Biomolecular Patterning via Photocatalytic Lithography

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ABSTRACT

We have developed a novel method for patterning surface chemistry: Photocatalytic Lithography. This technique relies on inexpensive stamp materials and light; it does not necessitate mass transport or specified substrates, and the wavelength of light should not limit feature resolution. We have demonstrated the utility of this technique through the patterning of proteins, single cells and bacteria.

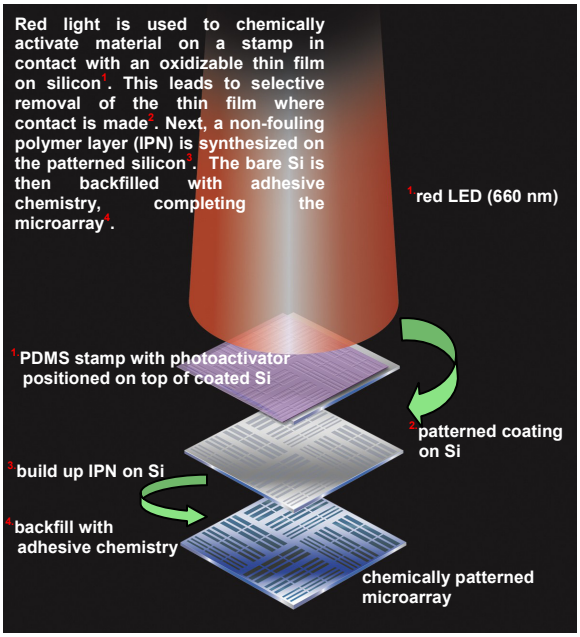
Keywords: microarray, surface modification, lithography

1 INTRODUCTION

Deterministic collection and organization of proteins, DNA and cells into ordered arrays at surfaces holds enormous potential for materials science, synthetic chemistry, biology and medicine. Our research investigates the development of novel hybrid materials, as well as the development of innovative photocatalytic techniques for patterning surfaces. Our long term goal is to pattern surface arrays having nanometer-scale features quickly and inexpensively.

2 METHODS AND MATERIALS

Silicon and glass substrates were cleaned and modified with a silane base layer of a non-fouling coating; clean gold substrates were modified directly with non-fouling polyethylene glycol (PEG) thiol-based coatings[1]. Next, photocatalyst, deposited on a stamp, was selectively positioned on top of the coatings. Controlled patterning and removal of material then was achieved by local oxidation via activation of the photocatalyst with 660nm red LED light for a few seconds (Figure 1). On silicon or glass, a non-fouling coating of P(AAm-*co*-EG)[2], an interpenetrating network (IPN) chemistry, was then built up on the regions that retained the base chemical layer. Freshly patterned (bare) regions of silicon and glass substrates then were modified with adhesive silanes. Gold substrates conveyed non-fouling and adhesive regions immediately after the initial photocatalytic patterning step.



Materials and substrates were first characterized by microdroplet experiments (Figure2). Briefly, optical images of substrates were collected with a Nikon D100 camera mounted on a Nikon Labophot 2 microscope. The substrates were then exposed to a stream of water vapor and imaged again. Ultraviolet / Visible Absorption Spectroscopy (UV-VIS) was used to identify appropriate photocatalysts that would activate at 660 nm. Time-of-flight Secondary Ion Mass Spectrometry (Tof-SIMS, Figure 3) confirmed chemical patterning and Atomic Force Microscopy (AFM, Figure 4,5) conveyed topographic, deflection, as well as friction based measurements.

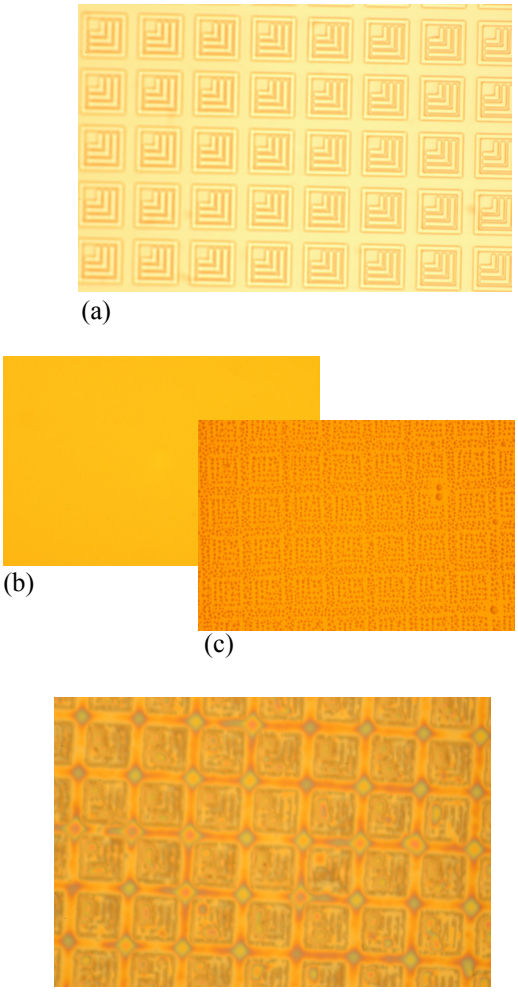
To biologically test chemically patterned silicon, surfaces were exposed to fluorescently-tagged protein, FITC-NeutrAvidin (Figure 6) or HeLa cells (Figure 7). Adhesion of the single cells and proteins was limited to regions of the adhesive chemistry, as confirmed through optical microscopy or fluorescence. Some experiments tested the ability of arrayed cells to be transfected with green fluorescent protein (GFP, Figure 8).

Presently, we are pursuing patterning at the nanoscale (smaller than the wavelength of light) via patterning of individual bacterium (prokaryotic cells) on substrates. We plan to array individual rod shaped bacteria (approximately 1 micron x 100 nanometers) in a high throughput manner. Experimental goals include analyzing bacterial expression

as a function of culture conditions, gene silencing experiments and nano-SIMS experiments.

To begin this work, we have started with larger (10 μm x 10 μm) patches of adhesive chemistry on which to covalently tether e-coli, surrounded by a non-fouling chemical matrix (Figure 9).

3 RESULTS AND DISCUSSION



(a)

(b)

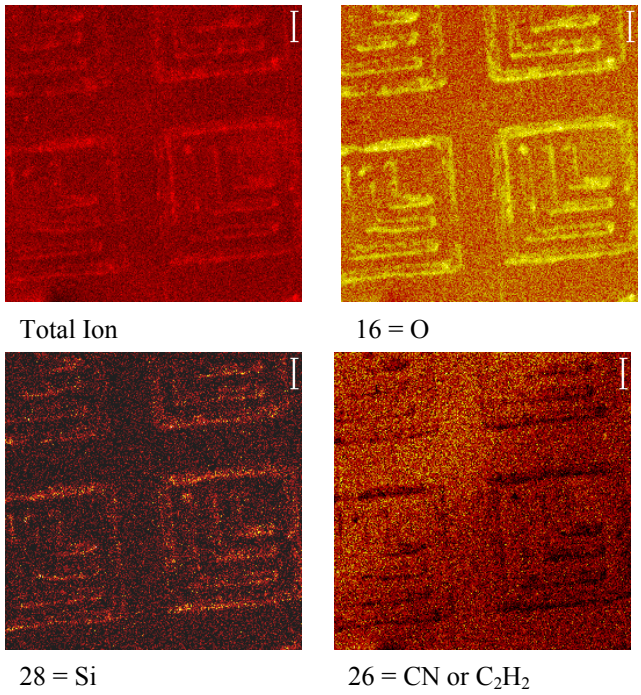
(c)

(d)

Figure 2. Optical image of stamp pattern bearing LLNL logo (a); optical image of Au substrate patterned photocatalytically with PEG thiol (chemistry is too thin to visualize) (b); optical image of same substrate as in (b) exposed to water vapor to reveal chemical pattern via variation in surface energy of different regions (c); optical image of silicon substrate patterned photocatalytically showing non-fouling IPN matrix[1] and adhesive regions (darker Ls). Line width of each L is 4 μm.

In Figure 2, an optical image of the stamp pattern bearing the LLNL logo used to demonstrate photocatalytic patterning is shown. A PEG thiol patterned gold substrate is then shown, before and after exposure to water vapor. Such microdroplet experiments are useful for conveying differences in surface energy of thin, contrasting chemical layers; in this case hydrophilic (PEG), hydrophobic (Au). Finally, a chemically patterned silicon substrate is shown after exposure to water vapor. In this case, the two chemistries used are both hydrophilic, so droplets do not condense on the surface and optical clarity of the image is enhanced.

A Trift III Time-of –flight secondary ion mass spectroscopy (Tof-SIMS) was employed to collect both positive and negative scans of a chemically patterned silicon substrate. A thin layer of acrylamide (AAm, ~15 nm) was grafted to the matrix of the silicon; the L regions were bare SiO_2. Figure 3 shows a few peaks from the negative fragment Tof-SIMS spectra.



Total Ion

16 = O

28 = Si

26 = CN or C_2H_2

Figure 3. Tof-SIMS conveys chemical proof of concept for photocatalytic oxidation (m/z neg. fragment spectra shown): AAm chemistry on silicon rich in hydrocarbons and nitrogen (matrix); return to bare SiO_2 on patterned Ls where photocatalytic oxidation took place. Scale bars are 10 μm.

A Digital Instruments Veeco Metrology, Dimension 3100 Atomic Force Microscope (AFM) was used to characterize topography and friction response of silicon and gold substrates, respectively, that underwent photocatalytic patterning. Figure 4 shows the same substrate consisting of an AAm matrix contrasted with SiO_2 L regions from Figure 3. Figure 5 reveals the friction response of a photocatalytically patterned PEG thiol substrate, as seen in Figure 2 b and c. Viewing past the diagonal warble lines of the AFM image, one can see that the hydrophilic PEG thiol

matrix appears lighter, while the hydrophobic gold L regions appear darker.

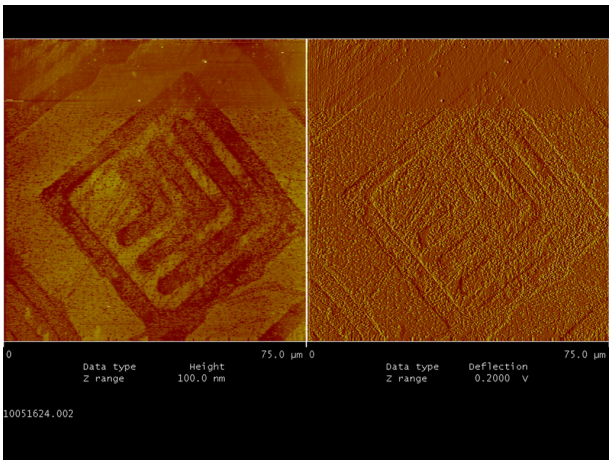


Figure 4. AFM height (left) and deflection (right) data taken on same patterned silicon sample shown in Figure 3.

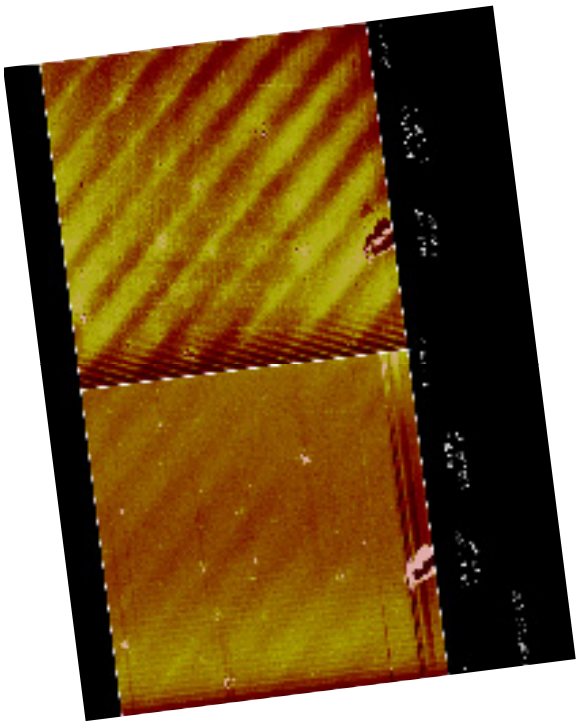


Figure 5. AFM friction (top) and height (bottom) data from gold substrate coated with PEG thiol and photocatalytically patterned. Friction image reveals difference in surface energy between PEG (matrix) regions and Au (L) regions. Note that the PEG thiol is so short (~1 nm) that it is difficult to detect in height image.

Biological surface testing commenced with protein adsorption experiments. A stamp bearing the LLNL logo was used to photocatalytically pattern a silicon substrate, allowing the synthetic build-up of a non-fouling IPN[2] matrix, followed by subsequently back-fill with aminopropylsilane (APS) onto the L regions. Following incubation with an aqueous solution of fluorescein-labeled Neutravidin, surfaces were rinsed in buffer solution and then imaged on a Zeiss Axiovert fluorescence microscope. Protein attached to adhesive L areas and was repelled by the IPN (Figure 6).

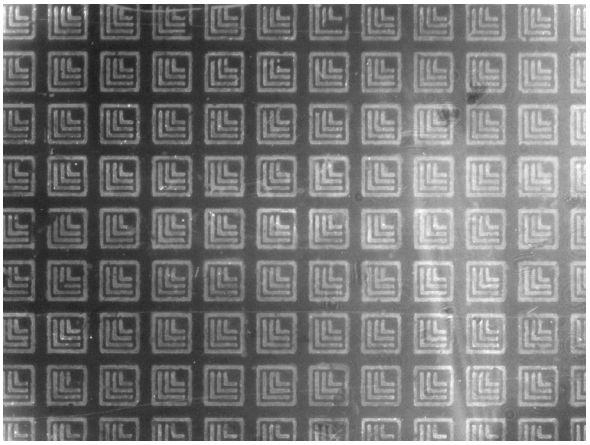


Figure 6. Fluorescence micrograph showing selective protein adsorption to adhesive APS regions, protein is repelled by the surrounding, non-fouling IPN. Magnification = 20x; line width of L= 4 µm.

As protein adsorption drives many time of eukaryotic cell adhesion events, we next sought to pattern cell clusters and single cells on patterned substrates. Figure 7 reveals HeLa cells (previously transfected with green fluorescent protein (GFP)) patterned on IPN/APS templated surfaces from the mm scale (top), to 100 µm patches (middle), to the single cell level (bottom).

HeLa cells in their native state were also plated on patterned substrates for 24 hours and then transfected with Lipofectamine reagent, Invitrogen. The cell array was imaged via fluorescence microscopy at 18, 24 and 96 hrs post-transfection. Apart from demonstrating the ability to manipulate cells after capture on the patterned substrate, an increase in the number of transfected cells with time also indicates continued cell viability.

We plan to employ this new experimental platform to identify molecular regulators of proliferation and differentiation in individual stem cells, and determine the relative roles of intrinsic versus extrinsic signals in modulating stem cell fate commitment.

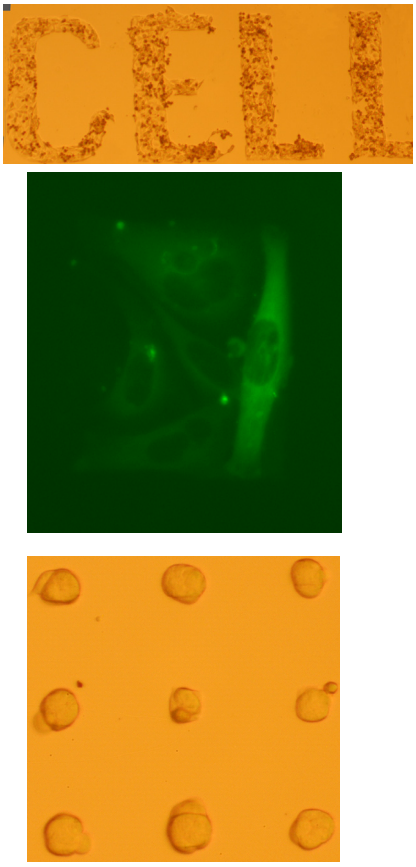


Figure 7. Reflectance images of HeLa cells plated on chemically patterned microarray: top image 1.5x, middle image, 20x, GFP-Tubulin transfected HeLa cells adhering to 100 μm square island, lower image 30 μm circles.

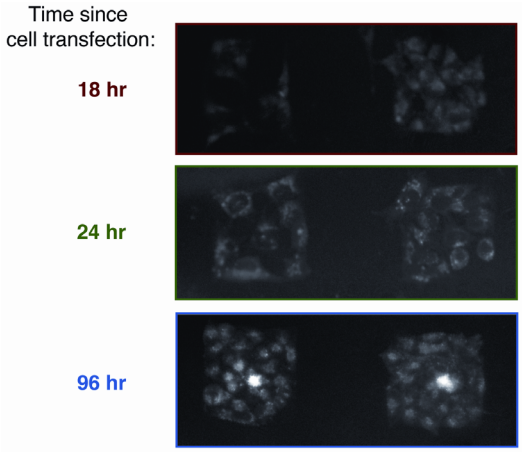


Figure 8. HeLa cells were arrayed on a patterned silicon substrate (back-filled with APS) for 24 hrs prior to aided transfection. Arrayed cells incorporated (GFP)-tubulin vectors over time. All micrographs show 100 μm square islands at 20x magnification.

To begin work in arraying prokaryotic cells, a stamp with 10 μm x 10 μm patches was used to pattern silicon substrates with the same IPN/APS chemistry used to pattern cells. Additional tethers linked to fluorescent protein were linked to the APS regions and then e-coli, washed in PBS, were covalently coupled to the tethers (Figure 9). Although a fluorescent protein layer was not critical to the attachment of bacteria, it aided in identifying target binding regions.

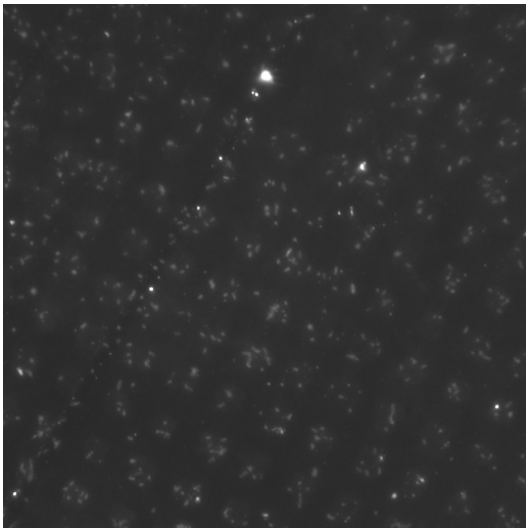


Figure 9. E coli bound to silicon substrate on adhesive, fluorescent protein-based square islands (10 μm x 10 μm); background is non-fouling IPN.

Initial data suggests that Photocatalytic Lithography may overcome resolution limitations inherent to traditional photolithography and allow rapid lithographic processing with inexpensive optic systems and substrates. The technique also avoids pinholes that may form when patterning via mass transport bonding of chemistry to a surface. Furthermore, Photocatalytic Lithography is not substrate or chemistry dependent, and enables the study of biological functions interfacing with synthetic materials.

4 CONCLUSION

Photocatalytic lithography enables deterministic collection and organization of proteins, eukaryotic and prokaryotic cells into ordered arrays on surfaces.

5 ACKNOWLEDGEMENTS

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