Fabrication of Three-Dimensional Microfluidic Systems by Soft Lithography

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Introduction

Two-dimensional (2D) methods for transferring patterns to planar substrates have enabled the technological revolution in microfabrication that has marked the last 40 years.[1] The overall trend toward increased miniaturization has led to the development of new types of devices in areas unrelated to conventional microelectronics: analytical tools, chemical reactors, microelectromechanical systems (MEMS), optical systems, and sensors.[2] The widespread use and high level of technological development associated with photolithography has also made the methodologies for microelectronics—patterning photosensitive polymers, etching and deposition of thin films, and liftoff—ubiquitous in the fabrication of these new classes of microsystems. These new systems have specialized requirements, however, and are not simple extensions of microelectronics technologies. They often require materials—especially organic polymers—that are not commonly used in microelectronic systems, they must have low cost, and they may need 3D structures in order to implement complex designs. These requirements have stimulated the development of new methods for microfabrication.

Mastering and Replication

The process of microfabrication generally can be divided into two steps: (1) the generation of a master and (2) the use of the master to produce replicas. In photolithography, a mask writer is used to create a pattern of chromium on a glass or silica plate for each lithography step; the pattern subsequently is transferred onto a coating of photosensitive polymer on each wafer in production. The process used to generate the original mask is a serial, high-precision technique that uses a beam of photons or electrons to transfer a pattern from the computer into a resist layer. This process is expensive, but the high cost of the original master is offset if the number of replicas generated from the master is large and the value of the final device is high.

Soft Lithography

Soft lithography is a collection of low-cost techniques for replicating patterns—from masters generated by photolithography, machining, or other methods—onto a range of substrates.[3–5] The term "soft" is taken from condensed-matter physics, and we used it originally to refer to organic elastomers used as stamps or molds. We now use it more generally to refer to organic and organometallic materials; the "soft" component can refer to either the system used in pattern transfer or the materials being patterned, but particularly the former. In most applications, soft lithography uses an elastomeric, topographically patterned replica or stamp—typically fabricated from poly(dimethylsiloxane) (PDMS), an elastomeric organic polymer—to transfer the original pattern of a master by molding or printing. PDMS is inexpensive, homogeneous, optically transparent, nontoxic, and commercially available. The flexibility of the PDMS replicas also allows patterning by microcontact printing or micromolding on nonplanar structures, either by wrapping thin stamps around modestly curved substrates or by rolling a curved substrate over a stamp (see the article by Rogers in this issue).[3,6,7]

Soft lithography can replicate structures with feature sizes ranging from 1 mm to less than 10 nm (Figure 1).[3] Soft lithography also includes a set of techniques for transferring the edges of topographically patterned features onto a substrate with critical dimensions ranging from 50 nm to 200 nm. These techniques include topographically directed etching (TODE),[8] near-field phase shifting,[9] maskless lithography,[10] and controlled undercutting.[11]

Rapid Prototyping

The goal of rapid prototyping is to shorten the cycle from concept to working prototype. For many of the emerging applications of microfabrication (and especially those in microfluidic systems and micropatterned substrates for bioanalysis and cell biology), feature sizes are relatively large (~50 μm); organic polymers are often the materials of choice for these applications, since they must be inexpensive. For these sorts of systems, soft lithography provides an efficient and convenient method for prototyping such structures rapidly. An initial design is sketched in a CAD design program, and a patterned mask for photolithography is generated using a high-resolution image setter (in essence, high-resolution commercial printing). With a 5080-dpi image setter, minimum linewidths of the order of 40 μm (~25 μm with slight distortions) are easily attained on transparent film;

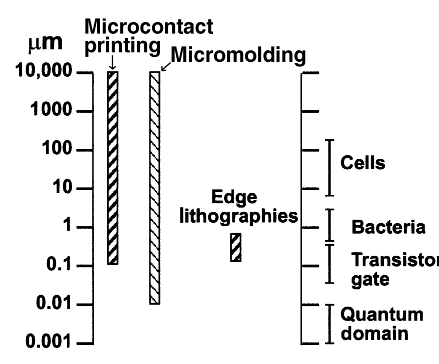

Figure 1. Scale bars indicating the ranges of feature sizes that have been demonstrated for the subclasses of techniques within the suite of soft lithography.

these printed transparencies can be used for photolithography. The film masks cost 20–100 times less than a chromium mask of the same patterns, and they take hours to produce rather than days or weeks. With further optical reductions of the film masks, feature sizes of the order of 1–10 μm are achieved over limited areas of exposures.[12] Photolithography transfers the pattern of the mask into a photoresist layer; after development, the bas-relief pattern of the photoresist serves as the master for generating stamps/molds in PDMS. A negative replica of the photoresist master is generated by casting an uncured prepolymer of PDMS against it.[3,4] This soft replica is the stamp or mold used for subsequent pattern transfers.

Polymeric Microfluidic Systems

Soft lithography, and especially replica molding, is well suited for generating microfluidic channels in PDMS with cross sections of the order of 50 μm $\times$ 50 μm.[13,14] Polymers provide an alternative to the materials first used for microfluidic systems, namely, silicon and glass. The polymer channels have advantages over silicon or glass microfluidic channels in that they are inexpensive, they are flexible and durable, and they are simple to prototype; they have the disadvantage that they are not stable in contact with some organic solvents and at high temperatures. They are, however, entirely appropriate for use with aqueous samples important in biology.

Single-Level Fabrication

A 2D, elastomeric channel system typically is fabricated by replica-molding of the channels in PDMS, starting with a photoresist (SU-8) master generated by photolithography (Figure 2). The inlets and outlets are bored into the monolithic elastomer using a small drill. In order to enclose and seal the microfluidic system, the channels are treated in an air plasma and then quickly placed into contact with another oxidized surface for support (silicon wafer, glass, PDMS).[14] The plasma treatment is believed to form open Si-OH groups on the surface of the PDMS that can bind irreversibly to the substrate by the loss of water.[15]

Laminar Flow Etching

The small size scale of microfluidic systems renders all fluidic flow laminar, that is, without turbulence.[16] In multiphase laminar flow, streams of different fluids—introduced from a "Y" or "T" junction—flow side by side with only diffusional mixing. This controlled mixing can be used to create metal wires, luminescent lines, patterned crystals, selectively dyed cells, and a system for microelectrochemical detection.[17–20]

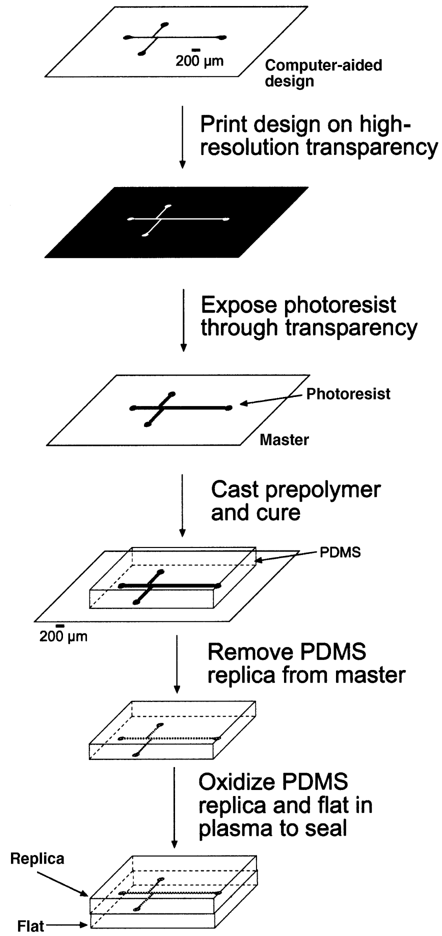

Figure 2. Fabrication scheme for single-level polymeric microfluidic channels. A computer-assisted design file is printed onto a transparency photomask, which is then used in photolithography to generate a photoresist master. A prepolymer of poly(dimethylsiloxane) (PDMS) is cast onto the master, and the inverse replica is removed from the master and sealed onto a solid support (flak) (e.g., glass, silicon, or polymer).

One application of microfluidic channels fabricated by soft lithography uses laminar flow to create surface topography inside the channel (Figure 3). Different topographical features from 10 μm to 100 μm can be formed by controlling the extent of etching, by adjusting widths of the etching stream(s), or by using obstacles in the channels to redirect the etchant flow.[19] Additional steps of laminar flow etching along different axes within the channel system can be aligned to form more complex or compartmentalized features. Topographical micropatterning is a technique that is well suited for studying the response of cells to microenvironments and for growing cell cultures in microchambers.

Gradient Mixer

One application that illustrates the fabrication and use of planar microfluidic channels is the generation of gradients in the composition of a solution or a surface.[21] Gradients are important in both chemistry and biology and are difficult to create reproducibly. Soft lithography generates appropriate systems easily (Figure 4). A branched treelike network of channels produces a gradient in the flow by dividing, mixing, and recombining a small number of inputs several times before rejoining all of the mixed channels into a single output stream. The relative flow rates of the input streams control the width of the gradient in the channel and the shape of the lateral profile of the gradient; thus, the width and the profile can be altered dynamically.

Methods for Fabricating 3D Microfluidic Systems

Two-dimensional channels, which utilize patterned laminar flow or gradient flow, are suitable for simple applications in microfluidics. Other applications may require more complex geometries.[20,22]

Crossing Channels

One approach to generating a 3D channel system is to seal a replica mold with channels indented in its surface to another similar mold with crossing channels. In the resulting two-level channel system, the fluids from both channels split at the junction where the two channels cross. The paths split according to the pressure gradient, which is determined by the aspect ratio at the channel crossing.[23] The splitting is easily observed using parallel, laminar flows of dye (Figure 5). Applying pressure to the elastomeric mold at the crossing point alters the aspect ratio, changes the choice of path of the dye stream, and creates a microfluidic switch.

Membrane Sandwich

Two-level systems do not allow channels to cross without intersecting and mixing. The ability to fabricate an isolated channel crossing requires three levels of fabrication; the ability to cross channels allows *any* channel system to be constructed, and a general method for fabricating

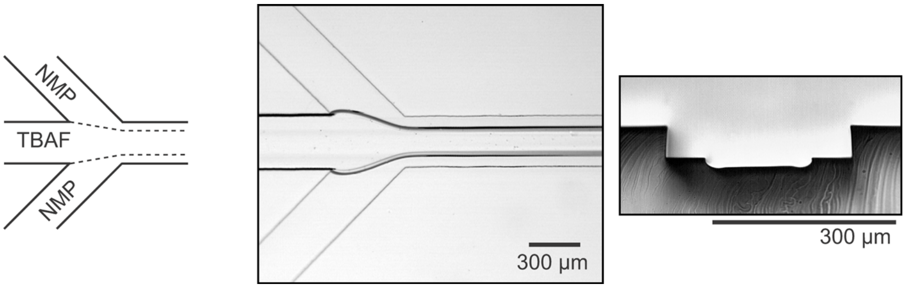

Figure 3. Microfluidic channel with three-dimensional (3D) topographical profile generated by laminar flow etching. The etchant (tetrabutyl ammonium fluoride, TBAF) and nonetching solution (N-methylpyrrolidinone, NMP) were allowed to flow from the designated inlets on the left of the branched channels. The relative volumetric flow rates of the etchant and the nonetching solution control the lateral size and the depth of the features created.[19]

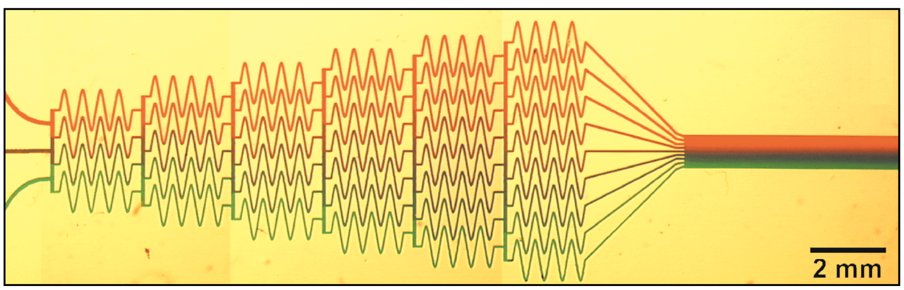

Figure 4. An optical micrograph of a branched network of channels used to output a gradient in the solution flowing through the system. The inputs at the left of the image contain three differently colored dyes that are evenly divided throughout the branched network, mixed, and recombined in the output to give the color gradient.

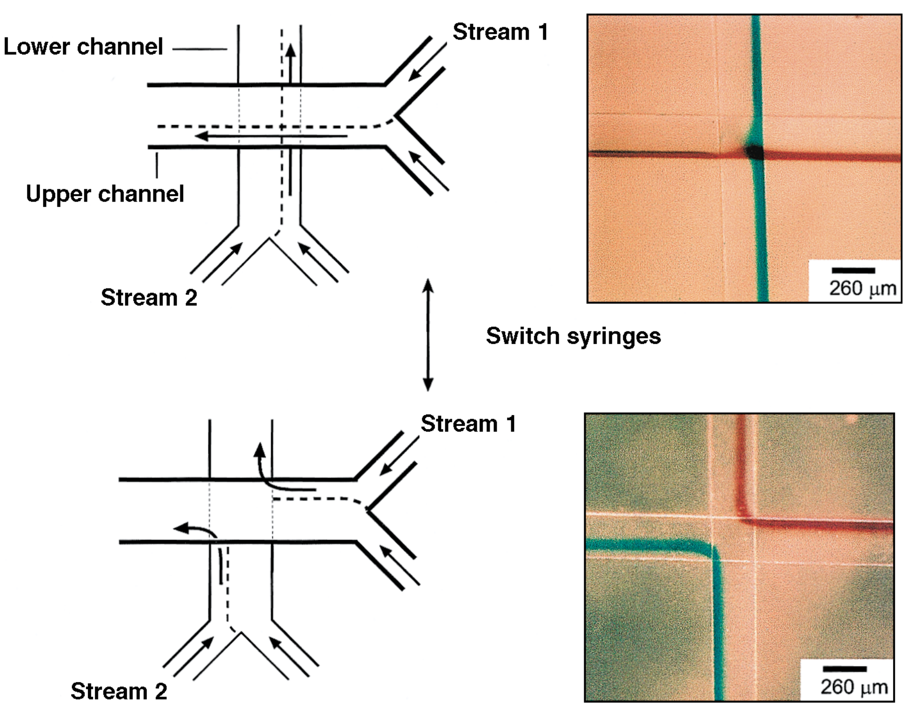

Figure 5. Two-level system of crossing single-level channels in which the fluid flow turns or continues straight, depending on the pressure gradient and the aspect ratio of the intersection.[23]

three-level systems enables the fabrication of microchannel systems of arbitrary connectivity, which is necessary as microfluidic devices become more complex and integrated.

The "membrane sandwich" method is capable of fabricating an arbitrary number of channel crossings in parallel.[17] Replica-molding of PDMS, compressed between two patterned masters, creates thin, flat, and flexible membranes containing channels (Figure 6). When the protruding features of a top and bottom master are aligned and in contact, a three-level channel system within a single membrane is formed. Alignment tracks on the masters simplify the process of registration and alleviate the need for microscopes or other alignment tools. By mechanically supporting the membrane, the stepwise transfer of the membrane from each master to a flat slab of PDMS encloses the open channels without tearing or distorting the features of the channels.

The ability to generate a membrane with a flat surface (except for the pattern) makes it possible to stack an arbitrary number of membranes and to seal them to other patterned slabs or membranes to form even more complex channel systems.[17] One application of a multilayer, microfluidic system is as parallel, fluidic problem-solvers. For example, to solve the maximal clique problem (a representative so-called "NP complete" problem) for a graph with six vertices requires 3D fabrication of a 16-level microfluidic device, but offers parallel optical readout of all solutions.[24]

Components for Valving and Pumping

The control of the direction and speed of fluid flow inside a microfluidic system requires a force applied by an external device, by components inside, or both.[25] The membrane sandwich method makes possible a range of functional components that include a small part of the membrane or a flap to passively control fluid flow. One example of such a component is a valve that imposes directionality on the fluid flow without requiring an external energy source. A 3D flapper valve, for example, uses a small piece of the membrane to block the flow path under back pressure but allows free flow under forward pressure.[26]

Because a valve acts as a resistor in a microfluidic channel, a few valves can be connected in series and the individual resistances summed. Multiple valves in series increase the difference between the resistance under forward flow and under back flow (Figure 7a). Furthermore, fabricating a 3D chamber in the middle of a

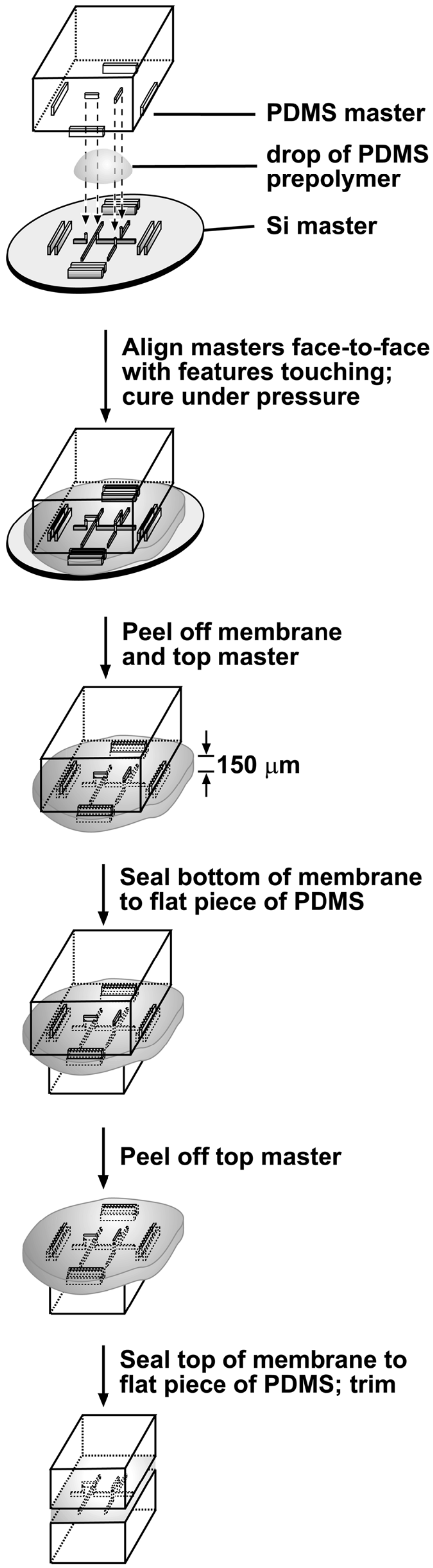

Figure 6. Membrane sandwich method for fabricating thin membranes of PDMS that may contain multiple levels of channels and may be stacked to form complex 3D microfluidic systems.

series of similarly oriented valves generates a reciprocating pump. By applying light pressure to the chamber, fluid is forced in one direction (Figure 7b). Because PDMS is elastomeric, releasing pressure on the chamber pulls fluid into the chamber from the other end.

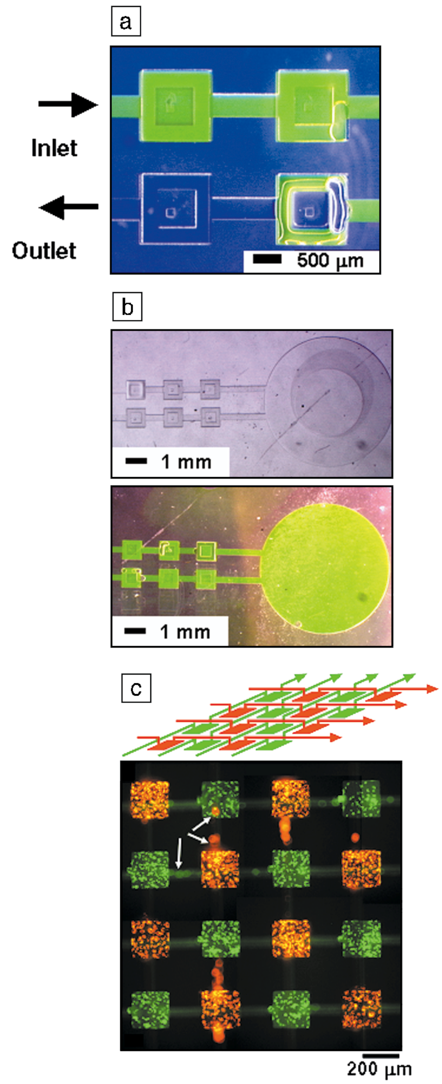

Figure 7. (a) One-way passive flapper valves fabricated by stacking membranes in an integrated 3D microfluidic channel system. (b) Multiple valves in series, with a chamber having an elastomeric membrane as one face, form a reciprocating pump. (c) An interlaced basket weave of 3D microfluidic channels applies two different fluorescent dyes to a cell culture on the surface. The channel system was fabricated using the membrane sandwich method; the bottom layer of the channels in the stamp has openings through which the fluid flow passes over the surface.[28]

Patterning Surfaces Using 3D Microfluidics

Techniques such as photolithography and microcontact printing require multiple steps of registration or overlay to pattern a surface with different materials in discrete areas.[27] The membrane sandwich method has been used to create a 3D "microfluidic stamp" for addressing discrete areas on a surface with different inks in independent channels. Cells deposited on a surface were stained with two different dyes using an array of crossing channels (Figure 7c).[28] Each area was addressed with an open area of the channel in contact with the surface.

Other 3D Microfabrication

A PDMS stamp can be used for microcontact printing on curved surfaces such as glass capillaries and hemispheres. For example, when a capillary is rolled over a patterned PDMS stamp coated with an alkanethiol "ink," its surface is printed with the pattern. By electroplating, the cylindrical surface pattern is built up into a sturdy, metallic microstructure. Metallic microstructures with unusual structural features,[29] medical applications,[30] and topological complexity[6] have been generated using this method.

Such microstructures can serve as templates for 3D microfluidic channels. When a PDMS stamp with a simple 100-μm line pattern is coated with alkanethiol and rolled over a capillary at an angle slightly offset from perpendicular, a helix is printed. Wet chemical etching removes the exposed metal, and a subsequent step of electroplating silver yields a helix 70 μm thick. The capillary was molded with PDMS, removed, and another glass capillary was inserted and sealed in its place to yield a smooth, helical channel (Figure 8a).[31]

A cylindrical surface can be printed by rolling a 2D PDMS stamp over it, but a spherical surface requires the stamp to conform to its shape. A PDMS membrane under vacuum is pulled into contact with a metal-coated sphere to transfer a pattern of self-assembled monolayers (SAMs). Glass spheres with radii of curvature of ~1.5 cm have been printed with feature sizes of 50 μm (Figure 8b).[31]

We have shown that this method for patterning spherical surfaces can be extended to 100-nm-scale patterns using two soft lithographic techniques: near-field phase-shifting lithography and topographically directed photolithography. In the former technique, centimeter-scale spheres were coated with photoresist and exposed while under vacuum and in conformal contact with a patterned PDMS membrane; the edges of the topographical pattern led

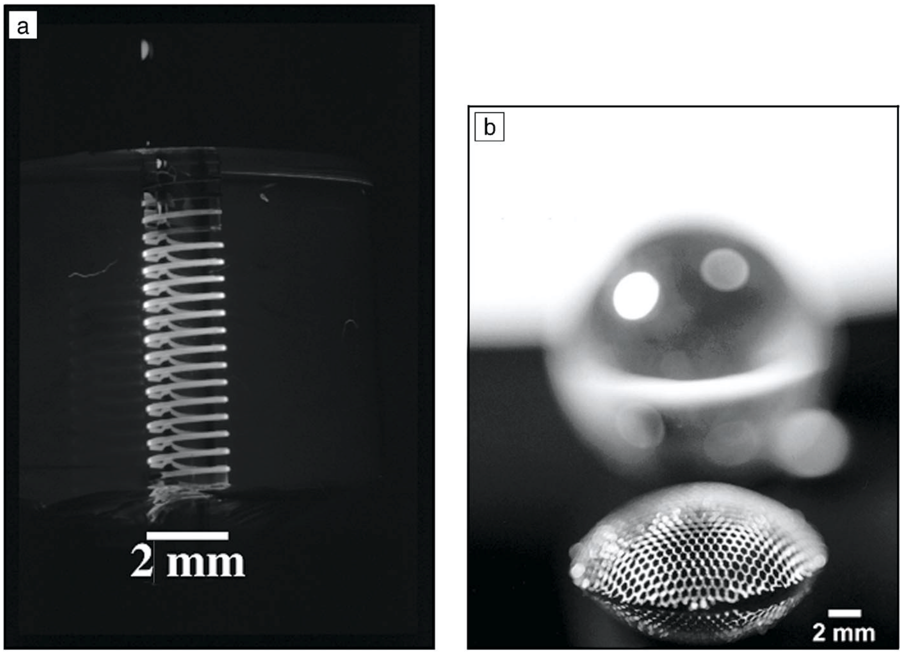

Figure 8. (a) A spiral channel in PDMS filled with fluorescent fluid. A metal helix was first formed by microcontact printing, wet etching, and electroplating. This microstructure was then molded in PDMS, removed, and sealed. The cross section of the helical channel is 100 μm wide by 70 μm deep. (b) A free standing, hemispherical, silver structure with a hexagonal pattern formed by microcontact printing using a PDMS membrane as a template for electrodeposition. The sphere used is in the background.

to lines in the photoresist.[32] In the latter technique, a patterned PDMS membrane wetted with isopropanol contacted the photoresist coating on a sphere and molded its surface; the topography of the photoresist alone focused UV light to make a pattern that was then developed.[10]

Future Directions

The methods presented here for fabricating 3D microfluidic systems, combined with others, constitute a set of sophisticated but convenient procedures for fabricating complex microsystems. These tools will facilitate fundamental studies in cell biology and other fields and will provide low-cost alternatives to current devices for micrototal analysis (μTAS) and high-throughput screening (HTS). The ability to design in 3D will offer the capability to generate microfluidic systems with compact designs and small areas. The ability to fabricate complex 3D microfluidic systems will enable new systems that mimic certain biological functions (e.g., the circulation of blood). Miniaturization of the critical channel dimensions to the nanoscale will make it possible to explore fluid flow on these scales; examples of subjects that are particularly interesting include fundamental postulates of fluid dynamics, entropic effects on polymers in confined spaces,[33] and the effects of overlapping ionic double layers.

Acknowledgments

This research was supported by DARPA NSF ECS-9729405 and NSF ECS-0004030. J.C. Love gratefully acknowledges the U.S. Department of Defense for support in the form of a graduate fellowship.

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