

## Dynamic Interfaces between Cells and Surfaces: Electroactive Substrates that Sequentially Release and Attach Cells

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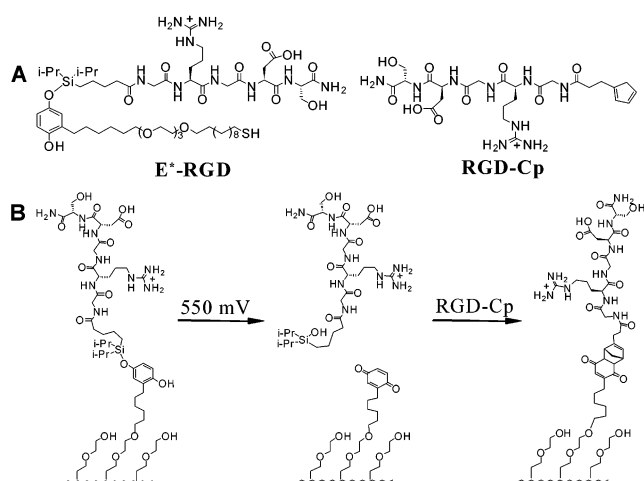
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An important theme in surface chemistry is the design of interfaces between cells and materials. This work has had an important impact in basic studies to understand the adhesion of cells and in many applied areas, including biomaterials and tissue engineering.<sup>1</sup> An exciting recent focus has been the development of substrates that are dynamic and offer real-time control over the presentation of ligands to attached cells. Early examples of dynamic substrates have relied on the use of thermally responsive<sup>2</sup> and photoactive<sup>3</sup> gels to manipulate the adsorption of proteins and therefore the adhesion of cells. We have previously described electroactive substrates that directly switch ligand activities on or off in response to electrical potentials.<sup>4</sup> In this communication, we describe an important advance by demonstrating a substrate that combines two dynamic properties: the release of one ligand followed by the immobilization of a second ligand.

We demonstrate our approach using a self-assembled monolayer (SAM) that incorporates an *O*-silyl hydroquinone moiety to present a peptide ligand (E\*-RGD, Figure 1). The Arg-Gly-Asp peptide is a ligand for cell surface integrin receptors and serves to mediate the adhesion of cells while the surrounding tri(ethylene glycol) groups serve to prevent the nonspecific attachment of cells to the monolayer.<sup>5</sup> The *O*-silyl hydroquinone ether is electroactive and provides for selective release of the peptide from the substrate. The release is triggered by applying an electrical potential to the substrate, effecting the oxidation of this group to yield the corresponding benzoquinone with hydrolysis of the silyl ether. The resulting benzoquinone group undergoes a selective immobilization reaction with a diene-tagged peptide (RGD-Cp, Figure 1A) by way of a Diels–Alder reaction and, therefore, provides the basis for a second dynamic activity. The benzoquinone is redox active and can be reduced to the hydroquinone, which prevents immobilization of the diene-tagged ligand. In this work, we demonstrate these two dynamic properties by observing the effects of ligand modulation on the adhesion of cells.

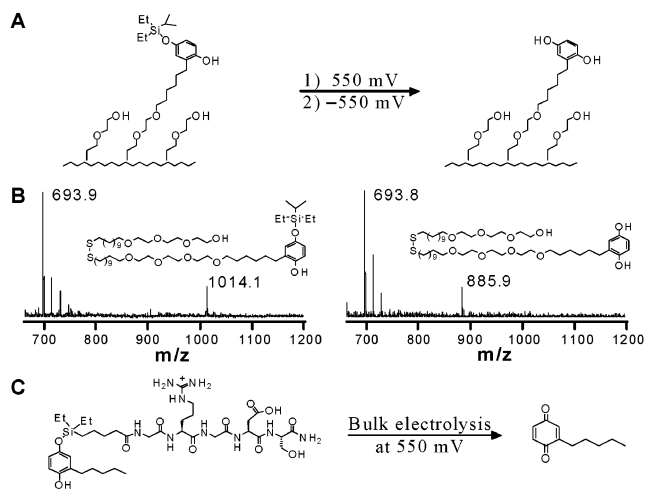
We first characterized the electrochemical modulation of the monolayer. We prepared a self-assembled monolayer comprising a diethylisopropylsilyl hydroquinone-terminated alkanethiolate and a tri(ethylene glycol)-terminated alkanethiolate in a ratio of 1:3 (Figure 2A). MALDI-TOF mass spectrometry of this monolayer showed two major peaks at  $m/z$  694 and  $m/z$  1014 (Figure 2B, left).<sup>6</sup> The first peak corresponds to the symmetric disulfide derived from a tri(ethylene glycol)-terminated alkanethiolate, and the second peak corresponds to the mixed disulfide derived from a diethylisopropylsilyl hydroquinone-terminated alkanethiolate and a tri(ethylene glycol)-terminated alkanethiolate. An electrical potential of 550 mV was applied to an identical monolayer for 5 min to generate the benzoquinone followed by a potential of  $-550$  mV for 30 s to reduce the benzoquinone to the hydroquinone.<sup>7</sup> MALDI-TOF revealed a new peak at  $m/z$  886 corresponding to the mixed disulfide derived from a hydroquinone-terminated alkanethiolate and a tri-



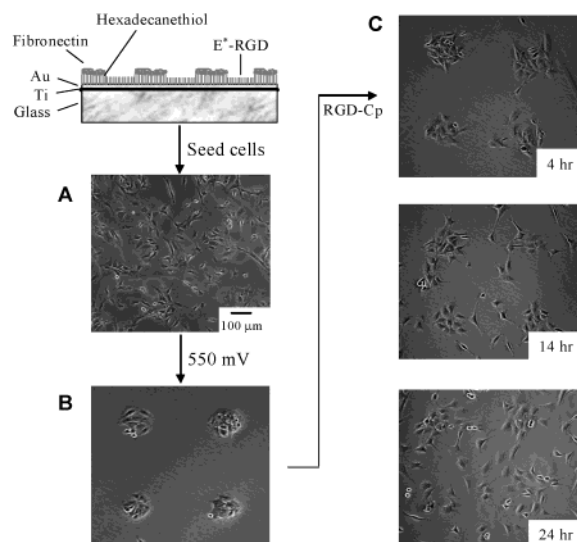
**Figure 1.** (A) Structures for the functionalized alkanethiol used to prepare dynamic substrates (E\*-RGD) and the cyclopentadiene moiety (RGD-Cp) used to selectively immobilize ligand. (B) A monolayer presenting the *O*-silyl hydroquinone undergoes electrochemical oxidation to give a benzoquinone, with hydrolysis of the silyl ether and selective release of the RGD ligand. The resulting benzoquinone reacts with RGD-Cp by way of a Diels–Alder reaction, which selectively immobilizes the second ligand. The RGD peptide mediates the adhesion of cells.

(ethylene glycol)-terminated alkanethiolate (Figure 2B, right). The electrochemical generation of benzoquinone was verified with cyclic voltammetry, which showed the voltammetric waves for the benzoquinone redox pair and the loss of the benzoquinone on addition of cyclopentadiene (see Supporting Information).<sup>8</sup> This result confirms that benzoquinone groups were generated by the electrical potential and consumed through Diels–Alder reaction with cyclopentadiene. As a final control experiment, we synthesized an analogue of E\*-RGD that does not contain the alkanethiol moiety and performed a bulk electrolysis in phosphate-buffered saline (PBS) at pH 7.4. Extraction of the aqueous solution with dichloromethane gave the corresponding benzoquinone in  $\sim 90\%$  yield (Figure 2C).

To demonstrate the application of this strategy to manipulating the adhesion of cells, we patterned a monolayer into circular regions ( $220 \mu\text{m}$  in diameter) with hexadecanethiol and modified the remaining regions with E\*-RGD at a density of 0.02% mixed with a tri(ethylene glycol)-terminated alkanethiolate (Figure 3).<sup>9</sup> This patterned substrate was treated with a solution of fibronectin—a protein that contains the RGD peptide and mediates cell adhesion—which resulted in the adsorption of protein only to the circular regions. Swiss 3T3 fibroblast cells were incubated on the substrate for 2 h.<sup>10</sup> An optical micrograph showed that cells adhered to the monolayer and were evenly distributed across the regions presenting fibronectin and RGD peptide (Figure 3A). To selectively release RGD peptides outside the circular features, we applied an electrical potential of 550 mV to the monolayer for 5 min.<sup>11</sup> Cells on these



**Figure 2.** Characterization of the electrochemical oxidation of *O*-silyl hydroquinone groups on the monolayer. (A) A monolayer presenting diethylisopropylsilyl hydroquinone groups was treated with an electrical potential of 550 mV for 5 min followed by a potential of  $-550$  mV for 30 s to give the hydroquinone. (B) A MALDI-TOF mass spectrum for the initial monolayer displayed a peak at  $m/z$  1014 corresponding to the mixed disulfide derived from a diethylisopropylsilyl hydroquinone-terminated alkanethiolate and a tri(ethylene glycol)-terminated alkanethiolate (left). After electrochemical treatment, the original peak was absent and gave rise to a new peak at  $m/z$  886 corresponding to the hydroquinone-terminated disulfide (right). (C) Bulk electrolysis of a model compound gave the corresponding benzoquinone.



**Figure 3.** Demonstration of a dynamic substrate that combines two dynamic properties: (i) the release of RGD ligands and, thus, the release of cells, (ii) the immobilization of RGD ligands and, hence, migration and growth of cells. A monolayer was patterned into circular regions that present fibronectin and surrounded by RGD ligands tethered by way of an electroactive linker ( $E^*$ -RGD). (A) Swiss 3T3 fibroblast cells adhered and spread evenly over entire substrate. (B) An electrical potential of 550 mV was applied to the substrate for 5 min, and the substrate was incubated for 4 h. Cells were efficiently released only from the  $E^*$ -RGD regions. (C) Treatment of the monolayer with RGD-Cp resulted in ligand immobilization and initiated cell migration from fibronectin regions onto remaining regions. After 24 h, cells were distributed evenly over the substrate.

regions immediately began to adopt a round shape and detach from the monolayer. An optical micrograph showed that most cells on  $E^*$ -RGD regions were released, while cells on the fibronectin-coated circular regions were not affected by the electrical potential and remained on the monolayer (Figure 3B). This result indicates that the RGD ligand was released from the *O*-silyl ether tethered SAM

by the electrochemical treatment as described in Figure 1B. We next replaced the cell medium with the serum-free medium containing RGD-Cp at a concentration of 1 mM for 1 h and then replaced the medium with serum-containing medium. Following the Diels–Alder mediated immobilization of the peptide, cells began to migrate from fibronectin-coated circular regions onto surrounding regions of the substrate (Figure 3C). After 14 h of culture, the original circular pattern was no longer evident, and after 24 h, cells were distributed evenly over the entire surface. When the quinone was electrically reduced to give the hydroquinone before addition of RGD-Cp, cells did not migrate and remained localized to the circular pattern. Further, cells did not respond to the immobilization of an inactive RGE peptide (see Supporting Information). This example demonstrates that the electrochemical strategy for both releasing and attaching ligands from/to the monolayer is effective in complex cell culture media.

The demonstration of a dynamic substrate that combines two functions makes a significant advance on previous reports of substrates that can modulate ligand activities. The ability to effect multiple changes in the bioactivity of a substrate will be especially useful in studies of heterotypic cell–cell interactions where the fate of a given cell depends on the identities and periods of exposure to neighboring cells. The dynamic substrates will also find use in microfluidic lab-on-a-chip systems by allowing active use of channels to process analytes in a sample. More broadly, this example illustrates a flexible strategy for using synthetic and physical organic chemistry to design and prepare surfaces that display complex functions.

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**Supporting Information Available:** Synthesis and experimental details for  $E^*$ -RGD, cyclic voltammograms showing electroactive cleavage, and a control experiment with RGE-Cp (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References

- (1) (a) Mrksich, M. *Chem. Soc. Rev.* **2000**, *29*, 267. (b) Hubbell, J. A. *Curr. Opin. Biotechnol.* **1999**, *10*, 123.
- (2) (a) Okano, T.; Yamata, N.; Sakai, J.; Sakurai, Y. *Biomaterials* **1995**, *16*, 297. (b) Collier, T. O.; Anderson, J. M.; Kikuchi, A.; Okano, T. *J. Biomed. Mater. Res.* **2002**, *59*, 136. (c) Shimizu, T.; Yamato, M.; Kikuchi, A.; Okano, T. *Biomaterials* **2003**, *24*, 2309.
- (3) (a) Hern, D. L.; Hubbell, J. A. *J. Biomed. Mater. Res.* **1998**, *39*, 266. (b) Elbert, D. L.; Hubbell, J. A. *Biomacromolecules* **2001**, *2*, 430.
- (4) (a) Hodneland, C. D.; Mrksich, M. *J. Am. Chem. Soc.* **2000**, *122*, 4235. (b) Yeo, W.-S.; Hodneland, C. D.; Mrksich, M. *ChemBioChem* **2001**, *2*, 590. (c) Yousaf, M. N.; Houseman, B. T.; Mrksich, M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5992. (d) Yousaf, M. N.; Houseman, B. T.; Mrksich, M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1093.
- (5) *Chemistry and Biological Applications of Polyethylene Glycol*; Mrksich, M., Whitesides, G. M., Eds.; ACS Symposium Series 680; American Chemical Society: Washington, DC, 1997; pp 361.
- (6) The monolayers were analyzed on a Voyager-DE Biospectroscopy mass spectrometer using 2,5-dihydroxybenzoic acid (1  $\mu$ L of 10 mg/mL solution in acetonitrile) as a matrix. Sodium adducts of disulfides are the major species observed in MALDI spectra of SAMs: see (a) Su, J.; Mrksich, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 4715. (b) Su, J.; Mrksich, M. *Langmuir* **2003**, *19*, 4867–4870. (c) Trevor, J. L.; Lykke, K. R.; Pellin, M. J.; Hanley, L. *Langmuir* **1998**, *14*, 1664.
- (7) Electrochemistry was performed with a Bioanalytical Systems CV-50W potentiostat using PBS (pH 7.4) as the electrolyte. All experiments used a custom-designed electrochemical cell with the monolayer as the working electrode, a Pt wire as the counter electrode, and an Ag/AgCl reference electrode.
- (8) (a) Yousaf, M. N.; Mrksich, M. *J. Am. Chem. Soc.* **1999**, *121*, 4286. (b) Kwon, Y.; Mrksich, M. *J. Am. Chem. Soc.* **2002**, *124*, 806.
- (9) The preparation of patterned substrates is described in ref 4d.
- (10) Swiss Albino 3T3 cells (ATCC, Rockville, MD) were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin. All cultures were maintained at 37  $^{\circ}$ C in a humidified 5%  $\text{CO}_2$  atmosphere.
- (11) DMEM media containing serum, pH 7.4, used as solvent and electrolyte.

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