Surface Chemistry

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Using an Electrical Potential to Reversibly Switch Surfaces between Two States for Dynamically Controlling Cell Adhesion**

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Because of the importance of cell adhesion to a diverse range of physiological processes, including wound healing, tissue repair, and cancer metastasis, there has been enormous research interest in developing molecularly well-defined surfaces that can control cell adhesion and migration.[1] Most frequently these surfaces are functionalized with the tripeptide sequence arginine-glycine-aspartate (RGD), a peptide motif found in many proteins of the extracellular matrix (ECM).[1,2] By varying the RGD ligand density, distribution, and the underlying topography these surfaces have provided insights into the mechanisms of cell adhesion.[1,2] However, in vivo, cells respond to an environment where expression of adhesive cues is frequently remodeled. Hence recently attention has turned to the fabrication of model surfaces where the cell adhesive properties can be dynamically switched.[2b,3]

Switchable surfaces for cell biology[2b,3a] are based on thermal,[3b,4] chemical,[5] electrochemical,[6] and optical[7] stimuli to alter the surface characteristics. The majority of these surfaces are irreversible in their switching behavior.[2b,3a,7b] The exception is the optically switchable surface that employs azobenzene. Exposure of UV light causes a *trans* (E)–*cis* (Z) photoisomerization of a surface-bound azobenzene derivative to which an RGD peptide was attached.[7] The *cis* to *trans* isomerization causes an increase in the accessibility of the RGD ligand and enhanced cell adhesion. The surfaces can be switched back to the *cis* isomer, and cells are removed by addition of soluble RGD peptide. The timescales for switching from cell-resistant to cell-adhesive is an hour or more.

Electrical potentials that stimulate a geometry change in the surface-bound molecules have been shown to facilitate rapid and reversible switching. This was first reported by Lahann and Langer[8] who switched a surface from hydrophilic to hydrophobic. The idea has been extended to biointerfaces by Yeung et al.[9] to modulate protein binding to surfaces. These reversibly switchable surfaces work by modifying an electrode surface with a self-assembled monolayer (SAM) that has charged moieties on its distal end. Application of the same potential as the charged moiety causes the molecules to be repelled from the surface. Conversely, application of a potential of opposite polarity attracts the distal moiety to the electrode, and hence immerses it in the organic monolayer.

Herein we report the extension of this reversible switching concept to control cell adhesion by developing a surface that can switch from cell-repulsive to cell-adhesive and back again. This is more complex than reversibly switchable surfaces described previously,[8,9] as a multi-component SAM is required. The SAM is composed of a protein-resistant hexa(ethylene glycol) species (EG_6), which contains a charged moiety on its distal end, and a component terminating in GRGDS peptides to which cellular adhesion receptors, integrins, can bind (Scheme 1).[10] These SAMs are formed on a silicon electrode surface with which we have previously conducted a variety of cell adhesion studies.[11] If the electrode possesses a potential of the same polarity as the charged moiety, the EG_6 molecules project out from the surface and conceal the RGD peptides from the cells, hence resisting cell adhesion. Switching the potential to the opposite polarity causes the EG_6 molecules to flip towards the surface and exposing the RGD peptides, thus allowing cells to adhere. As we have prepared two different EG_6 molecules, one with a sulfonate (anionic) distal moiety and the other with an ammonium (cationic) distal moiety, patterned surfaces can be made where toggling the potential from negative to positive will cause some regions to become cell-resistant and others cell-adhesive (Scheme 1).

The fabrication and characterization of the surface is outlined in detail in the Supporting Information (see Scheme SI2). In brief, a hydrogen-terminated silicon surface was first modified with a mixed monolayer of 1,8-nonadiyne and 10-undecenoic acid in a ratio of 5×10^2:1 (to result in a 10^3:1 ratio on the surface[12]) or 5×10^5:1 (giving 10^6:1 ratio on the surface). The reason the surface is modified with a mixed monolayer of 1,8-nonadiyne and 10-undecenoic acid is the carboxylic acid on the 10-undecenoic acid allows simple attachment of the GRGDS peptide using standard carbodiimide coupling chemistry, whereas the custom synthesized, charged EG_6 molecules (see Scheme SI1), which possess an azido moiety at the distal end, are "clicked" onto the alkyne-terminated species through the copper-assisted Huisgen-1,3-dipolar cycloaddition reaction. The coupling yield for the click reaction is typically around 50 %[13] which ensures there

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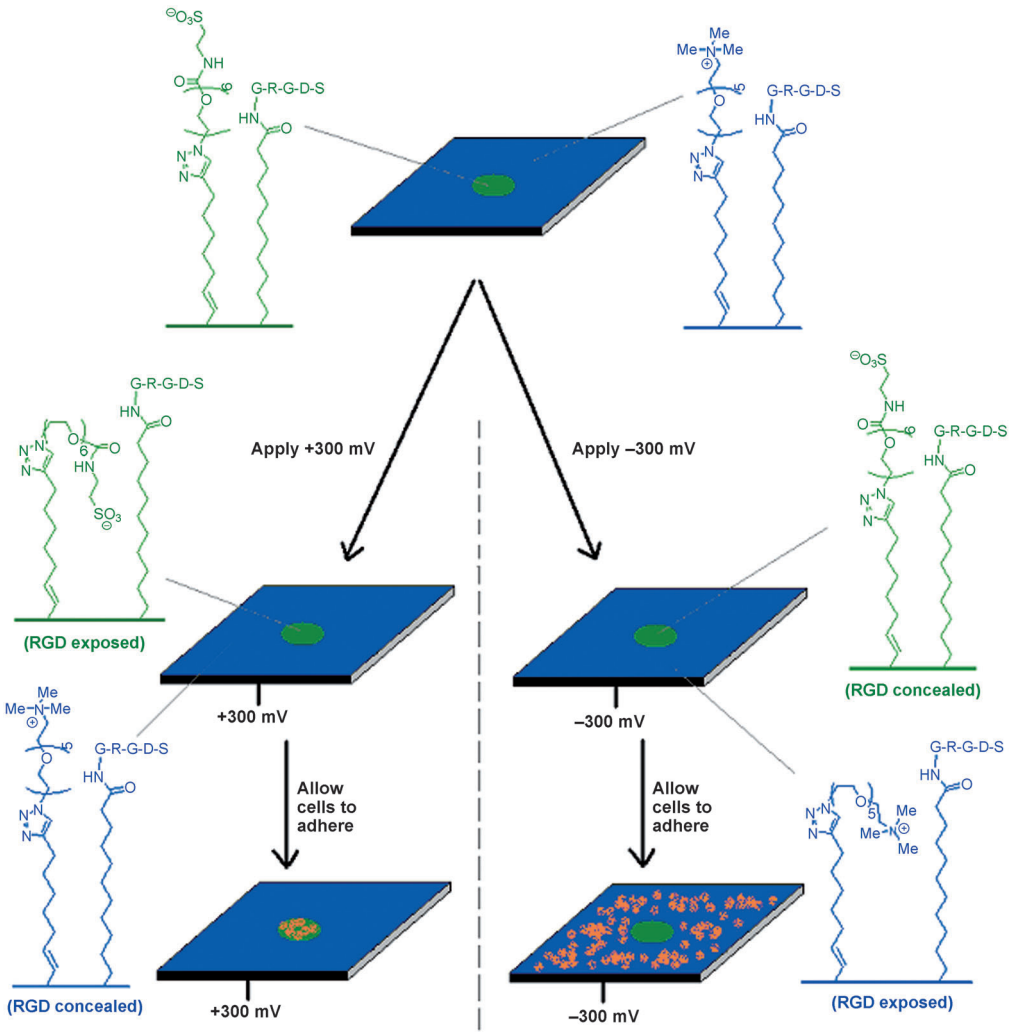

Scheme 1. The design and operation principle of the proposed "switchable surfaces".

is sufficient space for the EG_6 molecules to flip towards the surface on application of a potential of opposite polarity. There are a couple of aspects that warrant a comment on this surface modification strategy. First, the reason why a base layer is first formed before EG_6 and other functionality are attached is that we achieve a far better layer, in terms of passivating the silicon, in this stepwise approach as previously shown.[14] Second, this design is an unique use of orthogonal click reactions, allowing species to be sequentially attached to a base monolayer without any cross-coupling reactions to form mixed functionality layers. To form patterned surfaces of oppositely charged EG_6 species, the entire surface was first modified with the GRGDS peptide and then one of the EG_6 species was placed in a defined region by pipetting a 1 μL drop onto the surface. Then the rest of the surface was modified with the opposing EG_6 molecule.

After confirming the successful attachments of EG_6-sulfonate and EG_6-ammonium species, the "switchable surfaces" were tested for cell adhesion using bovine aortic endothelial cells (BAEC), which are contact-dependent for survival,[15] under either +300 or −300 mV. Two different ratios of RGD to EG_6 were used for each type of "switchable surface"—$1{:}10^3$ and $1{:}10^6$—as these average densities of RGD ligands were previously shown to yield the maximum cell adhesion.[11] The densities of the BAEC on these "switchable surfaces" were normalized to the positive control (100% GRGDS grafted directly onto 10-undecenoic acid SAM), and compared to the negative control surfaces (either 100% EG_6-OH, 100% EG_6-sulfonate, or 100% EG_6-ammonium grafted directly onto the 1,8-nonadiyne SAM with no RGD peptide present; see Scheme SI2 in the Supporting Information).

Focusing first on the sulfonate "switchable surface", RGD ligands become exposed, and cells can adhere, when a potential of +300 mV was applied. The number of adherent cells under these conditions was 75% of the number that adhered to 100% RGD surfaces for the RGD:EG_6-sulfonate surface at a ratio of $1{:}10^3$ and 73% at a ratio of $1{:}10^6$. However, if the surfaces were poised at −300 mV, such that the RGD ligands were concealed, the number of cells that adhere was 85% lower than at +300 mV for the $1{:}10^3$ surface (to <10% of the number of cells on the 100% RGD surface) and 94% lower than at +300 mV for the $1{:}10^6$ surface (see Figure 1). For the $1{:}10^6$ surface, when switched to cell-resistant, the number of cells that adhere was <10% of that observed on the 100% RGD surface. In contrast, if the RGD peptide was absent (e.g. on the 100% EG_6-sulfonate surface) there was no statistically significant change (95% confidence levels), and the overall cell density was low compared to both sulfonate "switchable surfaces" containing RGD peptides when cell-adhesive (+300 mV). Hence under positive potentials, cell adhesion was promoted, as depicted in Scheme 1, suggesting that changes in molecular conformation that expose RGD peptides can be used to dynamically control cell adhesions. Controls were performed to demonstrate that the change in cell adhesion was not caused by the potential influencing the antifouling ability of the EG_6 molecules (100% EG_6-OH in Figure 1) and the potential did not influence cell adhesion per se or the ability of the peptide to bind to cells (Figure SI3).

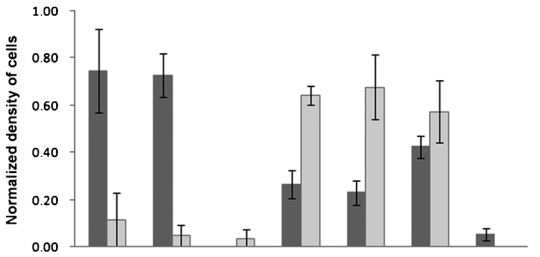

Figure 1. Density of adhered cells on various "switchable surfaces", normalized against the density of cells adherent onto 100% RGD surfaces under +300 (dark gray) and −300 mV (light gray). On each type of surface, cell counts from at least four different regions were averaged.

Next, we focus on the ammonium variety of the "switchable surfaces". Note that the EG_6-ammonium surfaces were more fouling than the EG_6-sulfonate surfaces, because many proteins and cells are electrostatically attracted to cationic surfaces as demonstrated previously.[16] When −300 mV was applied to the ammonium "switchable surface", the cell density on the $1{:}10^3$ and $1{:}10^6$ surfaces were relatively high (64% and 68% of the 100% RGD-terminated surface, respectively), indicating that at −300 mV the RGD peptides were exposed. However, if +300 mV was applied to the ammonium "switchable surface", there was a about 59% reduction in cell adhesion with a RGD:EG_6-ammonium ratio of $1{:}10^3$ compared to that on −300 mV, and a about 66% reduction of cell adhesion for the surface with RGD:EG_6-ammonium ratio of $1{:}10^6$ compared to that on −300 mV. Hence with the ammonium "switchable surfaces", a positive potential makes the surfaces more resistant to cell adhesion. Note, for the 100% EG_6-ammonium surface (no RGD) a change from negative to positive potential did not affect cell adhesion. An important control here was to ensure that the ammonium moiety was not cytotoxic to cells, as quaternary ammonium compounds are often used as disinfectants. As shown in Figure SI4 in the Supporting Information, the number of unhealthy rounded cells compared with healthy spread cells was lower on the ammonium surface, whether the surfaces were in cell-adhesive or cell-repulsive modes, than the sulfonate surface. This shows that our surfaces with ammonium moieties are no more harmful to cells than surfaces bearing sulfonate moieties.

The rapid switching of the surface allowed us to determine the influence of exposure time of cells to the adhesive surface on the number of adhered cells. An array of silicon electrodes was prepared that presented a RGD:EG_6 ratio of $1{:}10^3$ in a single cell culture dish. Individual surfaces were switched to cell adhesive at intervals of 10 minutes. As expected the longer cells were exposed to an adhesive surface, the more cells were adhered (see Figure SI5 in the Supporting Information). We also investigated whether switching the surfaces from cell-adhesive to cell-resistant would cause cells to detach from the surface. Unsurprisingly, with contact-dependent cells such as BAECs, adherent cells did not detached when surfaces were switched to antifouling (see Figure SI6).

Having two EG_6 derivatives with different charges, presents the possibility to form patterned surfaces with positive and negative charged regions. Hence switching potentials will cause regions to toggle between cell-adhesive and adhesion-resistant. Two different patterned surfaces were fabricated: EG_6-ammonium inside the spot and EG_6-sulfonate outside the spot (N-in), and vice versa (S-in). Both types of surfaces consisted of RGD moieties with a ratio of RGD to total EG_6 of $1{:}10^3$. Cells were allowed to adhere on the patterned surfaces with potentials of −300 or +300 mV. Figure 2 a–c shows the distribution of cells on one of such surface, N-in, with +300 mV applied. The difference in cell adhesion inside and outside the spot is clearly discernable. The cell numbers for the four different surface combinations are shown in Figure 3 a–d. Although the absolute number of cells is different in each case (because of the differences in the total number of cells in the culture), the ratio of the number of cells adhered onto antifouling regions to that of cells adhered onto adhesive regions ranges from 1:5 to 1:12.

Following the success of patterning "switchable surfaces" we next performed cell migration experiments. In these experiments, differentiated HL60 (dHL60) cells, a neutrophil-like cell line, were used because these cells are intrinsically motile and less contact-dependent than BAECs. Cells were allowed to adhere onto two identical patterned "switchable surface" with the inner region containing EG_6-ammonium species, while the outer region was backfilled with EG_6-sulfonate moieties (Figure 4). The ratio of RGD to total EG_6 was $1{:}10^3$. Both surfaces were incubated in a dHL60 cell suspension, under +300 mV, for 45 mins. Non-adherent cells were removed after incubation and then a potential of −300 mV was applied to the surface in Figure 4 b for 45 minutes. This meant that the inner region switched from cell-resistant to cell-adhesive. Hence, cells were able to infiltrate the previously nonadhesive zone, presumably by migrating from the outer to the inner region as we saw no appreciable cell loss. In the absence of the switching potential, the inner zone remains resistant to cell adhesion (Figure 4 a).

In conclusion, a "switchable surface" that could control cell adhesion was successfully fabricated. Sulfonate and ammonium EG_6 derivatives were synthesized, attachment to alkyne-terminated silicon surfaces and the control over cell adhesion examined under various configurations. The "switchable surface" was tested under positive and negative potentials, and it was found that the ammonium "switchable surface" switched from antifouling to cell-adhesive when the potential switched from +300 to −300 mV; while the sulfonate "switchable surface" switched from antifouling to adhesive when the potential switched from −300 to +300 mV. Since the applied potentials per se neither affected the antifouling properties of the EG_6 moieties nor the cell adhesion to RGD peptides, we conclude that the change in potential causes rearrangement of the molecular confirmation on the surfaces such that the peptide ligands are either exposed to, or concealed, depending on the molecules used and potentials applied. Patterned "switchable surfaces" containing the sulfonate-EG_6 moiety in one region and the ammonium-EG_6 moiety in the other region were able to be switched, allowing cells to migrate into previously antifouling

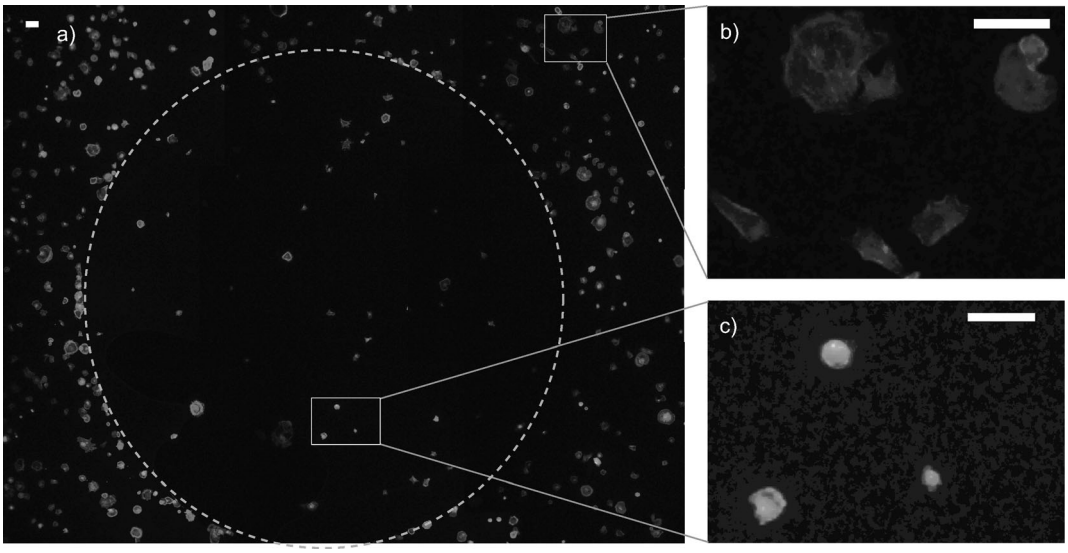

Figure 2. a) Fluorescence image of adhered BAEC (F-actin staining) on a surface presenting a mixture of RGD and EG_6 moieties, with RGD:(total EG_6) = 1:1000, with the outer region (outside of the white dashed circle) containing EG_6-sulfonate moieties and the inner region containing EG_6-ammonium moieties. A potential of +300 mV was applied to the surface during cell incubation. The two zoomed images (rectangular regions on the main image) compared b) adhered cells spreading on adhesive region of the surface (EG_6-sulfonate, +300 mV), and c) nonadhered cells showing a round shape on the antifouling region of the surface (EG_6-ammonium, +300 mV). Scale bar = 20 μm on all three images.

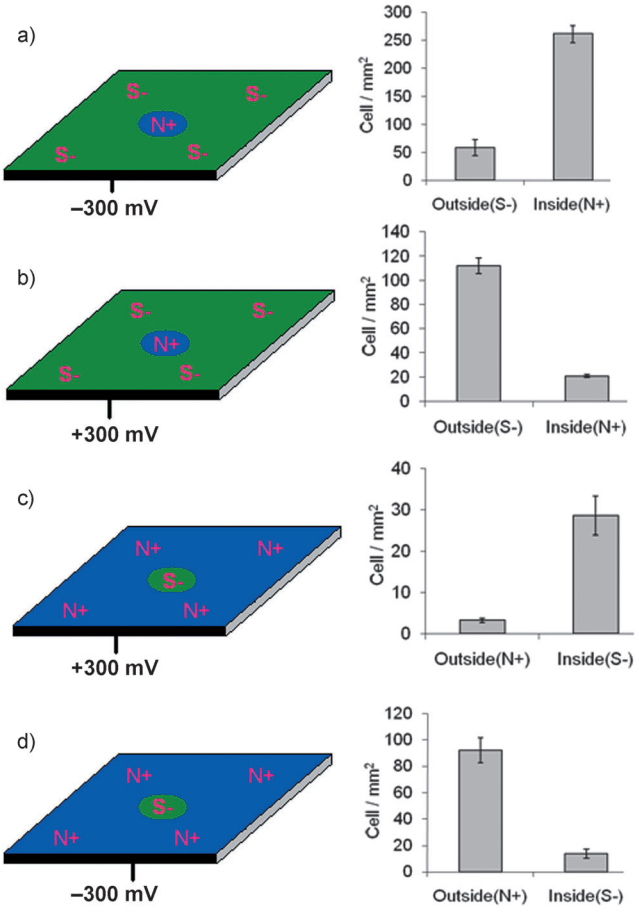

Figure 3. a–d) Antifouling performance difference between different regions (EG_6-ammonium, "N+" or EG_6-sulfonate, "S-") on a patterned "switchable surface" under +300 or −300 mV.

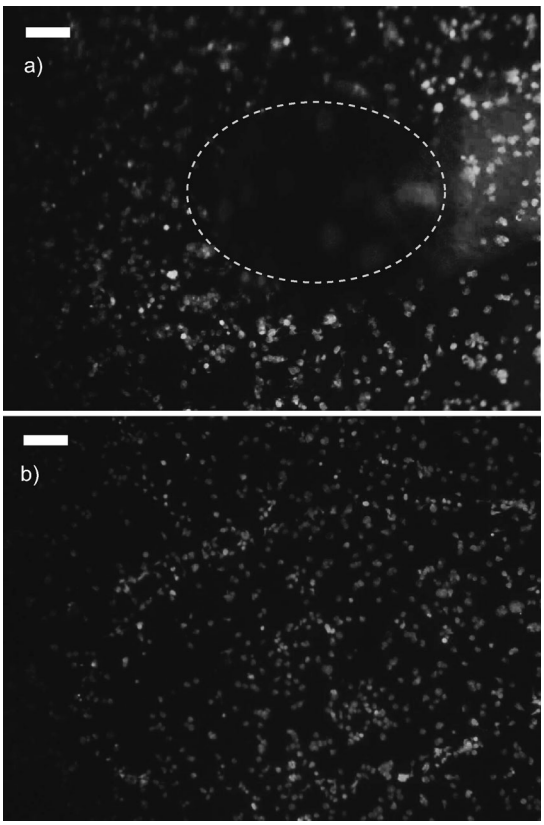

Figure 4. HL60 cells adhered (Celltracker staining) onto two independent surfaces presenting a mixture of RGD and EG_6 moieties (RGD:(total EG_6) = 1:1000) with the inner spot (highlighted by a dashed circle) consisting of EG_6-ammonium moieties while the remaining surface consists of EG_6-sulfonate moieties. Cells were adhered for 45 minutes at +300 mV applied to both surfaces. After nonadherent cells were removed, the surfaces were either a) disconnected, or b) the potential was inversed to −300 mV and incubated for a further 45 minutes. Scale bar = 100 μm.

regions on such surfaces. These "switchable surfaces" may find applications in different areas of cell biology, such as selective capture and release of cells, controlling the location of cell adhesion and cell migration on surfaces, and potentially allowing the control of the availability of other biomolecules on surfaces.

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