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Fabrication of Topologically Complex Three-Dimensional Microfluidic Systems in PDMS by Rapid Prototyping

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This paper describes a procedure for making topologically complex three-dimensional microfluidic channel systems in poly(dimethylsiloxane) (PDMS). This procedure is called the "membrane sandwich" method to suggest the structure of the final system: a thin membrane having channel structures molded on each face (and with connections between the faces) sandwiched between two thicker, flat slabs that provide structural support. Two "masters" are fabricated by rapid prototyping using two-level photolithography and replica molding. They are aligned face to face, under pressure, with PDMS prepolymer between them. The PDMS is cured thermally. The masters have complementary alignment tracks, so registration is straightforward. The resulting, thin PDMS membrane can be transferred and sealed to another membrane or slab of PDMS by a sequence of steps in which the two masters are removed one at a time; these steps take place without distortion of the features. This method can fabricate a membrane containing a channel that crosses over and under itself, but does not intersect itself and, therefore, can be fabricated in the form of *any* knot. It follows that this method can generate topologically complex microfluidic systems; this capability is demonstrated by the fabrication of a "basketweave" structure. By filling the channels and removing the membrane, complex microstructures can be made. Stacking and sealing more than one membrane allows even more complicated geometries than are possible in one membrane. A square coiled channel that surrounds, but does not connect to, a straight channel illustrates this type of complexity.

The complexity of microfluidic systems is increasing rapidly as sophisticated functions—chemical reactions and analyses, bioassays, high-throughput screens, and sensors—are being integrated into single microfluidic devices.[1−7] Complex systems of channels require more complex connectivity than can be generated in a single level, since single-level design does not allow two channels to cross without connecting. Most methods for fabricating microfluidic channels are based on photolithographic procedures and yield two-dimensional (2D) systems.[8] There are a number of more specialized procedures—stereolithography,[9] laser−chemical three-dimensional (3D) writing,[10] and modular assembly[11]—that yield 3D structures, but these methods are not ideally convenient either for prototyping or manufacturing and are not capable of making certain types of structures. Better methods for generating complex 3D microfluidic systems would accelerate the development of microfluidic technology. This report presents a procedure we call the "membrane sandwich" method for fabricating, transferring, registering, and fusing multiple elastomeric membranes of poly(dimethylsiloxane) (PDMS) that contain one or more in-plane systems of microfluidic channels. This procedure provides a convenient route to complex microfluidic systems.

Representation and Analysis of Systems of Microfluidic Channels as Knots. We can extend the analogy between a microfluidic nonbranching channel and mathematical non-self-intersecting, curved line; the capability of the membrane sandwich method to fabricate the physical realization of knots is evidence that it is a general route to making topologically complex channel systems. In mathematical terms, a knot is a closed, non-self-intersecting, curved line in three dimensions (a homeomorphic image of the unit circle).[12] Knots are typically described in

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mathematics in terms of their projections to a plane. For nontrivial knots, these projections contain "double points"—that is, points where the projected curve crosses itself. A knot can always be slightly perturbed in 3D so that, in projection, it has no triple- or higher-order points: that is, points that the projected curve crosses three or more times. Hence, knots can be described completely by giving such a projection, together with the information about which piece of the curve crosses over or under the other piece, at each double point.

The problem of designing a method for making arbitrary 3D systems of channels can therefore, in principle, be reduced to developing a method of fabricating the physical realization of the double point—that is, a fabrication method that allows one piece of a channel to cross over or under another can, in principle, produce a physical realization of any knot. Likewise, such a method can produce physical realizations of any arrangement of interlinked knots and of arbitrary 3D networks whose projections to the plane can contain many crossings. For systems of microfluidic channels based on stacked planes to avoid intersection at the point of crossing, there must be three identifiable levels: a bottom level that contains the channel that crosses "under", an upper level that contains the channel that crosses "over", and a center level that isolates and connects the bottom and top levels. No additional levels are needed because triple- or higher-order points in the projection are not necessary.

The membrane sandwich method has the capability to fabricate a membrane in one step that contains three levels. The features present in the three levels are divided between two "masters"—a "top master" and a "bottom master"—that we fabricate by a rapid prototyping methodology based on high-resolution commercial printing.[13−15] This method produces patterns of channels in the upper and lower levels, with vias between them, all contained within a single membrane. Because any number of crossings can be fabricated in parallel using this procedure, it has the capability to generate a channel system of arbitrarily many crossings—that is, the physical realization of any mathematical knot or network—in a single membrane. We demonstrate that our method indeed has this capability in practice by fabricating a basketweave structure, since its highly interwoven structure challenges the ability of any fabrication methodology to handle crossings.

RESULTS AND DISCUSSION

Fabrication of Microfluidic Channel Systems by the Membrane Sandwich Method. (a) Fabrication of the Bottom Master of Photoresist on Silicon by Multilevel Photolithography. We fabricated two levels of features that are "high relief"—that is, raised above the surface of the silicon wafer—by using two-level photolithography. Two-level photolithography comprises two, registered steps of photolithography generating two-level structures in photoresist after development (Figure 1). We first patterned the short (in the *z*-direction) features of the master by spin-coating a layer (typically 50−150 μm thick) of negative photoresist onto the wafer and exposing it to UV through a high-resolution transparency that acts as a photomask.[13−15] *Without developing the un-cross-linked photoresist*, we spin-coated a second layer of photoresist (also about 50−150 μm thick) on top of the first. We aligned a second photomask to the cross-linked features and exposed the photoresist. This photomask contained the pattern for the tall features: that is, the interconnects that would eventually link the channels of the upper and lower levels, and also tall alignment tracks that surrounded the channel system. We then developed the exposed photoresist; both layers of photoresist developed at the same time.

There are two reasons for using two-level photolithography in making the membrane that provides the basis for the channel

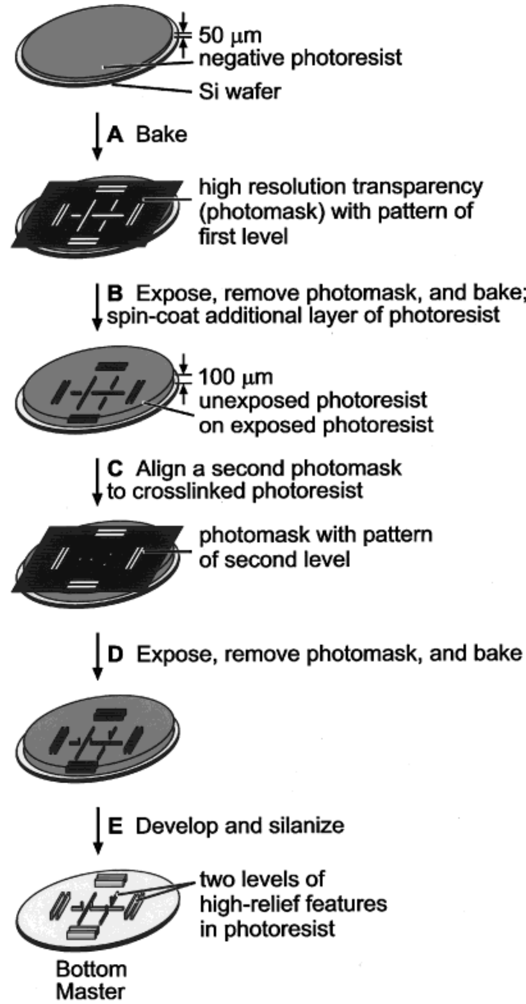

Figure 1. Scheme describing the fabrication of the bottom master of two levels of photoresist on silicon by multilevel photolithography. (A) A layer of negative photoresist, 50−150 μm thick, was spin-coated on a silicon wafer and soft-baked for 5−15 min. (B) A high-resolution transparency containing the lowest level of the channel system and alignment tracks was used as the mask in photolithography. The photoresist was hard-baked and a second layer of negative photoresist, also 50−150 μm thick, was spin-coated on top of the first. (C) A second high-resolution transparency was aligned to the exposed features under the unexposed photoresist. This transparency contained the design for the middle level of the channel system (the interconnects between channels in the lowest and uppermost levels) as well as the alignment tracks. (D) The resist was exposed and soft-baked for 10−20 min. (E) The master was developed to yield two levels of high-relief features and passivated by vapor-phase silanization.

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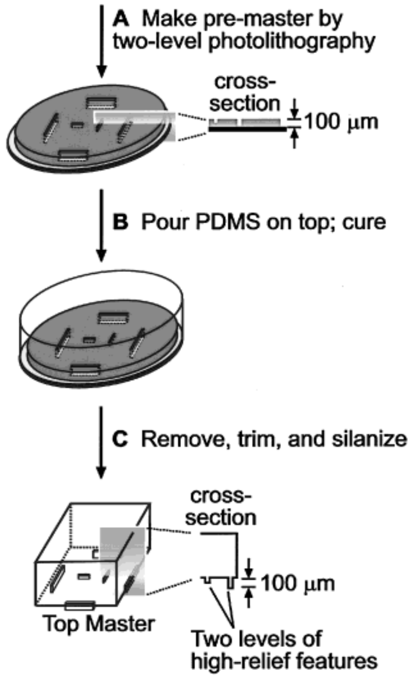

Figure 2. Scheme describing the fabrication of the top master of PDMS by replica molding. (A) Multilevel photolithography was used to generate a premaster: two levels of low-relief features in a photoresist layer on a silicon wafer. The shallow level went only halfway through the double layer of photoresist (100−300 μm thick in total); it included features for the uppermost level of the channel system. The deeper level went through the entire double layer of photoresist to the silicon surface and included no part of the channel system, only the alignment track. (B) Prepolymer of PDMS was poured onto the premaster and cured at 75 °C for 1 h. (C) The PDMS was peeled off the premaster, trimmed to a convenient size, oxidized for 1 min in an air plasma, and passivated by vapor-phase silanization to give the top master of PDMS with high-relief features.

system. First, every registration step in the production of the photoresist-on-silicon master eliminates two steps—a registration step and a sealing step—later in the assembly of the system. For example, two levels, once aligned when fabricating a master, are automatically aligned in every replica mold of it. Second, photolithography in photoresist supported on silicon is a well-understood technology, and because the substrate is rigid, registration of 50-μm features is straightforward. Registration of PDMS structures is technically more complicated since they can stick, slide, or distort.

(b) Fabrication of the Top Master in PDMS by Two-level Photolithography and Replica Molding. We made the top master of PDMS by first fabricating a two-level structure in photoresist on silicon—the "premaster". The premaster contained features in "low-relief"—that is, below the level of the bulk surface—so that molding PDMS to it produced features in high relief, as shown in Figure 2. To generate low-relief features, we fabricated a premaster that was covered with a thick layer of photoresist everywhere except the features of the channel system and those of the alignment tracks. The features of the channel system extended to a level below the surface of photoresist but did not traverse it completely; these features were all on one level. Alignment features that we designed to fit between the alignment tracks on the bottom master were in deeper low relief and went all the way through the photoresist to the silicon wafer. We formed the top master by casting PDMS on the premaster. When cured, the PDMS was removed, oxidized, and silanized. This top master had two levels of high-relief features: tall alignment tracks and short features for the channel system. The bottom master also had tall alignment tracks, but its features for the channel system were both tall and short. When the top and bottom masters are put together, the channel system comprises three levels.

(c) Fabrication of the Membrane Sandwich by Replica Molding of the Membrane and Two Cycles of Successive Steps of Transfer and Sealing. We placed the two masters face to face with a drop of PDMS prepolymer between (Figure 3A). We aligned the features of the masters quickly and without magnification by manually sliding the top master over the prepolymer and bottom master until its tall alignment tracks slipped between the tall alignment tracks of the bottom master. Using PDMS for the top master enabled us to observe these features and made alignment straightforward: a microscope was not necessary because the alignment tracks were macroscopic. In addition to facilitating the alignment of the segments of the channel system quickly and without magnification, the tracks balanced the top master and prevented the registered masters from shifting positions in response to physical disturbance or pressure.[16]

We applied pressure to the top master and elevated the temperature to cure the PDMS in place. Channels in the *z*-direction formed at the points where the features of PDMS master conformally contacted the features of the bottom master and excluded prepolymer. If sufficient pressure was not applied to the top of the sandwich to exceed the critical pressure of ~100 g/mm^2 (1000 kPa), prepolymer seeped between the features and a thin film of PDMS blocked the via (the vertical channel) that was intended to connect channels in the upper and lower levels.

Using this procedure, we are able to make membranes less than 20 μm in thickness. These membranes are, however, relatively fragile. More importantly, because PDMS is a relatively soft elastomer, if it is handled unsupported, distortions or sagging can seriously deform the channels. We therefore required a method of transferring the membrane to the PDMS slabs that served as facing structures and a method of sealing the resulting "membrane sandwich" (Figure 3B−E). To accomplish these tasks, we developed a procedure for manipulating and sealing the membrane to a slab of PDMS that took advantage of the subtle gradations in noncovalent interfacial adhesion between PDMS and other substrates.

The PDMS membrane did not bond permanently to either the bottom (photoresist on silicon) master or top (PDMS) master, but adhered more weakly to silanized photoresist/silicon structure than to the silanized PDMS. We first separated the silicon master by simply peeling off the PDMS master and membrane. Before separating the PDMS master and membrane, we sealed the bottom surface of the membrane irreversibly to a flat, featureless slab of PDMS by oxidizing them both in an air plasma for 1 min and immediately bringing them into conformal contact. This process results in irreversible sealing by a process in which we

(16) Manual alignment was accurate to ~10 μm. In some cases we observed that the PDMS of the top master shrank by ~1% during curing and this shrinkage limited the accuracy of registration, especially over larger areas of 1 cm^2 or more. U.S. Patent Pending.

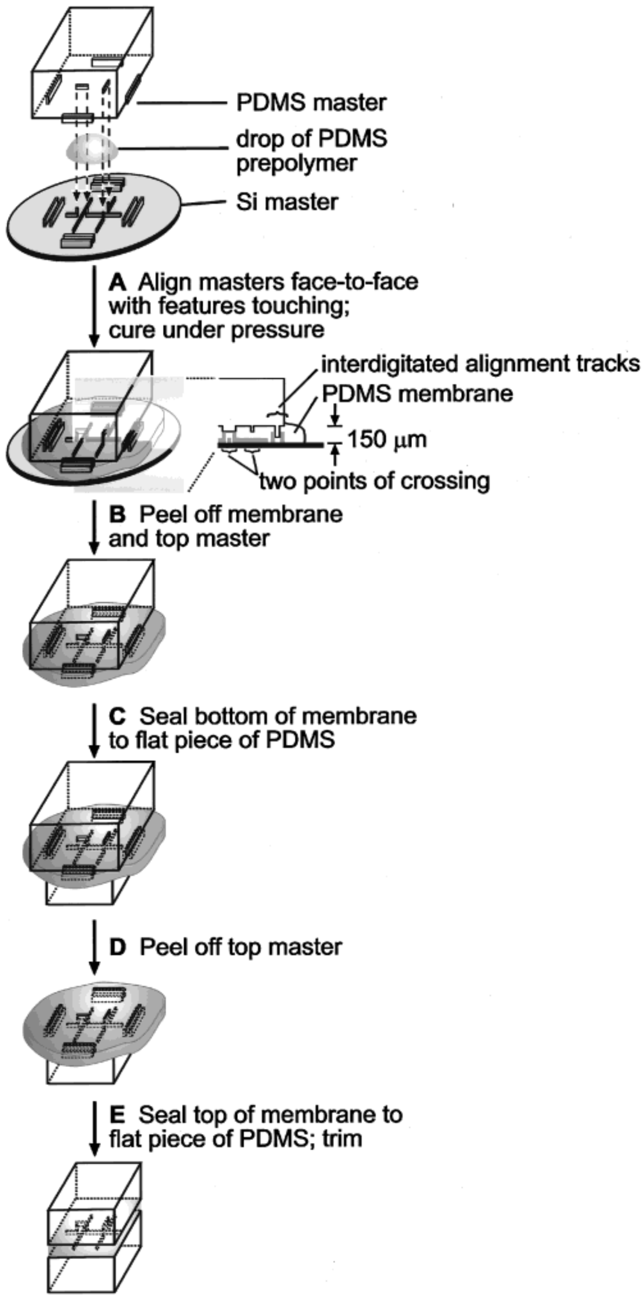

Figure 3. Scheme describing the membrane sandwich method. (A) The top master was placed facedown on top of the bottom master with a drop of PDMS prepolymer in between. The top master slid on the prepolymer until its tall alignment track fell into the tall alignment tracks of the bottom master and the segments of the channel system from both masters were aligned. Sufficient pressure was applied to the top master so that prepolymer did not seep between features that were in contact, and the PDMS was heated to 75 °C and cured in place. (B) The PDMS membrane and the top master were peeled off the bottom master. (C) The bottom of the membrane and a flat slab of PDMS were oxidized in an air plasma for 1 min and brought into contact to seal. (D) The top master was peeled off. (E) The top surface of the membrane was sealed to a flat slab to enclose the channel system and trimmed to a convenient size.

believe that the Si−OH groups formed by the plasma condense to form Si−O−Si.[17−20] With the membrane thus supported, we then peeled off the PDMS master and sealed the top surface of the membrane to another slab of PDMS to completely enclose the channel system of the membrane.

DEMONSTRATIONS OF CAPABILITY

Microfluidic Basketweave. To illustrate the capability of the membrane sandwich method to make topologically complex systems of channels, we fabricated an interwoven network of channels we call a "basketweave" channel system (Figure 4A). The membrane of PDMS incorporated eight channels in the *x*-direction and eight in the *y*-direction, all of $70 \times 100\ \mu$m cross section, each alternating between crossing over and under the perpendicular channels (Figure 4B). Thus we fabricated, in parallel, without interface or sealing, 64 crossovers in an area of 30 mm^2. Since the membrane sandwich method easily handles large numbers of channel crossings, it provides a general route to the fabrication of networks and channel systems of complex topology within a single membrane.

We filled the channels by capillarity to demonstrate that every channel we formed was continuous. In addition, we used different colors of fluid, as shown by shading differences in Figure 4C, to show without ambiguity which channel crosses over which and to demonstrate that the channels do not intersect at any point. We conclude that curing PDMS around the features of the top and bottom masters that are in contact is a method of fabricating channels in the *z*-direction precisely and reproducibly.

A microfluidic channel system provides a route to topologically complex micro*structures*—that is, the physical realization of the 3D knots—in polymer. We filled the channels with photocurable epoxy prepolymer, exposed it to ultraviolet light for 20 min through the PDMS casing, and dissolved the PDMS using tetrabutylammonium fluoride (Figure 4D).

Coiled Channel Surrounding a Straight Channel. The membrane sandwich method produces a membrane that is flat (except for its features) on both its top and bottom surfaces. Once the first membrane is sealed to a solid support, we can align and seal a second membrane to the first. This capability of the method makes it possible to align the features of the membranes and stack them to make more elaborate 3D channel systems.[21,22] We routinely stack five membranes with 200-μm features and a channel structure extending over an area of 4 cm^2.[23]

As we have shown, only one membrane is required for any topology of channels that pass over and under one another, but the requirements for specific geometries may also impose constraints in some applications. Examples of microfluidic systems that benefit from a multimembrane strategy include a configuration in which a coiled channel surrounds a straight channel (for efficient dissipation of heat or countercurrent extraction of small molecules across the PDMS wall with the PDMS acting as a membrane), devices for sorting and binning particles, and systems that have specific volumetric constraints.[24]

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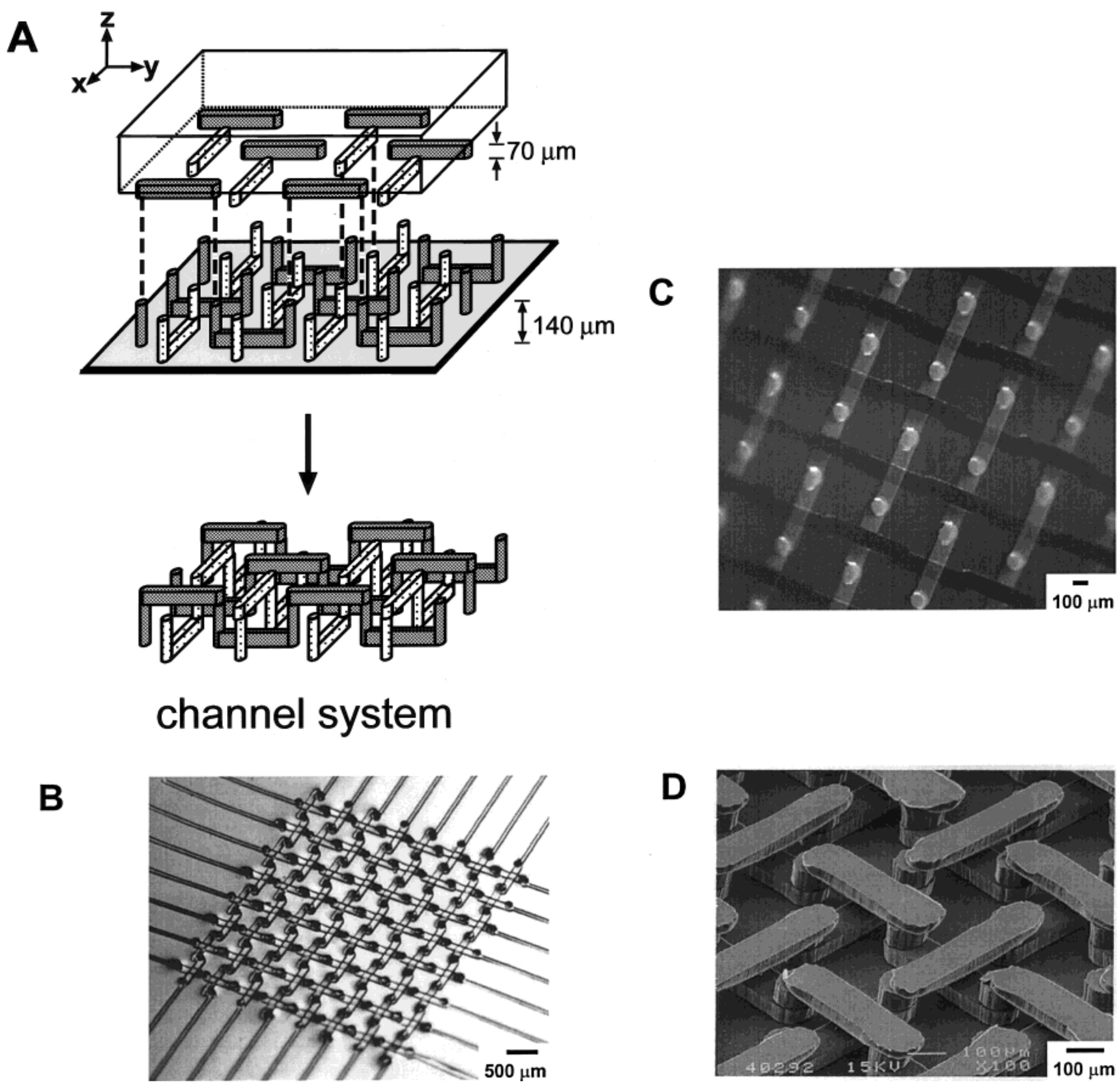

Figure 4. A microchannel system in a basketweave pattern. (A) Schematic illustration of a portion of the structure of top and bottom masters and the channel system that results from using them in a membrane sandwich. For clarity, features in photoresist oriented in the *y*-direction are depicted with a darker pattern than those in the *x*-direction. (B) Optical micrograph (looking down the *z*-axis) of the PDMS membrane alone, which contains the entire 8 × 8 channel system. The channels are 100 μm wide (*x*- or *y*-direction), and each of the three levels used in the fabrication is 70 μm high (*z*-direction) but is not enclosed. (C) Optical micrograph (looking down the *z*-axis) of a portion of the enclosed, fluid-filled channels. Channels in the *y*-direction are filled with a solution of fluorescein and channels in the *x*-direction are filled with a solution of Meldola's Blue dye. (D) Scanning electron micrograph of the microstructure in epoxy polymer. It was formed by filling the basketweave channel system with epoxy prepolymer, curing under ultraviolet light for 10 min, and dissolving the PDMS casing in tetrabutylammonium fluoride.

To demonstrate this capability of stacking, registering, and sealing membranes, we fabricated a straight channel enclosed by thin (∼65−100 μm) PDMS walls; this channel was isolated from the coiled channel that surrounds it. For the peripheral, coiled channel to surround a straight, central channel without connecting to it, the system must have five levels of detail and (at least) two patterned membranes. Figure 5A outlines the design and fabrication of these two membranes, one with two and one with three levels of features.

A registration step was required to assemble the stacked system. We aligned the membranes together using an alignment stage[25] before plasma oxidation. We backed one membrane away from the second by a few millimeters in the *z*-direction, exposed the surfaces of both membranes to an air plasma, and brought the membrane back into contact with the other membrane. Figure 5B shows that the five levels are properly aligned and connected so that the coiled channel is continuous itself but isolated by a thin layer of PDMS (∼65 μm in the smallest *z*-direction) from the straight channel within it.

CONCLUSIONS

The membrane sandwich method provides a route to making, within a single membrane, any network of channels that pass over

(25) The alignment stage consists of two pieces. The first piece is a *xyz* micrometer that has a glass slide immobilized onto the *x*-axis stage. The second piece has a glass slide attached to a rotational stage, which is mounted on a two-axis tilt platform. Both pieces are set on a rail carrier, by which the distance between the two pieces can be adjusted along a dovetail rail. The two glass slides are used to attach conformally the two PDMS layers for alignment. This entire alignment assembly measures less than 3 in. in width.

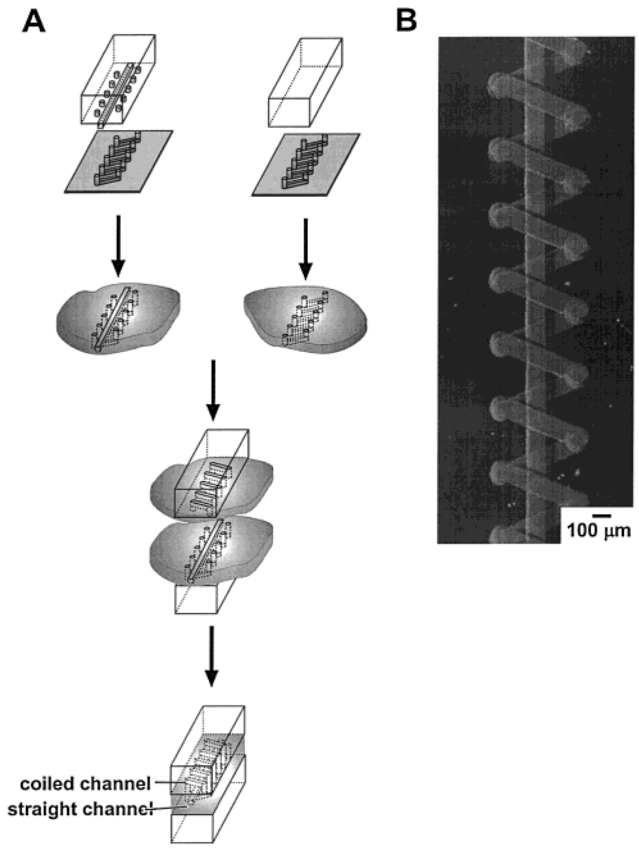

Figure 5. A coiled channel surrounding a straight channel. (A) Schematic illustration of the fabrication of the five-level channel system comprising two membranes. The top and bottom masters shown on the left were used in the membrane sandwich method to make a three-level membrane. The top and bottom masters shown on the right were used in the membrane sandwich method to make a two-level membrane. The bottom masters were removed from the membranes and flat slabs of PDMS sealed in their place. The top masters were then peeled off and the two membranes were aligned face to face on the stages of micromanipulators. This orientation required that the two-level membrane (right) be flipped over. The membranes were backed apart by 3–5 mm, oxidized in an air plasma, and rolled back into aligned contact. (B) Optical micrograph of a portion of the fluid-filled channel system. The two channels were filled with fluorescein.

and under one another; the method has the flexibility to integrate several membranes to make possible the fabrication of more complex or compact systems. As such, it can satisfy a need to prototype 3D systems of channels. Other routes to 3D microfluidic systems that are based on the scheme of stacking layers are limited by the necessary inclusion and alignment of additional substrates (often silicone elastomer) to serve as gaskets and by the difficulty of making effective interconnects between layers that are small enough to be truly microfluidic.[26–28]

The membrane sandwich method combines a convenient process for microfabrication—rapid prototyping based on high-resolution printing—with multilevel photolithography, replica molding, and supported membrane transfer. This combination remains experimentally straightforward but gives the membrane sandwich method the simplicity and flexibility to be an exceptionally useful methodology for prototyping microfluidic devices. These devices may be also used to mold topologically complex epoxy microstructures, as an alternative or complementary method to other established methods of fabricating 3D nonfluidic microstructures.[29–31] With development, it may also be suitable for production, since the steps are intrinsically uncomplicated and inexpensive, although derived from an unfamiliar technology base. We believe that this method will have applications in micro total analysis systems, high-throughput screens, and bioassays.

EXPERIMENTAL SECTION

Fabrication by Multilevel Photolithography. Designs for channel systems were generated in a CAD program (Freehand 8.0, Macromedia, San Francisco, CA). High-resolution (3386 dpi) transparencies were produced by a commercial printer from the CAD files. Negative photoresist (SU 8-50, Microlithography Chemical Corp., Newton, MA) was spin-coated (~5000 rpm, 20 s) on a silicon wafer and soft-baked to drive off solvent (105 °C, 5 min). The transparency was used as a photomask and the photoresist exposed (45 s) to make features ~50 μm high.

The procedure was then repeated using another transparency to make the second level of features ~100 μm high. The transparency was aligned (using the Karl Suss mask aligner) to the exposed photoresist, exposed (1 min), and hard-baked (5 min). It was developed in propylene glycol methyl ether acetate, and silanized (in vacuo, 2 h) with a few drops of tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (United Chemical Technologies, Inc., Bristol, PA). Silanization of the master facilitates the removal of the PDMS replica after molding.

To make the top master, a premaster of photoresist on silicon, made as described above, was covered with PDMS prepolymer and cured at 75 °C for 1 h. It was trimmed, oxidized in a plasma cleaner (PDC-23G, Harrick, Ossining, NY) for 1 min, and silanized (in vacuo, 8 h) with a few drops of tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane.

Molding of the Membrane. The top master was placed face down on top of the bottom master with a drop of PDMS prepolymer between and was registered by hand by sliding the tall alignment tracks of the top master into the tall alignment tracks of the bottom master. The segments of the channel system were thus aligned and the top master balanced on the bottom master. Pressure was applied by placing glass slides on top of the top master, until prepolymer was excluded from between features on both masters that were in contact. Approximately 100 g/mm^2 (1000 kPa) was required.

The membrane was cured in place (75 °C, 1 h). Flat pieces of PDMS were formed by casting prepolymer against a silanized silicon wafer and curing. To transfer the membrane, the membrane and top master were peeled off the bottom master, the bottom surface of the membrane and flat pieces of PDMS were oxidized in an air plasma (1 min), and they were brought together

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immediately. The oxidized PDMS surface remains reactive for a few minutes after plasma treatment—after this time, it will no longer seal. (It eventually returns to its hydrophobic state.) We believe that this change is due to the rearrangement of polymer chains below the surface.[32,33] We have found that we can prolong the reactivity of the surface by covering it with a hydrophilic liquid such as water, methanol, or trifluoroethanol.[34] A protected surface will still seal more than 30 min after oxidation. The transfer was repeated by replacing the top master with a slab of PDMS.

Fabrication of Microstructures. After the channel system was fabricated, epoxy prepolymer (Epo-tek, Epoxy Technology, Billerica, MA) was placed at the channel openings. After ~1 h standing at ambient pressure, the epoxy had degassed and filled the channels completely. The channels could be filled when they were enclosed (membrane sealed between two pieces of PDMS) or unenclosed (membrane alone, unsealed). The filled channels were exposed to ultraviolet light for 20 min through the PDMS. The surrounding PDMS then dissolved in tetrabutylammonium fluoride (1.0 M in tetrahydrofuran).

Stacking of Membranes. Two membranes, each with solid support on one surface, were attached to stages of micromanipulators facing each other and aligned. One membrane was back away from the other about 3−5 mm. The entire apparatus was placed in a plasma cleaner and oxidized for 1 min as described above. The membranes were immediately brought into contact by adjusting the manipulators.

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