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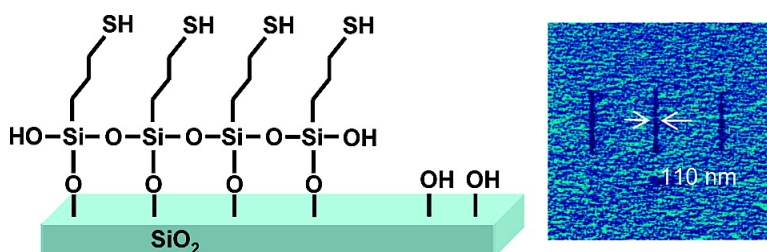
Dip-Pen Nanolithography of Reactive Alkoxysilanes on Glass

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Dip-pen nanolithography (DPN),[1] which uses an "inked" atomic force microscopy (AFM) tip to deposit molecules on a substrate, has been used to create nanoscopic patterns of many different materials including solid-state materials,[2] DNA,[3] and proteins.[4] The majority of DPN experiments have used gold surfaces as the substrates and thiol molecules as the inks, such as alkanethiols, arylthiols, and thiol-modified DNA and proteins. However, the conductivity of gold can limit the ability to optically or electronically address nanostructures.

Recently, specific ink-substrate chemistries have been developed for patterning different materials on insulating and semiconducting surfaces with DPN, such as alkylsilazanes,[5] dendrimers,[6] and inorganic salts.[7] Organic dye molecules and fluorescently labeled polymers and antibodies have been directly patterned onto glass and imaged with an optical microscope.[8] However, the specific ink-substrate chemistry used for a particular application must be selected with care. The efficiency of activated mass transfer of ink molecules from the tip, as well as their reactivity with coadsorbed solvents, with each other and with the substrate can limit the number of ink-substrate systems that can be successfully used with DPN.[5,9]

A successful strategy for attaching biologically active molecules to glass, oxidized silicon, or fused silica surfaces involves reaction with organofunctional silanes followed by subsequent coupling reactions of biomolecules to the pendant functional groups introduced on the surface.[10] This method is widely used for immobilizing biomolecules in fabricating DNA, small-molecule, and protein microarray chips.[11] The success of this approach lies in its flexibility and generality; in principle, any biomolecule, including proteins, can be anchored to the silanized surface without loss of functionality, provided it can be appropriately modified.

Many of the details of how reactive trialkoxysilanes or trichlorosilanes form thin siloxane films on glass surfaces are not clear. It is commonly believed that film formation involves hydrolysis of the reactive groups of physisorbed silanes by surface water on hydrated glass, followed by condensation reactions that leave the silane covalently bound to the oxide surface. However, polymerization reactions can also occur, at free silanol groups on the surface as well as between silane molecules, which can result in a heterogeneous, highly polymeric network. For this very reason, trichloro- and trialkoxysilanes have previously been reported not to be suitable inks for DPN.[5] Nevertheless, strategies are available to limit polymerization of alkoxysilanes.[10]

Here, we show that by taking steps to minimize the degree of polymerization during DPN writing, it is in fact possible to write nanoscopic patterns of reactive trialkoxysilanes on glass, and that the patterned areas are fully functional for subsequent immobilization of fluorescently labeled streptavidin via covalent attachment of biotin. A stepwise procedure of functionalizing DPN-written self-assembled monolayers of 16-mercaptohexadecanoic acid on gold with biotin-streptavidin has been reported recently.[12] Analogous molecular recognition chemistry can be applied to patterning glass substrates as well. Large protein molecules that may be difficult to
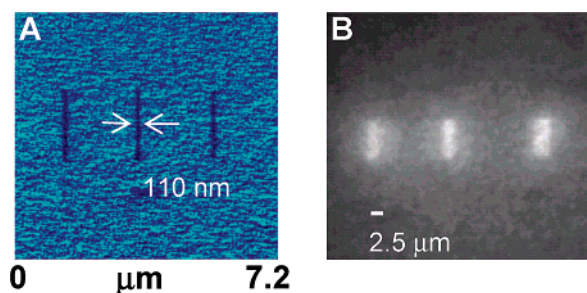

Figure 1. (a) Lateral force microscopy (LFM) image of 2 μm long lines of MPTMS written with DPN on glass at 22% relative humidity. (b) Fluorescent image of three 2.5 μm $\times$ 10 μm regions of MPTMS conjugated with biotin-maleimide and Cy3-streptavidin.

deposit onto a surface directly from an AFM tip, or that may be at risk of losing their functionality if they are not maintained in a fully hydrated state during the writing process, may be captured in their buffered environment by molecular recognition onto previously written reactive nanoscopic regions.

Polymerization was minimized by the choice of trialkoxysilane used as the ink and the control of relative humidity during the process of inking and writing with the AFM tip. We used 3′-mercaptopropyltrimethoxysilane (MPTMS) as the ink because the thiol functional group of MPTMS does not interfere with anchoring reactions at the surface.[13] Commercially available AFM tips (silicon nitride cantilever, 0.06 N/m, Digital Instruments) were cleaned in "piranha" solution (3:7 (v/v) mixture of 30% H_2O_2 and H_2SO_4) (*caution: this mixture reacts violently with organic materials*) for 30 min at room temperature, rinsed copiously with deionized water (Millipore Gradient), and dried at 120 °C. MPTMS (Aldrich) was coated onto the tips by evaporation from a 0.5−5.0 μL drop of the neat liquid at 120 °C for 30 min in a 70 mL sealed glass jar that had been purged with dry nitrogen gas passed through a desiccant. Coated tips were used for writing immediately. Glass substrates (VWR #1 coverslips) were cleaned according to standard published methods.[14]

DPN experiments were performed using a Multimode AFM from Digital Instruments in a large glovebag that was purged with dry nitrogen gas. The relative humidity, measured with a digital hygrometer, could be controlled from ∼0% up to ambient values (35−45%). All experiments were performed at room temperature between 22 and 23 °C. Writing was performed in contact mode using negative tip−substrate contact forces (−4 nN).

Patterns were imaged by lateral force microscopy (LFM) immediately after writing. Figure 1a shows an LFM image of 2 μm long lines of MPTMS written at 5 μm/s. The relative humidity was 22%. The average width of the patterns was 110 nm. Written areas were darker in the LFM images than the more hydrophilic glass background, indicating a decrease in friction between these areas with the tip. Cross-talk between the vertical bending of the probe used for topographic imaging and frictional forces acting on the AFM cantilever made reproducible height determinations of
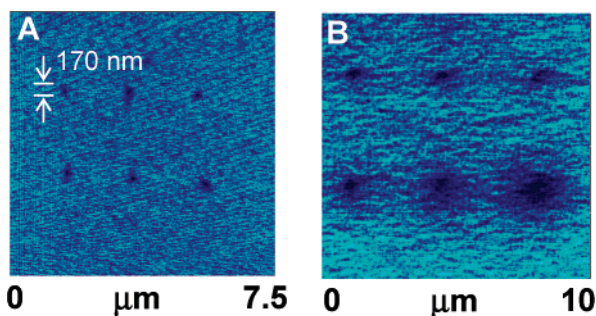
Figure 2. LFM images of MPTMS dots on glass for tip−surface contact times of 1, 15, 30 s (top row, left to right) and 60, 120, 240 s (bottom row, left to right). (a) 22% relative humidity. (b) 0% relative humidity.

the written areas impossible in contact mode. However, tapping mode imaging in water of MPTMS-written areas indicated that the heights of patterned regions were consistent with the reported thickness of a monolayer of MPTMS[15] (see Supporting Information).

With the inking conditions reported above, patterns could be written for several hours with no noticeable changes in feature appearance or size. The limiting factor for extended DPN writing in this case appears to be the time needed for the tip to run out of ink. For relative humidity between 25% and 30%, patterns became broadened and irregular, and the LFM contrast changed from dark to bright, indicating an increase in friction relative to the glass background. We believe this is due to the formation of more disordered polymeric films. Topography images taken simultaneously with LFM showed large height increases consistent with multilayer formation during polymerization. Above relative humidity values of about 30%, DPN writing with MPTMS was impossible due to strong adhesive or frictional forces on the tip.

The availability of the pendant thiol groups of DPN-patterned MPTMS for further coupling reactions was tested by introducing biotin and Cy3-streptavidin.[12] Nonpatterned areas were passivated against nonspecific adsorption of protein with a trialkoxysilane containing poly(ethylene glycol). Fluorescence was observed using an inverted epi-fluorescence microscope with a Hg/Xe arc lamp (Eclipse TE300, Nikon). Images of fluorescent patterns were captured with a high-resolution, Peltier-cooled CCD camera (Cool-Snap-HQ, Roper Scientific). Figure 1b is a fluorescence image of three 2.5 μm × 10 μm regions patterned with Cy3-streptavidin (corresponding LFM image not shown).

Figure 2 contains LFM images that show the results of varying the tip contact time, from 1 s to 4 min, in writing dot patterns of MPTMS on glass at two relative humidity values, 22% (Figure 2a) and 0% (Figure 2b). At 22% relative humidity, there was no discernible time dependence in dot growth, unlike the case of thiol writing on gold. For thiol writing on gold, ink molecules are not chemically reactive with each other; molecules that are deposited onto previously chemisorbed regions can freely diffuse across these regions until they become bound at the periphery to reactive gold sites, resulting in isotropic growth.[16]

In the case of MPTMS at 22% relative humidity, chemical cross-linking of silane molecules in the thin water layer at the surface limited the importance of isotropic surface diffusion and constrained the feature size and shapes of dot patterns. Other DPN experiments have shown deviations from isotropic diffusional growth when intermolecular interactions can compete with surface binding.[17]

Although mostly circular, the dot shapes were nonuniform. Notably, the diameters of the dots, from tip−surface contact times ranging from 1 to 240 s, were all roughly the same, indicating that the lateral extent of growth was quickly quenched (within a few seconds).

Even at 0% relative humidity, residual water condenses at the contact point between the tip and the substrate[16] and can initiate hydrolysis reactions of the silane ink. Under these conditions, however, surface diffusion played a greater role, as seen by increasing dot sizes with contact time, although feature growth was still anisotropic. Dot patterns were irregular in shape and consisted of a dark disk surrounded by a diffuse halo. Halo effects have been seen in many self-assembled monolayer structures and attributed to a reorientation of adsorbed molecules from a vertical to a prone orientation below a critical surface concentration at the periphery of a growing dot.[18] Whether or not this transition occurs for MPTMS written with DPN is uncertain, but the patterns do indicate that diffusive growth competes more effectively with lateral cross-linking reactions at lower humidity values.

The highest degree of spatial control of MPTMS patterns on glass written with DPN was obtained at low, but nonzero, values of the relative humidity. An optimal amount of adsorbed water is necessary to initiate strong covalent bond formation between the silane ink and the glass substrate, while limiting the extent of polymerization-induced disorder.[19] DPN writing of reactive trialkoxysilanes is possible when the silane is evaporated onto the AFM tip under anhydrous conditions followed by patterning at low humidity values. These patterns can then be chemically and biologically functionalized with the same general techniques used to immobilize many biomolecules on glass and oxidized surfaces.

Supporting Information Available: Tapping mode AFM image of MPTMS monolayer, methods used for biotin/streptavidin conjugation, LFM, and corresponding fluorescent images (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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