Cell Adhesion

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Using Azobenzene-Embedded Self-Assembled Monolayers To Photochemically Control Cell Adhesion Reversibly**

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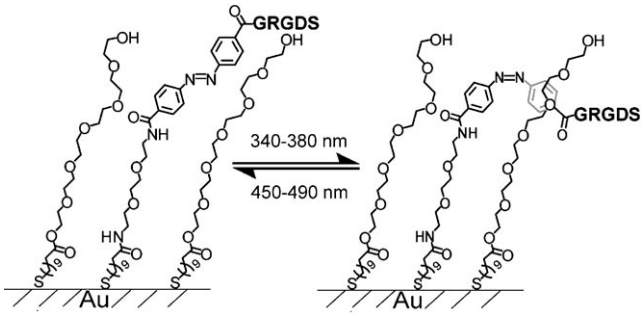
This Communication provides a method for the reversible control of cell adhesion on a molecularly well-defined substrate. Cell adhesion is an important physiological process.[1] Recent advances in surface chemistry, in particular, self-assembled monolayers (SAMs) of alkanethiols on gold, have permitted unprecedented control of cell adhesion on molecularly defined surfaces.[2] Most adherent mammalian cells employ integrin receptors on their membranes that can recognize and noncovalently bind with the extracellular matrix (ECM).[3] Much work has demonstrated that the tripeptide sequence arginine–glycine–aspartate (RGD, initially isolated from ECM proteins[1]) grafted on SAMs allows biospecific adhesion of mammalian cells that mimic natural adhesion. Dynamic control of cell adhesion on surfaces is critical to many applications in biology.[4] Using external trigger such as light,[5] voltage,[6] heat,[7] microelectrodes,[8] microfluidic systems,[6b,9] and surface gradient of nanotopography,[10] researchers have been able to dynamically control cell adhesion on different substrates. With few exceptions, existing technologies have mostly relied on irreversible control, that is, once the original structure for controlling cell adhesion on the surface is altered, it cannot be regenerated for further use.[5–10] This limitation has hindered the development of molecularly defined surfaces that can reversibly control cell adhesion. We are also interested in a technique that allows easy access to spatial control of chemistry and think that a technique utilizing the light on an optical microscope might provide the simplest approach.

Azobenzenes undergo reversible photoinduced isomerization rapidly at different wavelengths of light,[11] and therefore potentially allow the reversible control of cell adhesion on SAMs. A recent demonstration that azobenzene grafted on poly(methyl methacrylate) surfaces that allows some level of control of cell adhesion has encouraged us to design SAMs to reversibly control cell adhesion.[12] The motivation to carry out this reversible conversion on SAMs is that the molecularly well-defined nature of SAMs allows much flexibility in designing the surface for many avenues of future applications.

Herein, we aim to generate a surface that allows the azobenzene unit to reversibly present a ligand that contains RGD peptide on SAMs. Many previous studies have shown that presenting this ligand in a background of poly(ethylene glycol) (PEG) terminated SAMs allows specific interactions between cells and surfaces, and prevents nonspecific adhesion.[13] We reasoned that the *E* isomer of azobenzene would present the RGD peptide for cell adhesion, while the *Z* isomer of azobenzene would mask the RGD peptide in PEG-terminated SAMs to prevent cell adhesion (Scheme 1). The



Scheme 1. The azobenzene moiety can be converted photochemically between the *E* and *Z* configurations to either present or mask the RGD ligand and hence modulate biospecific cell adhesion.

interconversion between *E* and *Z* can be achieved with two wavelengths of light (UV light, 340–380 nm, for the *E* to *Z* conversion, and visible light, 450–490 nm, for the *Z* to *E* conversion). Because the *E*-to-*Z* isomerization is completely reversible, this method provides the only means we know to control cell adhesion reversibly on a molecularly well-defined surface.

To construct a mixed SAM as described above, we have two options. The first is to directly form mixed SAMs with two kinds of thiols, **1** and **2**, in different ratios. The second option is first to form a mixed SAM presenting an *N*-hydroxysuccinimide(NHS)–azobenzene moiety by introducing **2** and **3** (**3** is an alkanethiol incorporating azobenzene terminated with an NHS-activated ester group, which can

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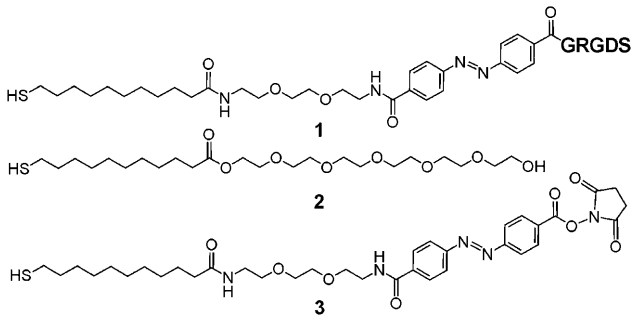
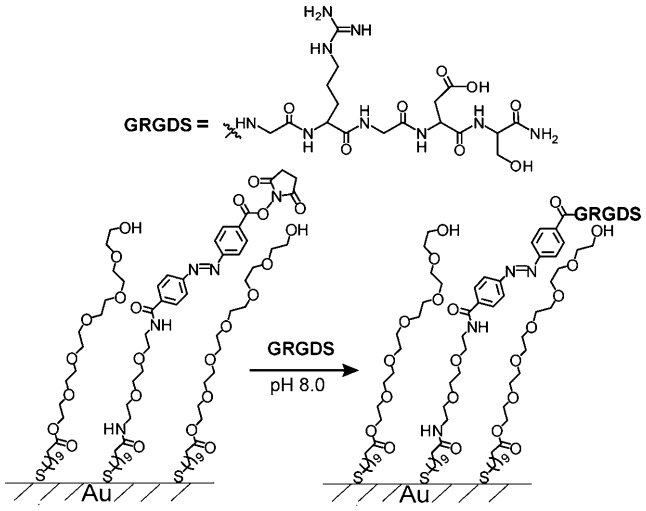


immobilize RGD peptide once it forms SAMs on a gold surface) onto the gold surface, and then introduce the RGD peptide in situ.[14] After a very arduous effort of synthesizing **1**, we found that it was difficult to obtain SAMs directly from **1** because of its poor solubility in almost all kinds of solvents.

We therefore used the second option by synthesizing compounds **2** and **3** initially (their syntheses are outlined in Schemes S1 and S2 in the Supporting Information). Coating the gold surfaces with **2** and **3** in appropriate ratios to form SAMs followed by coupling the RGD peptide with NHS-activated ester at the end of azobenzene (*E* configuration) resulted in cell-adhesive SAMs (Scheme 2).



Scheme 2. Strategy for immobilizing GRGDS peptide onto SAMs presenting an azobenzene unit terminated in an NHS-activated ester, which couples with the primary amine group on the glycine residue of the GRGDS peptide. The reaction took place in a phosphate buffer (pH 8.0).

We tested the optimal ratio of **3** and **2** in mixed SAMs for supporting cell adhesion after **3** was coupled with glycine–arginine–glycine–aspartate–serine (GRGDS) peptide. Before forming mixed SAMs, we irradiated the solution of **3** in ethanol (2 mmol L^{-1}) with visible light for 5 h and stored it for 3 days in the dark to ensure that all azobenzene moieties were in the *E* configuration. We mixed **3** and **2** with different molar ratios (0.01 %, 0.1 %, and 1 % **3** in total alkanethiol) in ethanol (2 mmol L^{-1}) to form SAMs. We immersed these SAMs into an aqueous solution of GRGDS (2 mg mL^{-1}) in a phosphate buffer (pH 8.0) for 20 min to allow the reaction of the activated NHS ester groups with primary amines on GRGDS.[15] These processes were carried out in the dark to ensure the conformation of azobenzene moieties was *E*. After tethering GRGDS with the azobenzene moieties, we obtained SAMs presenting GRGDS peptides through azobenzene units in the *E* configuration. These surfaces supported cell adhesion. To demonstrate that these surfaces can also resist cell adhesion, we irradiated a set of mixed SAMs in the above three ratios with UV light for 10 h to change the conformation of azobenzene from *E* to *Z* (Scheme 1). When we cultured murine NIH 3T3 fibroblasts on these SAMs, the number of cells adhered to surfaces presenting the *E* configuration was far greater than that for those presenting azobenzene in *Z* configuration. We found that compound **3** in a ratio of 0.1 % of total alkanethiolates formed SAMs (coupled with GRGDS) that completely supported cell adhesion when the azobenzene moieties were in the *E* configuration and resisted cell adhesion when they were in the *Z* configuration (Figure 1).

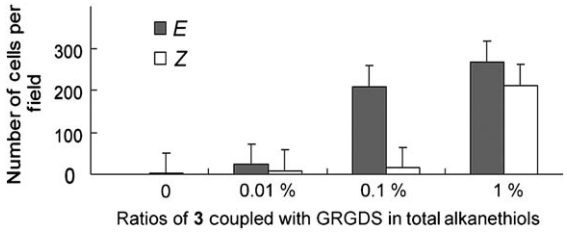


Figure 1. Number of NIH 3T3 fibroblasts adhered to mixed SAMs presenting azobenzene groups terminated in GRGDS peptides in a background of hexa(ethylene glycol) groups.

We next showed that the same substrates can reversibly control cell adhesion. After cells have adhered and begun to spread on mixed SAMs presenting GRGDS with *E* isomer of azobenzene in a ratio of 0.1 % of total alkanethiolates (Figure 2a), we used a soluble GRGDS peptide (1 mg mL^{-1}) to detach the cells from the surface (this process needs about 30 min), because the soluble peptide competed with immobilized RGD for the cell surface integrin receptors.[13,14] Irradiating these cleaned surface with UV light for 10 h converted the *E* configuration of azobenzene into the *Z* form, and the GRGDS ligand was masked in PEG to give a cell-resistant surface (Figure 2b). Using a solution of

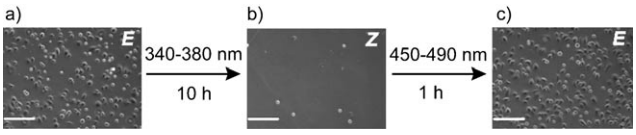


Figure 2. Reversible modulation of the surface for cell adhesion (phase contrast images of NIH 3T3 fibroblasts on SAMs in culture medium). a) Cells adhered onto SAMs with the azobenzene group in the *E* configuration (terminated with the GRGDS peptide). b) Few cells adhered to the same SAMs as (a) with azobenzene in the *Z* configuration. c) Cells adhered to the SAMs again when the conformation of azobenzene was changed from *Z* to *E*. Scale bars are 200 μm.

GRGDS peptide (1 mg mL^{-1}) to detach the few cells from the inert surface and treating the inert surface with visible light for 1 h allowed the SAMs to support cell adhesion again as a result of the conformational switch of azobenzene from *Z* to *E* (Figure 2c and Figure S1 in the Supporting Information). This surface, therefore, allows completely reversible control of cell adhesion.

In conclusion, SAMs allow us to design and quickly obtain a surface that allows reversible control of cell adhesion on a molecularly well-defined surface. The surface can either accommodate or resist cell adhesion depending on the conformation of the azobenzene embedded in SAMs; the two states of the surface are reversible at different wavelengths of light. This technique makes it possible to dynamically modulate patterns of cell adhesion by using light generated by the mercury lamp of a standard fluorescence microscope. Because cell migration essentially comprises a series of steps of adhesion and detachment of the cell, we believe that by combining with the ability to spatially address surface chemistries, the technique presented herein can be used to control the mobility of parts of a single cell (such as lamellapodia or neurites), a single cell, or groups of cells directionally at will.[1] Furthermore, the rapid conversion between the two configurations that azobenzene allows, and the adjustability of the density of the azobenzene on solid surfaces when grafting to a SAM, could facilitate the rational design of new types of photomemory devices.[16]

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