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Dip-Pen Nanolithography in Tapping Mode

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Abstract: Dip-pen nanolithography (DPN) is becoming a popular nano-patterning technique for depositing materials onto a substrate using the probe of an atomic force microscope (AFM). Here, we demonstrate the deposition of a short synthetic peptide by DPN using the Tapping Mode of AFM rather than the commonly used contact mode. DPN in Tapping Mode requires drive amplitude modifications for deposition, yet allows for gentle imaging of the deposited material and enables deposition on soft surfaces.

Introduction

Various forms of scanning probe microscopies (SPM) have been explored in recent years to pattern surfaces using lithography and to deposit molecules on solid surfaces. SPM-based techniques offer a much higher resolution as compared to microcontact printing[1] or microfluidic devices[2]. In particular, the ability of SPM to manipulate and image at the nanoscale makes it an obvious method of choice for biomolecules.[3−5] For instance, the SPM probe has been used to etch away and reattach desired biomolecules, in a process known as nanografting.[6] Bruckbauer[7] et al. have used a form of SPM called scanning ion-conductance microscopy and developed a nanopipet for the controlled delivery of biomolecules on substrates. SPM has also been used to push (slide) and arrange nanoparticles on solid surfaces.[8−9]

Dip-Pen Nanolithography (DPN) is a SPM-based deposition technique developed in recent years to accomplish nanoscale deposition.[10] This technique employs an atomic force microscope (AFM) to deposit ("write") compounds onto a substrate in precise nanoscale patterns in a fashion similar to an old-style quill pen, where the compound is the ink and the substrate is the paper. The process is quick, has nanoscale resolution, and multiple compounds can be deposited on a single substrate.[11] Traditionally, the substrate is gold and the deposited compound is a hydrocarbon with attached thiols for gold binding.[10] The palette of inks/substrates has been expanding lately and organic (hexamethyldisilazane[12]), inorganic (metals[13]), and biomolecules (collagen,[14] lysozyme, and immunoglobulin,[15] DNA[16]) have been deposited on semiconductor surfaces using DPN.

Contrary to most previously published works, which utilize contact mode AFM for DPN[10−13,15−16], we have been able to systematically demonstrate DPN using the Tapping Mode. This enables one, not only to deposit, but subsequently image and/or manipulate biomolecules using a gentler mode of AFM more conducive to biological imaging.[17] Although others[14] have explored the use of Tapping Mode for DPN, this is the first detailed analysis of how the technique is performed using this mode.

We designed a synthetic peptide MH2 for deposition (Scheme 1). This peptide contains an N-terminal c-Myc tag (an antigenic epitope), and a C-terminal histidine (His) tag. The c-Myc tag allows one to detect the peptide using indirect immunofluorescence while the C-terminal His-tag can be used in binding to Ni-chelating substrates.

The flexibility of using designer peptides allows one to subsequently detect and/or build upon the initially deposited pattern. Thus, we envision the DPN technique as a tool for nanoscale structure fabrication by allowing the placement of biological building blocks in specific patterns.

Experimental Section

Materials. The peptide was chemically synthesized by New England Peptide, Inc. (Fitchburg, MA). Nickel nitrilotriacetic acid (Ni−NTA) slides were purchased from Xenopore (Hawthorne, NJ). Muscovite mica, grade 1 was from S & J Trading Inc, NJ. Gold coating on mica

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Scheme 1. Amino Acid Sequence of the Peptide MH2

MH2: H2N-CKEQKLISEEDLGHHHHHH-COOH

Myc tag His tag

and AFM probes was done using a two-ion-beam sputter coater (Southbay Inc., San Clemente CA) from a gold target with 99.99% purity; 10−20 nm thick coating for AFM probes and 50−200 nm for mica. Nanoscope IIIa Multimode Scanning Probe Microscope (Digital Instruments, Santa Barbara, CA) was used for all AFM/DPN experiments. NP-20 probes were from Digital Instruments, and NSC15 probes were purchased from MikroMasch (Estonia).

Sample Preparation. The peptides were prepared in 10 mM sodium phosphate (pH 8.0) at a concentration of 20−100 μg/mL. NP-20 probes were used for contact mode and gold-coated NSC15 probes were used for Tapping Mode DPN. Probes were dipped into peptide solution for 15−30 s and air-dried. Gold substrates were prepared by template stripping as described elsewhere.[18−19] Briefly, 50−200 nm of gold was sputter-coated onto freshly cleaved mica and the gold-coated surface was attached to the specimen disk with double-sided tape. The mica was peeled off just before use to expose an atomically flat gold surface. The surface roughness of the gold surface thus prepared was <0.1 nm.

DPN/AFM Experiments. All Dip-Pen Nanolithography and subsequent imaging were performed in the Tapping Mode unless otherwise stated. Relative humidity during deposition ranged from 10 to 40%. Scanner type used was J; scanning rate was 2−3 Hz for imaging with 256 lines per scan direction. Images were recorded in height and phase modes for Tapping Mode, and height and lateral force mode for contact mode. For Tapping Mode, the Si cantilevers (of nominal spring constant 40 N/m, tip curvature radius ~10 nm) were tuned prior to imaging at a set point between 1.0 and 2.0 V and preset target amplitude. The drive amplitude and resonance frequency values (ranging from 295 to 370 kHz) thus obtained were used for imaging. Increasing the drive amplitude by at least a factor of 2−5 and reducing the scan rate to a range of 0.02−1.0 Hz enabled us to achieve DPN in Tapping Mode. Pattern formation using DPN was accomplished by changing the scan angles and aspect ratios of the scanned areas. The Nanoscope IIIa software enabled scanning areas with aspect ratios (the ratio of height to width) ranging from 1:1 to 1:128 and scan angles from 0° to 180°. Thus, lines of various widths could be written at desired angles in a region. All imaging was done with scan regions having an aspect ratio of 1:1.

Results

Tapping Mode Deposition. Our initial attempts to employ the His-tag of the peptide MH2 for deposition onto Ni-chelating slides using contact mode AFM resulted in scratching of the Ni−NTA layer on the glass slides (Figure 1a−c). The scratching is evident by the dark regions in the height image (Figure 1a) and the dip in the section profile (Figure 1b). The scratched regions exhibit an increased friction as observed by the bright regions in the corresponding lateral force image (Figure 1c). No deposition of the peptide could be observed due to absence of any increased height in the DPN regions. Thus, a gentler mode of DPN was necessary, and Tapping Mode was explored for deposition. Figure 1, parts d and e, shows the height and phase images, respectively, of a region after employing DPN in Tapping Mode. No scratching of the surface or deposition of the peptide is seen in these experiments. The lack of deposition is likely due to nonuniformity of the Ni−NTA coating and high surface roughness of the slides.

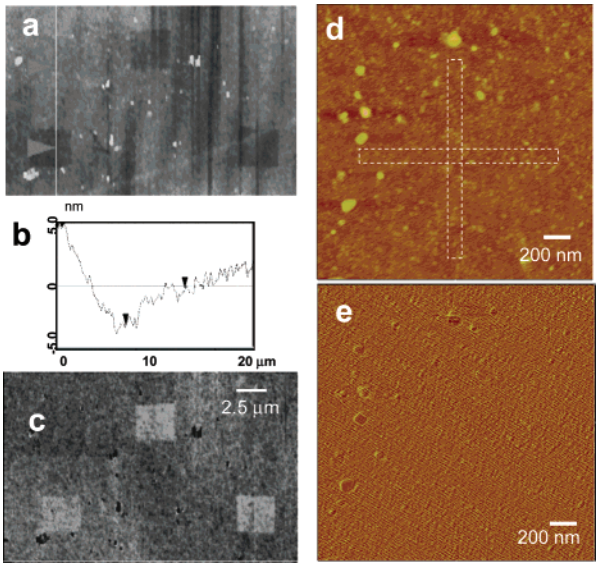

Figure 1. DPN carried out in contact mode on Ni chelated slides using the peptide MH2. Scratching of the Ni-NTA slides occured as seen by (a) dark areas in height images and (b) dip in height profile. (c) Lateral force images exhibited the difference in friction coefficients created by scratching. Writing of a "+" (indicated by dotted lines) attempted using Tapping Mode DPM does not show any scratching in (d) height and (e) phase images. However, no deposition is observed due to surface roughness and nonuniformity of slides.

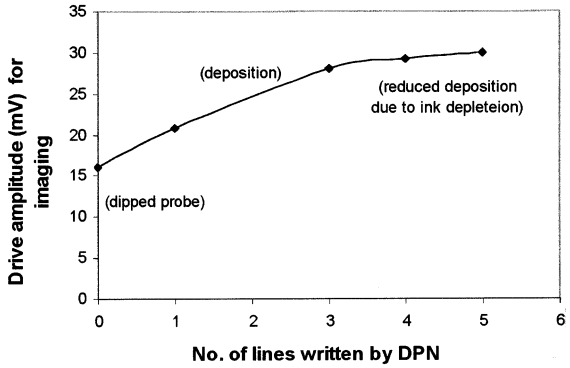

Figure 2. Drive amplitude plays an important role in Tapping Mode DPN. The drive amplitude required for imaging with a peptide−coated probe increased as the peptide was depleted from the probe when successive 1 μm lines (aspect ratio 1:32) were written by DPN.

We therefore explored the deposition of peptide MH2 by utilizing its cysteine residue on a molecularly smooth gold surface. In the process, we discovered that the drive amplitude of the probe is a critical factor for achieving DPN in Tapping-Mode. Repeated experiments demonstrated that the drive amplitude of the probe decreased significantly from its native value when coated with peptide and tuned at the same preset target amplitude. This provided a way to determine if the probe had been coated with peptide. Second, deposition required a drive amplitude at least 2−5 times higher than the value used for imaging. In addition, as the peptide was depleted from the probe, the drive amplitude required for imaging increased until the probe regained its native drive amplitude value, at which point most of the peptide had been deposited. Figure 2 illustrates the increase in drive amplitude required for imaging as successive lines were written with an MH2 coated probe on a gold surface. When all the peptide had been depleted, no deposition was observed even when the drive amplitude was increased

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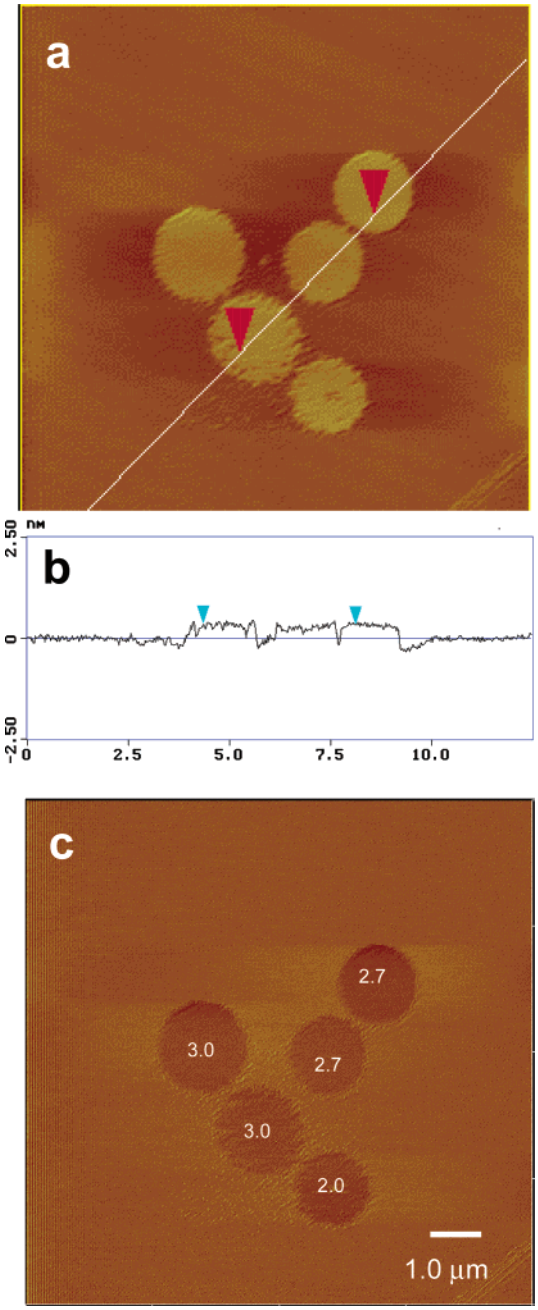

Figure 3. Diffusion of the peptide MH2 on a gold substrate using Tapping Mode DPN. (a) Height, (b) height profile and (c) phase images of MH2 deposition on gold. The peptide diffuses into circular spots on the substrate with an increase in the spot size with diffusion time (indicated in minutes on phase image). Height profile showed a height range of 0.2−0.5 nm for the diffusion spots.

further. Additionally, uncoated probes exhibited no deposition when using high drive amplitudes and low scan rates (data not shown). These observations, along with the results described below, confirmed that deposition and not surface scratching of the substrate occurred during Tapping Mode DPN.

Diffusion Experiments. To verify the affinity of the ink (peptide) to the substrate (gold), diffusion experiments were performed, where the AFM probe was held at a specific point on the surface and material on the probe was allowed to diffuse. These experiments resulted in distinct diffusion spots similar to those observed by others using contact mode DPN[10,16] and verified deposition in Tapping Mode (Figure 3a). The 0.2−0.5 nm vertical height of the diffusion spots suggests that the peptides are lying horizontally along the substrate (Figure 3b). The patterned regions were darker (lower phase angle) in phase images (Figure 3c). An increase in spot size with diffusion time was observed.

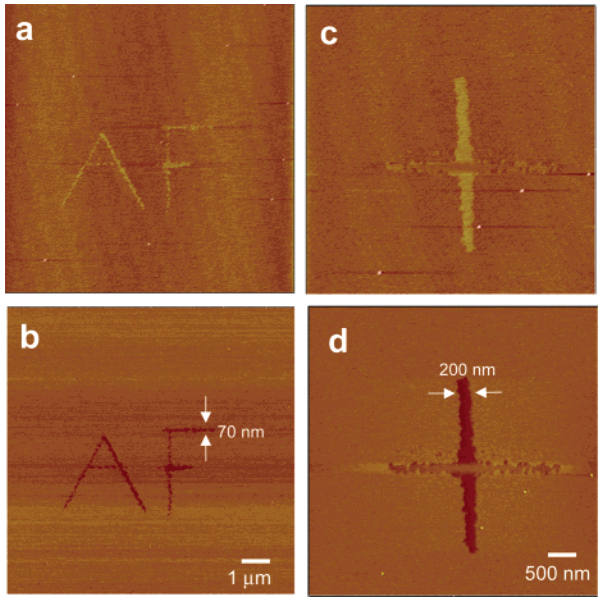

Figure 4. Nanoscale patterns written with the peptide MH2 on a gold surface using Tapping Mode DPN. (a) Height image of "AF" written using an aspect ratio of 1:64 and various scan angles. The corresponding phase image (b) shows a darkened patterned region. (c) Height and (d) phase image of "+" written with aspect ratio of 1:32. Line widths of patterns are indicated.

Nanowritings. We next performed a more controlled set of experiments where thin lines and patterns could be written by changing the scan angles and aspect ratios of the scanned region. Figure 4 shows patterns written with MH2 peptide on gold using a scan speed of 0.5 Hz. Height images exhibited a 0.2−0.5 nm profile and DPN was verified by phase contrast imaging**.** Line widths ranging from 70 to 200 nm could be obtained when using aspect ratios of 1:64 to 1:32 in the above experiments.

Discussion

We have been able to achieve deposition of a synthetic peptide on a molecularly smooth gold substrate using the DPN technique in Tapping Mode. In the Tapping Mode, the cantilever is made to oscillate near its resonant frequency instead of grazing the sample as in contact mode. While oscillating, the AFM probe is only in intermittent contact with the sample surface and interacts only once in every cycle. In contrast to contact-mode AFM, the interaction time between the probe and the sample and lateral forces between them, are reduced.[20−22] For DPN experiments, it is important that the cantilever remains in contact with the substrate for extended periods of time. A key to achieving deposition using Tapping Mode was to increase the drive amplitude by a factor of 2−5 from its imaging value. At increased drive amplitude, the cantilever becomes a case of a nonlinear driven oscillator system, which results in increasing the force exerted by the probe and an increase in the contact

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time of the probe with the substrate.[23] This facilitates diffusion of the ink from the probe to the substrate through the water meniscus formed due to capillary condensation. Despite the greater forces exerted upon increasing the drive amplitude in Tapping Mode, the forces in this mode are still much less than those in contact mode. This is evidenced by our results where the Ni−NTA slides are scratched in contact mode but not in the Tapping Mode.

After each successive writing, we observed that a higher drive amplitude is required for imaging even though the drive frequency remained constant. This could be due to the effect that during DPN there is a water meniscus surrounding the probe. After writing with DPN is complete, there could still be water/ink solution adhered to the probe. This can create a viscous drag on the probe when used for imaging and a slightly higher drive amplitude value is needed to overcome these forces. Second, using a high drive amplitude during DPN can blunt the AFM probe. The blunt probe will have more frictional contact area requiring more force to be exerted to write/image.

The deposition of MH2 was verified using height and phase imaging with AFM. Our height data revealed a vertical profile of 0.2−0.5 nm, which is consistent with the peptide (or layers of peptide) lying horizontally on the substrate. Phase contrast images in Tapping Mode AFM are a recording of the phase shift, which is the difference between the phase angles of the driving signal and the deflection of the cantilever oscillation. Darker regions correspond to greater phase lag and vice versa. Various factors can contribute to the phase shift, such as, the interaction between probe and substrate[24], energy dissipated in the probe-sample contact,[25] and composition and material properties of a sample.[26] Magonov et al.[24,27] have related the phase shift ($\Delta\Phi$) between a free and interacting cantilever to the force derivatives for probe-sample interactions (σ) as

$$\Delta\Phi \propto Q\sigma/k$$

for the case when $\sigma \ll k$, where k is the force constant of the cantilever, and Q is the quality factor. Therefore, for attractive forces ($\sigma < 0$), the phase shift will be negative, i.e., greater phase lag. In our experiments the DPN regions appear dark in phase-contrast images when imaged with a peptide-coated probe. Our observation of greater phase lag in the DPN regions indicates that the interaction between the MH2 coated probe and the deposited peptide is possibly greater than that between the peptide coated probe and the bare substrate (gold). This may be due to disulfide attractions between exposed cysteine residues and/or electrostatic interactions between other residues.

Efforts to detect the deposited MH2 peptide via immunolabeling were unsuccessful. This may be due to the gold substrate quenching the fluorescently-labeled antibody and/or a weak signal. Height scans indicated the peptide was laying flat on the substrate partially contributing to gold-mediated quenching and/or unsuitable epitope presentation. As a result, we are exploring more desirable methods of peptide deposition, including nickel-histidine (Ni−His) binding. Further experiments to employ the stronger Ni−His binding of MH2 peptide are underway through the use of electrochemical DPN in Tapping Mode on metallic nickel surfaces.[28]

DPN is a powerful tool for the placement of small and large proteins in specific patterns and locations. We have shown here that Tapping Mode can be employed for DPN by driving the AFM cantilever at high amplitudes. The Tapping Mode does not exert harsh disruptive forces on surfaces as in contact mode, which can result in surface scratching or dragging of biomolecules. This opens up possibilities for depositing and subsequently imaging biomolecules on soft substrates and in cases where the molecules are not strongly immobilized.

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