NANOSHAVING AND NANOGRAFTING OF WATER-SOLUBLE POLYMERS ON GLASS SURFACES

by

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ABSTRACT

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Bachelor of Science

Chemical surface patterning at the nanoscale is an important component of the chemically directed assembly of sensitive biological molecules or nanoscale electrical devices onto surfaces. Here we present a scanning probe lithography technique that allows for patterning of aqueous polymers on glass or silicon dioxide surfaces.

The surfaces were functionalized by covalently bonding a silane monolayer with a known surface charge to either a borosilicate glass slide or thermal oxide on a silicon wafer. A polymer layer less than 2 nm in thickness was then electrostatically bound to the silane layer, passivating the functionalized surface. An Atomic Force Microscope (AFM) probe was used to mechanically remove a portion of the polymer layer, exposing the functional silane layer underneath.

Employing this method we made chemically active submicron regions. These regions were backfilled with a fluorescently-tagged polymer. Chemical differentiation

was verified through tapping mode AFM and optical fluorescence microscopy. Lines with a pitch as small as 20 nm were observed with AFM height and phase mode data. Scribing forces were measured as low as 0.3 μN. Scribing was successful in ambient conditions as well as in aqueous solution, thus allowing patterning of sensitive biological molecules in their native environments. No instabilities in the created patterns were noted during observation periods of several months.

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I would like to thank all others who contributed to this project by means of ideas, suggestions, and preliminary work. Especially of note are Dr. Adam Wooley and Dr. Matthew Linford whose labs we frequented often, and Jodi Knoble and Katie Hurd whose preliminary work laid the foundation for this project's success.

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1. Introduction

Patterned surfaces are of great interest to scientists in many fields as they are the foundation for such widespread applications as the fabrication of nanoelectrical devices and microelectromechanical devices (MEMS), the study of crystal structure, surface science, microfluidics, and more. Specifically, the "precise patterning of biomolecules on surfaces with [nanometer] resolution" is of interest because such patterns have "great potential in many medical and biological applications ranging from molecular diagnostics to advanced platforms for fundamental studies of molecular and cell biology."[3] (These "advanced platforms," more commonly known as labs-on-a-chip, could be used by employers for drug testing, by forensic teams to process crime scene evidence, or by medical personnel for blood typing, in addition to fundamental studies of molecules and cells as noted above).

Despite the variety of uses for such surfaces, current surface patterning techniques have limitations of spatial resolution and shape, and some impose harsh conditions to biomolecules such as extreme pH or a dry environment. There is currently no known general method to pattern functional bio-polymers at high resolution.

A technique known as nanografting permits the high resolution patterning needed, though it has its own set of limitations. Nanografting is a scanning probe lithography technique first demonstrated in 1997 using thiols on a gold surface.[1] The process works by using an Atomic Force Microscope (AFM) tip to scribe away weakly bound thiol groups from a gold surface immersed in a solution of different thiols that will bind to the surface as the weakly bound layer is scribed away. Features less than 30 nm have been

reported in the literature using this method.[2] Nanografting has almost been exclusively done using this gold-thiol chemistry.[3] Nanografting has been further developed so that some biological molecules can be linked to the thiol molecules on the gold substrate.[4] However, the gold-thiol chemistry is not desirable for many applications that patterned surfaces might be used for. Thiol molecules are easily removed in water, the native environment of many biological molecules. Gold is conductive, which causes quenching effects for work requiring fluorescent differentiation; an electrically insulating substrate is also a must for creating nanoelectrical devices. Furthermore, the gold-thiol chemistry does not provide the long-term stability required for such devices. In a nutshell, nanografting provides the desired high resolution, but the gold-thiol chemistry it was developed with is not suitable to pattern functional bio-polymers or nanoelectrical devices, both of which are of high interest at BYU and abroad.

Work has been done at BYU under the direction of Dr. Linford and Dr. Davis to adapt nanografting to other chemistries. The first extension of nanografting involved use of semiconducting surfaces[5], but the technique required large scribing forces which caused wear on the AFM tip. This is significant because tip wear quickly causes the process to lose its high-resolution pattern-making capability. Work was also done with nanografting on insulating surfaces[6], using a chemistry compatible with DNA patterning. While the environment was much more friendly toward biological molecules, and the work was done on a nonconducting surface, the tip wear issue wasn't resolved.
This work describes nanografting water-soluble polymers on a functionalized oxide surface, which differs from previous work due to a non-covalently bonded protective layer on top of the substrate. This polymer layer solves previous problems hindering the

high-resolution patterning of biomolecules as it simultaneously allows patterning on a non-conducting substrate in a bio-friendly aqueous environment, with smaller scribing forces yet greater stability than previous methods.

2. Methods

2.1 Overview

A glass surface is first functionalized with a silane coupling agent, which allows the inorganic substrate to be linked to an organic compound. We chose carboxyethyl-silanetriol, which forms a covalent bond to the glass surface resulting in a strong cross-linked monolayer about 0.5 nm thick*. A polymer layer less than 2 nm in thickness is then electrostatically bound to the negatively-charged carboxyl groups of the silane layer, passivating the functionalized surface. We chose Poly-L-Lysine, a positively-charged polymer with many common biological applications. Following the deposition of the polymer layer, an Atomic Force Microscope (AFM) probe is then used to mechanically remove select portions of the polymer layer, exposing the functional silane layer underneath. These functional areas (or "holes") can then be backfilled with the desired bio-polymer; in this work, we chose fluorescently tagged Poly-L-Lysine to assist in the verification process. Figure 1 shows a cartoon depiction of this process.

2.2 Chemical Preparation

The silane solution was prepared by mixing carboxyethylsilanetriol (sodium salt, 25% in water, Gelest) in deionized water (4% w/v) and titrating the solution with glacial acetic acid (Mallinckrodt) to $5.5 < pH < 6.0$. Borosilicate glass microscope cover slips (Fisherbrand) were piranha cleaned, rinsed in deionized water, and immersed for 24 h in the silane solution in tightly-sealed jars at 70 °C. After rinsing in water and drying in a stream of N_2, substrates were cured at 100°C for 30 min in air. They were then immersed

* One nanometer (nm) is one-billionth of a meter. For reference, a nanometer is roughly one-thousandth the size of a single bacterium, or about ten times the radius of a typical atom.

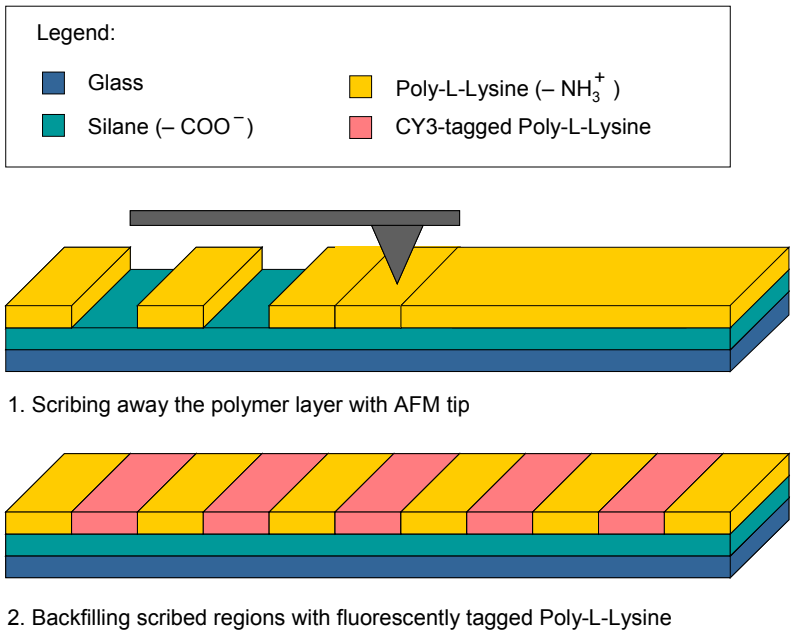


Figure 1: Overview of the water-soluble polymer nanografting process

in 1% (w/v) aqueous Poly-L-Lysine solution (Ted Pella, Inc., used as received) for 10 min, rinsed in deionized water, and dried in a stream of N_2.

2.3 Polymer Nanoshaving

Lines, patches, and patterns were created using a set force on a Dimension V AFM (Digital Instruments, with Nanoscope V software) in contact mode. Lines were scribed by scanning a (nominally) 50nm radius tip (NSC12/Pt50/AlBS, Mikromasch) back and forth on 128 lines in a 3 μm square area as if imaging in contact mode, with a set force of approximately 0.3μN. Patches of various sizes were created by increasing line density in scribed regions. After scribing the surface was immersed in 0.01% (w/v) CY3-tagged Poly-L-Lysine solution (Nanocs, Inc.) for 10 min to backfill the scribed regions, rinsed in deionized water, and dried in a stream of N_2.

2.4 Polymer Nanografting

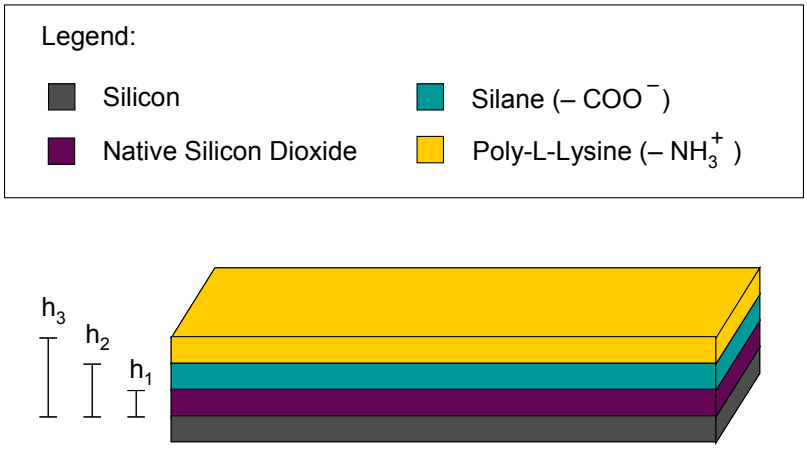
Nanografting is simply when the nanoshaving step is done simultaneously with the backfilling step; that is, the surface is scribed while immersed in the fluid to be patterned (as opposed to scribing in air). Patches were created using a PicoPlus 2500 AFM (Agilent Technologies, with PicoScan 5 software) in contact mode. Samples were scribed in deionized water. After removing the AFM head, 1% (w/v) CY3-tagged Poly-L-Lysine solution (Nanocs, Inc.) was added to the water (until it was ~ 0.01% (w/v)). The sample was immersed for 10 min in this solution then rinsed in deionized water.

2.5 Verification process

Layer Deposition. Because the chemical layers are shorter than the wavelength of visible light, there will be no change in samples' appearance before and after deposition. Thus, verification of layer deposition was performed by use of an M-2000D ellipsometer (J. A. Woolam), which uses polarized light of multiple wavelengths to calculate layer thicknesses. As ellipsometry is difficult to perform on transparent glass, a side-by-side experiment was performed using silicon wafers with natively grown silicon dioxide, which essentially has the same surface chemistry as glass. (A side-by-side experiment is when two different surfaces are subjected to identical procedures; though only one surface is measured, results will be the same for both surfaces. In this case, the results of ellipsometry on the silicon dioxide surface indicate either the success or failure of layer deposition on the glass surface).

An ellipsometer only takes objective data about the polarization of reflected light for a given wavelength; to interpret the data and determine layer thicknesses, the data must be fit to model of known optical constants. While on the macroscale every material

has different optical constants, thin films such as the layers in this work share similar optical properties. Consequently, the same model (1 mm of silicon (si_jaw.mat) covered by a fitted-thickness layer of silicon dioxide (sio2_jaw.mat), both of which files were included in the factory-distributed software for the M-2000D) was employed to fit the data acquired for all ellipsometry measurements. Data was taken before and after each deposition to calculate the thickness change at each step. Thus, the layer thickness after the silane treatment minus the thickness of the native silicon dioxide layer alone is the thickness of the deposited silane layer; the thickness after the polymer deposition step minus the measured thickness after silane treatment is the thickness of the polymer layer. See Figure 2 for a cartoon depiction of this process.



The thickness of the SiO_2 layer is h_1. Following silane deposition, the total layer thickness will have risen to h_2; the calculated thickness of the silane layer alone is found by subtracting h_1 from h_2. Similarly, the thickness of the Poly-L-Lysine layer is found by subtracting h_2 from h_3.

Figure 2: Calculating thin-film layer thicknesses using an ellipsometer

Scribing. An AFM allows several modes of operation, two of which were used in this work: contact mode, where the tip is dragged along the surface of the sample (used to dig holes), and tapping mode, where the tip barely comes in contact with the sample surface (used to determine topography without modifying the layers on the sample surface). Following the nanoshaving step, the AFM was used in tapping mode to verify that the shape of the created patterns matched expectations and the depth of the scribed holes matched the layer thickness data from the ellipsometer. This verification step was omitted for the nanografting process as the intent of the nanografting process is to avoid removing the molecules' native environment. In this case, verification of chemical functionality serves as simultaneous verification of the scribing process.

Chemical Functionality. It is possible for only partial scribing to occur, in which the polymer layer is not sufficiently removed to produce chemical distinction in the patterned regions. Thus, to explicitly demonstrate chemical differentiation, scribed surfaces were exposed to a solution containing a fluorescently tagged polymer that would adhere to any exposed carboxyl groups on the silane layer but would be easily rinsed off of the untouched polymer layer elsewhere. Using a Nikon Eclipse TE300 confocal microscope, images were taken and compared to earlier AFM topography images to determine whether scribed regions were chemically different than unscribed areas.

Relocation of Patterned Regions. Before taking the tapping-mode topography data, the AFM head must be removed to install a sharper tip than the one used for scribing to provide higher resolution for captured images. Upon reinstalling the head, the patterned area will not be directly underneath where the tip used to be. Furthermore, after applying the fluorescently tagged polymer, the sample must be removed from the AFM

for rinsing. Without some sort of alignment system to help in the relocation process, the odds of randomly finding the previously patterned area on a 1 cm^2 sample are just better than two in one million. Thus, an alignment system was developed to avoid false negatives in the AFM and fluorescence microscopy verification steps.

For the nanoshaving process, alignment marks were manually scribed into samples using a diamond-tipped scribing pen, following the silane layer deposition. To avoid large amounts of glass dust, marks were created with minimal pressure in the shape of a greater-than sign (i.e., ">"), beginning at the tip of the pattern and scribing outward to the left. The sample was then immediately rinsed to remove dust before the application of the polymer layer. AFM scribing began 100 μm to the right of the tip of the alignment mark to avoid any residual dust. Following the scribing and backfilling procedure, a very small drop of deionized water was placed on a cleaned glass slide. The sample was placed onto the slide as a normal coverslip would be except with the patterned side face-up. Due to the surface tension of the water droplet, the coverslip would stay adhered while the slide was inverted for use in the confocal fluorescence microscope. See Figure 3 for a pictoral description of this process. Once the sample was on the fluorescence microscope, the alignment marking was used to locate the patterned region.

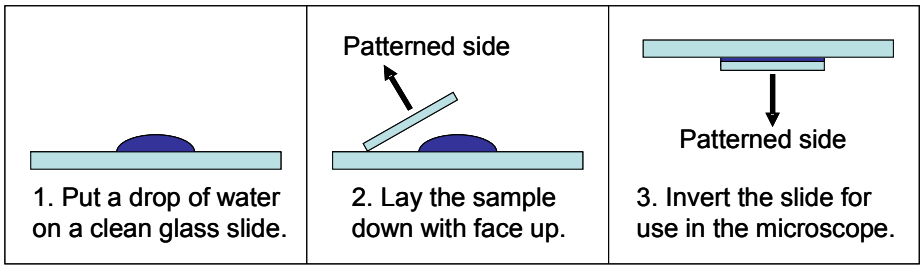


Figure 3: Preparing a patterned sample for confocal microscopy

As noted earlier, since the intent of the nanografting process is to be able to create patterns of molecules without removing their native environment, it is not desirable to include the scribing verification step, where AFM topography data is taken. Because of this, alignment restrictions are not as limiting, and manual alignment marks are notneeded. Instead, to find the nanografted pattern, the PicoPlus AFM was adapted to fit directly over the stage of the fluorescence microscope. Conveniently, the tip holder of our AFM fluoresces, so the fluorescence microscope was aligned to the AFM by searching for the shadow of the non-fluorescent tip against the background of the fluorescing tip holder. Once the two microscopes were aligned, the sample could be removed from and returned to the microscope without losing its position (roughly) with respect to the viewing window it had before. To guard the sample against movement inside the sample holder when rinsed, it was fastened under the O-ring in the AFM's standard fluid imaging cell.

Finally, to ensure that we were able to unmistakably resolve our created features under the fluorescence microscope, we developed a pattern shape that is clearly distinct from the assortment of dust and crud normally found on samples. Each pattern consisted of five boxes in a pattern like that on a 6-sided die.

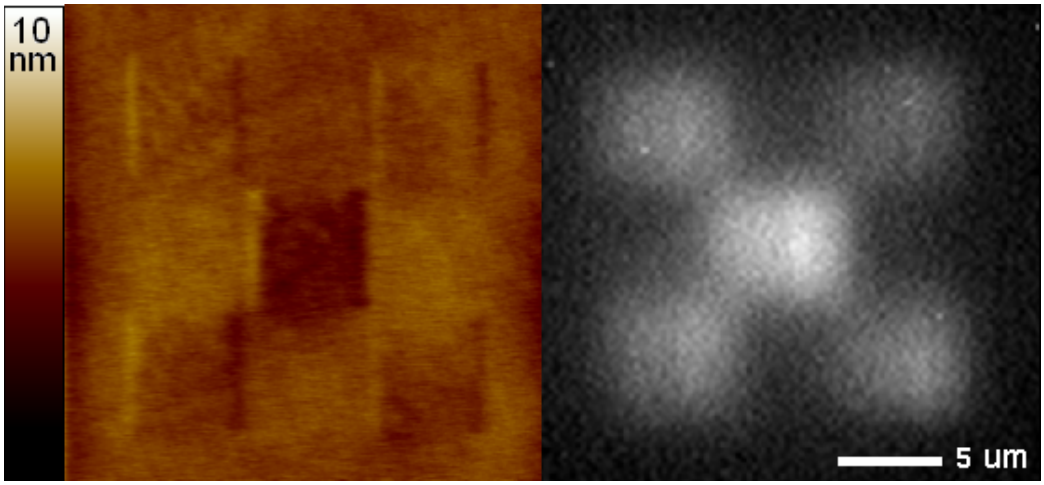
3. Results

Ellipsometry results indicated that a silane layer averaging 0.5 nm consistently adhered to the glass slide when the procedure was strictly followed. A Poly-L-Lysine layer of approximately 2 nm likewise adhered to the silane layer.

AFM topography data indicated that large scribed regions had a difference in height roughly matching expected values from the data collected with ellipsometry.

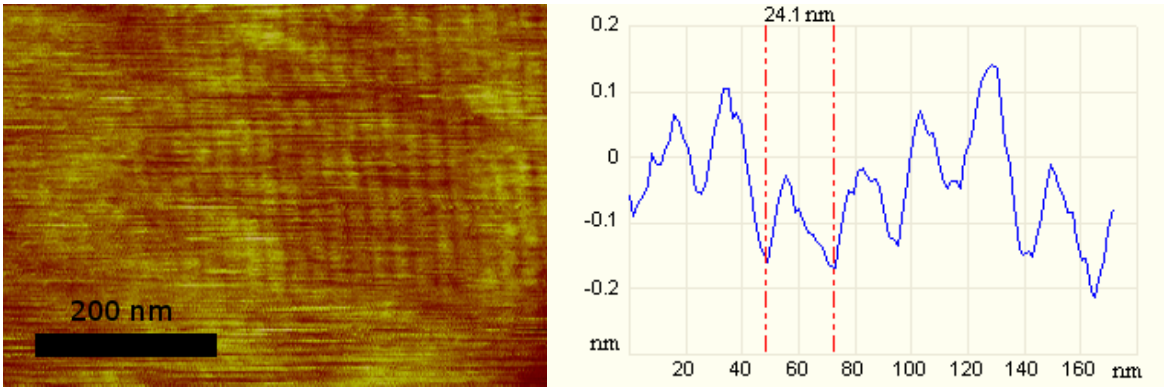
Patterns of various shapes and sizes found with fluorescence microscopy were identical to the AFM topography images. This indicates that the scribed regions were chemically functional. See Figure 4.

Lines with a pitch of 24 nm were observed, matching the expected value for dividing a 3 μm box into 128 lines (given a small amount of thermal drift during the scribing process). See Figure 5.



AFM topography image Fluorescence microscopy image

Figure 4: Composite image verifying chemical differentiation



AFM topography image | Corresponding section cut

Figure 5: High-resolution lines with a pitch of 24 nm

The lines in Figure 5 were created by scanning the AFM 128 times in a 3 μm area, but that square was itself only the lower-left corner of a die pattern. Before taking the data that produced Figure 5, a large square region enclosing all five boxes was first imaged in tapping-mode. Then a region enclosing the entire lower-left box was imaged at 45°. Finally, a section of the lower-left box was imaged at 90° in high resolution to show the lines in detail. After all the tapping-mode AFM data was taken, the sample was exposed to the fluorescently tagged polymer, and the image shown as Figure 6 was taken.

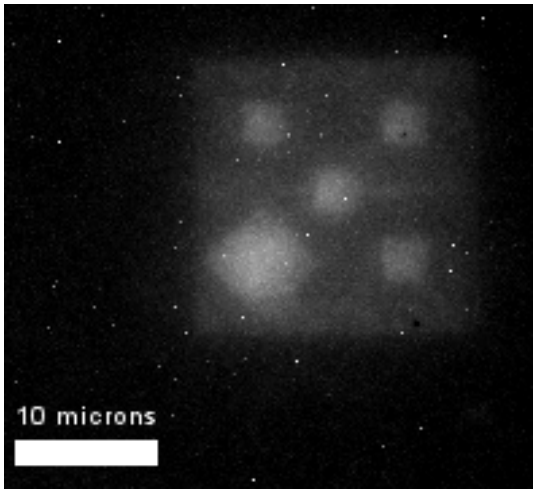


Figure 6: Fluorescence microscopy image corresponding to Figure 5

4. Discussion

4.1 Bio-friendly

The scribing and patterning can be performed in air or in aqueous solution, providing the capability to keep most biological molecules in their native environment during the patterning process. This avoids the harsh conditions of drying or extreme pH often imposed in other patterning techniques, and provides the capability to control the concentration and other conditions of the biomolecules in solution.

4.2 Non-conducting substrate

This work was performed on borosilicate glass slides, which allows for easy fluorescence measurements and has the same basic surface chemistry as silicon dioxide. While the patterning technique was not explicitly tried on a silicon wafer, it is highly expected that it would work just as well as it would with the glass surfaces.

4.3 Low scribing forces

While scribing was done with a set force of 0.3 μN, Figure 6 indicates that even the light tapping-mode forces were sufficient to partially scribe some of the polymer layer away. This indicates that even lower forces may be possible. Low scribing force is critical in controlling tip wear, which adversely affects patterning resolution since the minimum pattern size is only limited by probe-substrate contact area.

4.4 Stability

No instabilities in the created patterns were noted during observation periods of several months. The high stability of the patterns is likely due to the nature of the polymer chains: though individual molecules may naturally become loose from the

surface, they remain tethered to the surface through the polymer backbone. It is similar to a chain-link fence: even if several links are broken here and there, the fence nonetheless retains its shape and integrity due to its thorough cross-linking.

Continuing this analogy, it is interesting to note that a hole in a chain-link fence is easily cut by a few easy snips in a deliberate pattern, just as the polymer is removed by deliberate use of an AFM tip. To summarize, though each link itself is very weak and a hole is easily cut (low scribing forces), the remaining part of the chain-link fence is still strong despite the hole in it (enhanced stability).

4.5 Complementary electric polarization

The technique described thus far only allows patterning of polymers with a positive electric charge due to the negatively-charged carboxyl groups of the silane layer. Preliminary work has been done using an Aminopropyltriethoxysilane (APTES) layer on natively-grown silicon dioxide, using Polystyrene Sulfonate (PSS) as the complementary negatively-charged polymer layer. Results indicate that scribing in air produced only partial nanoshaving, though scribing in aqueous solution seemed to work well. Recently, data demonstrating chemical differentiation was taken. This complementary system would allow any charged polymer to be patterned, provided that it does not unselectively adsorb to the barrier polymer layer as well as to the exposed silane layer underneath.

4.6 Advantages of polymers

Polymers are well suited for patterned chemical surfaces because of "the diversity of existing synthetic and biological polymers, and the ability to 'design' new types of polymers"[7] – polymers can be custom-chosen to suit a wide range of needs.

5. Conclusion

We have demonstrated nanoshaving and nanografting with an aqueous polymer. Nanoshaving was characterized by tapping mode AFM and the presence of adsorbed polymers was verified by optical fluorescence microscopy. Patterns of boxes and lines were generated. High resolution patterning of lines and spaces with half pitch features of 12 nm were observed with AFM height and phase mode data. Scribing forces were measured as low as 0.3 μN. Patterns were stable over the course of several months.

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Appendix A

Standard Operating Procedure: Carboxyl Silane Deposition

1. Materials:
    a. Clean vials with caps (I usually use scintillating vials)
    b. 1 plastic and 1 (cleaned) glass petri dish, with lids
    c. Syringe or other large volume measuring thing
    d. pH paper (4-7ish range)
    e. Micropipette and tips
    f. Blow dryer (N_2)
    g. Piranha cleaning stuff
    h. Glass or silicon chips
    i. Deionized H_2O (from Millipore tap) or HPLC water (from Chem. Stockroom)
    j. Carboxyethylsilanetriol (25% salt in water) - Gelest Inc.
    k. Acetic Acid (I used glacial - Mallinckrodt Inc. (from Chem Stockroom))
    l. (optional) Poly-L-Lysine, 0.1% (w/v), aqueous - Ted Pella, Inc.

2. Make 1% silane solution: (this is scaled for a dip bath enough to coat 10 chips)
    a. Place a clean vial on a scale and zero it.
    b. Use the syringe to add 0.8g (800mg) silane to the vial.
    c. Add 19.2g deionized or HPLC water to vial.
    d. Using the micropipette, titrate the solution to 5.5 < pH 6.0 with acetic acid.

    Note: If you are using glacial acetic acid, it is so concentrated that the titration only requires 50-60 **micro**liters or less. A good tip for titrations in general is to only add half as much acid as you think you need and check the pH, repeating until the pH is in range. Use a new pipette tip to check the pH each time as any residual acid left in the tip will make the reading much lower than it should be.

3. Silane deposition:

    a. Piranha clean a few pieces of glass or silicon.

        Note: You can find a good piranha cleaning SOP taped to the fume hood in Dr. Wooley's lab if you need it.

        Note: Piranha cleaning is essential to hydroxylate the surface to prepare silanol groups to form good covalent bonds. Plasma cleaning won't cut it.

    b. Divide the silane solution into the glass vials (one per chip to be coated).

    c. Rinse the chips in deionized or HPLC water then put them in the jars with the silane and screw the lid on tightly. Immerse them overnight at 70 °C.

    d. Rinse the chips in deionized or HPLC water then dry in the N_2 stream.

    e. To cure, put them in the glass dish in the oven at 100°C for 30 min.

4. Poly-L-Lysine deposition (optional):

    a. Remove the chips from the glass dish (after cooling) and put them in a room temperature petri dish to prevent evaporation of the polymer solution.

    b. Immerse the chips in a polylysine bath for 10 min.

        Note: Alternatively, you can just wet the surface with enough drops to cover it to save solution. Just don’t let it evaporate! Evaporation causes dirty samples.

    c. Rinse the chips in deionized or HPLC water then dry in the N_2 stream.