Hydrophilic/Hydrophobic Patterned Surfaces as Templates for DNA Arrays

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Addressable DNA arrays are created by photopatterning self-assembled monolayers to form hydrophilic and hydrophobic regions on a gold surface. The hydrophilic regions act to contain small volumes of different DNA solutions placed on them using an automated pin-tool loading strategy. This method allows for efficient attachment, manipulation, and hybridization of pure DNA strands on the surface.

I. Introduction

The rapid development and implementation of DNA arrays on solid supports in recent years have been driven by their potential for the high-throughput analysis of DNA sequences, genetic variations, and gene expression. Methods to create DNA arrays on surfaces fall into two broad categories: (1) attachment of tethered DNA strands at different sites in the array and (2) attachment of different complete strands at individual array sites.

The former approach is exemplified by work from Affymetrix, where Foder et al.1 have developed a photolithographic technique to create DNA arrays through a series of exposures through masks to control the addition of bases to specific sites on the surface. This technique has been reported to have a repetitive yield of ~0.95 per base pair attachment,2 considerably limiting the length of the oligonucleotide that can be synthesized with fidelity. Piezoelectric printing of each base followed by standard washing and deprotection for the subsequent deposition of another base onto the anchored DNA strand is a second example of base-by-base DNA synthesis on a surface. Piezoelectric printing has a reaction success rate of ~0.99, similar to controlled pore glass (CPG) synthesis, a common traditional oligonucleotide synthesis technique. Methods of DNA strand synthesis on the surface do not allow for oligonucleotide purification after synthesis to eliminate incompletes strands or ones with an excess number of bases, considerably reducing the overall array reliability.

Shalon and Brown have created arrays using the second method, attachment of complete strands of DNA to a surface.3 A pin tool is used to load the array mechanically. The oligonucleotides can be rigorously purified before surface attachment to allow for longer strands of high fidelity, something not possible with in situ synthesis. After CPG synthesis of DNA strands, a standard cartridge filter process is used to eliminate the failed sequences. Further purification can include HPLC, ion exchange, size exclusion, or electrophoresis gel procedures to achieve highly pure DNA samples. Nanogen’s technique, electrically polarizing an array site so that the DNA sample is attracted to that site only, is another example of attachment with presynthesized DNA onto a surface. Finally, ink jet technology has also been used to print previously synthesized oligonucleotides onto an active surface.4

In these latter methods, while the attachment chemistry of the DNA strands to the surface is vital, it is the delivery system, such as the ink jet, the pin tool, or the photolithographic technique, that controls the precise placement of the oligonucleotides onto the array element. Blanchard and Hood proposed a technique in which the surface would assist in the placement, through the use of a polyfluorinated hydrophobic background and hydrophilic active areas on a silicon support. A patent on DNA microarrays, issued to ProtoGene, also demonstrates an application of chemical patterning to aid in placement with a glass support and fluorooalkylsilane chemistry.6

In this paper, we demonstrate the use of alkanethiol chemistry to create a patterned hydrophobic/hydrophilic array to confine minute volumes (100–200 nL) of DNA solutions. The alkanethiol system is a stable, well-characterized chemistry with a fast reaction rate that creates well-formed self-assembled monolayers and can be used to create a patterned surface. We create an array pattern on the surface with ultraviolet photolithography to control the placement of the different alkane thiols. Methyl-terminated alkane thiol molecules are attached to a gold film on a solid support as a hydrophobic background layer. DNA is attached to active hydrophilic regions, linked to hydrophilic alkane thiols, arranged in an array. We control the placement of the DNA onto the surface using a computer-programmable automated pin tool. The hydrophobic interaction with the aqueous DNA solutions prevents DNA attachment between the elements of the array and thus acts as a barrier for diffusion of DNA between the elements. Furthermore, any DNA that is placed on a hydrophobic region near a hydrophilic element

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diffuses into this region. With our strategy, we can attach completely synthesized and purified DNA strands to create high-purity arrays with high positional fidelity, excellent stability, and minimal cross talk between elements.

II. Experimental Section

In this section, we first describe aspects of the DNA attachment to surfaces that are general and not specific to the existence of a laterally defined pattern. We then describe the materials used in our procedure. Finally, we describe surface patterning and aspects of the attachment chemistry that are specific to the existence of a pattern.

A. Uniform Surface. (1) Overview. A glass slide or silicon wafer is prepared by evaporating on it 50 Å of chromium followed by 1000 Å of gold. 11-Mercaptoundecanoic acid (MUA), a hydrophilic alkanethiol, is attached to the surface and orders into a self-assembled monolayer (SAM).7 Poly-L-lysine hydrobromide (PL) is attached to the MUA surface.8 The sample is then covered with a solution of sulfosuccinimidyl 4-[(N-maleimidomethyl)-cyclohexane-1-carboxylate (SSMCC) followed by placement of 5'-thiol-modified DNA solutions onto the surface. The DNA reacts with the SSMCC, forms a bond, and becomes anchored to the surface.9 DNA attached onto the SSMCC areas is detected by exposing the surface to a solution of the fluorescently tagged complement of the DNA and by imaging the surface with a fluorescent imaging technique.10

(2) Surface Modification Chemistry. The gold surface is immersed in 1 mM ethanolic MUA for at least 18 h and is then covered with 1 mg/mL of PL of pH 8.0 for 30 min. An amine group of the PL reacts with a carbonylic acid group of the MUA to form an electrostatic bond.9,10 Subsequent reaction with SSMCC occurs for 30 min; during this time the remaining amine groups of the PL react with the carbonylic acid groups of the SSMCC to form a covalent bond. The surface is then ready for DNA attachment.9,10

(3) Contact Angle Measurements. To investigate the surface free energies of the several levels of the chemically modified surfaces, we measured the contact angles of water at room temperature with a model 100-00 contact angle goniometer (Ramé-Hart, Inc.). The contact angles are recorded immediately, after dispensing 10 µL droplets of water onto the surface. The reported values for the alkane-thiols are an average of 20 different measurements taken on three individually prepared samples. The MUA and MUA + PL data are for 14 measurements on each of two samples. The MUA + PL + SSMCC data are for 18 measurements each from three different samples. The results are discussed below.

(4) DNA Attachment. A glass slide or silicon wafer is chemically modified for DNA attachment as described above. A purified 1 mM aqueous solution of 5'-thiol-modified DNA is placed onto the slide in 0.8 µL droplets and allowed to react for at least 6 h in a humid chamber at room temperature, during which time the alkene of the SSMCC pyrrole group reacts with the thiol-modified DNA to tether the DNA strand to the surface at the 5' terminus. The surface is rinsed with water and soaked for 1 h in a solution of 300 mM NaCl, 20 mM sodium phosphate, 2 mM EDTA, and 6.9 mM sodium dodecyl sulfate (2X SSPE/0.2% SDS) at room temperature, after which the sample is ready for hybridization.9,10

(5) Hybridization. Hybridization to the attached DNA probe molecules is accomplished by exposure to a 2 µM solution of 5'-fluorescein-labeled target oligonucleotides in 2X SSPE/0.2% SDS buffer. A 30 µL drop of the complement solution is placed onto the sample and then spread over the entire surface by placing a clean cover slip on top of the sample. Hybridization adsorption is allowed to proceed for 30 min, after which the sample is rinsed with water and is immersed in a solution of 2X SSPE/0.2% SDS buffer without the 5'-fluorescein-labeled complements for 10 min.9,10

(6) Surface Fluorescence Measurements. We use fluorescence to detect hybridization, with a Molecular Dynamics FluorImager 575. The sample is placed face down on top of a glass scanner tray, with a 30 µL droplet of buffer between the sample and the glass tray, and then scanned at 530 nm. A signal is measured on the slide at each level of chemistry to get background and noise figures. An image of the surface is obtained.9,10

(7) Subsequent Hybridizations. After the fluorescence signal is recorded, the sample is placed in 8.3 M urea solution at 37 °C for 20 min to remove the fluorescent complement and is washed thoroughly with water. The sample is then ready for a second hybridization for the detection of another target sequence.9,10

B. Materials. The chemicals, 11-mercaptoundecanoic acid (MUA) (Aldrich), n-octadecyl mercaptan (ODM) (Aldrich), poly-L-lysine hydrobromide (PL) (Sigma), sulfo succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SSMCC) (Pierce, Biorad), and triethanolamine hydrochloride (TEA) (Sigma), were all used as received. Gold substrates were prepared by vapor deposition of 50 Å chromium followed by 1000 Å of gold onto a microscope slide cover (Fisher No. 2, 18 x 18 mm) or silicon surfaces (3 in. diameter, n-doped wafer, 1–10 Ω cm, 100, Wafer World).

Millipore filtered water was used for all aqueous solutions and for rinsing. All oligonucleotides were synthesized on an ABI DNA synthesizer at the University of Wisconsin Biotechnology Center. Glen Research 5'-thiol modifier C-6, and ABI 6-FAM were used for 5'-thiol-modified and 5'-fluorescein-modified oligonucleotides, respectively. Before use, the oligonucleotides were purified by reverse-phase binary gradient elution HPLC (Shimadzu SCL-6A). However, if the HPLC column or the injecting needle has not been sufficiently cleaned, the next DNA sample that is run through the HPLC may have some slight contamination.11 We can see this contamination if the needle has not been sufficiently cleaned. The next DNA sample that is run through the HPLC may have some slight contamination.11 We can see this contamination if the needle has not been sufficiently cleaned. The next DNA sample that is run through the HPLC may have some slight contamination.11 We can see this contamination if the needle has not been sufficiently cleaned.
For a copy of the Turbo Pascal code, contact the authors.

Figure 1. The process of patterning a surface. (a) A solid support coated with gold is placed into a solution of 11-mercaptooundecanoic acid (MUA) for at least 18 h. The surface is subsequently covered with poly-L-lysine (PL), which binds to the MUA, creating a hydrophilic surface. (b) When the MUA–PL surface is exposed to UV light through a quartz mask, the gold–sulfur bond between the MUA and the gold surface is oxidized. (c) Those regions where the UV light was blocked by the mask are preserved. Rinsing the surface completely removes the MUA–PL from the gold surface in the exposed areas. (d) n-Octadecyl mercaptan (ODM) fills the bare gold regions and transforms the exposed areas into hydrophobic regions. (e) The surface is then reacted with sulfosuccinimidyl 4-(N-maleimido-1-carboxyate) and finally with 5’-thiol-modified DNA. The SSMCC reacts only with the MUA–PL regions of the surface, not to the ODM areas, and the 5’-thiol-modified DNA only attaches to the MUA–SSMCC sites. The ODM regions are hydrophobic while the MUA–PL–SSMCC areas are hydrophilic. The DNA solutions are confined to the specific hydrophilic regions.

Table 1. Contact Angles of Water on a Gold Surface That Has Been Chemically Modified

<table>
<thead>
<tr>
<th>Surface</th>
<th>Contact Angle (°)</th>
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<tbody>
<tr>
<td>1. 11-mercaptooundecanoic acid (MUA)</td>
<td>58.1 ± 3.54°</td>
</tr>
<tr>
<td>2. MUA + poly-L-lysine (PL)</td>
<td>34.3 ± 2.44°</td>
</tr>
<tr>
<td>3. MUA + PL + SSMCC</td>
<td>41.5 ± 1.54°</td>
</tr>
<tr>
<td>4. n-octadecyl mercaptan (ODM)</td>
<td>91.8 ± 1.75°</td>
</tr>
</tbody>
</table>

a (1) A bare gold surface exposed to a solution of 11-mercaptooundecanoic acid (MUA) for 18 h, and after rinsing with water and ethanol. (2) Further exposure of the surface to a solution of poly-L-lysine (PL) for 30 min. (3) Further exposure of the surface to sulfosuccinimidyl 4-(N-maleimido-1-carboxylate) and finally with 5’-thiol-modified DNA. The SSMCC reacts only with the MUA–PL regions of the surface, not to the ODM areas, and the 5’-thiol-modified DNA only attaches to the SSMCC sites. The ODM regions are hydrophobic while the MUA–PL–SSMCC areas are hydrophilic. The DNA solutions are confined to the specific hydrophilic regions.


(13) For a copy of the Turbo Pascal code, contact the authors.
Controls, 102 mm; z, 5000 series Daedal Positioning Systems and Controls, 56 mm). To transfer the DNA solution onto the sample, the robot manipulates a 0.5 mm mechanical pencil tip and places it in a microtiter plate well and ~0.5 μL of solution is transferred into the tip. After depositing ~100–200 μL of solution through capillary action by touching the tip onto a hydrophilic portion on the sample, the robot rinses the tip for 10 s in a water bath, dries it in a nitrogen gas (N₂(g)) stream for 10 s, and then touches the tip into another DNA solution on a microtiter plate to repeat the process.

Finding an absolute home, with a magnetic marker without relying on the mechanical motion of the robot and before placement of a DNA solution onto the patterned surface, allows the robot to perform precise, repeatable motions. The smallest step size within the robot's capability is specified to be ~0.2 μm, but absolute precision is approximately ~50 μm, likely due to backlash in the mechanical motion. The positioning capabilities of the robot used in these investigations limited the array density and size that we were able to load.

(4) Hybridization. The process used for hybridization on a patterned surface is similar to hybridization on an unpatterned surface, as described above. The surface is flooded with a solution of complementary fluorescently labeled DNA strands and then covered with a clean cover slip. The hydrophobic regions repel the solution and do not allow it to spread homogeneously over the surface. After a reaction time of 30 min, the slide is immersed in 2X SSPE/0.2% SDS at 37 °C for 5 min, and then the step is repeated with fresh SSPE/0.2% SDS at 37 °C, which removes any nonspecifically bound fluorescent DNA solution.

(5) Surface Fluorescence Measurements. A patterned surface is treated exactly the same as an unpatterned surface in fluorescence measurements. The resolution is limited by the CCD camera pixel size, and as a consequence, the highest resolution of the FluorImager is 100 μm x 100 μm. While our smallest feature in the present data is much larger than this limit, we do see some pixelation in the images.

III. Results

The premise of using a hydrophilic/hydrophobic patterned surface to create arrays is that the chemical patterning aids to confine small quantities of the DNA solutions to prescribed areas, from which they do not bleed to contaminate other areas. The success of this premise depends on the degree to which a particular DNA solution prefers to go to and stay on the specific element onto which it is placed. This preference depends on surface free energy differences between the hydrophilic and hydrophobic regions. As we decrease the array element size, the magnitude of the difference in the surface free energies of the hydrophilic and hydrophobic regions becomes increasingly important.

Using contact angles, we can determine the difference in interfacial free energies between the surface/air interface and the surface/water interface from Young's equation:

\[ \gamma_{LV} \cos \Theta = \gamma_{SV} - \gamma_{SL} \] (1)

where \( \gamma_{LV} \), \( \gamma_{SV} \), and \( \gamma_{SL} \) are the specific interfacial free energies for the water (solution)/air, surface/air, and surface/water interfaces at 25 °C, respectively. The value for \( \gamma_{LV} \) is 71.97 dyn/cm;\(^{14} \) using the measured contact angles, the difference of \( \gamma_{SV} - \gamma_{SL} \) is 53.9 dyn/cm for the hydrophilic MUA + PL + SSMCC surface and ~2.25 dyn/cm for the ODM surface. As already shown, an aqueous solution on ODM will have a tendency to bead up while an aqueous solution on an SSMCC surface will wet. We exploit these differences with our patterned surface. For a feature size of 500 μm, the hydrophilic and hydrophobic areas can be seen with the unaided eye in a humid environment when the surface is cooled. The larger the differences in \( \gamma_{SV} - \gamma_{SL} \) for the two array surfaces (hydrophilic/hydrophobic), the greater the driving force for the solution to remain on the hydrophilic region.

We have fabricated and successfully loaded a 7 x 7 array of 500 μm x 500 μm squares. Figure 2 shows that DNA attaches on the energetically favorable regions. We see a well-formed array with discrete array sites. Loading the entire array with the same DNA sequence as in Figure 2 illustrates the effectiveness of the hydrophobic matrix and hydrophilic sites in containing DNA to regular sites without bleeding. The DNA sequence is 31 base pairs long, synthesized via CPG, and subsequently purified. For comparison, a 31 base pair long strand synthesized in situ would have a 20% fidelity.

The image in Figure 2 is plotted using arbitrary fluorescence units. A value of 0 is a scan without a sample and a signal of 10 000 is the saturation limit of the detector. A scan of a sample with a MUA surface has an average signal of 158 ± 11 units, a MUA + PL surface has an average signal of 158 ± 13, and a MUA + PL + SSMCC surface has an average signal of 157 ± 12, indicating that for all purposes these surfaces are identical. For a patterned surface, there is no discernible difference in the fluorescence reading between the hydrophilic areas and the hydrophobic background even though the pattern is easily detectable by washing the sample with water or by cooling the surface in a humid environment. A scan of a patterned sample shows an average signal of 155 ± 13. The samples with single-stranded DNA without fluorophores attached to the patterned surface produce a signal of 158 ± 11 units. The intensity for a sample with the fluorescent complement is usually over 1000 ± 150. In Figure 2, this background signal of 155 has been subtracted. The error in this background, ±13, is, of course, maintained in the signal that we show but is negligible compared to the magnitude of the signal and to the other sources of error described below.

DNA solutions placed on the array elements remain confined to those sites and do not spread into adjacent array elements. A proof-of-principle experiment is illustrated in Figure 3. Two different DNA sequences, A and B (Figure 3e), have been placed on adjacent 500 μm squares in the arrangement shown in Figure 3a. After hybridization with both complementary sequences, all array sites on which DNA sequences had initially been placed fluoresce (Figure 3b). When the surface is hybridized with only one of the complementary sequences, signal is detected only from the corresponding location of that sequence in the array (Figure 3c,d).

Figure 3 shows that we occasionally see a signal for elements in which we did not deliberately place any DNA.

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The signal is never higher than 20% of the minimum signal for a correct site. There are two possible reasons for the existence of these stray signals. One, inaccuracies in the loading procedure may have caused solutions to run to adjacent elements. All elements with adventitious signal lie next to ones in which we have deliberately placed DNA solution. Our basic premise is thus sound. This first reason, however, cannot be the complete cause, because if it were, all elements showing a signal in Figure 3b should show a signal in either panel c or d of Figure 3. They do not. The second possibility is that the DNA solutions we loaded in the array are not entirely pure. If A contained some B, then one would observe an A signal in the sites nominally containing B. If B contained some A, then the reverse would be true. If either were true, we should consistently observe the error on all corresponding sites in panels c and d of Figure 3. We do not. The fact that all adventitious elements lie next to a deliberately loaded element suggests that some combination of the above causes must apply. We cannot, at this stage, determine which and thus conclude that our present experiments have a maximum uncertainty of 20% of the minimum real signal and as little as 8–10% of the average signal.
the array and simple modifications in the computer-controlled automated pin tool to place DNA solution in the array in a variety of different arrangements. The achievable array density is presently limited by the quality of the mechanical loading tool, but arrays with pixel sizes of the order of 50 \( \mu \)m are easily foreseeable, making a 1000 element array in 1 cm\(^2\) an achievable target. The application of such patterned surfaces is not limited to DNA arrays but can be modified to create a surface with a variety of terminal groups separated by an inert background for surface chemistry experimentation.

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