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Electroactive Monolayer Substrates that Selectively Release Adherent Cells

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KEYWORDS:

biosurfaces · cell adhesion · dynamic substrates · electrochemistry · monolayers

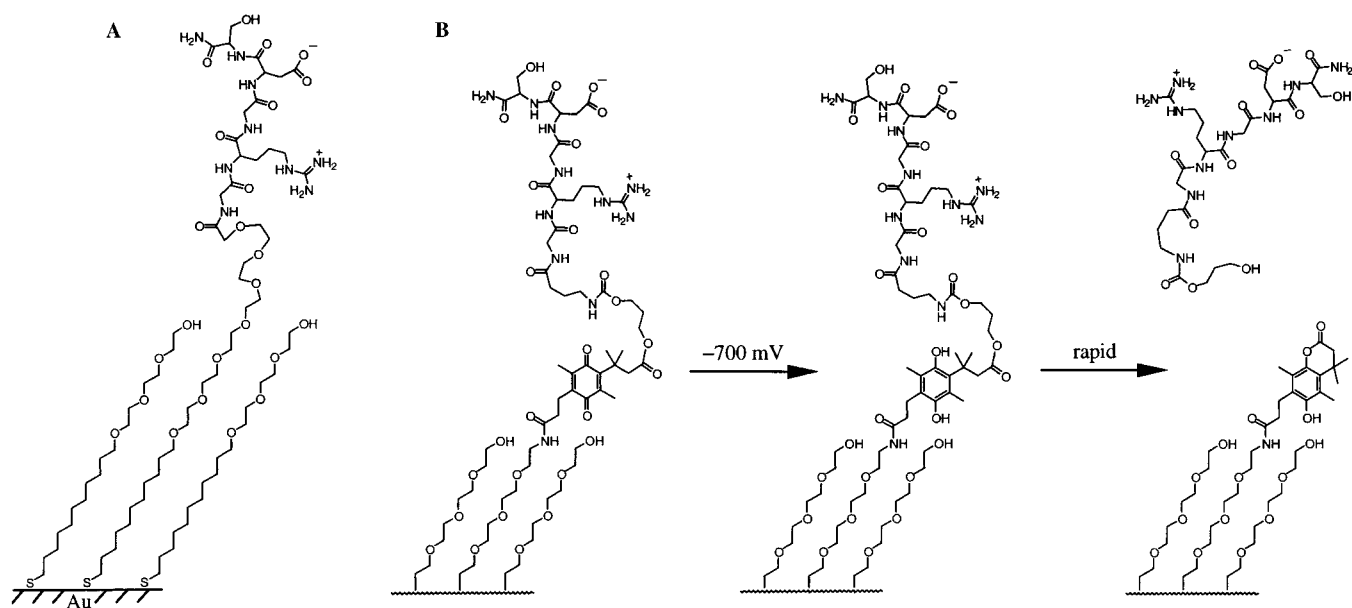
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This communication describes a dynamic substrate that can selectively release immobilized ligands and hence can regulate, in real-time, the ligand–receptor interactions between a cell and the substrate to which it is attached. The aim of this work is to provide model substrates for mechanistic studies of cell adhesion and migration. The adhesion of cells is mediated by the binding of cell-surface receptors—often, the integrin family of receptors—to ligands of the insoluble protein matrix (also known as the extracellular matrix).^[1] In many cases, cells respond to changes in the composition of ligands presented within the matrix. Examples are found in the growth or differentiation of cells,^[2] and in tumor metastasis, where malignant cells migrate through the endothelial barrier.^[3] The development of dynamic substrates which can modulate the composition of ligands that interact with adherent cells would provide new opportunities for studying many important cellular processes. In this paper, we describe a chemical strategy to develop a dynamic substrate that can selectively release immobilized ligands under electrochemical control.

Our approach is based on a self-assembled monolayer (SAM) of alkanethiolates on gold that presents peptide ligands tethered to the monolayer through an electroactive quinone ester moiety (Scheme 1 B). The quinone ester undergoes a two-electron reduction on application of an electrical potential to the underlying gold substrate to give the corresponding hydroquinone, which then rapidly cyclizes to give a lactone with release of the peptide ligand.^[4, 5] We use as a model system, a monolayer that can release the tripeptide Arg-Gly-Asp (RGD). This peptide is a ligand found within many extracellular matrix proteins and which mediates cell adhesion through integrin receptors.^[1] With this system, the application of an electrical potential results in the release of RGD and, therefore, of cells that are attached to the monolayer. This model system has the benefits that the dynamic property can be easily visualized, and that it establishes the compatibility of the electroactive substrate with the conditions of cell culture. The tri(ethylene glycol)

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Scheme 1. Structures of self-assembled monolayers of alkanethiolates on gold that were used to demonstrate the selective release of adherent cells. A) Monolayer presenting the peptide RGD that supports cell adhesion but is not affected by electrical potentials because it does not contain the electroactive tether. B) Monolayer that was designed to release RGD on application of an electrical potential to the underlying gold substrate. Upon electrochemical reduction of the quinone to the corresponding hydroquinone, a cyclization reaction ensues to give a lactone with release of the RGD ligand. In both monolayers, RGD is present at a density of less than 0.1% and is surrounded by tri(ethylene glycol) groups which impart resistance to nonspecific protein adsorption.

groups are critical to the design because they make the substrate inert to nonspecific protein adsorption and to nonspecific cell adhesion—a strict requirement for biological studies at interfaces.^[6]

We prepared a substrate that was patterned into two regions that each presented the RGD peptide, at a density of less than 0.1%, mixed with tri(ethylene glycol) groups.^[7] The two regions differed only in the linkage used to tether the peptides to the monolayer. In one region, the peptide was tethered by way of the quinone ester and, therefore, could be released with application of an electrical potential (Scheme 1B).^[8] In the second region, the peptide was tethered with a linker that is not electrically active (Scheme 1A).^[9] We used this patterned substrate because it simultaneously allows characterization of the selective release of cells, and it shows that the electrical potential does not affect cells attached to the adjacent region.

This substrate was placed into cell-culture media and a suspension of 3T3 Swiss fibroblast cells was added.^[10] Figure 1A shows that cells efficiently attached to both regions of the patterned monolayer. The cells were evenly distributed on the surface and adopted a spread morphology over the entire substrate. After cells were cultured at 37 °C for 30 minutes, an electrical potential of -700 mV (versus an Ag pseudo-reference) was applied to the gold substrate for 4 minutes.^[11] Immediately after the electrochemical treatment, more than 70% of the cells on the electroactive region reverted to a rounded morphology and rested unattached on the substrate. After the substrate was incubated at 37 °C for 10 minutes, the media was replaced with fresh media and the substrate was photographed. Figure 1B shows that no cells remained on the region of the electroactive monolayer. Cells that were attached to the region of the

monolayer presenting the nonreleasable RGD, by contrast, were not affected by application of the electrical potential.

As a final control experiment, we next replaced the cell media with fresh media that contained the soluble peptide Gly-Arg-Gly-Asp-Ser (GRGDS) at a concentration of 2 mM.^[12] The substrates were kept at 37 °C for 20 minutes and then removed from the incubator and photographed. Figure 1C shows that greater than 95% of the cells were released from the surface. This final experiment is important because it shows that the adhesion of cells was mediated only by the RGD peptide, and that the electrochemical treatment did not compromise the inert property of the substrate.^[13] A similar inhibition experiment for cells attached to the patterned substrate prior to electrochemical treatment also resulted in near complete detachment of cells from both regions. Overall, these results show that cell adhesion to monolayers presenting RGD tethered through electroactive quinone groups is biospecific and that the RGD ligand can be released from the substrate selectively, without consequence to cells attached to other ligands.^[14]

This work provides an early example of a dynamic substrate that can regulate, in real-time, the ligand–receptor interactions between a cell and the substrate to which it is attached. The most significant aspect of this method is that it provides control at the molecular level and, therefore, can be used to modulate specific receptor–ligand interactions. Previous examples of substrates that can release attached cells have relied on a thermally responsive poly(*N*-isopropylacrylamide) film.^[15] This polymer is hydrophobic and therefore a good substrate for cell adhesion, but at temperatures below the lower critical solution temperature the polymer adopts a solvated structure which is a poor substrate for cell adhesion. While this thermally responsive

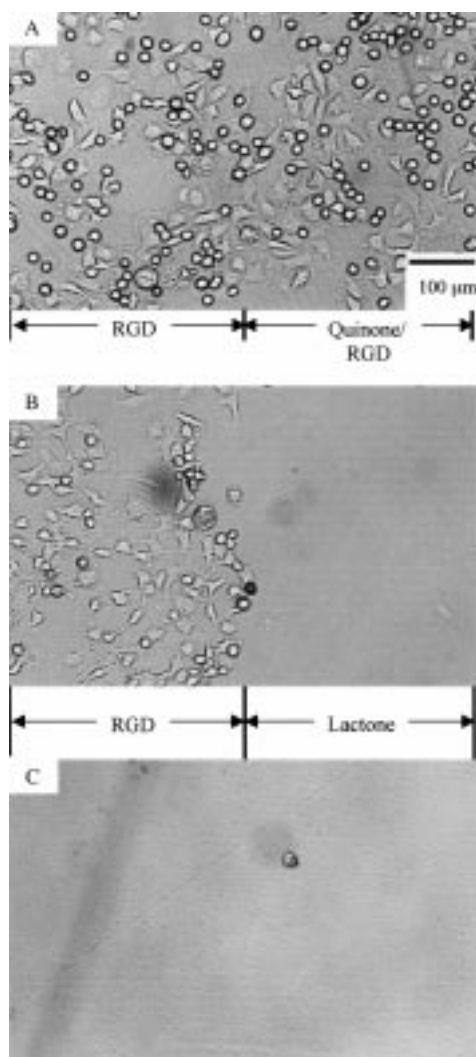


Figure 1. Optical micrographs of 3T3 Swiss fibroblast cells interacting with a patterned self-assembled monolayer. The left side of the pattern comprises the monolayer shown in Scheme 1A while the right side comprises the monolayer shown in Scheme 1B, which contains the electroactive quinone group and can release the RGD peptide. A) Cells adhere and spread uniformly on both sides of the substrate. B) After application of a potential of -700 mV for 4 minutes, the quinone groups were reduced to the hydroquinone with subsequent lactonization and release of RGD. Only the cells attached to the region presenting RGD tethered to the electroactive quinone groups were released from the substrate. C) Addition of the peptide inhibitor GRGDS (2 mM) to the cell culture resulted in the detachment of cells from the left side of the monolayer, which shows that the properties of the monolayer were not affected by the electrochemical treatment.

gel is an important substrate for the harvesting of cells and tissues, it does not offer the molecular-level control necessary for studies of cell behavior. The method we describe in this communication is significant because it can release specific ligands and, hence, can be adopted to examine the pathways by which cells respond to the loss of specific receptor–ligand interactions. The electrical potentials used are noninvasive, selective, and compatible with the conditions of cell culture. Furthermore, the use of patterned substrates or microelectrode arrays can localize the ligand release to a designated region of the substrate.

This work provides a new approach to investigate the chemical biology of cell adhesion and migration. The preparation of dynamic substrates that can selectively release ligands—even when multiple ligands are presented—gives highly controlled, well-defined model systems for investigating the responses of cells to changes in the composition of ligands of the substrate. The strategy outlined here can be generalized to the development of other classes of dynamic substrates, including those that turn on the activity of immobilized ligands and that modulate the affinities of immobilized ligands.^[16] We believe that this method will be useful for mechanistic studies in cell biology and for applications in biotechnology.

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- [8] The ethanolic solutions used for formation of the monolayer^[7] contained a mixture of an alkanethiol substituted with tri(ethylene glycol) and an alkanethiol substituted with either the RGD peptide or the RGD peptide–quinone ester conjugate in a ratio of 999:1. The peptide–quinone ester conjugate was synthesized in 29 steps from commercially available reagents. All intermediates gave satisfactory ¹H NMR and mass spectra.
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- [10] 3T3 Swiss Albino cells (ATCC, Rockville, MD) were grown in Dulbecco’s Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and penicillin/streptomycin. All cultures were maintained at 37 °C in a humidified 10% CO₂ atmosphere.
- [11] Electrochemistry was performed in a custom-designed electrochemical cell with the monolayer-coated gold as the working electrode, a Pt wire as the counter electrode, and an Ag wire pseudo-reference electrode. All studies were performed with DMEM cell media containing fetal bovine serum (pH 7.4) as the solvent and electrolyte. DMEM contains many components, but the major electrolyte is NaCl (109 mM).
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Bionic Catalyst Design: A Photochemical Approach to Artificial Enzyme Function

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Successful imitation of the environmentally benign processes mediated by natural biopolymers remains a difficult challenge for future green-chemistry technologies. Among the most important industrial and synthetic goals that rely on the exploitation of sustainable resources are the controlled activation of atmospheric dioxygen^[1, 2] and the ecological conversion of alcohols into carbonyl compounds.^[3] Several native enzymes catalyze the transformation of primary alcohols under aerobic conditions, producing aldehydes and hydrogen peroxide. To mimic the regulation mechanisms and the selective chemistry of enzymes in the absence of a protein environment, the novel strategy of bionic photocatalysis tries to impose light-induced electronic, energetic, and geometric changes on artificial substrate recognition sites.^[4] As an example of this bio-inspired catalyst design leading to photocatalytic artificial enzymes (photozymes), a robust synthetic oxidoreductase for the photo-assisted two-electron oxidation of alcohols and the catalytic accumulation of hydrogen peroxide is presented. It is concluded that photochemical modeling of enzymatic key functions has the potential to offer new pathways for a pollution-free production of industrial chemicals driven by solar energy under ambient conditions.

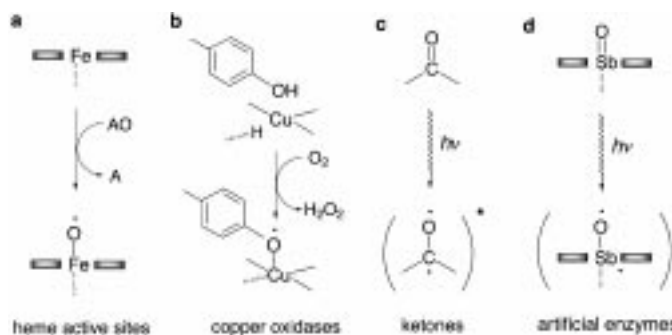
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Free radicals are aggressive species that tend to cause irreversible degradation of biological tissue and synthetic materials. Therefore, the survival of living cells and chemical systems with a dynamic function critically depends on the control mechanisms for reactive radical intermediates. Nevertheless, there is an increasing number of reports on biocatalytic transformations involving protein radicals,^[5] and there are substantial efforts to understand and mimic these processes for synthetic applications.^[6–8] In many cases hydrogen atom abstraction from the substrate (S) is considered as one of the mechanistic key steps catalyzed by radical enzymes (E) as shown in Equation (1):



Scheme 1 illustrates some of the design principles that have been followed for the construction of a simple photon-driven functional model of native oxidoreductase enzymes that are capable of catalyzing H-atom transfer processes. The synthetic



Scheme 1. How are molecules activated for hydrogen atom transfer chemistry? Different ways to induce the desired catalytic reactivity at a given site are shown. The common link is a species with unpaired spin density at an oxygen atom accessible to the substrate. a) Formation of the so-called compound I intermediates^[9] in heme-enzyme mechanisms (AO = oxygen atom donor). b) Coordination of a protein-bound tyrosyl radical cofactor in the active form^[10] of the copper-containing enzyme galactose oxidase. c) The radical character of $n \rightarrow \pi^*$ -excited states of carbonyl compounds, which enables a photochemical hydrogen abstraction^[11, 12] without multiple turnovers. d) Photocatalytic enzyme counterparts (photozymes) combine and modify the most efficient strategies described.

photocatalyst $SbO(tpp)OH$ reported here (Scheme 1 d) consists of an antimony metal center with a terminal oxo group ($Sb^V=O$) which can mediate hydrogen abstraction, a macrocyclic ligand ($tpp = meso$ -tetraphenylporphyrin) acting as the light-harvesting and radical-stabilizing subunit, and a further axial ligand (OH^-) to form a coordinatively saturated compound. The monoprotanated catalyst precursors^[13] of the type $[Sb(tpp)(OH)_2]^+ X^-$ are rather stable coordination compounds that can be stored for several years in the dark without decomposition.

As an example of a catalytic substrate transformation that is considered to involve hydrogen abstraction as the rate-determining mechanistic step,^[5, 10] the two-electron oxidation of primary alcohols to aldehydes with dioxygen acting as the electron acceptor was chosen [Eq. (2)]:

