XPS peaks of silicon (binding energy: 105.5 eV), nitrogen (binding energy: 404 eV), oxygen (binding energy: 537.1 eV), and carbon (binding energy: 290 eV) were clearly detected in all these SAMs. The results of the elemental composition on surfaces by XPS analysis are shown in Table S1. The C, N, and O elemental composition of the α-CD SAMs and α-CD/azo-GRGDS SAMs upon UV irradiation were almost the same, indicating that α-CD

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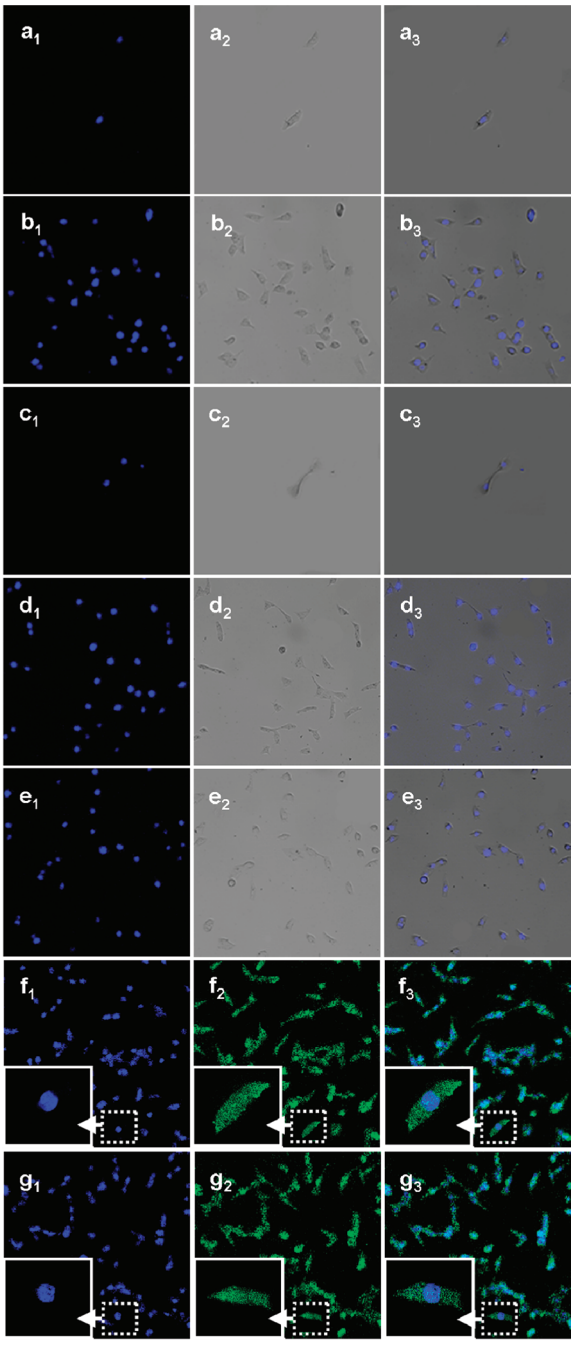

Figure 3. CLSM images of HeLa cells on different substrates: (a_1, a_2, a_3) α-CD SAMs, (b_1, b_2, b_3, f_1, f_2, f_3) α-CD/azo-GRGDS SAMs, (c_1, c_2, c_3) α-CD/azo-GRGDS SAMs (UV), (d_1, d_2, d_3) clean quartz substrates, (e_1, e_2, e_3) clean quartz substrates (UV), (g_1, g_2, g_3) fixed GRGDS SAMs. (a_1, b_1, c_1, d_1, e_1, f_1, g_1) fluorescence images of cell nuclei stained blue by Hoechst 33258; (a_2, b_2, c_2, d_2, e_2) bright field images; (f_2, g_2) fluorescence images of cell cytoskeletons stained green by Oregon Green; (a_3, b_3, c_3, d_3, e_3, f_3, g_3) overlapped images.

SAMs could modify the quartz substrates successfully and the presence of RGD could be controlled through UV light.

To further confirm that α-CD/azo-GRGDS SAMs can be regulated for supporting or resisting cell adhesion by UV irradiation, Hela cells were seeded and cultured on different surfaces. It is well-known that a strong UV irradiation will cause the cell death. In our studies, we tried to culture Hela cells on substrates and exposed them under UV irradiation with different strengths and time periods to identify the appropriate irradiation condition. It was found that the viability of Hela did not obviously change under the UV irradiation (365 nm, 15 W) for 10 min, while azobenzene could change its configuration during this period of time. On the basis of this result, we then cultured the cells on α-CD SAMs, α-CD/azo-GRGDS SAMs, α-CD/azo-GDSRG SAMs, and fixed GRGDS SAMs for 24 h at 37 °C. The α-CD/azo-GDSRG SAMs and fixed GRGDS SAMs were controls, and GDSRG was a scrambled GRGDS peptide without the function of supporting cell adhesion. The number of Hela cells adhered to the surface with the presence of azo-GRGDS was much greater than that with the presence of α-CD only and azo-GDSRG. The cell viabilities of these four different SAMs were 34.2%, 92.5%, 13.1%, and 94.3%. The α-CD/azo-GRGDS SAMs completely supported cell adhesion when azobenzene was in the trans form. After 24 h culture, we put these cell-adhered SAMs under UV light with the suitable irradiation condition and subsequently cultured cells for 1 h in the dark. To make sure that the conformation of azobenzene had almostly changed to cis and azobenzene ligands had detached from the surfaces, we repeated aforementioned process three times, and the viability of α-CD/azo-GRGDS SAMs reduced to 36.5%. At the same time, the α-CD/azo-GDSRG SAMs increased to 30.7%. The viabilities of the surfaces containing the fixed GRGDS did not change obviously, just from 94.3% to 93.8%. Figure 2 shows the viabilities of Hela cells on different substrates. One reason for this phenomenon may be that the α-CD modified on substrates did not cover the surfaces entirely; thus, the uncovered silicon surface leaded the little adhesion. That is why there were still a few cells adhering to the α-CD and α-CD/azo-GDSRG surfaces. On the other hand, it is crucial that azobenzene group cannot change its configuration entirely as a photochemically switchable unit, so it was possible that there were some residual ligands existing on the surfaces upon the UV light. For these reasons, the viability of α-CD/azo-GDSRG SAMs increased after the UV irradiation. From the viabilities of Hela cells, we believed that the density of azobenzene ligands had reduced to a sufficient level with the UV light.

In order to visualize the Hela cells on substrates, nuclei and cytoskeletons of cells were subsequently stained by blue molecular probe Hoechst 33258 and green molecular probe Oregon Green 488 Taxol bis-acetate and then viewed carefully under confocal laser scanning microscope (CLSM). Figure 3 shows the CLSM images of the cells on different SAMs. Only a few cells adhered to α-CD SAMs and α-CD/azo-GRGDS (UV) SAMs with the similar cell density distributions. The cell numbers on α-CD/azo-GRGDS SAMs, blank (clean quartz substrates), and blank (UV) were nearly the same. These images were consistent with the above cell viability conclusion. From cell morphology and cell numbers, we noticed that α-CD/azo-GRGDS SAMs and fixed GRGDS SAMs had no significant differences. Therefore, this α-CD/azo-GRGDS SAMs was demonstrated successfully that it could control cell adhesion reversibly.

CONCLUSION

In conclusion, we have designed and constructed a new "smart surface" which consisted of photosensitive SAMs containing α-CD terminal alkanesilane and azo-GRGDS. This surface can control cell adhesion reversibly upon different irradiation based on the interconvertion of azobenzene's trans and cis configuration.

The "smart template" containing α-CD can assemble with different ligands to obtain different uses. This surface which has the ability in response to external trigger reversibly makes it possible to dynamically regulate biological functions and subsequently modulate biomolecule activity. For instance, by incorporating other types of bioactive signals on this SAMs, we can mimic some properties of natural ECM; thus, the surface can employ to cell adhesion and promote cell migration. Furthermore, it may be used in cell micropatterning, biochips, and tissue engineering. This surface has great potential application in diverse medical and biological fields.

■ ASSOCIATED CONTENT

Supporting Information. Synthesis of azo-GRGDS, azo-GDSRG, and α-CD SAMs, ESI-MS spectrum of azo-GRGDS, NMR spectra of azo-COOH and compound **1**, assembly process of azobenzene ligands and α-CD SAMs, cell experiments, and XPS analysis of different surfaces. This material is available free of charge via the Internet at http://pubs.acs.org.

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