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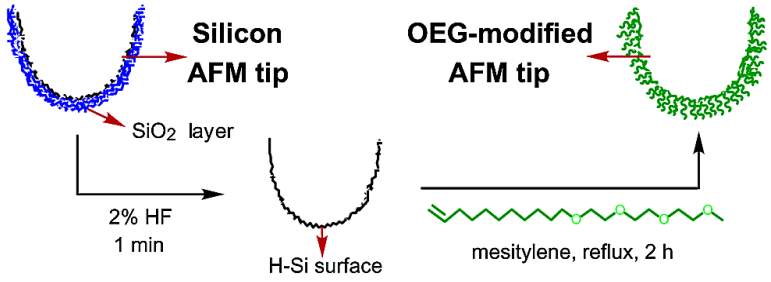

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Modification of Silicon AFM Cantilever Tips with an Oligo(ethylene glycol) Derivative for Resisting Proteins and Maintaining a Small Tip Size for High-Resolution Imaging

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Atomic force microscopy (AFM) has become a powerful tool for biophysical study at nanoscale.[1] AFM uses a cantilever spring with a sharp tip to measure the interactions between the surfaces of the tip and the sample. Currently, most of the AFM tips are made of silicon or silicon nitride (Si/SiN). A major problem that results from using such tips to study biological samples is the ability of proteins and cellular debris to absorb onto the tips, thus lowering the image resolution and generating artifacts.

We are interested in developing techniques for anchoring a few or even one functional molecule at the apex of AFM tips. These modified tips may allow accurate measurement of specific molecular interactions and mapping of these interactions with a high resolution and contrast.[1c] The first step toward this goal is to modify the bulk tip surface with an ultrathin layer of molecules, such as oligo(ethylene glycol)s (OEGs), that resist the nonspecific interaction of the tip surface with proteins.[2] Currently, most chemical modifications of AFM tips involve the growth of a self-assembled monolayer (SAM) of thiolates on gold-coated tips.[1b] Although the gold-coated tips are commercially available, they are very large—the tip radii ranging from 35 to 100 nm. The reason is that such tips are prepared by coating Si/SiN tips first with a chromium adhesive layer and then a gold layer that is tens of nanometers thick;[1b] thinner gold films tend to form isolated gold islands rather than to fully cover the surface. The large tip size significantly decreases the resolution of AFM imaging. Therefore, it is highly desirable to develop techniques for direct coating of Si/SiN tips with a robust monolayer of functional molecules. Growth of a siloxane SAM directly on the hydrophilic Si/SiN tip surfaces is possible.[3] It is, however, difficult to control the conditions to prevent deposition of multilayers or large particles on the tip surface.

Herein, we present a practical method to modify silicon AFM tips based on hydrosilylation reaction of 1-alkenes with hydrogen-terminated silicon surfaces to form strong Si−C bonds.[4] Specifically, Ultrasharp NSC12 silicon AFM cantilevers (Silicon-MDT, Moscow) mounted on a Teflon holder were cleaned with Piranha solution at 80 °C to remove organic contaminates. (*Caution:* since Piranha solution (H_2SO_4/30% H_2O_2, 7:3) reacts violently with many organic compounds, extreme care must be taken when handling it.) This was followed by removal of the oxide layer on the silicon surfaces by immersion of the cantilevers in ∼2% HF solution prepared by diluting Buffer-HF Improved (Transene Co, Danvers) with 5 parts of Millipore water. After 1 min, the cantilevers were taken out, rapidly rinsed with Millipore water, and immediately dried with a flow of pure nitrogen. This etching process generates a hydrophobic hydrogen-terminated silicon surface.[5] The surface hydrosilylation was performed by immersion of the etched cantilevers in a degassed, 0.1 M solution of $CH_3O(CH_2CH_2O)_3(CH_2)_9$-$CH{=}CH_2$ (**1**)[3b] in freshly distilled mesitylene[4c] under N_2, and gently refluxed for 2 h. The cantilevers were then thoroughly washed sequentially with petroleum ether, ethanol, and CH_2Cl_2, and blow-dried with N_2. To evaluate the quality of the OEG-modified tips, tapping-mode AFM images of a Nioprobe tip calibration sample (Aurora NanoDevices, Inc.) were acquired. Figure 1, a−d, shows the typical images obtained with a silicon tip before (Figure 1a) and after cleaning with Piranha solution (Figure 1b) and then etching with the HF solution (Figure 1c), followed by reaction with the OEG-alkene **1** (Figure 1d). Comparison of these images indicates a slight decrease in resolution upon cleaning with Piranha solution and OEG modification. Blind reconstruction[6] of the tip shape based on the images in Figure 1, a−d, using the software provided in ref 6 leads to the estimated tip size: 4 nm for the as-received tip, 10 nm after Piranha cleaning, 9 nm after etching, and 11 nm after OEG modification. Therefore, the processes for removal of the oxide layer and surface hydrosilylation barely affects the tip sizes that are far less than the present gold-coated AFM tips. The tip enlargement from 4 to 10 nm by the cleaning step is probably due to the growth of the oxide layer on the tip surface in Piranha solution at 80 °C.

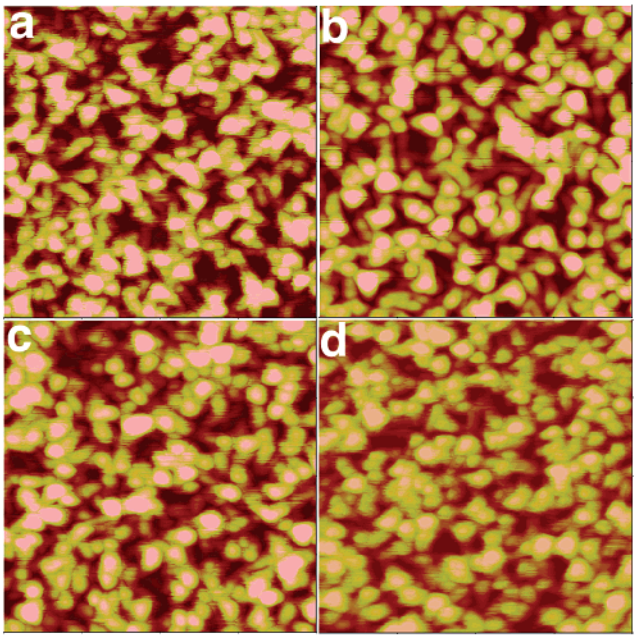

Figure 1. Tapping-mode AFM images of a Nioprobe tip calibration sample, obtained with the same AFM tip before (a) and after (b) cleaning with Piranha solution, etching in HF solution (c), and then reaction with the OEG-alkene **1** (d). The images were acquired with a MultiMode IIIa AFM (Digital Instrument) under identical ambient conditions. Image size: 400 nm; *z*-scale (contrast): 10 nm; scan rate: 1 Hz.

Force-versus-distance ($F-D$) curves were acquired to probe the interactions between the tip and the films of fibrinogen and bovine serum albumin (BSA) on alkyl-terminated SAMs.[2b] The $F-D$ curves were recorded first with a Piranha-cleaned tip and a protein

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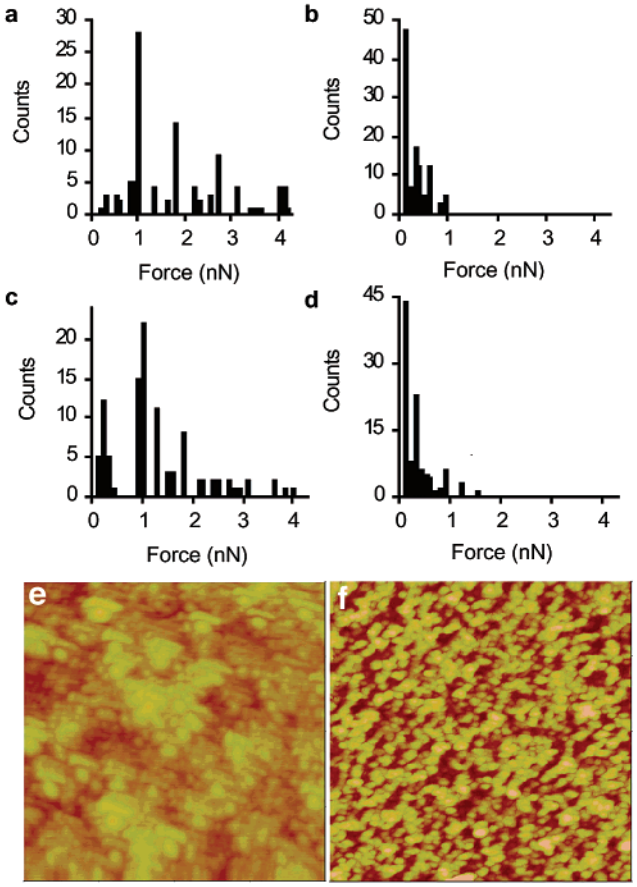

Figure 2. (a−d): Histograms of the adhesion forces between a fibrinogen film and a bare silicon tip (a) and the same tip upon OEG modification (b), and between a BSA film and a bare silicon tip (c) and the same tip upon OEG modification (d). The fibrinogen and BSA films, 6 and 2 nm in thickness, were deposited on a SAM derived from octadecyltrichlorosilane on flat silicon surfaces. All measurements were performed with a cycle frequency of 1 Hz in PBS buffer (0.05 M, pH 7.4, Sigma) at 23 °C, and the spring constants of the tips were ∼0.3 N/m. (e, f): Selected tapping-mode topographic AFM images (800 nm scan and 10 nm *z*-scale) of a fibrinogen film with an as-received tip [(e) tip size 7 nm] and an OEG-modified tip [(f) tip size 11 nm], both with a cantilever spring constant of ∼7.5 N/m and resonance frequency of ∼100 kHz, in PBS buffer (0.05 M, pH 7.4).

film, and the same tip was cleaned again, etched, and modified with OEG before acquiring the $F-D$ curves of the same protein film. For each system, the adhesion forces (F_a) were measured from 100 consecutive $F-D$ curves obtained at several spots of the film to provide the histograms (Figure 2, a−d).

The data clearly show a significant improvement of protein resistance for OEG-modified tips. The distribution of adhesion forces between an unmodified silicon tip and fibrinogen or BSA was irregular, and strong adhesions (F_a > 2 nN) occurred quite often as shown in Figure 2, a and c; the average F_a obtained from 100 $F-D$ curves was 1.77 nN for fibrinogen and 1.30 nN for BSA. This suggests that imaging of proteins with such tips may lead to artifacts and destruction of the soft sample. Upon modification of the same tips with OEG, the average adhesion force dropped to 0.34 nN for fibrinogen and 0.32 nN for BSA, and the adhesion forces seldom exceeded 1 nN (Figure 2b,d).

Indeed, the OEG-modified tips greatly improved the contrast and resolution for imaging high coverage films of fibrinogen. Figure 2, e and f, represents the best images obtained with a bare silicon tip[7] and an OEG-modified tip. In addition to the better resolution and contrast, the imaging with OEG-modified tips was much easier to perform than with the unmodified tips (by which maintaining a stable feedback during imaging was difficult), consistent with the strong, random adhesion between the silicon tip and the protein (Figure 2a).

The above measurements have been repeated with many tips. Although unmodified tips did show deviating adhesion forces likely due to the variation in tip size, most of them exhibited a significant reduction of adhesion forces with the proteins upon OEG modification. We did observe that some of the modified tips were very adhesive to the proteins (average F_a, > 3 nN). We believe that these tips were damaged or contaminated with large particles during the modification process, as indicated by the low image resolution using these tips. Indeed, careful handling of the cantilevers, using solvents freshly distilled in glassware that had been precleaned with Piranha solutions, and using other means to minimize contamination are crucial to the preparation of high-quality tips. Such tips can be stored in the dark for at least one month. We also carried out a blank experiment where the tips were subjected to the same modification procedure but in the absence of the alkene **1**. The adhesion forces between fibrinogen and the resulting tips remained nearly unchanged upon the treatment, indicating that OEG film on the tip is necessary for reducing the interaction with the protein.

In conclusion, we describe a new approach to modify silicon AFM tips that is based on direct chemisorption of 1-alkenes on hydrogen-terminated silicon surfaces. Compared to the conventional method based on deposition of thiolate SAMs on gold-coated tips, this technique allows the small tip size to be maintained for high-resolution imaging. We showed that the silicon tips thus modified with the OEG-alkene **1** effectively reduced the nonspecific interactions with proteins while maintaing capability for high-resolution imaging. Development of other OEG-based coatings on silicon to improve protein resistance and unraveling the origin of the high imaging contrast for the OEG-coated tips, possibly associated with the ionic strength of the buffer solution, are currently underway.

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References

(1) (a) Jena, B. P., Horber, H. J. K., Eds. Atomic Force Microscopy in Cell Biology. In *Methods in Cell Biology*; Academic Press: Amsterdam, 2002; Vol. 68. (b) Takano, H.; Kenseth, J. R.; Wong, S. S.; O'Brien, J. C.; Porter, M. D. *Chem. Rev.* **1999**, *99*, 2845. (c) Hinterdorfer, P.; Gruber, H. T.; Kienberger, F.; Kada, G.; Riener, C.; Borken, C.; Schindler, H. *Colloid Surf. B* **2002**, *23*, 115. (d) Ortega-Vinuesa, J. L.; Tengvall, P.; Lundström, I. *Thin Solid Films* **1998**, *324*, 257.
(2) (a) Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 10714. (b) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605.
(3) Lee, S.-W.; Laibinis, P. E. *Biomaterials* **1998**, *19*, 1669.
(4) (a) Linford, M. R.; Fenter, P.; Eisenberger, P. M.; Chidsey, C. E. D. *J. Am. Chem. Soc.* **1995**, *117*, 3145. (b) Buriak, J. M. *Chem. Rev.* **2002**, *102*, 1271. (c) Sieval, A. B.; Vleeming, V.; Zuilhof, H.; Sudholter, E. J. R. *Langmuir* **1999**, *15*, 8288.
(5) Okorn-Schmidt, H. F. *IBM J. Res. Dev.* **1999**, *43*, 351.
(6) Todd, B. A.; Eppell, S. J. *Surf. Sci.* **2001**, *491*, 473.
(7) The resolution of Figure 2e is similar to that of the best reported images of high-coverage fibrinogen films, obtained under similar conditions. See ref 1d.

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