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PL, a continuous wave (cw) He–Cd laser (325 nm) with an output power of 20 mW was used as the excitation source. The PL was dispersed through a SPEX Triax 500 spectrometer with a 600 lines/mm grating and was detected by a nitrogen-cooled charge-coupled device (CCD) camera.

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[1] M. Huang, S. Mao, H. Feick, H. Yan, Y. Wu, H. Kind, E. Weber, R. Russo, P. Yang, *Science* **2001**, *292*, 1897.
[2] K. Kempa, B. Kimball, J. Rybczynski, Z. P. Huang, P. F. Wu, D. Steeves, M. Sennett, M. Giersig, D. V. G. L. N. Rao, D. L. Carnahan, D. Z. Wang, J. Y. Lao, W. Z. Li, Z. F. Ren, *Nano Lett.* **2003**, *3*, 13.
[3] X. Feng, L. Feng, M. Jin, J. Zhai, L. Jiang, D. Zhu, *J. Am. Chem. Soc.* **2004**, *126*, 62.
[4] S. Zhou, L. Zhao, G. C. Schatz, *Proc. SPIE-Int. Soc. Opt. Eng.* **2003**, *5221*, 174.
[5] S. Malynych, G. Chumanov, *J. Am. Chem. Soc.* **2003**, *125*, 2896.
[6] T. W. Odom, V. R. Thalladi, J. C. Love, G. M. Whitesides, *J. Am. Chem. Soc.* **2002**, *124*, 12 112.
[7] Z. K. Tang, G. K. L. Wong, P. Yu, M. Kawasaki, A. Ohtomo, H. Koinuma, Y. Segawa, *Appl. Phys. Lett.* **1998**, *72*, 3270.
[8] Y. C. Kong, D. P. Yu, B. Zhang, W. Fang, S. Q. Seng, *Appl. Phys. Lett.* **2001**, *78*, 407.
[9] E. A. Meulenkamp, *J. Phys. Chem. B* **1998**, *102*, 5566.
[10] C. Liu, J. A. Zapien, Y. Yo, X. Megn, C. S. Lee, S. Fan, Y. Lifshitz, S. T. Lee, *Adv. Mater.* **2003**, *15*, 838.
[11] P. Yang, Y. Haoquan, S. Mao, R. Russo, J. Johnson, R. Saykally, N. Morris, J. Pham, R. He, H.-J. Choi, *Adv. Funct. Mater.* **2002**, *12*, 323.
[12] M. Huang, Y. Wu, H. Feick, N. Tran, E. Weber, P. Yang, *Adv. Mater.* **2001**, *13*, 113.
[13] X. Wang, C. J. Summers, Z. L. Wang, *Nano Lett.* **2004**, *4*, 423.
[14] T. W. Odom, J. C. Love, K. E. Paul, D. B. Wolfe, G. M. Whitesides, *Langmuir* **2002**, *18*, 5314.
[15] J. C. Love, K. E. Paul, G. M. Whitesides, *Adv. Mater.* **2001**, *13*, 604.
[16] B. Liu, H. C. Zeng, *Langmuir* **2004**, *20*, 4196.
[17] J.-J. Wu, S.-C. Liu, *J. Phys. Chem. B* **2002**, *106*, 9546.
[18] A. L. Burin, H. Cao, G. C. Schatz, M. A. Ratner, *J. Opt. Soc. Am. B* **2004**, *21*, 121.

Electroactive Substrates that Reveal Aldehyde Groups for Bio-Immobilization**

By *Woon-Seok Yeo* and *Milan Mrksich**

The development of surfaces that have switchable properties offers a new dimension in the design of advanced materials.[1–5] For biological applications, the recent development of surfaces that allow the modulation of ligand activity, protein immobilization, and cell adhesion has enabled studies of cell–cell communication, provided routes to engineered tissue, and is motivating the design of hybrid microsystems that integrate cells as functional components.

Our approach to dynamic substrates has emphasized the design of self-assembled monolayers that respond to applied potentials by modulating the activities of immobilized ligands.[6–12] Our strategy is based on monolayers that can be switched to initiate the selective immobilization of a ligand. An earlier example from our laboratory used an electrochemical oxidation of hydroquinone to benzoquinone, which then participates in a Diels–Alder reaction with a diene-tagged ligand to immobilize the ligand.[6,8,10] While this scheme benefits from a rapid and very selective immobilization reaction, it does require the synthesis of a diene-tagged ligand. To generate an analogous strategy that can be used for the immobilization of a variety of unmodified proteins and ligands, we report here a surface that reveals an aldehyde functionality in response to an oxidative potential, and which allows immobilization of amino-functionalized ligands.

Our approach begins with a self-assembled monolayer (SAM) on gold that presents a 4-*H*-benzo[*d*][1,3]dioxinol group.[13] The acetal functionality in this molecule masks an aldehyde functional group. Application of an oxidative potential to the monolayer causes the oxidation of the aromatic ring with hydrolysis of the acetal to yield the corresponding aldehyde group. The resulting aldehyde can be used in several formats for immobilizing ligands, including condensation with hydrazide-tagged ligands (Fig. 1). In this paper, we demonstrate a monolayer having this dynamic property and illustrate its use by first immobilizing a ligand and subsequently immobilizing a corresponding protein to the ligand. Further, we show this dynamic substrate can be applied to studies of cell migration.

We used cyclic voltammetry to characterize the electrochemical conversion of the protected aldehyde. We first pre-

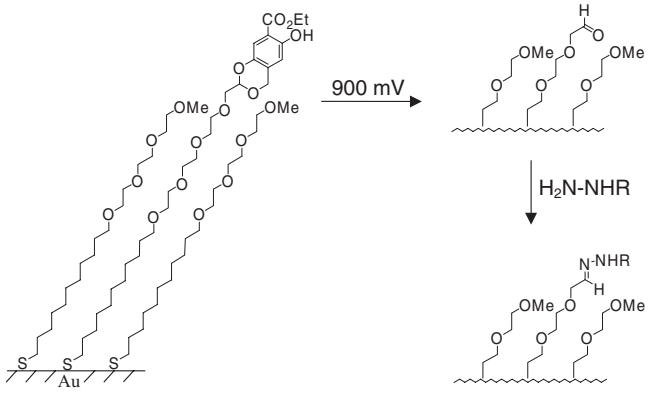

Figure 1. Strategy for preparing a dynamic substrate that reveals aldehyde groups. A monolayer presenting the 4-*H*-benzo[*d*][1,3]dioxinol group is subjected to an electrical potential at 900 mV to yield an aldehyde group. This group reacts with hydrazide-tagged ligands to give immobilization of the ligand by way of a hydrazone linkage.

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pared a self-assembled monolayer from a 4-*H*-benzo[*d*][1,3]-dioxinol-terminated alkanethiol and 6-mercapto-1-hexanol in a ratio of 1:3 (Fig. 2A). Cyclic voltammetry showed an anodic peak at 800 mV on the first scan and a dramatic decrease of this peak on the second scan, indicating that the electrochemical deprotection was nearly complete within one scan. Subsequent scans showed no electroactivity because the resulting aldehyde is not electroactive in this range of potential. As a control experiment, a monolayer presenting 4-*H*-benzo[*d*][1,3]dioxinol was treated with an electrical potential at 800 mV for 1 min and analyzed by cyclic voltammetry. As expected, cyclic voltammetry showed only a non-Faradaic current verifying that there were no electroactive molecules present on this monolayer (data not shown).

We next used matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS) to establish that the electrochemical deprotection gave the intended product. A monolayer presenting the protected aldehyde against a background of a methoxy-terminated alkanethiolate (as shown in Fig. 1) at a density of 20 % displayed two major peaks at *m*/*z* 721.6 and *m*/*z* 943.5 (Fig. 2B).[14] These peaks correspond to the symmetric disulfide derived from a methoxy-terminated alkanethiolate and a mixed disulfide derived from a 4-*H*-benzo[*d*][1,3]dioxinol-terminated alkanethiolate and a methoxy-terminated alkanethiolate, respectively. An identical monolayer was treated with an electrical potential of 900 mV for 90 s and analyzed by MALDI-ToF MS. A new peak at *m*/*z* 749.6 was observed, which corresponds to the disulfide containing the product aldehyde (Fig. 2B). Further, the peak for the protected aldehyde was absent, showing that the electrochemical deprotection proceeded in high yield.

To verify that the resulting aldehyde could serve to immobilize ligands by way of a hydrazone linkage, we treated the monolayer with 4-nitrobenzhydrazide (500 μL, 5 mg mL^{-1} in acetonitrile for 30 min). MS analysis showed a new peak at *m*/*z* 912.9 corresponding to the disulfide containing the hydrazone adduct (Fig. 2B, bottom). We immobilized six different hydrazides. All mass analyses gave satisfactory product peaks (data not shown).

To demonstrate biologically relevant applications of this dynamic substrate, we immobilized the biotin ligand to make a surface that could selectively bind the protein streptavidin.[15] The monolayer was treated with a solution of biotin hydrazide (100 μL, 1 mg mL^{-1} in phosphate-buffered saline (PBS) at pH 7.0 for 30 min). Mass analysis verified that biotin was immobilized to the surface by way of a hydrazone linkage (Fig. 3A). An identical monolayer was then treated with a solution of streptavidin (100 μL, 25 μg mL^{-1} in PBS at pH 7.0 for 30 min). MS analysis of this monolayer revealed peaks corresponding to the bound protein, including a doubly charged monomer (6.8 kD), monomer (13.5 kD), dimer (27 kD), trimer (40.5 kD), and tetramer (54 kD) (Fig. 3B). Two control experiments verified that protein binding to the monolayer was specific. In the first, a monolayer was sub-

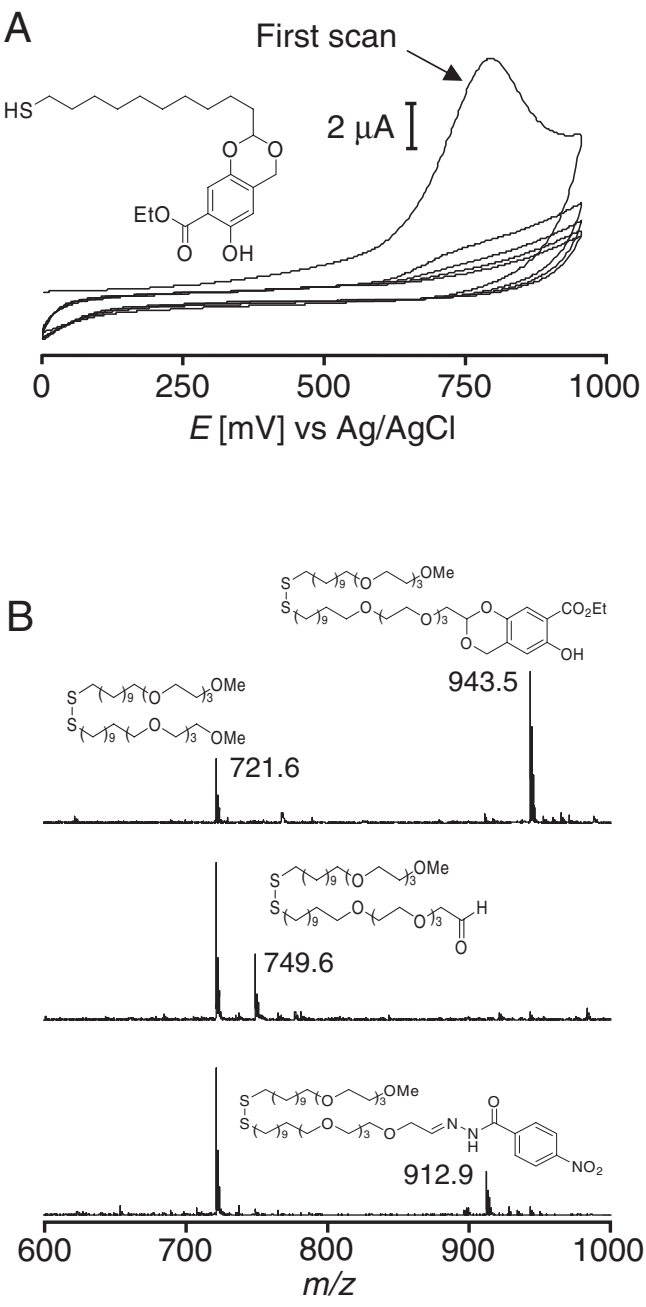

Figure 2. Characterization of the electrochemical conversion of the 4-*H*-benzo[*d*][1,3]dioxinol group to the corresponding aldehyde on the monolayer. A) Cyclic voltammetry of a monolayer presenting 4-*H*-benzo[*d*][1,3]-dioxinol groups shows an anodic peak at 800 mV on the first scan. This wave decreased dramatically on the second scan, indicating that the electrochemical deprotection was almost complete after the first scan. B) The mass spectrum of the monolayer as shown in Figure 1 displayed two major peaks at *m*/*z* 721.6 and 943.5, corresponding to the disulfides displayed above the peaks (top). After electrochemical treatment, the original peak was absent and gave rise to a new peak at *m*/*z* 749.6 corresponding to the disulfide containing the product aldehyde (middle). An identical monolayer was treated with 4-nitrobenzhydrazide and analyzed by MALDI-ToF MS, which showed a new peak at *m*/*z* 912.9 corresponding to the disulfide containing hydrazone adduct (bottom).

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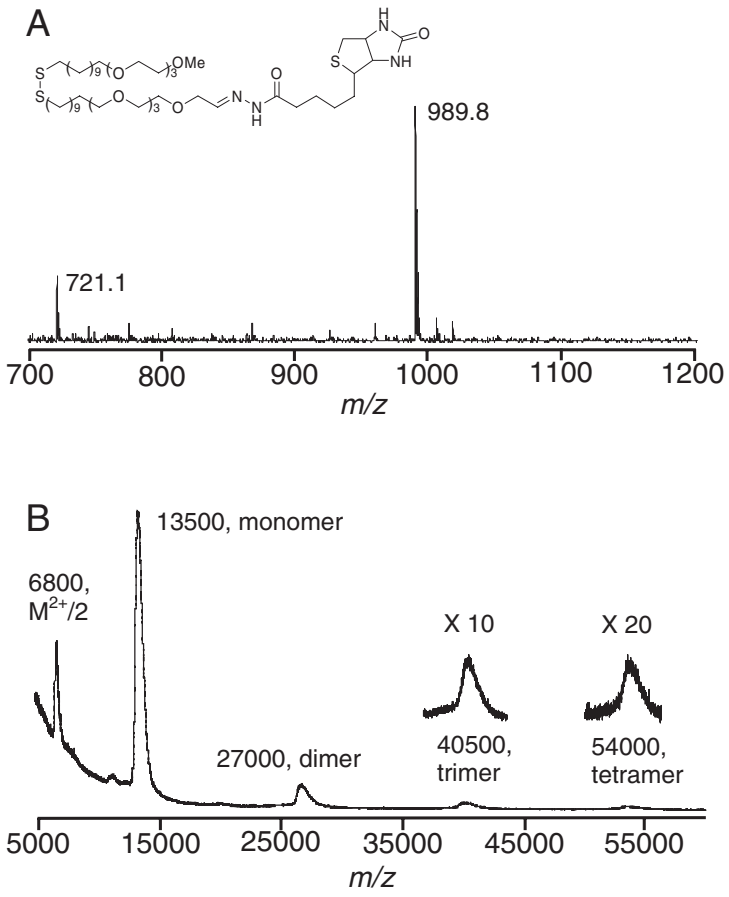

Figure 3. Application of the dynamic substrate to protein immobilization. A) The aldehyde-presenting monolayer was treated with a biotin hydrazide. Mass analysis showed a new peak at *m/z* 989.8 verifying that biotin was immobilized to the surface. B) The monolayer was then treated with a solution of streptavidin. Analysis by SAMDI revealed peaks corresponding to doubly charged monomer (6.8 kD), monomer (13.5 kD), dimer (27 kD), trimer (40.5 kD), and tetramer (54 kD). Masses are rounded to the nearest 100 D.

jected to an electrical potential at 900 mV and treated with streptavidin, but without immobilization of the biotin ligand. In the second, a monolayer was not subjected to the electrical potential but otherwise treated with biotin hydrazide and streptavidin. Mass spectra of both monolayers gave no protein ion peaks (data not shown). Taken together, these results establish that streptavidin associated with the surface-bound biotin biospecifically. A second conclusion is that the combination of SAMs and MALDI-TOF MS—in a technique we refer to as SAMDI[16]—is well-suited for the direct observation of proteins that are selectively bound to the surface.

We next demonstrate the application of this strategy to the preparation of patterned surfaces for studies of cell migration. We used microcontact printing to pattern a monolayer into circular regions (220 μm in diameter) with hexadecanethiol and then modified the remaining regions with 4-*H*-benzo[*d*][1,3]dioxinol-terminated alkanethiolate at a density of 2 % mixed with a tri(ethylene glycol)-terminated alkanethiolate (Fig. 3).[10] Fibronectin was allowed to adsorb to the patterned regions (15 μg mL^{-1} in PBS, pH 7) to give a substrate that directed the attachment of Swiss 3T3 fibroblast cells to the circular regions (Fig. 4). After 4 h of culture, we applied an electrical potential of 900 mV for 10 s to the monolayer. Cells began to migrate from circular regions onto surrounding regions of the substrate. After 10 h, the original circular pattern was no longer evident and after 20 h, cells were distributed evenly over the entire surface. We carried out two control experiments. First, an identical monolayer was prepared as described above and seeded with cells, but not subjected to electrical potential treatment. Second, we prepared a monolayer which had same circular patterns but modified the remaining regions only with a tri(ethylene glycol)-terminated alkanethiolate, which prevents non specific adsorption of proteins and cells. This second monolayer was subjected to an electrical potential of 900 mV. As expected, circular patterns remained intact for both monolayers for 20 h.[17] This result indicates that application of an oxidative potential to the monolayer revealed aldehydes, which presumably condensed with amine groups of matrix proteins in the media, and hence, initiated cell migration onto surrounding regions.

This work describes a new electroactive substrate that reveals aldehyde groups in response to electrical potentials. We have shown that this approach can be used to activate a substrate for immobilization of a ligand and then used in subsequent experiments, and that the electrochemical conversion is compatible with the conditions of cell culture. This strategy makes a significant advance over previous dynamic substrates that initiate immobilization of ligand because it reveals a functional group that does not require labelling of a ligand: for example, aldehydes can be used for immobilization of amino-tagged ligands with a reductive amination reaction. This example also serves to demonstrate a molecular strategy for creating dynamic substrates. By combining physical organic chemistry with synthetic chemistry, the former to design surfaces having tailored properties and the latter to realize the designs, this molecular approach should find wider use in preparing advanced materials for a range of applications.

Experimental

Preparation of SAMs: The monolayers presenting the protected aldehydes were prepared according to standard procedures [18]. Briefly, gold-coated cover slips were immersed in an ethanol solution of 4-*H*-benzo[*d*][1,3]dioxinol-terminated alkanethiol and a methoxy-terminated alkanethiol in a ratio of 1:4 for 6 h (total concentration of thiol was 1 mM.). The monolayer was rinsed with ethanol and dried under a stream of nitrogen.

Electrochemistry: Electrochemistry was performed with a Bioanalytical Systems CV-50W potentiostat using phosphate-buffered saline

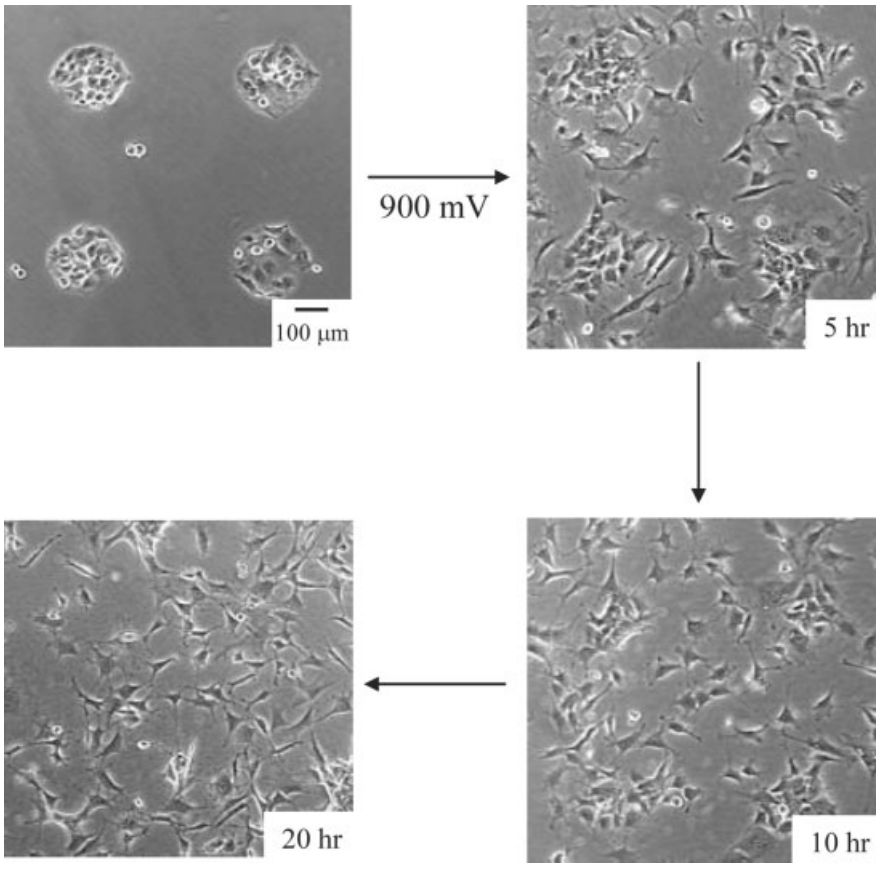

Figure 4. Application of the dynamic substrate to studying cell migration. A monolayer was patterned into circular regions that present fibronectin and surrounded by 4-*H*-benzo[*d*][1,3]dioxinol group. Swiss 3T3 fibroblast cells adhered only to circular regions. An electrical potential of 900 mV was applied to the substrate for 10 s and cells began to migrate from circular regions onto surrounding regions of the substrate. After 10 h of culture, the original circular pattern was no longer evident and after 20 h, cells were distributed evenly over the entire surface.

(10 mM phosphate and 150 mM NaCl) as the electrolyte at pH 7.0. A custom-designed electrochemical cell was used for measurement with the monolayer as the working electrode, a Pt wire as the counter electrode, and an Ag/AgCl reference electrode. Cyclic voltammetry was carried out with scanning from 0 mV to +950 mV at 100 mV s^{-1}.

MALDI-ToF Analysis: Mass analysis was performed as described previously [14,16], using a Voyager DE-PRO mass spectrometer (Applied Biosystems, Framingham, MA) operating with a 337 nm nitrogen laser desorption-ionization source. The mass spectra of alkanethiols were acquired with 20 kV accelerating voltage using reflector mode in positive ions with a 2,4,6-trihydroxyacetophenone (1 μL, 5 mg mL^{-1} in acetonitrile) as a matrix. The mass spectra for streptavidin were acquired with 25 kV accelerating voltage using linear mode in positive ions with a sinapinic acid (1 μL, 5 mg mL^{-1} in acetonitrile/water (50:50), 0.1 % trifluoroacetic acid (TFA)) as a matrix.

Cell Culture: 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. 50 000-100 000 cells were seeded on monolayers and all cultures were maintained at 37 °C in a humidified 5 % CO_2 atmosphere.

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[1] T. P. Russell, *Science* **2002**, *297*, 964.
[2] R. J. Thibault, P. J. Hotchkiss, M. Gray, V. M. Rotello, *J. Am. Chem. Soc.* **2003**, *125*, 11 249.
[3] N. Nath, A. Chilkoti, *J. Am. Chem. Soc.* **2001**, *123*, 8197.
[4] T. Shimizu, M. Yamato, A. Kikuchi, T. Okano, *Biomaterials* **2003**, *24*, 2309.
[5] R. Blonder, E. Katz, I. Willner, V. Wray, A. F. Bückmann, *J. Am. Chem. Soc.* **1997**, *119*, 11 747.
[6] M. N. Yousaf, M. Mrksich, *J. Am. Chem. Soc.* **1999**, *121*, 4286.
[7] C. D. Hodneland, M. Mrksich, *J. Am. Chem. Soc.* **2000**, *122*, 4235.
[8] M. N. Yousaf, B. T. Houseman, M. Mrksich, *Angew. Chem. Int. Ed.* **2001**, *40*, 1093.
[9] W.-S. Yeo, C. D. Hodneland, M. Mrksich, *ChemBioChem* **2001**, *2*, 590.
[10] W.-S. Yeo, M. N. Yousaf, M. Mrksich, *J. Am. Chem. Soc.* **2003**, *125*, 14 994.
[11] X. Jiang, R. Ferrigno, M. Mrksich, G. M. Whitesides, *J. Am. Chem. Soc.* **2003**, *125*, 2366.
[12] Kwak and co-workers have reported an example of an electrochemical deprotection of carboxylic acid groups from the corresponding acylhydroquinones. K. Kim, H. Yang, E. Kim, Y. B. Han, Y. T. Kim, S. H. Kang, J. Kwak, *Langmuir* **2002**, *18*, 1460.
[13] L. Novák, P. Kovács, G. Pirok, P. Kolonits, E. Szabó, J. Fekete, V. Weiszfeiler, C. Szántay, *Synthesis* **1995**, 693.
[14] Sodium adducts of disulfides are the major species that are observed in MALDI spectra of SAMs of alkanethiolates: see a) J. Su, M. Mrksich, *Angew. Chem. Int. Ed.* **2002**, *41*, 4715. b) J. L. Trevor, K. R. Lykke, M. J. Pellin, L. Hanley, *Langmuir* **1998**, *14*, 1664.
[15] Biotin hydrazide and streptavidin were purchased from Sigma (St. Louis, MO). A monolayer presenting 4-*H*-benzo[*d*][1,3]dioxinol was treated with biotin hydrazide (100 μL, 1 mg mL^{-1} in PBS at pH 7.0) for 30 min at room temperature and rinsed with PBS, distilled water, and ethanol. The monolayer was then treated with matrix (2,4,6-trihydroxyacetophenone, 1 μL, 5 mg mL^{-1} in acetonitrile), dried, and analyzed by MALDI-ToF MS. A monolayer presenting biotin was treated with streptavidin (100 μL, 25 μg m L^{-1} in PBS at pH 7.0) for 30 min at room temperature and rinsed with PBS and distilled water. The monolayer was then treated with matrix sinapinic acid, 1 μL, 5 mg m L^{-1} in acetonitrile/water (50:50), 0.1 % TFA), dried, and analyzed by MALDI-ToF MS.

ADVANCED MATERIALS

[16] D.-H. Min, W.-J. Tang, M. Mrksich, *Nat. Biotechnol.* **2004**, *22*, 717.
[17] For the second control experiment, we did observe a slight loss of the patterned cells, indicating that the electrical potential of 900 mV compromised inertness somewhat.
[18] C. D. Bain, E. B. Troughton, Y. T. Tao, J. Evall, G. M. Whitesides, R. G. Nuzzo, *J. Am. Chem. Soc.* **1989**, *111*, 321.

Using Soft Lithography to Pattern Highly Oriented Polyacetylene (HOPA) Films via Solventless Polymerization**

By *Hongwei Gu*, *Rongkun Zheng*, *Xixiang Zhang*, and *Bing Xu**

This paper describes the use of soft lithography;[1] which is used to pattern highly oriented polyacetylene (HOPA) thin films onto substrates via solventless ring-opening metathesis polymerization (ROMP)[2] and the subsequent pyrolytic conversion of the HOPA film into a diamond-like carbon film. The term soft lithography refers to a set of simple microfabrication techniques that were developed during the last decade. It is a useful and convenient procedure for creating patterned surfaces on the micro- or nanometer scales for applications in chemistry and biology and in microelectronics and engineering. For example, soft lithography has been used to make microfluidic devices for cell patterning,[3] in order to selectively deposit proteins on substrates for cell adhesion,[4] to build microstructures of metal oxides for waveguides,[5] to create microstrutures of colloids for photonic bandgap materials,[6] and to pattern electroactive polymers for organic transistors and light-emitting diodes.[7] In these patterning processes, water or hydrophilic solvents are generally used in order to prevent the swelling of poly(dimethylsiloxane) (PDMS) stamps that is caused by organic solvents (e.g., hexane, chloroform, and tetrahydrofuran), which limits the scope of the polymers that can be patterned on substrates by soft lithography. To avoid this complication, we have developed a process that combines soft lithography and solventless polymerization and allows patterning of a variety of polymers, including poly(norbornene).[8,9] To explore the scope of the combination of soft lithography and solventless polymerization, we used this method to pattern HOPA.

Polyacetylene,[10] a serendipitously discovered polymer that led to the establishment of a new research field of conducting polymers,[11] exhibits improved mechanical and electrical properties when it is highly oriented.[12] Various methods can produce highly oriented polyacetylene (HOPA), such as mechanical stretching of polyacetylene embedded in a matrix or synthesis of polyacetylene in a liquid-crystalline medium.[12,13] Despite the success of these methodologies, a more convenient and simpler process would be advantageous, particularly for incorporating HOPA into microdevices or forming patterned microstructures by further conversion of polyacetylene.

To extend our previous work, we provide here a simple protocol for the above need. This process involves defining the position of a catalyst on the substrate by using soft lithography and solventlessly generating polyacetylene films that are based on ROMP. We found that this solventlessly generated polyacetylene exhibits a higher orientation order than films that were prepared from the solution phase. In addition, pyrolysis converts this highly oriented polyacetylene film into a diamond-like carbon film with a hardness of 65 GPa. Thus, this process not only generates patterned HOPA for potential uses in microdevices, but also promises a new route to create ultra-hard films in selected areas that are defined by soft lithography, which has not been explored previously.

Figure 1A shows the ROMP reaction utilized to generate polyacetylene. We use 1,3,5,7-cyclooctatetraene (**1**) as the monomer and tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene] [benzyldine] ruthenium(IV) dichloride as the catalyst, following the reaction reported by the Grubbs and co-workers.[14] The polymerization proceeds in both a solventless manner and in the solution phase (as the control). To generate patterned lines of polyacetylene on a gold surface, we use the procedure illustrated in Figure 1B. After generating alternating hydrophobic and hydrophilic lines achieved by microcontact printing (μCP),[15] we dipped the substrate into the solution of the catalyst, and the catalyst adheres only to the hydrophilic line after the substrate is drawn out from the solution. By placing this substrate in a chamber containing **1** at reduced pressure, we create the patterned lines of polyacetylene. Using the procedure shown in Figure 1C, we produce the linear pattern of polyacetylene on a silicon wafer: after the surface of the silicon is covered uniformly with the catalyst, a PDMS stamp is applied on the surface to selectively expose the catalyst. When the stamp-covered substrate is put into a chamber containing a vial of **1** at reduced pressure, polyacetylene grows only on the areas to which the catalyst was exposed.

Figures 2A and 2B, respectively, show the patterned HOPA on the surface of a gold-coated silicon wafer and on the surface of a silicon wafer. Even though the optical images show that the gaps between the lines are smaller than 10 μm, the mapping of time-of-flight secondary ion mass spectra (ToF-SIMS) indicates that both the gaps and the lines have widths

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