Chapter 3

Rapid Prototyping of Microstructures by Soft Lithography for Biotechnology

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Abstract

This chapter describes the methods and specific procedures used to fabricate microstructures by soft lithography. These techniques are useful for the prototyping of devices useful for applications in biotechnology. Fabrication by soft lithography does not require specialized or expensive equipment; the materials and facilities necessary are found commonly in biological and chemical laboratories in both academia and industry. The combination of the fact that the materials are low-cost and that the time from design to prototype device can be short (< 24 h) makes it possible to use and to screen rapidly devices that also can be disposable. Here we describe the procedures for fabricating microstructures with lateral dimensions as small as 1 μ m. These types of microstructures are useful for microfluidic devices, cell-based assays, and bioengineered surfaces.

Key words: Soft lithography, poly(dimethylsiloxane), microfluidics, microfabrication, rapid prototyping, cell-based assays.

1. Introduction

Most research in microfabrication is focused on applications in microelectronics. Applications in biotechnology are, however, rapidly emerging: these include tools for cell-based assays (1–5), molecular (DNA sequencing on microarrays) (6–12) and clinical (enzyme-linked immunosorbant assay (ELISA) on microchips) diagnostics (13–17), drug discovery (1–5), and chemical and biological defense (microchip-based sensors of chemicals and pathogens) (18–24).

There are four general steps in the process of microfabrication:

- (1) fabrication of a master (i.e., the pattern used to make replicas),
- (2) replication of the master, (3) transfer of the pattern in the

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replica into functional materials (e.g., polymers, metals, ceramics, or biologics), and (4) registration of a master (the original one or a different one) with the patterned material in Step 3 to form multilevel structures. The materials patterned by the techniques used in the microelectronics industry (e.g., photolithography and electron-beam lithography) are limited to photosensitive polymers. These techniques can be used to pattern DNA (10–12), but they have limited capability to pattern other biologically relevant materials (e.g., proteins and cells) directly.

Soft lithography is set of techniques that provides alternatives to photolithography for Steps 2–4; these techniques are useful for the prototyping of devices useful in biology, chemistry, and physics (1, 25–29). They use a topologically patterned stamp (or mold) to transfer a pattern to a substrate. There are two types of soft lithography: (1) the replication of a pattern defined in a soft (elastomeric) stamp into organic materials or onto the surface of metals, ceramics, and semiconductors and (2) the replication of a pattern defined in a hard (rigid) stamp into a thin layer of soft organic molecules (e.g., thermoplastic polymers). In this chapter we discuss the use of the first type of soft lithography in the fabrication of microstructures useful in biology.

The types of microstructures useful for biological applications have requirements different from those in microelectronics. Microfluidic systems, for example, are relatively simple in design and have design rules that accept large features (i.e., feature sizes of 100 µm and areas of >5 cm²). It is also useful to be able to try a large number of designs of microfluidic systems and bioanalytical tools rapidly and at low cost for each device. Soft lithography also makes it possible for researchers to fabricate prototype devices rapidly (i.e., from design to prototype in 24–48 h) using equipment found commonly in most scientific laboratories. The capabilities of soft lithography overlap well with the requirements of the fabrication of microdevices needed in biology and biochemistry.

A number of reviews and papers describe the types of biological experiments that have been performed using devices fabricated by soft lithography (1, 25–28, 30, 31). Here we describe how to fabricate membranes with holes, microfluidic channels, and engineered surfaces by using rapid prototyping and soft lithography.

2. Materials

Note: The companies listed below are not the sole providers of these materials.

2.1. Fabrication of Masters

- 1. Computer drawing software (e.g., Adobe Photoshop, Macromedia Freehand, WieWeb Clewin)
- 2.1.1. Fabrication of Transparency-Based Photomasks
- 2.1.2. Contact Photolithography Using Transparency-Based Photomasks
- 1. Contact mask aligner equipped with a mercury-arc lamp (found in most cleanroom facilities)
- 2.1.3. Microscope Projection Photolithography (MPP)
- 1. Upright microscope equipped with a mercury-arc lamp

2.1.4. Microlens Array Photolithography (MAP)

- 1. Standard overhead projector equipped with a broadband light bulb
- 2. Aqueous hydrogen peroxide (20%vol) (see Note 1)
- 3. Concentrated sulfuric acid (see Note 1)

2.1.5. Materials Common to All of the Photolithographic Techniques

- 1. Substrates silicon wafers (test grade, <100>), glass slides, gold-coated glass slides (Platypus Technologies, Madison, WI). Note: Cracked silicon wafers can be very sharp. Please use caution when handling cracked wafers.
- 2. Cleaning solution trichloroethylene (TCE), acetone, and methanol (Aldrich Chemical Co., St. Louis, MO)
- 3. Primer hexamethyldisilazane (HMDS) (Aldrich Chemical Co., St. Louis, MO)
- 4. Positive photoresist Microposit 1813, 1805 (Shipley Co., Inc., Marlborough, MA)
- 5. Negative photoresist SU-8 50 (Microchem Corp., Newton, MA)
- 6. Developer Microposit 351 (Shipley Co., Inc., Malborough, MA); Propylene glycol methyl ether acetate (Aldrich Chemical Co., St. Louis, MO)

2.2. Fabrication of Poly(dimethylsiloxane) (PDMS) Replicas

- 1. Surface treatment Tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (CF₃(CF₂)₆(CH₂)₂SiCl₃) (United Chemical Technology, Bristol, PA)
- 2. Glass vacuum dessicator (VWR Scientific, West Chester, PA)
- 3. Elastomeric materials Poly(dimethylsiloxane) Sylgard 184 Silicon Elastomer Kit (Dow Corning, Highland, MI)

2.2.1. PDMS Membranes

1. Spin coater capable of spinning at 500-5,000 rpm

2.3. Fabrication of Microfluidic Channels

- 1. 16-gauge needles (VWR Scientific, West Chester, PA)
- 2. Metal file
- 3. Sand paper
- 4. Sharp tweezers
- 5. Oxygen plasma cleaner (Harrick Scientific Corporation, Ossining, NY)
- 6. Polyethylene tubing (PE-60, VWR Scientific, West Chester, PA)

2.4. Fabrication of Engineered Surfaces

- 1. 1-octadecanethiol (Aldrich Chemical Company, St. Louis, MO)
- 2.4.1. Microcontact Printing of SAMs to Pattern Cells
- 2. Cotton swabs
- 3. Tri(ethylene glycol)-terminated undecanethiol (Prochima, Poland; Shearwater Polymers, Huntsville, AL)
- 2.4.2. Microcontact Printing of Reactive SAMs to Pattern Ligands
- 1. Hexa(ethylene glycol)-terminated undecanethiol (Prochima, Poland; Shearwater Polymers, Huntsville, AL)
- 2. 1-Ethyl-3-(dimethylamino)propylcarbodiimide (EDC) (Aldrich Chemical Company, St. Louis, MO)
- 3. Pentafluorophenol (Aldrich Chemical Company, St. Louis, MO)
- 4. Biotin cadaverine (Molecular Probes, Inc., Eugene, OR)

3. Methods

This section details the steps and procedures used to take a design to a functional prototype by rapid prototyping and soft lithography. The organization of this section follows that of the outline shown in Fig. 3.1.

3.1. Fabrication of Masters

3.1.1. Fabrication of Transparency-Based Photomasks The master contains the original pattern to be transferred into the elastomeric stamp by molding. The minimum size of the features necessary in the master is dictated by the specific application of the device. The master in photolithography – a fabrication technique used commonly in the microelectronics industry – is a photomask. Photoresist-based replicas generated by this technique are used as the masters for the fabrication of elastomeric stamps for soft lithography. The photomasks are generated by laser or electron-beam writing on photoresist that is coated on a chrome-coated sheet of float glass or quartz. This process exposes areas of the chrome; these regions of chrome are removed by wet-chemical etching.

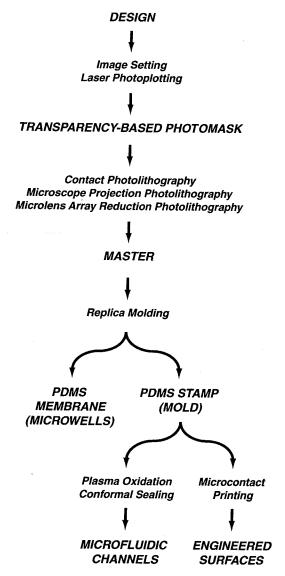


Fig. 3.1. Diagram of the flow of the process of microfabrication of elastomeric membranes with holes, microffuldic channels, and engineered surfaces by soft lithography.

Electron-beam and laser writing are capable of generating small features (e.g., <100 nm), but are expensive (~\$50/cm²) and slow (>4 h to pattern 5 cm²). Many applications in biology do not require such resolution, and appropriate masks can, therefore, be prepared using low-cost methods and materials. For example, the range of sizes of the smallest lateral dimension of the features used for microfluidic channels are 50–500 μ m, for single-cell assays are 5–50 μ m, and for subcellular assays are 1–5 μ m (1, 27, 30). There are few (if any) common applications in biotechnology

for <1-µm features. High-resolution printers are capable of generating transparency-based photomasks containing features with lateral dimensions of >10 µm (32, 33). Black ink printed on a standard transparency sheet is able to attenuate the transmission of light sufficiently to use as a photomask. Although these masks do not have the durability and dimensional stability required for use in the manufacturing of microelectronic devices, they are suitable for rapid prototyping of bioanalytical and microfluidic systems. Transparency-based photomasks are inexpensive ($\sim 0.15/\rm cm^2$) and can be produced rapidly (>200 cm²/min), and thus they are useful alternatives to chrome masks for applications in biotechnology.

Computer programs, such as Adobe Illustrator, Clewin, and Macromedia Freehand, are used to "draw" the patterns that will be on the photomask (see Note 2). These designs are printed onto transparencies using commercially available printers. The type of printer necessary depends on the minimum size of the features in the design. Standard laser printers are capable of printing with resolution of ~1,200 dpi (each dot is about 20 µm in diameter); these photomasks are acceptable for the fabrication of large (>150 µm) features, such as those in microfluidic channels (33). High-resolution printers are necessary for features that are <150 µm in width; such feature sizes are often necessary for cell-based assays. Commercial printing companies phat have these types of printers operate in most major cities. These companies use imagesetters (5,080 dpi; dot size \sim 5 μ m) and laser photoplotters (20,000 dpi; dot size ~1.25 µm); we have used masks generated by laser photoplotting successfully to generate high-quality features with 10-µm width by 1:1 contact photolithography (32). This minimum feature size is reduced to ~1 μm by projecting the patterns in transparency-based photomasks through reducing optics (e.g., microscope objectives or microlens arrays) in techniques such as microscope projection photolithography (34) and microlens projection photolithography (35-39).

3.1.2. Contact Photolithography Using Transparency-Based Photomasks Standard photolithography using transparency masks is used to fabricate masters that have a single pattern with feature sizes of $\geq\!10~\mu m$. The maximum area of the master is dictated by the diameter of the aperture of the light source; the maximum diameter is typically 75–100 mm (3–4 inches). Masters with these dimensions and feature sizes are useful for the fabrication of microfluidic channels and elastomeric membranes with holes.

The step-by-step procedure for photolithography includes the preparation of the substrate, the exposure of the photoresist, and the removal of the unwanted photoresist (Fig. 3.2). A substrate is coated with a thin layer of photosensitive polymer (photoresist) by

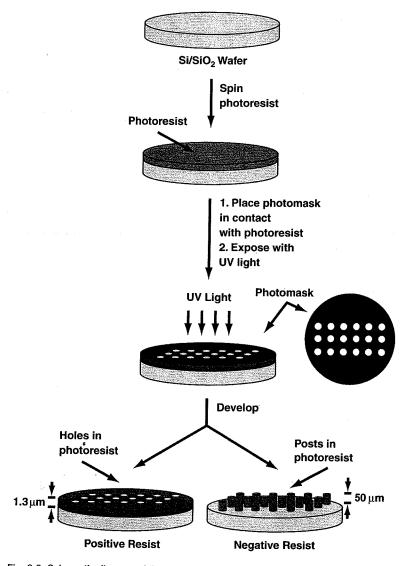


Fig. 3.2. Schematic diagram of the process of contact photolithography.

spin coating (see Note 3). The thickness of these layers can range from a few nanometers up to a millimeter depending on the type of resist that is used and the conditions used in spinning; SU-8 (Microchem Corp, Newton, MA) is used commonly for layers that are more than 10-µm thick. The transparency-based photomask is placed in contact with the photoresist-coated substrate such that the side with the ink is in contact with the surface of the photoresist (see Note 4). A transparent glass plate is placed on top of the photomask. Pressure is applied between the photomask and the substrate by clamping them between two rigid plates (e.g., metal plates with a hole in the center to allow light to pass) to press the photomask into good contact with the photoresist.

The photoresist is exposed using a UV-light source (see Note 3). The photoresist exposed to UV light becomes either more (positive resist) or less (negative resist) soluble in the developing solution (see Note 3). In either case, the pattern of the photomask is replicated into the photoresist. The following procedure describes the methods used to generate a patterned relief structures in a layer of positive photoresist with thickness of 1.3 µm:

- 1. Sonicate a substrate in TCE, acetone, and methanol for 10 min in each solvent, sequentially.
- 2. Dry the substrate in an air oven at 180°C for 10 min.
- 3. Spin coat the substrate with primer (HMDS) at 4,000 rpm for 40 s.
- 4. Spin coat the substrate with a positive photoresist (Microposit 1813) at 4,000 rpm for 40 s.
- 5. Bake the photoresist-coated substrate on a hot plate at $105^{\circ}\mathrm{C}$ for 3.5 min.
- 6. Expose photoresist through the photomask. The exposure time is 12 s for a lamp intensity of 10 mJ cm⁻²s⁻¹ at 405 nm.
- 7. Develop the photoresist for 1 min in dilute developer (Micro $posit 351:H_2O = 1:5 \text{ vol/vol})$

The following procedure describes the fabrication of a patterned relief structures in a layer of photoresist with thickness of greater than 10 µm using negative photoresists:

- 1. Sonicate a substrate in TCE, acetone, and methanol for 10 min, sequentially.
- 2. Dry the substrate in an oven at 180°C for 10 min.
- 3. Spin coat the substrate with primer (HMDS) at 4,000 rpm for 40 s.
- 4. Spin coat the substrate with a negative photoresist (SU-8-2050) at 3,000 rpm for 30s to obtain a layer of photoresist that is 50-um thick.
- 5. Bake the photoresist-coated substrate on a hot plate at 65°C for 3 min and then at 95°C for 6 min.
- 6. Expose photoresist through the photomask. The exposure time is $40 \,\mathrm{s}$ for a lamp intensity of $10 \,\mathrm{mJ} \,\mathrm{cm}^{-2} \mathrm{s}^{-1}$ at $365 \,\mathrm{nm}$.
- 7. Develop the photoresist for 6 min in propylene glycol methyl ether acetate.

Microscope projection photolithography (MPP) is useful for generating features smaller than those possible using contact photolithography with transparency photomasks (i.e., 1-10 µm in lateral dimensions), but the technique is limited to patterning small areas $(\sim 4 \times 10^4 \ \mu m^2)$ per exposure (34). Photoresist-based masters with these dimensions and feature sizes are useful for fabricating microwells and elastomeric membranes with holes (40).

3.1.3. Microscope Projection Lithography (MPP)

Microscope projection photolithography (MPP) uses a standard upright microscope equipped with a mercury-arc lamp to reduce the size of features defined in a transparency photomask by up to $25 \times ($ Fig. 3.3). Mercury-arc lamps emit UV light at the wavelengths necessary for the exposure of photoresist (i.e., 436 nm). These light sources are commonly used for fluorescence microscopy. A transparency photomask is placed before the objective in a location that is a conjugate image plane to that of the surface of the substrate (see Note 5). The reduction factor of the features on the mask depends on the magnification power of the objective (see Note 5). Typical exposure times ranged from 5 to 20 s (80 W Mercury Lamp) depending on the thickness of the resist (see Note 6). Microscope projection lithography can produce features with widths of 1 µm and an edge roughness of 0.2 µm. A drawback of the technique is that it is limited to the patterning of areas of $\sim 40,000 \, \mu \text{m}^2 \, (0.4 \, \text{mm}^2)$ for a single exposure. Rastering of the stage with subsequent exposures permits the fabrication of arrays of features. This technique is also useful for the registration of multiple exposures with high accuracy. Figure 3.3d shows examples of features patterned in photoresist by this technique. A comprehensive procedure is described as follows (see Note 6):

- 1. A transparent photomask is placed in the microscope in a location between the light source and the back aperture of the objective that is the conjugate image plane of the surface of the photoresist of the microscope.
- 2. A photoresist-coated substrate is prepared as described previously. It is placed on the microscope stage underneath the microscope objective (see Note 7).
- 3. A high neutral-density filter is placed in front of the light source to prevent accidental exposure of the photoresist while focusing.
- 4. The neutral-density filter is removed to expose the resist.
- 5. The photoresist-coated wafer is rastered to an unexposed area and Steps 3 and 4 are repeated to fabricate arrays of the features.
- 6. The exposed resist is developed using appropriate developer solutions (see Note 3).

3.1.4. Microlens Array Projection Lithography (MAP)

Microlens projection photolithography (MAP) is a technique that is capable of patterning arrays of the same feature over areas larger than 1 cm² in a single exposure (35–39). Masters with these types of features are useful for the fabrication of microwells and elastomeric membranes with holes over areas larger than those possible with microscope projection photolithography.

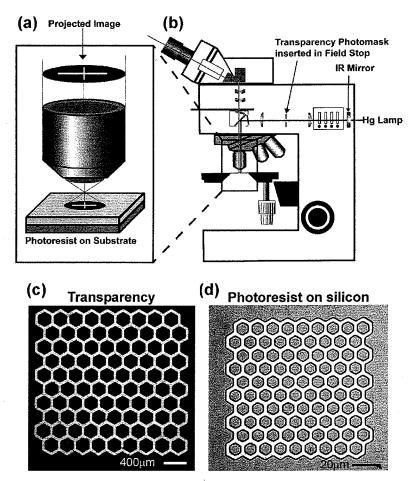


Fig. 3.3. (a and b) Schematic diagram of microscope projection photolithography. (c) Optical micrograph of transparency mask. (d) Optical micrograph of the pattern in (c) generated in photoresist by MPP. Images are reprinted with permission from Love et al. (34) © 2001 American Chemical Society.

Microlens array projection lithography (MAP) uses microlenses (3–100 µm in diameter) to reduce the size of a large image onto the photoresist by a factor of $\sim 800 \times$. The minimum feature size that can be patterned by this technique is ~ 600 nm. Figure 3.4a diagrams the process for the technique. A transparency mask is placed on the surface of a standard overhead projector. The projection optics are removed from the projector and replaced with the microlenses. The microlenses are made of photoresist posts that have been melted and reshaped into hemispheres on the surface of a glass slide (Fig. 3.5). The back of the microlens array is coated with a layer of poly(dimethylsiloxane) (PDMS) to allow for uniform contact of the photoresist-coated wafer with the lens array. The thickness of the PDMS layer is set at the focal length of the lenses; the focal length is determined by the diameter and the height of the lens. This technique is uniquely suited for the

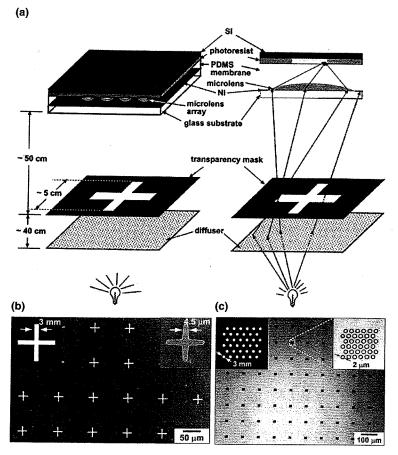


Fig. 3.4. (a) Schematic diagram of microlens array projection photolithography. (b and c) Scanning electron micrographs of arrays of features generated by the technique. The schematic diagram is reprinted from reference 36 with permission from the American Chemical Society. The images are reprinted from Wu et al., Generation of Chrome Masks with Micrometer-Scale Features using Microlens Lithography. *Adv. Mater.* 14, 1213–1216, 2002. Copyright Wiley VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

preparation of repetitive structures because it does so in a single exposure with minimal distortion (see Note 8). Figure 3.4b and c show examples of the types of features that have been fabricated by this technique. A detailed procedure for microlens projection photolithography using an array of 20- μ m-diameter lenses is given below:

1. A cleaning solution (piranha-etch solution) is prepared by slowly adding aqueous hydrogen peroxide (20%vol) to concentrated sulfuric acid at room temperature to reach a 1:2 ratio by volume. Note: The ingredients of this solution and the solution itself can cause severe burns, and contact with organic materials or solvents can result in explosions. See Note 1 for a discussion of the dangers of the piranha solution and proper handling procedures.

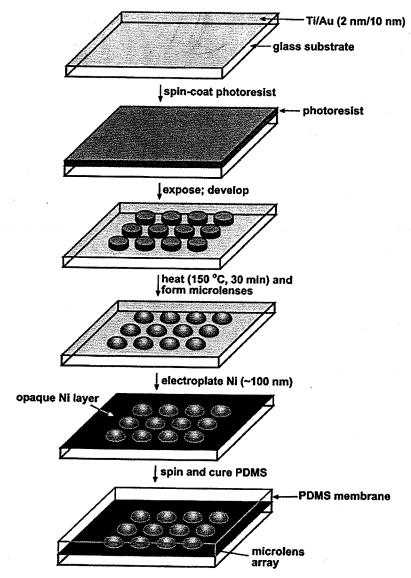


Fig. 3.5. Schematic diagram of the procedure used to make an array of microlenses. Scheme is reprinted permission from Wu et al. (36) © 2002 American Chemical Society.

- 2. Glass slides are immersed in the piranha-etch solution for 24 h (see Note 8).
- 3. A thin layer of titanium (2 nm), followed by gold (10 nm), is deposited by physical vapor deposition.
- 4. A 2-μm-thick layer of positive photoresist (Shipley S1818; Shipley Corporation) is spin coated (3,000 rpm) onto the gold-coated glass substrate.
- 5. A transparency mask with a 1×1 cm array of 20- μ m-diameter posts is prepared as described previously.

- 6. The photoresist is exposed by standard photolithography for 12 s and developed in dilute developer (Microposit 351:water = 5:1) for 1 min.
- 7. The posts are melted and reshaped into hemispheres by heating the array on a hot plate at 150°C for 30 min.
- 8. The focal length, f, of the microlenses is calculated using the following equation (36):

$$f = ((D/2)^2 + s^2)/2s(n_{\text{iens}} - n_{\text{PDMS}})$$

where D is the diameter of the lens (μ m), s is the height of the lens (μ m), n_{lens} is the refractive index of the photoresist (1.59), and n_{PDMS} is the refractive index of PDMS (1.405).

- 9. A fresh mixture of PDMS (prepolymer:curing agent = 10:1; Sylgard 184, Dow Corning) is spun (1,170 rpm) onto the lenses. The PDMS is cured for 2 h at 60°C.
- 10. A photoresist-coated wafer (500 nm thick; S1805 spun at 4,000 rpm) is prepared as described previously (see Section 3.1.2)
- 11. The microlenses are positioned at a distance of \sim 40 cm above the surface of the overhead projector. The photoresist-coated wafer is placed in contact with the PDMS-coated microlenses.
- 12. The overhead projector (equipped with a standard broadband light source ($\lambda = 400-1,200$ nm) is turned on for 1 min to expose the photoresist.
- 13. The photoresist is developed in dilute developer (Microposit 351:water = 5:1)

3.2. Fabrication of Poly(dimethylsiloxane) Replicas

Microstructures made in photoresist are usually not directly useful for applications in biotechnology; they cannot be transferred from one substrate to another easily, be handled independently from the substrate, or be modified chemically. Replication of the topography of the photoresist into an elastomeric polymer overcomes these limitations. Poly(dimethylsiloxane) (PDMS) is used commonly as the elastomeric material for replicas in soft lithography because it has eight useful properties: (1) it is inexpensive; (2) it is commercially available; (3) it is chemically inert; (4) it is non-toxic; (5) it has a low surface free energy (21.6 dyn/cm²) (25, 26); (6) it is optically transparent (25, 26); (7) it is intrinsically hydrophobic, but its surface can be made hydrophilic through brief treatment to an oxygen plasma; and (8) it is biocompatible (27, 30, 31).

Poly(dimethylsiloxane) replicas are prepared by molding of the liquid prepolymer against a photoresist master (Fig. 3.6). PDMS stamps (or molds) used for printing or microfluidics are thicker than the height of the features on the master. PDMS membranes are thinner than the height of the features on the

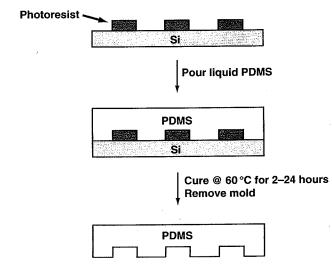


Fig. 3.6. Schematic diagram of replica molding of a master into PDMS.

master, so when released from the master these membranes have arrays of holes. Detailed procedures for the fabrication of each type of PDMS replica are described in the following subsections.

3.2.1. PDMS Stamps (or Molds)

Poly(dimethylsiloxane) stamps with thicknesses > 1 mm are prepared by pouring the liquid pre-polymer over the photoresist-based master. The thickness of the stamp does not have to be controlled precisely for applications in printing and in microfluidics.

- 1. Fabricate a "master" a patterned photoresist on a solid substrate by following the procedures described in Section 3.2.
- 2. Place the master in a vacuum dessicator along with a vial containing a few droplets of CF₃(CF₂)₆(CH₂)₂SiCl₃ under vacuum (~100 mTorr) for 20 min. The CF₃(CF₂)₆(CH₂)₂SiCl₃ will react with the exposed surface of the PDMS to modify the surface chemically, which decreases the surface free energy from 21.6 dyn/cm² to ~19 dyn/cm². Lowering the surface free energy of the master facilitates removal of the PDMS replica from the photoresist master without causing damage to the master or the replica.
- 3. Prepare a mixture of the PDMS prepolymer and the curing agent in a ratio of 10:1 by weight and mix thoroughly. Remove the gas bubbles that develop as a result of the mixing by placing the mixture in a dessicator under vacuum (~100 mTorr) for 45 min.
- 4. Pour the liquid PDMS over the master and cure to an elastomeric solid at 60°C for 3–4 h.
- 5. Use a scalpel or razor blade to cut around the features on the master and manually peel the PDMS stamp off the master (see Note 9).

3.2.2. PDMS Membranes

It is possible to prepare thin (<1 mm) membranes of PDMS by spin coating (40) and compression molding (31, 41). The thickness of the membranes is tailored to be less than the height of the features in the master so that an array of holes will exist in the membrane. These holes (when the membrane is placed in contact with a flat substrate) serve as microwells that are useful for biological experiments such as the patterning of cells on surfaces (Fig. 3.7) (40). Here we describe the procedure to prepare PDMS membranes by spin coating. This procedure is the same as that described in Section 3.4.1, up to Step 4. The subsequent steps are replaced with the following instructions:

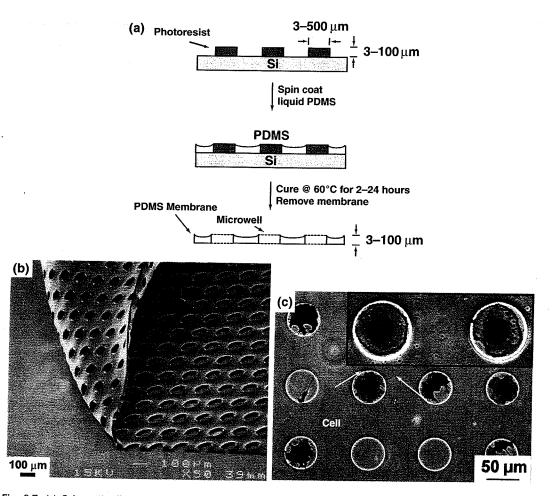


Fig. 3.7. (a) Schematic diagram of the fabrication of PDMS membranes by replica molding. (b) Scanning electron micrograph of a PDMS membrane with holes that was prepared by spin coating PDMS onto an array of photoresist posts. (c) Optical micrograph of cells patterned in the holes in a PDMS membrane. The surface of the PDMS was modified by physisorption of bovine serum albumin (BSA) to prevent the adhesion of cells to the membrane. The regions of the substrate exposed through the holes in the PMDS membrane were modified by physisorption of fibronectin to promote the adhesion of cells in these regions. The images in (b) and (c) are reprinted with permission from Ostuni et al. (40) © 2000 American Chemical Society.

- 1. Manually place the master onto the chuck of the spin coater. For membranes $> 30 \mu m$ thick use the following procedure:
- 2. Pour enough PDMS onto the master to coat the entire surface.
- 3. Spin the PDMS-coated master at the rate, r(rpm), calculated based on the thickness, τ , using the following equations:

$$r = (298790/\tau)^{0.8632}$$
; $\tau = 150-600 \,\mu\text{m}$
 $r = (158599/\tau)^{0.9563}$; $\tau = 30-150 \,\mu\text{m}$

For membranes <30 µm thick use the following procedure:

- 4. Dilute the PDMS with heptane at a 1:1 ratio by weight; pour enough of the diluted PDMS onto the master to coat the entire surface.
- 5. Spin the PDMS-coated master at the rate, r (rpm), calculated based on the thickness, τ (μ m), using the following equation:

$$r = (200.59/\tau)^{3.267}$$
; $\tau = 15-30 \,\mu\text{m}$

All thicknesses of PDMS membranes tear easily, so it is important to be careful when removing it from the photoresist-based master. Immersion of the PDMS-coated master in ethanol lowers the adhesive energy of the interface between the PDMS and the master and facilitates removal of the membrane from the master without causing damage to either one. See Note 9 for additional tips on how to handle PDMS membranes.

3.3. Fabrication of Microfluidic Channels

Examples of the use of PDMS-based microfluidic channels in microdevices for biological applications include immunoassay devices (13-15, 42-46), DNA and protein separators (47-51), cell sorters (52-55), and tools for cell biology (56-59). PDMSbased microfluidic devices are prepared by sealing the PDMS replica of a photoresist master against a topographically flat substrate; the surface of this substrate forms the fourth wall of the channel (27, 60). PDMS will seal reversibly (i.e., it is not chemically bonded) by van der Waals interactions when placed in contact with a clean surface; the reversible seal allows the separation of the PDMS replica and the substrate without causing damage to either surface (60). Oxidized PDMS will seal permanently (i.e., it is covalently bonded) to clean oxide surfaces (e.g., glass, oxidized PDMS, and quartz) (27, 60). Most applications that use pressure-driven flow require the PDMS to be sealed permanently to prevent rupture of the seal between the PDMS and the flat substrate. Figure 3.8 shows the preparation of a microfluidic channel by sealing a PDMS mold to glass (see Note 10). The procedure used to fabricate a 3-inlet, 1-outlet microfluidic network is outlined below:

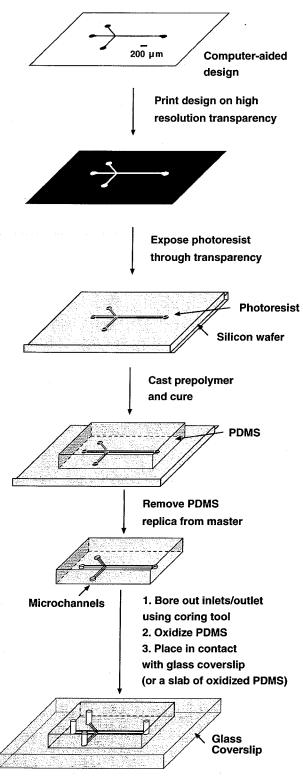


Fig. 3.8. Schematic diagram of the process of fabrication of PDMS-based microfluidic channels by soft lithography.

- 1. Design networks of microfludic channels such that three channels of $100~\mu m$ in width intersect into a channel of $300~\mu m$ in width. The inlets and outlets are drawn as circles of 2 mm in diameter. This design is printed onto a transparency to be used as a photomask as described previously. Note: The photoresist used for this procedure is a negative resist, so the photomask, when printed, should have the channels clear and the background black.
- 2. Prepare a photoresist-coated substrate by spinning Microposit SU-8-2050 (Microchem Corp., Newton, MA) at 3,000 rpm (see Note 2). Bake the photoresist-coated wafer on a hot plate at 65°C for 3 min and at 95°C for 6 min.
- 3. Expose photoresist through the photomask. The exposure time is 40 s for a lamp intensity of 10 mJ cm⁻²s⁻¹ at 365 nm.
- 4. Develop the photoresist for 6 min in propylene glycol methyl ether acetate.
- 5. Perform Steps 2–5 described in **Section 3.4** to prepare the PDMS replica of the master.
- 6. Cut off the end of a 16-gague syringe needle and file the inside and the outside using a pair of sharp tweezers and sand paper; this tool is used to form the inlets and outlets. Use the tool to make holes from the bottom to the top of the slab of PDMS in the location of the inlets and the outlets.
- 7. Clean the substrate that will form the bottom surface of the channel using a piranha solution described in **Section 2.3.2**Step 1 for glass substrates. Clean the surface of the PDMS replica with a piece of cellophane tape.
- 8. Place the PDMS and the substrate into an oxygen plasma cleaner (PDC-32G Harrick, or equivalent device) and oxidize for 1 min on high.
- 9. Carefully place the side of the PDMS with the channel in relief in contact with the substrate and allow the two surfaces to contact one another conformally. Place the sealed channel in an oven at 60°C for 1 h to improve the quality of the seal.
- 10. Carefully place the end of a PE-60 polyethylene tube into the inlets and outlet of the channel. The opposite end of the tube will fit onto a 21-gague syringe needle and permit sample introduction.

3.4. Fabrication of Engineered Surfaces

The ability to control surface chemistry is useful in biology to control the position and concentration of proteins or cells on surfaces. We and others have used self-assembled monolayers (SAMs) of alkanethiolates on gold, silver, copper, and palladium to tailor the properties of these surfaces, for example, to resist the adsorption of proteins or to present specific ligands for biospecific

binding of cells or proteins (61–76). Patterns of SAMs on these types of surfaces are generated by microcontact printing (μ CP) (Fig. 3.9a). In μ CP, a PDMS stamp is wet with a molecular "ink." The ink is transferred in the regions of contact between the stamp and the surface. This technique can also pattern proteins (77–82) and DNA directly (83, 84). Below we describe the protocol for engineering surfaces: (1) to control the location and shape of cell cultures for use in cell biology experiments (64) and (2) to display biologically relevant ligands for use in immunoassays (85). We limit our discussion to the rapid prototyping of these types of surfaces and refer the reader to the literature for instructions on how to carry out such experiments (57, 64, 74, 85, 86).

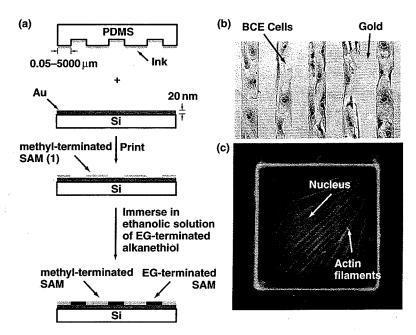


Fig. 3.9. (a) Schematic diagram of the procedure used to prepare SAMs for patterning cells by microcontact printing. (b) Optical micrograph of BCE cells grown on an Au substrate pattered with a SAM of methyl-terminated alkanethiols (regions where cells stick) and a SAM of EG-terminated alkanethiol (regions where the cells do not stick). (c) Fluorescence micrograph of a cell grown on a surface patterned by this technique. The actin and the nucleus were stained with fluorescently labeled molecules. These images are reprinted with permission from Ostuni et al. (64) © 2001 American Chemical Society.

3.4.1. Microcontact Printing of SAMs to Pattern Cells

Surfaces for cell cultures are prepared by patterning a methylterminated alkanethiol in the regions where adhesion of the cells is desired and an ethylene glycol (EG)-terminated alkanethiol in the regions where adhesion is not desired. A discussion of why ethylene glycol-terminated SAMs resist the non-specific adsorption of proteins is presented in detail elsewhere (87). Figure 3.9b

and c show examples of the use of such a surface to pattern the location and shape of cells (64, 86). The following procedure describes this process:

- 1. Prepare a photoresist master and a PDMS stamp as described in Sections 3.2 and 3.4.1. The stamp should be designed such that the raised features represent regions where cells will adsorb, and the recessed regions are where cells will not adsorb.
- 2. Prepare an ethanolic solution of 1-octadecanethiol (10 mM).
- 3. Prepare an ethanolic solution of tri(ethylene glycol)-terminated alkanethiol (2 mM).
- 4. Prepare a gold-coated silicon wafer (or glass slide) (20 nm gold/2 nm titanium as an adhesion layer) by physical vapor deposition or by purchasing from a commercial source (see Note 11).
- 5. Clean the PDMS stamp with a piece of cellophane tape.
- 6. Wet a cotton swab with the solution of octadecanethiol.
- 7. Ink the PDMS stamp by swiping the swab across the surface.
- 8. Dry the stamp in a stream of nitrogen for 30 s.
- 9. Place the stamp in contact with the gold surface for $5-10 \, \text{s}$.
- 10. Remove the stamp from the surface manually.
- 11. Immerse the patterned gold surface in the solution of the ethylene glycol-terminated SAM for 1–5 min.
- 12. Remove sample from the solution, wash thoroughly with ethanol, and dry under nitrogen.

3.4.2. Microcontact Printing of Reactive SAMs to Pattern Ligands

A useful method for engineering surfaces to present specific ligands is to use reactive SAMs and μCP (85). In this method, a mixed SAM of ethylene glycol-terminated alkanethiols and carboxylic acid-terminated alkanethiols is prepared on a gold surface. The carboxylic acid-terminated alkanethiol is activated chemically to allow reaction with amino groups at high yield. Ligands containing amino groups are patterned on the surface by μCP (Fig. 3.10a). These patterned surfaces are useful for biological experiments such as immunoassays (Fig. 3.10b and c) (85). The procedure to prepare a biotin-presenting surface by this technique is described in detail below:

- 1. Prepare an ethanolic solution containing both hexa(ethylene glycol)-terminated undecanethiol (2 mM) and tri(ethylene glycol)-terminated undecanethiol (2 mM).
- Prepare a gold-coated silicon wafer (or glass slide) (20 nm gold/2 nm titanium as an adhesion layer) by physical vapor deposition or by purchasing from a commercial source (see Note 11).

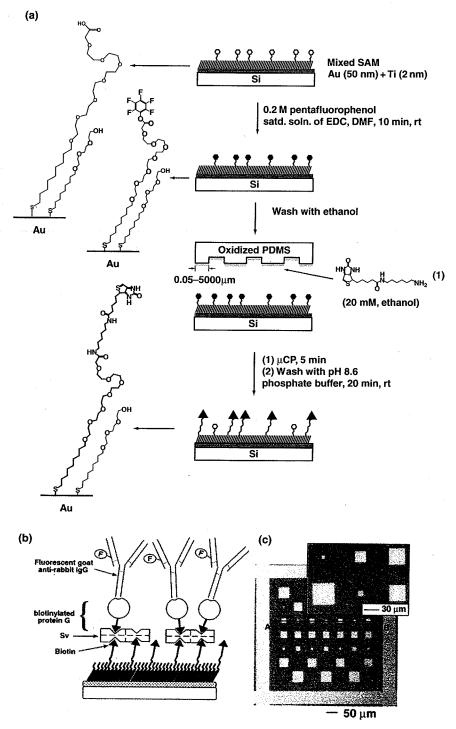


Fig. 3.10. (a) Schematic diagram for the patterning of reactive SAMs by microcontact printing. (b) Schematic diagram of an immunoassay performed on a sample patterned by this technique. (c) Fluorescence micrograph of patterned surfaces with fluorescently labeled antibodies bound to SAMs presenting biotin groups. The scheme in (b) and the fluorescence micrograph in (c) are reprinted permission from Lahiri et al. (85) © 1999 American Chemical Society.

- 3. Prepare a photoresist master and a PDMS stamp as described in Sections 3.2 and 3.4.1. The stamp should be designed such that the raised features represent where the ligands will be patterned on the surface.
- 4. Immerse the gold-coated wafer in the solution prepared in Step 1 for 2 h. Remove the sample from the solution, wash with ethanol, and dry under a stream of nitrogen. The samples should be used in the next step immediately.
- 5. Immerse the mixed-SAM-coated surface in a solution of DMF containing 1-ethyl-3-(dimethylamino)propylcarbodiimide (EDC) (0.1 M) and pentafluorophenol (0.2 M) for 10 min. Remove the sample from the solution, wash with ethanol, and dry under a stream of nitrogen.
- 6. Prepare an ethanolic solution of biotin cadaverine (1) (2 mM).
- 7. Oxidize the surface of the PDMS stamp in an oxygen plasma cleaner for 30 s.
- 8. Wet a cotton swab with the solution in Step 6 immediately following oxidation of the surface of the stamp. Dry the stamp under a stream of nitrogen.
- 9. Place the stamp in contact with the activated SAM for 5 min. Remove the stamp manually.
- 10. Immerse the patterned surface in phosphate buffer (pH 8.6, 25 mM) for 20 min to hydrolyze the remaining activated carboxylic acid groups.
- 11. Rinse the substrates with deionized water and ethanol and dry under a stream of nitrogen.

4. Notes

1. This solution and its ingredients are extremely corrosive. It will burn holes in clothing and cause severe skin burns on contact. It can also explode on contact with any significant quantity of organic solvent or material. It is safe when used (as here) to remove small quantities of organic impurities from non-oxidizable surfaces. Please follow appropriate safety protocol (i.e., using the solution in a fume hood and wearing thick rubber gloves, a lab coat, and goggles with side shields) while using this solution. Please read the Materials Safety Data Sheets (MSDS) for proper disposal instructions.

- 2. The files must be compatible with the plotters or printers used to make the transparencies. Check first with the commercial printing company to assure compatibility of your files with their printers.
- 3. The spin speeds, exposure conditions, and developing solutions vary for each type of photoresist. This information can be obtained from the companies that manufacture the resist. It is best to perform photolithography in a clean room facility to minimize contamination of the features by dust. Some experimentation is necessary to determine the proper exposure times; these times can vary widely from lamp to lamp.
- 4. Photolithography with the side without the ink in contact with the photoresist yields distorted features because of diffraction of light as it passes through the mask.
- 5. The mask and the surface of the substrate will be in focus at the same time only if the mask is in a conjugate image plane to that of the substrate. Most microscopes have a lens that expands the image to fill the back aperture of the objective. In a Leica DMRX microscope, this objective reduces the reduction power of the objective by a factor of 4.
- 6. The UV light in the room where this procedure is carried out must be minimized to prevent accidental exposure of the photoresist. The photoresist will not be exposed by short periods (<30 min) of illumination from standard fluorescent lighting.
- 7. This technique works with oil and water immersion lenses. A glass cover slip may be used to protect the surface of the resist from the oil, but is not necessary when using water.
- 8. Distortions in the size and shape of the photoresist features can occur at the edges of the lens array (>1 cm from the center of the array). It is therefore difficult to produce arrays of features by this technique that are uniform in shape and size over areas larger than 4 cm².
- 9. PDMS membranes can be difficult to handle because they are flexible and tear easily. It is useful to cure a thick (~1 mm) "ring" of PDMS outside of the area of the photoresist features. This ring of PDMS provides structural rigidity to the membrane, so the membranes can be handled easily using tweezers.
- 10. The sealing of PDMS to a slab of PDMS uses the same procedure as that of sealing PDMS to glass; it, however, requires plasma oxidation of *both* pieces of PDMS before they are placed in contact.
- 11. Gold substrates can be purchased from vendors such as Platypus Technologies (Madison, WI).

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