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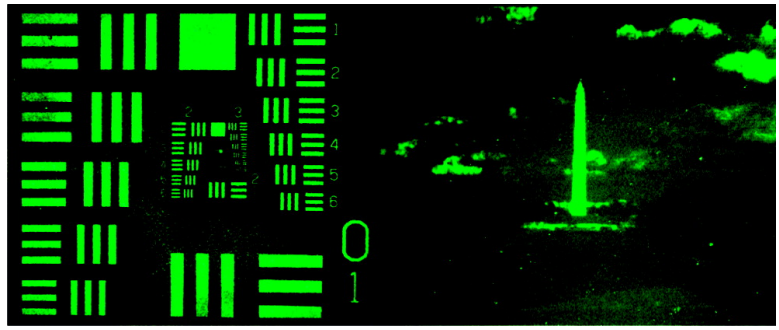
J. Am. Chem. Soc., **2008**, 130 (41), 13512-13513 • DOI: 10.1021/ja803999r • Publication Date (Web): 19 September 2008



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Published on Web 09/19/2008

Binary and Gray-Scale Patterning of Chemical Functionality on Polymer Films

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The ability to pattern chemical functionality on surfaces, essential for the fabrication of many optical, mechanical, and fluidic microdevices, is also important for addressing biological issues that include cell motility, chemical signaling, cell growth and aggregation and for biomedical applications such as tissue scaffolding. A number of methods exist for creating binary (on/off) patterns of chemical functionality on surfaces, but in many instances it is desirable to be able to create gray-scale patterns of functionalization with high dynamic range. Most approaches for gray-scale patterning of functionality rely upon effects such as diffusion to create simple gradient patterns.[1−6] Other methods can create more sophisticated patterns,[7] but generally not with high resolution and dynamic range. One exception is a technique developed recently by Bar-Ziv and co-workers[8] that is based on the creation of a self-assembled monolayer on glass using a specially synthesized molecule with photouncageable amine groups.

We have previously demonstrated that 3-D, acrylic polymer structures created with multiphoton absorption polymerization[9] (MAP) can be functionalized with amines.[10] The surfaces of structures created with MAP receive less light exposure than do the interior regions. Thus, if multifunctional acrylic monomers are used, then the surfaces include unreacted acrylate groups. Each surface acrylate can undergo Michael addition with ethylene diamine, leaving a free amine group on the polymer surface. Here we introduce a simple strategy, based on such Michael addition chemistry, for creating biocompatible, gray-scale amine-patterned surfaces with high resolution and dynamic range. Our technique is rapid, uses inexpensive, commercially available components, and avoids the use of photolabile protecting groups.

In bulk photopolymerization, the surfaces of the sample receive as much light exposure as does the interior. However, if a surface is exposed to a radical quencher such as oxygen during polymerization, then the radical chain reaction will proceed less efficiently there, potentially leaving unreacted acrylate groups. The surface density of unreacted acrylates in any region of the surface will depend upon the total light exposure, providing a means for photolithographic patterning of chemical functionality.

Our scheme for patterning of functionalization is illustrated in Figure 1. The substrates were glass microscope coverslips functionalized with (3-acryloxypropyl)trimethoxysilane to promote

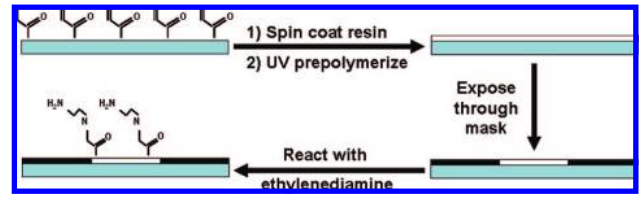

Figure 1. Strategy for binary and gray-scale patterning of amines on the surface of acrylic polymer films. The black regions of the polymer film represent areas that have received higher UV exposure.

adhesion. The prepolymer resin used was composed of 54 wt % dipentaerythritol pentaacrylate (Sartomer), 43 wt % tris(2-hydroxyethyl)isocyanurate triacrylate (Sartomer), and 3 wt % Lucirin TPO-L photoinitiator (BASF). Several drops of resin were spin coated on the substrate at 1500 rpm for 1 min to create a thin film. For ease of handling, the resin was prepolymerized with 365 nm light at an intensity of 10 mW/cm^2 for 10 s.

Photolithographic patterning can be accomplished with either a contact mask or a projection mask; here we present results obtained with the former, but both approaches yield equally good results. We have employed both commercial, reflective contact masks and absorptive contact masks created on laser printer transparencies. A contact mask was placed against the exposed surface of the prepolymerized sample, and the sample was UV cured for 45 s. Following exposure the mask was removed and the sample was immersed in a 20% v/v solution of ethylene diamine in water for 10 min. The sample was then washed three times in water for 5 min each.

X-ray photoelectron spectroscopy studies on unpatterned surfaces revealed a maximum surface density of about one primary amine per 2 to 3 nm^2. To assess the degree of surface functionalization on patterned samples we reacted them with dansyl chloride, which becomes strongly fluorescent after reaction with a primary amine. The dansyl chloride solution was prepared by adding 1 mL of dimethylformamide and 12.5 μL of diisopropylethylamine to 18 mg of dansyl chloride. The polymer sample was immersed in this solution for 2 min, and then it was rinsed with methanol.

Shown in Figure 2a is a fluorescent image of a binary pattern created with a reflective Air Force test pattern mask. The contrast between bright and dark regions in the pattern is excellent, with a signal-to-noise ratio greater than 100. The finest features in the pattern that are well resolved have a width of 10 μm, demonstrating the high resolution attainable.

Shown in Figure 2b is a gray-scale image of the Washington Monument created using a laser-printed mask. Mask regions of different optical density are well resolved in the image, as are fine

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10.1021/ja803999r CCC: $40.75

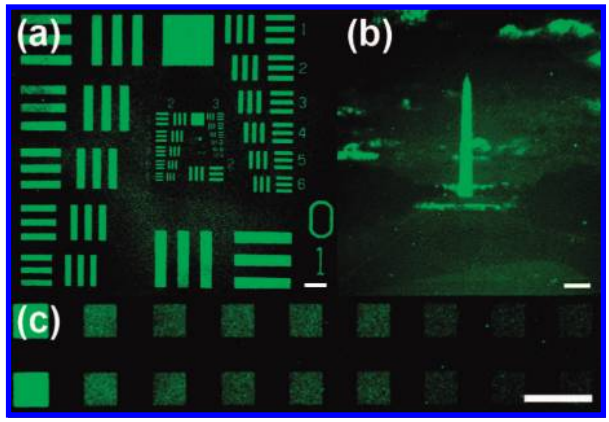

Figure 2. Representative fluorescent images of photolithographically patterned polymer surfaces: (a) dansyl chloride patterned with a binary Air Force test mask; (b) dansyl chloride patterned with a gray-scale image mask; (c) dansyl chloride patterned with a gray-scale mask. The mask transmission ranges from less than 1% for the squares on the far left to 71% for the squares on the far right. All scale bars are 1 mm.

features. In Figure 2c we show a pattern created with a reflective, gray-scale test mask in which consecutive panels have increasing transmission. The large variation in intensity going across the image demonstrates the dynamic range of this technique, which exceeds 20 dB (greater than that of color photographic prints).

To assess the biocompatibility of the amine-functionalized polymer, we employed the ameboid *Dictyostelium discoideum*, a model cell line used for the study of cellular motility.[11] *D. discoideum* cells do not feature integrins, which require specific surface chemistry and mechanics to generate strong focal adhesion complexes. Instead, cells propel themselves over surfaces via weaker, nonspecific interaction between cell and surface.

Figure 3a shows *D. discoideum* migrating and aggregating on gray-scale, amine-functionalized surfaces that were patterned with the mask used in Figure 2c. The cell speeds on amine-functionalized and native polymer surfaces are similar to those measured on glass. The cell migration and aggregation patterns are independent of surface amine density and are unaffected by boundaries between regions of different amine density (Figure 3b). As *D. discoideum* is a commonly used model cell line, our technique creates patterned surfaces that are also likely to be compatible with many cell types.

Since amine patterned surfaces themselves appear not to affect cell function, surface functionalization with biomolecules holds promise for generation of targeted interactions with cells. To demonstrate the capability for patterning biomolecules with our technique, we have synthesized peptides on amine-functionalized surfaces. Tripeptides composed of asparagine are known to bind Rhodamine B selectively from aqueous solutions.[12] We synthesized acetyl-capped, NNN-tripeptides with trityl-protected side chain amines using a protocol described previously.[13] Samples were then immersed in a dilute, aqueous solution of Rhodamine B for 60 h with agitation. The samples were washed with water and dried before performing fluorescence imaging. As can be seen in Figure 3c, binary patterning of these peptides can be performed with resolution comparable to that achieved in the dansyl chloride experiments. The gray-scale Washington Monument pattern shown in Figure 3d is also of comparable quality to the results obtained with dansyl chloride. As a control experiment, the amines on patterned substrates were capped with acetyl groups and exposed to the Rhodamine B solution. No fluorescent patterns were present on these samples.

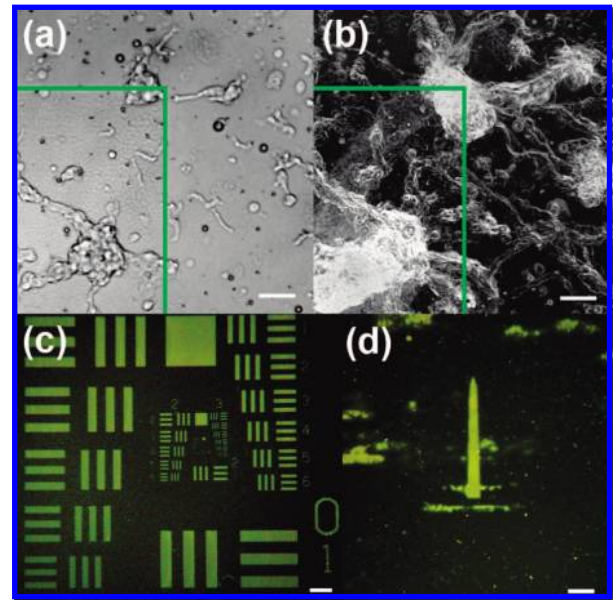

Figure 3. (a) and (b), *D. discoideum* cells aggregating on gray-scale, amine-functionalized patterned surfaces. The area bounded by green lines on the bottom left has a higher density of surface amines. (a) Snapshot of cells midway through aggregation. Both individual cells and cell aggregates are visible. (b) Time averaged image illustrates that cell tracks (lines) do not change significantly with the density of surface amines. (c) Rhodamine B bound to a tripeptide synthesized on a binary Air Force test mask pattern. (d) Rhodamine B bound to a tripeptide synthesized on a gray-scale image. The scale bars are 50 μm in panels a and b and 1 mm in panels c and d.

The method presented here can be extended readily to the patterning of a large variety of materials beyond peptides and fluorophores, including metals, oxides, nucleic acids, antibodies, and small molecules. By using more complex amine-containing molecules, it will also be possible to pattern different types of initial chemical functionality on the polymer surfaces.

Acknowledgment. We thank Laura Beachy for help with the *D. discoideum* experiments.

Supporting Information Available: Detailed experimental protocols, X-ray photoelectron spectroscopy data, analyses of amine density, and dynamic range. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA803999R