J|A|C|S
COMMUNICATIONS

Published on Web 06/12/2004

Nanometric Protein Arrays on Protein-Resistant Monolayers on Silicon Surfaces

Jianhua Gu, Chi Ming Yam, Sha Li, and Chengzhi Cai*

Department of Chemistry and Center for Materials Chemistry, University of Houston, Houston, Texas 77204

Received March 19, 2004; E-mail: cai@uh.edu

Nanometric biomolecular arrays may enable high-throughput screening of biomolecules at a single molecule level. Also, such arrays with a precise control on position and orientation of individual molecules may become a powerful tool for studying multivalent and multicomponent molecular interactions in biological systems.[1] To these ends, protein arrays with feature sizes smaller than 100 nm have been fabricated, mostly using dip-pen nanolithography and nanografting.[1,2] Herein we report a novel approach to preparing such arrays, based on binding of avidin molecules to templates generated by conductive AFM (c-AFM)[2,3] on oligo(ethylene glycol) (OEG)-terminated monolayers on silicon substrates.[4] The unique features of the approach include the following: (i) the OEG monolayers reduce nonspecific adsorption and denaturing of proteins on the templates; (ii) the monolayers are attached to the substrates via Si−C bonds, rendering the system highly robust; (iii) the lithography process is very rapid. The resulting avidin arrays may serve as templates for attaching proteins that are (site-specifically) labeled with biotin,[5] as demonstrated here with biotinated BSA (Figure 1).

Recently, we performed surface hydrosilylation[6] to attach a series of OEG-terminated alkenes to silicon surfaces through formation of Si−C bonds.[4] We demonstrated that the resulting monolayers terminated with more than six EG units effectively resisted nonspecific adsorption of proteins,[4] similar to the case of OEG-terminated alkylthiolate monolayers on gold surfaces.[7] In addition, the alkyl monolayers on silicon are much more robust than the thiolate monolayers on gold due to the stronger adsorbate−substrate binding (C−Si vs S−Au bonds).

The monolayers used in this study were readily prepared from α-hepta(ethylene glycol)methyl ω-undecenyl ether (**EG$_7$**) and conductive silicon (111) substrates with an atomically flat, H-terminated surface.[4] c-AFM lithography on these monolayers was performed under ambient conditions with a relative humidity of 40−55%, using a Nanoscope IIIa AFM (Digital Instrument) equipped with a pulse generator. During scanning of the film in contact mode with a conductive silicon cantilever tip, bursts of 1 μs pulses of +17 V were applied to the sample while the tip was grounded. After one scan (taking about a minute), the nanolithography was completed. Note that this process for generating a high density nanoarray is much faster than dip-pen or nanografting lithography that normally takes seconds to generate each nanospot.[1] The pulse generator was then disconnected, and the height and friction AFM images were simultaneously acquired in contact mode at a 90° scan angle with the same tip. We found that the height images of the patterned areas, e.g., Figure 2a, were nearly the same as those obtained before lithography. However, the corresponding friction image (Figure 2b) shows the presence of spots of ~90 nm in diameter where the friction is higher than the surroundings, indicating the presence of polar headgroups on the spots. It was suggested that AFM anodization of alkyl siloxane monolayers on silicon under certain conditions could oxidize the headgroups of

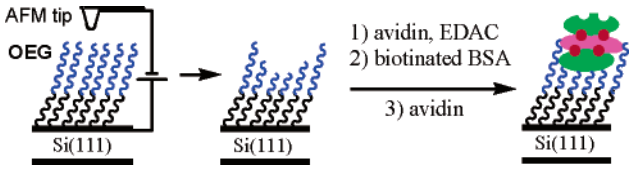

Figure 1. Illustration of c-AFM lithography on an OEG monolayer and the sequential attachment of proteins to the generated nanotemplate.

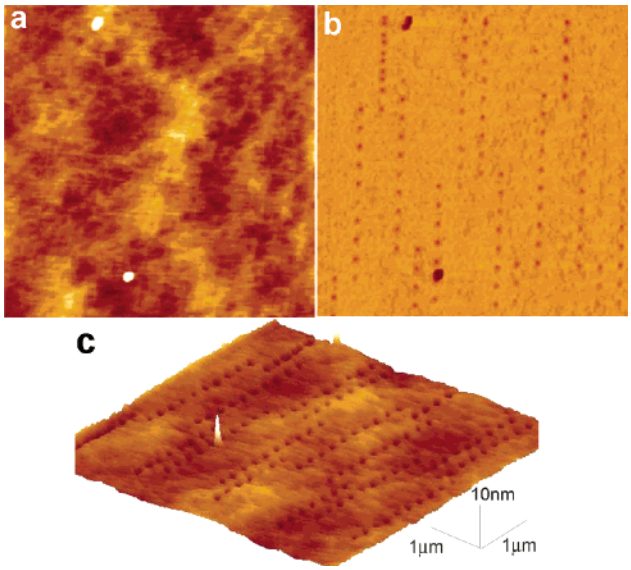

Figure 2. Height (a, 4 × 4 μm^2, 10 nm contrast) and friction (b, 0.2 V contrast) AFM images of an EG7 film on Si(111) after c-AFM lithography and three-dimensional image (c, 5 × 5 μm^2) of the same area upon treatment with succinic anhydride, DMAP, and pyridine. The lithography was performed by scanning the film surface with a silicon tip at 29.8 μm/s, during which 10 bursts of 1 μs pulses of +17 V relative to the tip with pulse interval of 8.33 ms and bursts interval of 4.29 s were applied to the substrate to generate rows of 10 spots with a separation of ~270 nm. Shorter separation and even overlapping of spots in a few rows are due to our current setup where the movement of the AFM scanner is not synchronized with the pulse generator, and thus a burst of pulses may be applied during both trace and retrace of the tip.

the monolayers into carboxylic acid groups.[3a] Nevertheless, recent study of a similar system using secondary ion mass spectroscopy showed no sign of COOH groups on the oxidized surfaces.[3b] This also appears to be the case in our system (see below).

Upon treatment of the sample with avidin in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) that mediates the formation of amide bonds between the surface COOH groups and the protein molecules, AFM images obtained during the first few scans showed that the absorbed molecules were readily removed by the scanning tip, indicating that they were not covalently bound to the surface. We speculated that, rather than COOH groups, the surfaces of the oxidized spots mainly consisted of hydroxyl groups that can be derivatized into COOH groups, e.g.,

10.1021/ja048405x CCC: $27.50

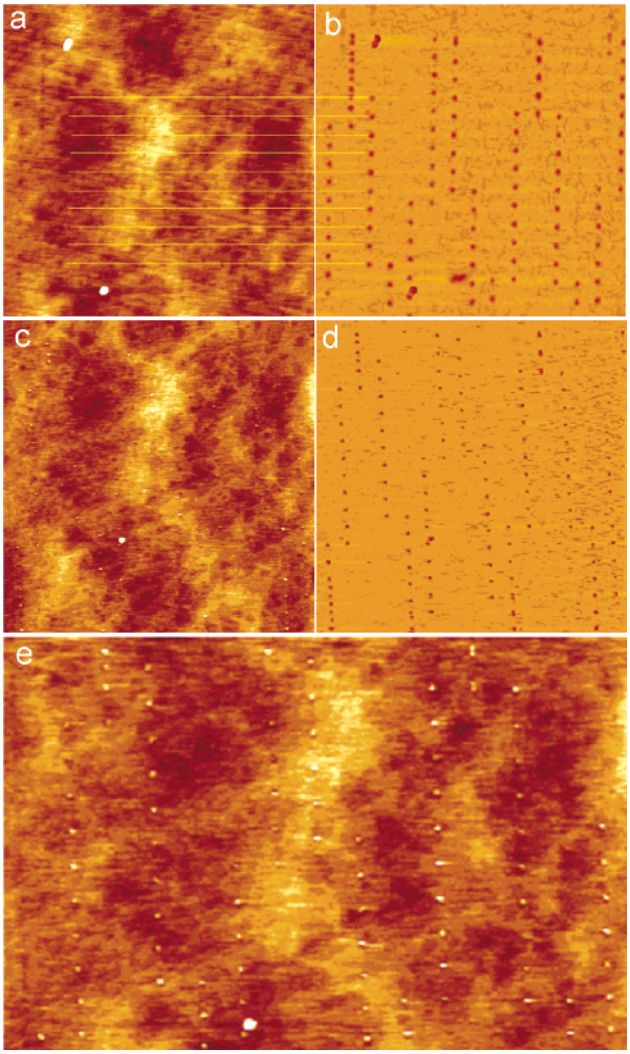

Figure 3. AFM height (a, c, e, 4 μm in width, 10 nm contrast) and friction (b, d, related to a, c) images of the similar area in Figure 2 upon sequential treatment with EDAC/avidin (a, b), biotinated-BSA (c, d), and avidin (e). The images are better viewed on screen with 500% magnification. The yellow lines in images a and b are used to guide the eyes.

by treatment with succinic anhydride, dimethylaminopyridine (DMAP), and pyridine. To our surprise, we found that the patterned spots were "etched" upon this treatment, forming nanoholes as shown by the three-dimensional AFM height image (Figure 2c). As measured by the line profile of about 100 patterned spots in Figure 2c, the diameter of the holes was 91 $\pm$ 6 nm, and the depth was 1.31 $\pm$ 0.12 nm, about one-third of the thickness of the film. The reason for the formation of the nanoholes as well as the chemical nature of the oxidized surface remains unclear. Upon incubation of the samples with EDAC followed by avidin in PBS solution, the nanoholes were nearly filled (Figure 3a) and barely recognized even by comparison with the corresponding friction image (Figure 3b). The depth of the holes decreased to 0.43 $\pm$ 0.06 nm, while the width remained nearly the same (87 $\pm$ 9 nm). The sample was then treated with BSA in PBS buffer. The depth of the holes remained the same, indicating that BSA did not bind to the molecules in the holes. To verify that the molecules in the holes were indeed avidin, the sample was treated with biotinated-BSA in PBS buffer. AFM height and friction images (Figure 3c and d) reveal that the patterned spots slightly protrude from the film surface. The height and half-height width of the spots were 0.14 $\pm$ 0.14 nm and 24 $\pm$ 3.5 nm, respectively. The measured heights for the assumed avidin (0.9 nm) in the holes and biotin-BSA (0.6 nm) are much lower than those of the native avidin (4 nm) and BSA (2 nm), most likely due to the compression by the AFM tip. The fact that the molecules in the holes bind biotinated-BSA but not native BSA strongly indicates that these molecules are avidin and they are capable of binding biotin. This conclusion is further confirmed by a study showing that fluorescent-labeled avidin selectively bound to micrometer-sized patterns generated by c-AFM under similar conditions and fluorescent-labeled biotin bound to the avidin-treated patterns; the results of the study will be published elsewhere.

The patterned biotinated-BSA with $\sim$ 9 biotin groups on each molecule should have free biotin groups available for binding of additional avidin onto the pattern. Indeed, upon incubation of the sample in a solution of avidin in PBS, nano-dot arrays were formed as shown by the AFM height image (Figure 3e). The height of the dots was 1.27 $\pm$ 0.37 nm, and the half-height width of the dots was 26 $\pm$ 3.4 nm. While the top avidin molecules could be removed by repeated scanning, the protein molecules in the holes were strongly bound and could not be removed by the scanning tip, neither by immersion in PBS for 6 h nor by immersion in detergent (SDS) solutions for 14 h. Remarkably, AFM images of the protein arrays remained nearly the same after four weeks under ambient conditions. Upon treatment with Proteinase K that catalyzes the hydrolytic fragmentation of the proteins, nanoholes very similar to those in Figure 2c were regenerated.

In conclusion, we demonstrated a new approach to preparing high density nanometric protein arrays on robust OEG monolayers. The method may be also used to position other nanometric bioentities on OEG surfaces. We are currently studying the AFM anodization process, aiming to reduce the diameter and depth of the patterned spots. Preserving sufficiently long OEG chains in the patterned spots may reduce possible denaturing of the immobilized protein.

Acknowledgment. This work was supported by the Welch Foundation, National Science Foundation (CTS-0210840), and University of Houston (GEAR, and TcSAM Special Funding).

Supporting Information Available: Preparation of **EG_7** and the films and procedures for the c-AFM lithography and derivatization of the patterned surfaces. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Lee, K.-B.; Park, S.-J.; Mirkin, C. A.; Smith, J. C.; Mrksich, M. *Science* **2002**, *295*, 1702. (b) Wilson, D. L.; Martin, R.; Hong, S.; Cronin-Golomb, M.; Mirkin, C. A.; Kaplan, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13660. (c) Liu, G.-Y.; Amro, N. A. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5165. (d) Hyun, J.; Ahn, S. J.; Lee, W. K.; Chilkoti, A.; Zauscher, S. *Nano Lett.* **2002**, *2*, 1203. (e) Lee, K.-B.; Lim, J.-H.; Mirkin, C. A. *J. Am. Chem. Soc.* **2003**, *125*, 5588. (f) Cheung, C. L.; Camarero, J. A.; Woods, B. W.; Lin, T.; Johnson, J. E.; De Yoreo, J. J. *J. Am. Chem. Soc.* **2003**, *125*, 6848. (g) Agarwal, G.; Naik, R. R.; Stone, M. O. *J. Am. Chem. Soc.* **2003**, *125*, 7408. (h) Pavlovic, E.; Oscarsson, S.; Quist, A. P. *Nano Lett.* **2003**, *3*, 779.

(2) Krämer, S.; Fuierer, R. R.; Gorman, C. B. *Chem. Rev.* **2003**, *103*, 4367.

(3) (a) Maoz, R.; Frydman, E.; Cohen, S. R.; Sagiv, J. *Adv. Mater.* **2000**, *12*, 725. (b) Pignataro, B.; Licciardello, A.; Cataldo, S.; Marletta, G. *Mater. Sci. Eng., C* **2003**, *23*, 7. (c) Li, Q. G.; Zheng, J. W.; Liu, Z. F. *Langmuir* **2003**, *19*, 166.

(4) Yam, C. M.; Lopez-Romero, J. M.; Gu, J.; Cai, C. *Chem. Commun.* Submitted.

(5) Lue, R. Y. P.; Chen, G. Y. J.; Hu, Y.; Zhu, Q.; Yao, S. Q. *J. Am. Chem. Soc.* **2004**, *126*, 1055.

(6) (a) Linford, M. R.; Fenter, P. M.; Eisenberger, P. M.; Chidsey, C. E. D. *J. Am. Chem. Soc.* **1995**, *117*, 3145. (b) Cicero, R. L.; Linford, M. R.; Chidsey, C. E. D. *Langmuir* **2000**, *16*, 5688. (c) Buriak, J. M. *Chem. Rev.* **2002**, *102*, 1271.

(7) Prime, K. L.; Whitesides, G. M. *Science* **1991**, *252*, 1164.

JA048405X