

Low-Contact-Angle Polydimethyl Siloxane (PDMS) Membranes for Fabricating Micro-Bioarrays

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Abstract - In efforts to design improved biological microarrays, we use a polydimethyl siloxane (PDMS) membrane[1] as a template that allows biological solutions of interest to interact with the surface only at discrete locations (the array sites). In current techniques, in which solutions are "spotted" on an unpatterned surface, cross-contamination and limited feature size (>50 μ m) cause limitations in the information density available on the microarray. Because of these issues and the need for mechanical loading in non-DNA applications (enzymes, other proteins, cells, polysaccharides, catalysts), in which other methods are not appropriate, microarrays with superior information density on a variety of substrates are desirable. PDMS membranes provide the means for doing so. The membrane can be applied to any surface without a chemical patterning methodology. When a surface with a patterned membrane affixed to it is exposed to an aqueous solution, the biological species in the solution attach only to the specific regions of the surface defined by the holes in the membrane. However, the high hydrophobicity of PDMS membranes becomes an obstacle when loading aqueous solutions into very small openings. The fluid is rejected even though the array elements are hydrophilic. Exposure of the PDMS membrane to an oxygen plasma, a common practice, provides only a temporary reduction in contact angle. To produce a permanent change, we have used a novel RF-plasma process, in which O₂-plasma-treated PDMS surfaces are exposed to a second plasma, which generates functionalities on the PDMS surfaces that are converted in the presence of moisture into low-mobility, large, -Si(OH)_x groups. This process stabilizes the PDMS surface and produces a permanent reduction in contact angle. Tuneability in the contact angle is possible by varying the process. We present XPS, FTIR, contact angle, and surface roughness measurements to support these conclusions. The process we describe for permanent modification of the wettability of PDMS can easily be extended to other applications of PDMS and to other polymeric substrates.

Keywords – PDMS, Plasma surface treatment, Biological microarrays

I. INTRODUCTION

In the past decade, bio-array fabrication techniques have been actively developed in both academic laboratories and the biotechnology industry, with numerous applications in high-throughput analysis for gene expression, gene variation, toxicology, and drug development. These efforts have led to the development of several different strategies for DNA attachment on the surface. Some laboratories employ an *in situ* synthesis on the surface to create the different DNA strands one base at a time. Others completely synthesize the entire strand before attaching it to the surface, an *ex situ* technique. Both methods have advantages but neither is ideal. Glass, silicon, and plastic have emerged as choices for substrates.

In the *in situ* approach, one base at a time is added to the surface via a cyclical surface chemistry scheme. The success rate is not 100% for the process and the error propagates with each cycles. This method works at present only for DNA and small peptide chains, and thus is quite limited. Advantages are small feature sizes in the array and the parallel synthesis of many different DNA strands.

The *ex situ* approach has a straightforward strategy. First, the DNA strands are completely synthesized and purified, and then small droplets of DNA solution are placed on a surface prepared for attachment of DNA. If the droplet volume is small enough and the spacing is large enough, then an array of DNA spots is easily created. However, with a uniform surface and undefined array sites, it is a common problem for the spots to bleed into each other. With current loading technology, the spacing is ~500 μ m between droplet spots. A similar approach can be taken with proteins. They must be synthesized, purified and allowed to fold into their correct 3-D configuration prior to their placement in an array.

Chemically patterning the substrate can overcome the limitations described above when complete strands of DNA or other molecules are attached to the surface to create an array.[2] A chemically patterned surface has an inert background, possibly hydrophobic in nature, and reactive array sites, possibly hydrophilic in nature. In the *ex situ* method with a patterned surface, aqueous solutions of DNA are placed onto the reactive sites and the droplets of solution become pinned on the array elements. The hydrophobic background acts as a barrier for diffusion of molecules between the elements. Combined with a covalent-

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bonding scheme for completely synthesized and purified DNA strands, high-purity arrays with high positional fidelity, excellent stability, small feature size, and minimal cross talk between elements can be fabricated. With a chemically patterned surface, the array element sites can be considerably smaller, $20\mu\text{m}$ in diameter or less. The density of array sites can then be equal to that of base-by-base attachment. But, because pure pre-synthesized strands are attached, the reliability can be greater than in base-by-base fabricated arrays. In addition, the total information density of the array can be higher than arrays fabricated by the *in situ* method.[3]

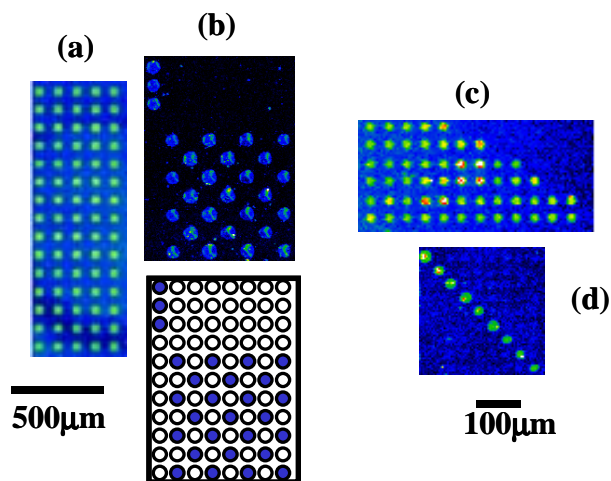


Figure 1: DNA microarrays on patterned gold-thiol substrates. Prior to DNA attachment, a gold film on a glass support has reacted with an alkane thiol that subsequently binds DNA to the surface and has a hydrophilic tail. The substrate is then exposed to UV light through a mask, which cleaves the gold-sulfur bond and creates a bare gold background. The surface is immersed in a second alkane thiol solution, which has a hydrophobic end group, and creates a patterned surface with a hydrophobic background and hydrophilic array sites. The thiol-modified, single-stranded DNA solutions interact with the array sites and become bound. For detection, the surface is exposed to a solution of its fluorescently tagged complement. Black and blue indicates low signal intensity, while yellow, red, and white indicate high intensity. (a) An array of $50\mu\text{m}$ squares spaced $100\mu\text{m}$ from center to center has been patterned on a gold film for DNA attachment. A solution of DNA is flooded onto the surface; the DNA attaches only at the array sites. (b) A surface has been prepared for DNA attachment with a pattern of an array of $50\mu\text{m}$ circles spaced $100\mu\text{m}$ from center to center. The DNA solution is placed on the array sites in the same pattern as the schematic diagram. The solutions are pinned at the specific locations. (c) same as (a) but with $20\mu\text{m}$ circles spaced $40\mu\text{m}$ center to center. (d) Same as (b) but with an array of $20\mu\text{m}$ circles spaced $40\mu\text{m}$ center to center.

In previous work, we have developed a chemical patterning technique for the *ex situ* method with gold and alkane-thiol chemistry via UV photopatterning. The array elements are hydrophilic, and the background is hydrophobic. The difference in wetting properties of the surface allows aqueous solutions to be pinned at specific locations and bound to the surface with minimal surface contamination, as seen in fig. 1.

Chemical patterning relies on specific surface chemistries that must be tailored to each attachment system. The use of a template to confine solutions to an array site would make possible high-density arrays on multiple surfaces. Templating has been explored with poly(dimethyl siloxane) (PDMS) membranes.[1] PDMS is formed into a film with holes in an array pattern and placed on a surface, creating a watertight seal with the substrate and producing a patterned surface without a chemical patterning methodology. When a surface that has been patterned with a membrane is exposed to an aqueous solution, the reactive species in the solution attach only to the specific regions of the surface defined by the holes in the membrane. However, the high hydrophobicity of PDMS membranes becomes an obstacle when loading aqueous solutions into very small features. The fluid is rejected even though the array elements (i.e., the exposed parts of the surface) are hydrophilic. To modify the wetting characteristics of the PDMS surface, we have explored the use of plasma chemistry.

II. EXPERIMENTAL

A. Membrane fabrication:

A master is created with standard semiconductor microfabrication technology. After a pattern of pillars ($20\mu\text{m}$ or greater height) has been created on a silicon substrate via standard etch procedures, a sacrificial layer of photoresist (1827 Microposit) is spun onto the master. Afterwards, a mixture of the polymer PDMS (poly(dimethyl siloxane), Corning Sylgard 184) and toluene (Fisher Scientific) in 1:1 volume ratio is spun onto the master and allowed to cure at 90°C for $\sim 12\text{h}$. The cured polymer mixture forms a thin ($5\text{-}10\mu\text{m}$) lace-like elastomer that has many uses as a patterning device. When the Si master mold with the membrane is placed in acetone (Fisher Scientific), the photoresist layer between the membrane and the silicon substrate dissolves and the membrane detaches from the master. Tweezers can then be employed to remove the membrane from the acetone to place it in a milder environment, such as ethanol. It is now ready for use.

B. Microarray Fabrication:

For PDMS-membrane microarrays on glass, solutions of Salmon Sperm DNA (Gibco BRL of Life Technologies Inc, Gaithersburg, MD) at concentrations

of 100mg/mL-500mg/mL DNA in distilled water are sonicated 5min prior to use. Solutions are stored at -20°C when not in use. DNA is placed on poly-l-lysine coated slides (used as purchased from the Gene Expression Center, UW-Madison) and allowed to react with the surface for $\sim 18\text{h}$ in a humid environment. The slide is then soaked in a solution of 70% by volume distilled water, 30% absolute ethanol for 1h prior to staining with Molecular Probes Sytox nucleic acid stain (488nm excitation/520nm emission) solution of $5\mu\text{M}$ distilled water and ethanol (1:1 volume ratio) for 30min-1h. After staining, the slide is then soaked in 70/30 water/ethanol for 30min-1h prior to scanning with a Scan Array 5000 Confocal Microscope Scanner at 488nm with an argon laser.

C. Plasma Processing:

Processing of the membranes with a SiCl_4 plasma is carried out in a home built system at 200W at 200mt using a 40KHz power supply after pre-treating the PDMS samples for 30s with an oxygen plasma at identical pressures and power setting for 30s. The PDMS samples were exposed to a SiCl_4 plasma for 30s, 2min, and 5min. The CCl_4 plasma processing is conducted in a LFE-PDS 501 Plasma Chrome Etcher at 100W at 4.5mt CCl_4 and O_2 mix (12:1) using a RF 13.56MHz power supply. The PDMS samples are exposed for 30s and 1min. For oxygen plasma treatment, the PDMS samples are processed in a Plasma Therm 74 system at 100W at 100mt using a 13.56MHz power supply.

D. Characterization:

To investigate surface modification, we analyze samples over time with several techniques. With attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy (Mattson RS1 FTIR with a Graseby Specac ATR attachment), we probe the chemical groups on the surface. We look at the root mean square (RMS) surface roughness with atomic force microscopy (AFM) in intermittent-contact mode (using a Digital Instruments Multimode AFM), and at the change in RMS values caused by different plasma processing conditions. Contact angle measurements (using a DataPhysics Contact Angle System OCA plus 15) show the change in wetting from the plasma treatments and over time. X-ray photoelectron spectroscopy (XPS) studies (conducted with a Perkin-Elmer Physical Electronics 5400 Small Area System; Mg source; 15kV, 300W; pass energy 89.45 eV; angle 45°) provide elemental analysis of the surface and chemical bonding differences that occur with plasma processing conditions.

III. RESULTS AND DISCUSSION

A. Characterization of Plasma-Processed PDMS Membranes

PDMS, when exposed to oxygen, nitrogen, helium, or argon plasmas at various pressures, powers, and times, responds in two typical ways related to the contact angle. [4,5] A silica-like layer forms on the surface of the PDMS, which cracks, creating unstable contact angles.[4] Alternately, after an initial change to a lower contact angle upon plasma treatment, over time PDMS returns to its native highly hydrophobic nature, because of the migration of low-molecular-weight polymer chains from the bulk to the surface and reorientation of the surface groups.[5]

For our application, stability is a necessity. A permanent change in wetting characteristics may be possible. Denes et. al. have demonstrated the attachment of SiCl_4 to polyethylene and polypropylene.[6] After a pre-treatment to an oxygen plasma, the SiCl_4 modifies the polymer surface via the SiCl_3^+ ion, which is highly reactive and adds to the carboxyl sites. The SiCl_3^+ cation forms a planar trigonal structure with a 3° distortion from the 120° ideal structure, which is extremely unstable and has been produced only in the gas phase. The instability of the cation in the plasma permits surface modification of inert polymer substrates.[7]

Our initial investigations show a permanent contact angle change to 74.1° from its original 112.9° when we expose PDMS films to SiCl_4 for 30s. This value is reached after 350 hr and remains stable. Figure 2 compares the evolution of contact angle with time after exposure to a SiCl_4 plasma with that for exposure to an O_2 plasma. When the films are exposed for longer periods of time, the PDMS samples form silica-like layers, an undesirable characteristic. Measurements show an increase in RMS surface roughness from 0.6nm to 1.5nm for all plasma exposures. XPS analysis indicates the development of the silica-like layer, with a marked increase in the silicon and oxygen peaks relative to the carbon peak, for samples exposed for longer than 30s. The chlorine peak becomes detectable when the plasma exposure time reaches 5 min. The ATR-FTIR data show characteristic peaks that confirm the surface modification and the regular bulk peaks, demonstrating that the plasma treatment does not affect the bulk.

We have also used carbon tetrachloride as a surface modifying gas. CCl_4 is commonly used to etch aluminum. Contrary to the etching mechanism of SiCl_4 , CCl_4 disassociates to form Cl^- ion and CCl_3^{\cdot} radicals in the plasma. The radicals recombine to form a number of species: Cl_2 , C_2Cl_4 , and chains of $(-\text{C}_{3-6}\text{Cl}-)_n$. In the aluminum etching process, Cl^{\cdot} and Cl^- act as etchants, while the C_nCl_m products passivate the surface. Passivation via carbon-chloride chains allows for anisotropic etching in the Al process, a useful characteristic for device fabrication.[8]

From our XPS data, it appears that a similar passivation process is occurring with the PDMS samples. Samples exposed for 30s to the plasma show an increase in carbon to silicon ratio compared to the untreated PDMS, and we see a strong Cl peak, indicating that Cl has become bound to the surface. PDMS samples exposed to the plasma for 1min show a lesser increase in the C and Cl peaks, revealing the formation of the silica-like layer. We see little change in the surface roughness compared to our standard, indicating that the surface becomes passivated during the process. For SiCl₄ and O₂ plasma treatments, the surfaces become increasingly roughened, with RMS values 2 to 3-fold higher than those of the untreated samples. The ATR-FTIR data again demonstrate that the bulk PDMS has not been modified.

Contact angle measurements shows a permanent wetting angle change, from 112° to 81.9° degrees, for samples exposed for 30s to CCl₄, and to 68.1° for samples exposed for 1 min. These values are reached after ~200 hrs.

TABLE I

Contact Angles of PDMS Subjected to Various Plasma Treatments

	Untreated	SiCl ₄ Plasma	
		30s	2min, 5min
Initial	112.9°±4.1°	47.5°±1.8°	Cracked
Final	114°±3.9° 1000 hrs	74.1°±6.3° 350 hrs	Cracked
	O ₂ Plasma	CCl ₄ Plasma	
		30s	1min
Initial	24.8°±4.8°	48.5°±9.2°	39.4°±6.5°
Final	97°±3.0° 1000 hrs	81.9°±6.2° 200 hrs	68.1°±6.2° 200 hrs

Table 1: Static contact angles with water are a measure of the hydrophobic/hydrophilic nature of the surface. A contact angle of less than 90° indicates water partially wets the surface. The “initial” values are recorded immediately after plasma treatment. The “final” values are those when the contact angle has reached a stable plateau. Oxygen plasma-treated surfaces are recorded only up to 1000 hrs but continue to drift upward. Times at which final values are recorded are shown.

Plasma Treated PDMS Samples v. Time

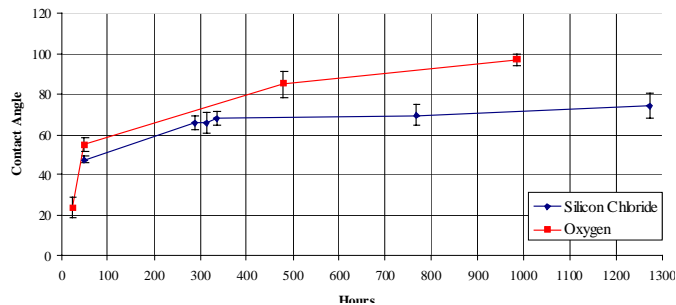


Figure 2: Evolution with time of contact angles of PDMS samples treated in SiCl₄ and O₂ plasmas. The contact angle of PDMS treated in SiCl₄ saturates within the range of error after 350 hrs, while the contact angle of samples treated in O₂ continues to rise.

TABLE 2

Surface Roughness of PDMS Subjected to Various Plasma Treatments

	Untreated	SiCl ₄ Plasma		
		30s	2min	5min
Initial	0.59±0.13nm	1.8±0.17nm	1.5±0.13nm	1.2±0.24nm
Final	0.49±0.13nm	1.5±0.36nm	1.2±0.13nm	1.0±0.13nm
	O ₂ Plasma	CCl ₄ Plasma		
		30s	1min	
Initial	1.8±0.13nm	0.5±0.15nm	0.4±0.13nm	
Final	1.7±0.28nm	0.4±0.13nm	0.3±0.13nm	

Table 2: The surface roughness (in terms of its rms value in nm) of PDMS processed in three plasmas for different times. Initial values are recorded immediately after plasma treatment. Final values are roughnesses when the contact angle has reached a stable plateau, see Table 1.

TABLE 3

Surface Composition of PDMS Subjected to Various Plasma Treatments

	Untreated	SiCl ₄ Plasma		
		30s	2min	5min
C	1.84	0.89	0.68	0.54
Si	1.00	1.00	1.00	1.00
O	1.45	2.35	2.50	2.41
Cl	0.00	0.00	0.00	0.03
	O ₂ Plasma	CCl ₄ Plasma		
		30s	1min	
C	1.36	1.36	1.18	
Si	1.00	1.00	1.00	
O	2.22	2.22	2.56	
Cl	0.16	0.16	0.10	

Table 3: XPS measurements of the surface composition of PDMS films and changes due to plasma treatments. Values are peak heights relative to Si. Trends can be determined by comparison to untreated sample.

TABLE 4

ATR-FTIR Comparison of PDMS Subjected to Various Plasma Treatments

ART-FTIR	Untreated	SiCl ₄ (30s)	CCl ₄ (30s)	O ₂
(cm ⁻¹)	702 (vw)	707(w)	704(w)	702(w)
Surface	763(sh)	763(sh)	763(sh)	763(sh)
	791(s)	787(s)	788(s)	789(s)
	846(sh)	847(sh)	845(sh)	847(sh)
	926(vw)	923(w)	914(w)	916(w)
	1013(s)	1014(s)	1014(s)	1010(s)
	1079(sh)	1076(sh)	1068(sh)	1074(sh)
Surface		1193.7(w)	1193.7(w)	1195.7(vw)
Surface	1259	1259	1257	1257
	1417(w)	1419	1404	1419
	1471(vw)	1471(w)	1456(w)	1465(w)
	2375(w)	2373(w)	2362(w)	2372(w)
	2967	2966	2964	2966

Table 4: Comparison of ATR-FTIR data for PDMS subjected to different plasma treatments. The peaks specific to the surface are indicated when compared to a bulk sample.[9] The abbreviations for the peak strength are as follows: sh=shoulder, s=strong, w=weak, vw=very weak.

B. Use of Membranes as Templates

PDMS membranes that have been plasma treated as above show a marked difference from untreated membranes when used as templates for DNA microarrays. Fluids are difficult to load into array elements (even 50 micron size) when PDMS membranes with no treatment are used. The arrays display uneven, ring-like features, as seen in Fig. 3. With O₂ plasma-

treated PDMS membranes, the loading of the array elements is easier. Such membranes do not have an extended “shelf life”, however, as the oxygen plasma-treated membranes become hydrophobic over a period of 1000 hr. Because the wettability of the surface decreases over time, loading will become increasingly difficult as

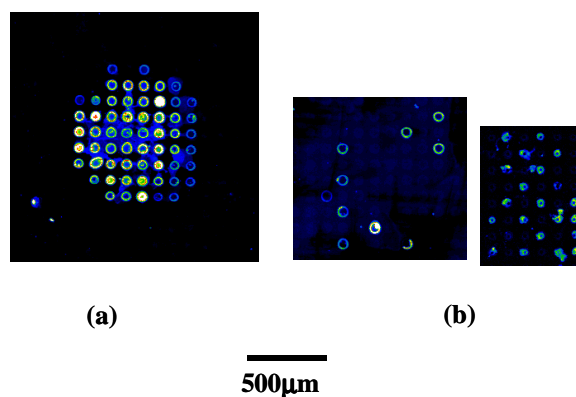


Figure 3: DNA microarrays made with unmodified PDMS membranes. A film of PDMS with 50µm circular holes spaced 100µm from center to center is placed on a poly-L-lysine coated glass slide ready for DNA attachment. The slide with DNA attached is exposed to a solution of the fluorescent nucleic acid stain Sytox, which binds to the DNA and allows for imaging via confocal microscopy. (a) A drop (0.5µL) of DNA solution is placed on the PDMS and allowed to interact with the membrane and the surface. The resulting array features are uneven with ring-like features due to the hydrophobic nature of PDMS, preventing the aqueous DNA solution from interacting with the underlying hydrophilic surface elements. (b) DNA droplets are loaded in the membrane in an attempt to create a DNA microarray. Ring-like features instead of evenly filled circles result.

the membrane ages. Even at a given time, different regions of the membrane can behave distinctly. Figure 4 shows array loading using CCl₄ plasma-treated membranes. Even with nonuniform loading (4a), the individual features upon removal of the membrane are uniform. As the feature sizes decrease, control over the membrane contact angles becomes increasingly important.

The ability to manipulate the wetting properties of the surfaces of polymeric materials can be a key factor in their use in a wide variety of applications. Our stencil for the fabrication of bioarrays is just one example. In particular, because PDMS is so ubiquitous, it not only serves as a good model system for studies of the control of polymer wettability, but will provide a range of

possible applications in the arena of microfluidics and biological interfaces, especially as devices shrink in size.

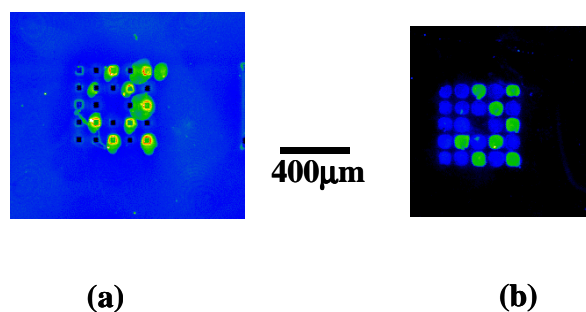


Figure 4: DNA microarray on a carbon tetrachloride plasma-treated PDMS membrane. A film of PDMS with 50 μ m square holes spaced 100 μ m from center to center has been exposed to a carbon tetrachloride plasma for 30 seconds and is placed on a poly-L-lysine coated glass slide that is ready for DNA attachment. After the double-stranded DNA has been bound to the surface, the slide is exposed to a solution of the fluorescent nucleic acid stain Sytox, which binds to the DNA and allows for imaging via confocal microscopy. DNA droplets are loaded onto the membrane in a regular pattern. (a) When the slide is stained, there is DNA present on the membrane resulting in a messy array. (b) When the membrane is removed and the slide is imaged again, the excess DNA is removed, leaving 50 μ m circular features that are fully filled. The membrane has fulfilled its function to mask off the non-uniform droplets and create regular features for the microarrays.

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