

Fabrication of Complex Three-Dimensional Microchannel Systems in PDMS

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Abstract: This paper describes a method for fabricating three-dimensional (3D) microfluidic channel systems in poly(dimethylsiloxane) (PDMS) with complex topologies and geometries that include a knot, a spiral channel, a "basketweave" of channels, a chaotic advective mixer, a system with "braided" channels, and a 3D grid of channels. Pseudo-3D channels, which are topologically equivalent to planar channels, are generated by bending corresponding planar channels in PDMS out of the plane into 3D shapes. True 3D channel systems are formed on the basis of the strategy of decomposing these complex networks into substructures that are planar or pseudo-3D. A methodology is developed that connects these planar and/or pseudo-3D structures to generate PDMS channel systems with the original 3D geometry. This technique of joining separate channel structures can also be used to create channel systems in PDMS over *large* areas by connecting features on different substrates. The channels can be used as templates to form 3D structures in other materials.

Introduction

Three-dimensional (3D) microfluidic systems are useful in many applications. Examples include patterning biomaterials (e.g., proteins, cells) onto surfaces,¹ solving problems in mathematics,² mixing solutions in microchannels,^{3,4} providing essential functions in micrototal analysis systems and microbiosystems,^{5–10} and manipulating light.¹¹ Several strategies have been used to generate 3D microstructures.^{12–20} The most

common strategy uses photolithography to fabricate two-dimensional (2D) layers; these layers are aligned, stacked, and sealed to produce 3D systems.^{2,9,13} Although this method has broad topographical generality, misalignment between adjacent layers¹³ usually creates kinks at the interface between connections. This stacking method is also time-consuming when the shapes and geometries for the 3D microfluidic network require many layers. Another strategy to generate 3D systems is to combine 2D systems with flexible tubing. A method based on mechanical connections may be unsatisfactory, however, for any of four reasons: (i) it does not easily make short connections (e.g., hundreds of microns); (ii) it does not make compact and densely interconnected networks; (iii) it usually uses tubing fabricated of materials that are different from those in the channels; and (iv) it normally uses tubing with a size and shape different from that of the channels (which are usually rectangular).

Here, we describe a simple method for fabricating 3D microfluidic networks in PDMS having complex geometries and topologies, by taking advantage of the fact that PDMS is an elastomer. We divide 3D microfluidic networks into two categories: pseudo-3D and true 3D. Pseudo-3D fluidic channels are systems that are topologically equivalent to noncrossing lines

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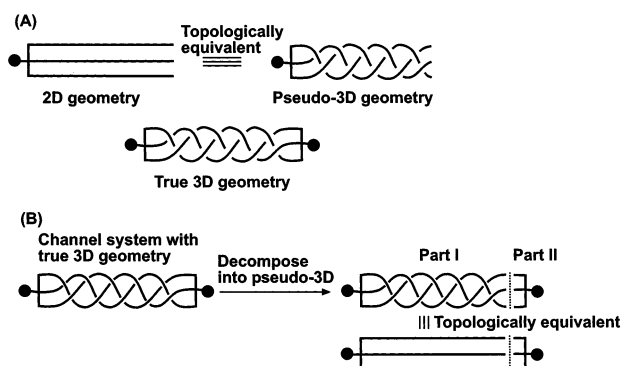


Figure 1. (A) Schematic illustrations of a planar (2D), a pseudo-3D, and a true 3D channel system. (B) Decomposition of the 3D system into two parts of substructures. Both substructures are topologically equivalent to planar structures.

in a plane, and true 3D channel networks are systems that are topologically more complex and, at minimum, require crossing lines when projected onto a plane.^{21,22} Figure 1A shows the difference among a planar (2D), a pseudo-3D, and a true 3D channel network. All true 3D networks can be decomposed into subsections of structures that are planar or pseudo-3D (Figure 1B).

In our approach, planar channels are fabricated in PDMS with a technique described previously.^{23–25} Pseudo-3D channels are generated by bending planar sheets of these channels, which have been cut into shapes of interest, into 3D geometries. Embedding the 3D systems of channels in PDMS freezes the geometries and protects the channels. True 3D channels cannot be formed by simply bending planar channels. Instead, we decompose a true 3D channel system into several substructures, planar or pseudo-3D. We then connect these substructures with a new technique, “plug-in and mold”, to reconstruct a network having the original 3D geometry (Figure 2). Two distinct parts of substructures are used for the reconstruction. The first part (part I) is planar or pseudo-3D channel systems in PDMS with certain ends open for connections. The second part (part II), connectors, starts with planar patterns of photoresist connected to vertical posts of photoresist with sizes chosen to fit into the channels fabricated in part I; these connectors are fabricated by two-layer photolithography on a silicon wafer.¹² After the open ends of the channels of part I are “plugged” into the posts of the connectors of part II, the entire structure is embedded in PDMS. Removal of part II (the wafer supporting posts) from the PDMS structure generates a structure having the required geometry, but with open channels corresponding to the photoresist features on the silicon wafer. The sealing of these open channels to a flat slab of PDMS encloses the entire 3D channel system in PDMS. Holes are drilled into the PDMS as inlets and outlets.

Using this plug-in-and-mold method, we have fabricated a variety of 3D structures including pseudo-3D structures (a figure-eight knot, a roll of channels, a spiral channel, a

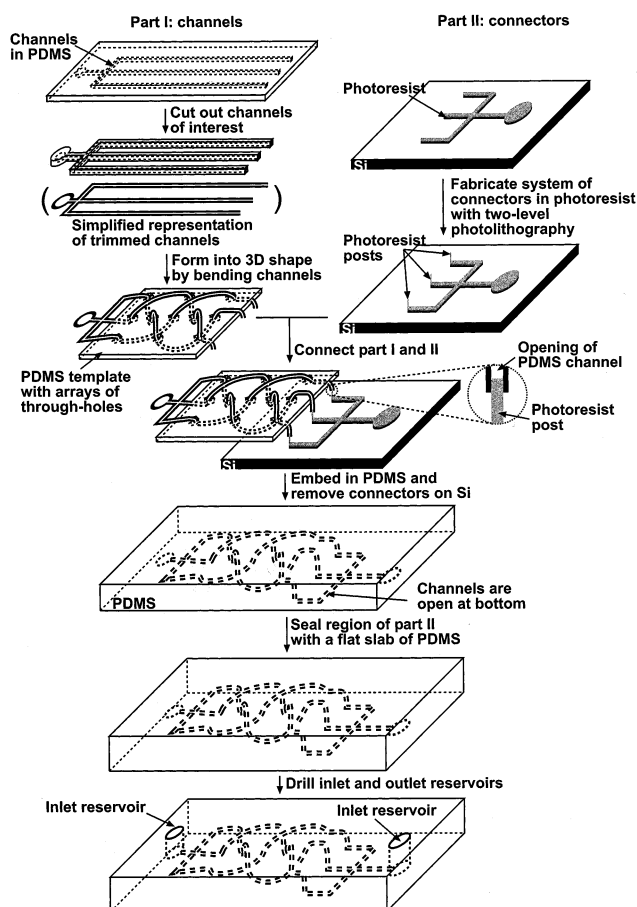


Figure 2. Schematic procedure to fabricate a complex, true 3D microchannel system: fabrication of the substructures (part I, pseudo-3D channels or planar channels, and part II, photoresist structures on a silicon wafer), connection of these structures into the original 3D shape, and embedding of the entire system in PDMS to form a connected, continuous 3D microfluidic system. For convenience, the channels are simplified in the schematic to show the process of braiding and connection afterward.

“basketweave” of channels, and a 3D chaotic advective mixer) and true 3D structures (a system of “braided” channels and a 3D grid of channels). These channels can be used to manipulate light¹¹ or as templates to generate structures in other materials, which may be used as scaffolds for culturing cells. We have also used this method to fabricate microstructures over large areas ($\sim 400 \text{ cm}^2$), since features on separate substrates can be joined and molded together.

Results and Discussion

Fabrication of Part I: Planar and Pseudo-3D Channels in PDMS. (A) **Fabrication of Planar Microfluidic Channels in PDMS.** A layer of negative photoresist (SU-8, micrometers to $\sim 1 \text{ mm}$ thick) was spin-coated onto silicon wafers, exposed to UV light through a high-resolution transparency photomask, and developed to produce masters for molding channels.^{24–26} A prepolymer of PDMS was poured or spin-coated (to make a thin PDMS membrane) on the masters and cured thermally. After peeling the PDMS mold from the master, we formed enclosed channels by sealing the mold to a flat piece of PDMS ($\sim 200 \mu\text{m}$ thick).

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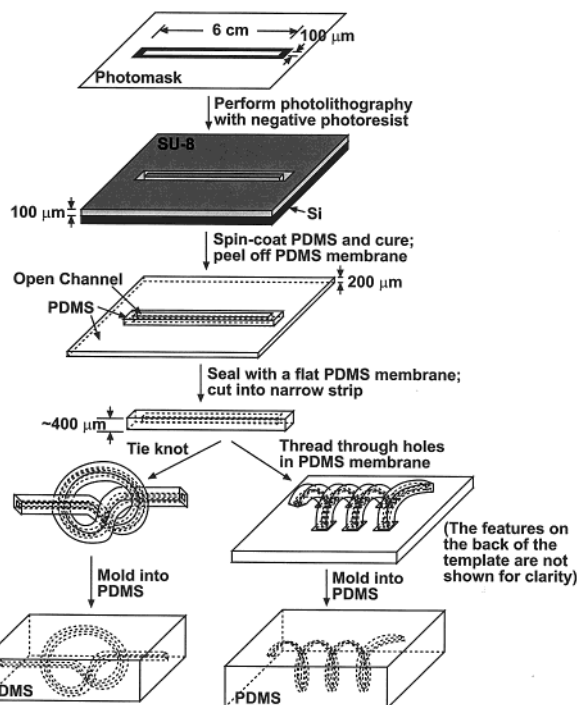


Figure 3. Schematic description of the procedure used to fabricate pseudo-3D microchannel structures by bending systems of planar microchannels in PDMS into 3D shapes, either with or without a template.

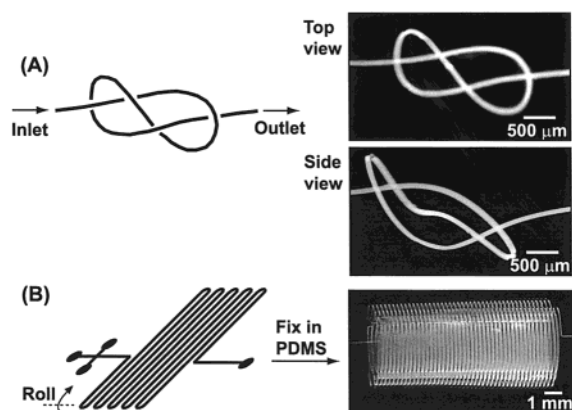


Figure 4. Microchannels produced by bending PDMS microchannels into pseudo-3D geometries. (A) Left: scheme of a figure-eight knot. Right: optical micrographs of the channel having the shape of a figure-eight knot. (B) Left: scheme of a 2D serpentine fluidic channel rolled into a pseudo-3D structure. Right: optical micrograph of the final structure. The channels were visualized by filling with aqueous solution of fluorescein and illuminated with UV light.

(B) Fabrication of Pseudo-3D Channels. Pseudo-3D channels can be fabricated by simply bending a planar PDMS channel system into a 3D shape (Figure 3). The 2D microchannels were fabricated by (i) molding a thin layer of PDMS against a master with photoresist patterns and sealing it to a thin PDMS membrane, and (ii) cutting the enclosed structure in PDMS into shapes corresponding roughly to the shape of the channels under a stereoscope. Strips of channels with walls of $\sim 100 \mu\text{m}$ width can be usually obtained by cutting manually using this procedure. These strips were bent into pseudo-3D channels either with or without a template.

(1) Formation of Pseudo-3D Channels without Templates.

Figure 4A shows a channel tied in the shape of a figure-eight knot; the size of the smallest knot was determined by the actual

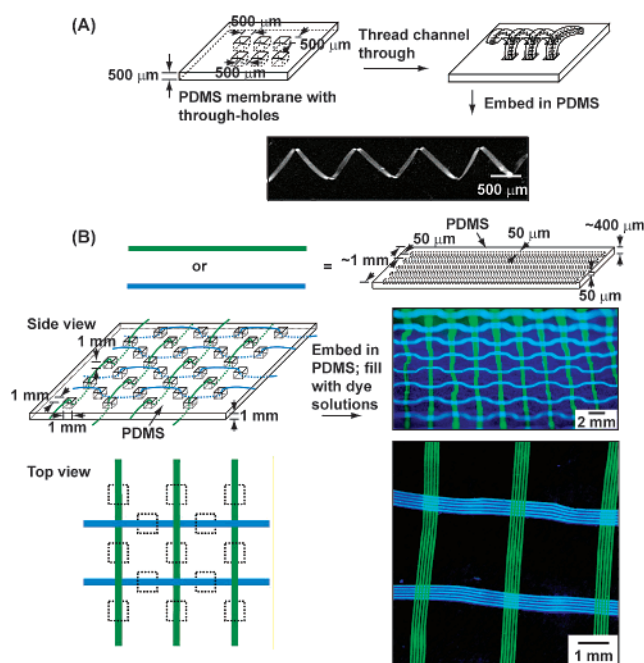


Figure 5. Pseudo-3D microfluidic systems produced by threading PDMS channels through a PDMS template having an array of through-holes. (A) A spiral channel. Top: scheme depicting a channel threaded through a PDMS membrane ($\sim 500 \mu\text{m}$ thickness) with two parallel lines of holes ($\sim 1 \text{ mm}$ spacing). Bottom: optical micrograph of the channel filled with an aqueous solution of fluorescein and illuminated with UV light. (B) A microfluidic network having the geometry of a basketweave. The template was a PDMS membrane ($\sim 500 \mu\text{m}$ thickness) having holes in a square array ($\sim 2 \text{ mm}$ spacing). The channels were filled with an aqueous solution of fluorescein (yellow) or Cascade Blue (blue) and illuminated with UV light.

size of the channel and the thickness of the PDMS strip enclosing the channel. The PDMS strip enclosing the channel ($100 \times 100 \mu\text{m}^2$) in Figure 4A had a cross-sectional area of $300 \times 300 \mu\text{m}^2$ and, when tied into a knot, occupied a volume of $1 \times 2 \times 3 \text{ mm}^3$. Other pseudo-3D structures can be fabricated by bending or rolling PDMS channels. Figure 4B shows an example of a structure formed by rolling a PDMS membrane patterned with microchannels and embedding it in PDMS. These channels were bent into curves with radii with dimensions much larger than the channels; this bending did not cause the channels to collapse or kink.

(2) Formation of Pseudo-3D Channels with Templates.

Templates can assist in positioning channels in specific 3D orientations. We used PDMS membranes with patterned through holes as templates for bending the strips of 2D channels. In Figure 5A, a single spiral channel was formed by threading a PDMS strip containing a single channel loosely in a zigzag through two parallel rows of the holes in a PDMS membrane; the spacing between these holes determined the periodicity of the resulting channel shape (approximately a spiral). We generated a basketweave pattern consisting of 10×10 strips of channels (with each strip enclosing five parallel microchannels) by threading 20 strips of channels through a PDMS template having holes in a square array (Figure 5B): 10 strips in one direction and 10 in the orthogonal direction. After this structure was embedded in PDMS, the separation between each crossover of the strips was equal to the separation of the holes (2 mm).

The example of the basketweave structure illustrates the strength of this method. Since parts of the final structure (here,

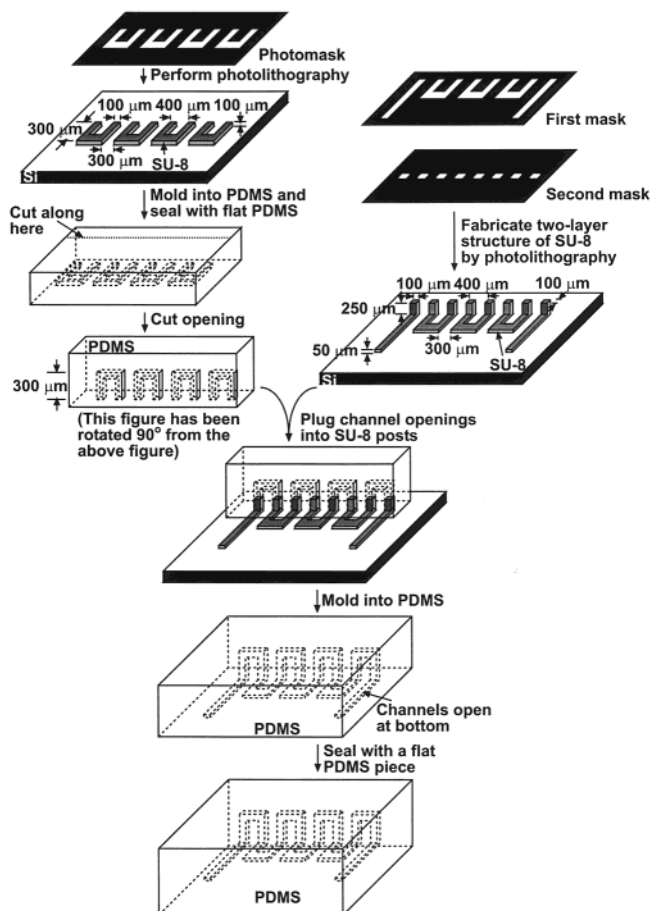


Figure 6. Schematic of the method plug-in and mold for the connection of a separate structure to form complex 3D channel systems. For convenience, the alignment marks in the fabrication of two-layer structures of SU-8 are not shown in the scheme.

arrays of parallel microchannels) can be fabricated by planar soft lithography, they can be quite complex. Their integration into a final structure can be straightforward, if it preserves the relationship generated in the planar lithographic steps.

Fabrication of Part II: Structures Used to Connect Channels in Topographically Complex Systems by Two-Layer Photolithography. A 3D connector consists of a two-level structure in photoresist: the lower level is a channel system patterned on a silicon wafer, and the upper level, a set of posts connected to the lower layer. Solid-object printing can produce two-level structures directly from CAD files for channels with large dimensions ($>250 \mu\text{m}$).¹⁸ The structures here were generated using two photolithographic steps whose procedure is described in detail in the Supporting Information.

Formation of 3D Microfluidic Networks: Connection of Separate Structures (Parts I and II). (A) **Pseudo-3D Channel Networks.** Figure 6 outlines the plug-in-and-mold method for connecting two sets of microchannels by fabricating a pseudo-3D structure, a chaotic advective mixer in PDMS (a system used to mix parallel laminar streams of liquid flowing in a microchannel). The design of this mixer is that of Beebe;¹³ we and others have described other structures for mixing fluids in microchannels.^{3,27,28} The micromixer consisted of “C”-shaped

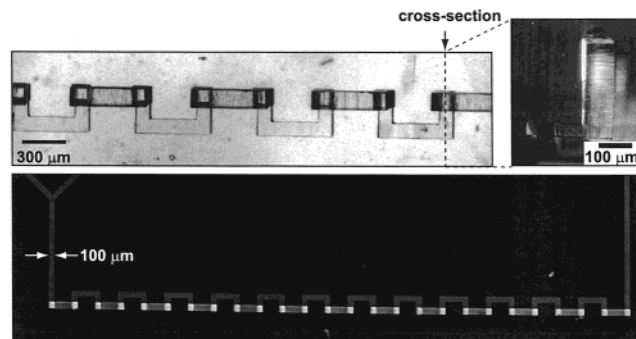


Figure 7. Micrographs of a chaotic advection mixer fabricated using the procedure sketched in Figure 6. Top left: optical micrograph of a section of empty channel. Top right: optical micrograph of the cross-section of the channel. Bottom: image of a channel filled with an aqueous solution of fluorescein and illuminated with UV light.

segments alternating in two orthogonal planes (Figure 6). This structure was decomposed into two substructures. The first substructure was a set of “C”-shaped channels fabricated by molding PDMS against features in photoresist on a silicon wafer. The ends of these channels were opened in a plane by cutting with a razor blade under a stereoscope. The second substructure was the 3D connector. It consisted of another set of “C”-shaped structures in photoresist; each of these structures had vertical, square posts at its ends. The cross-sectional dimensions of the posts were equal to or slightly (5%) larger than those of the PDMS channels in the first substructure to ensure a tight fit between posts and channels. The distance between the posts was equal to that between the channels.²⁹ Under a stereoscope, we aligned and pressed the openings of the PDMS channels into the photoresist posts. A prepolymer of PDMS was then poured over the entire joined structure and cured thermally; the tight fit of the elastomeric channels into the photoresist posts prevented the PDMS prepolymer from penetrating into these channels. After peeling the 3D structure from the wafer, we sealed the channel system against a flat piece of PDMS to form an enclosed, 3D network (Figure 7). The cross-section of the micromixer shows that the interfaces between parts I and II at connections were smooth and did not have kinks. We filled the channel with fluorescein by capillarity to demonstrate its continuity.

(B) **True-3D Channel Networks.** (1) **a 3D System of Braided Channels.** Figure 8 shows an example of a true 3D channel network. This network consisted of three braided channels; these three channels were joined to a single channel at each end. Figure 8A sketches the two substructures. Part I was a pseudo-3D channel system that can be formed by braiding three straight channels through an array of holes on a PDMS membrane. Part II was the 3D connector with square posts of photoresist on a silicon wafer. The connection between these two parts to form the final channel network is described in Figure 1. The photoresist posts connected the two parts when inserted into the channels. After molding both parts in PDMS and removing the PDMS structure from part II (the photoresist features on silicon wafer), we closed the PDMS channels that were molded from part II of the structure by sealing them to a

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(29) During cooling from high temperature (e.g., 60 °C) to room temperature, PDMS shrinks ~3%. To ensure the distance between the channels to be as designed after peeling from masters, the PDMS prepolymer was cured at room temperature overnight to prevent the shrinkage of PDMS.

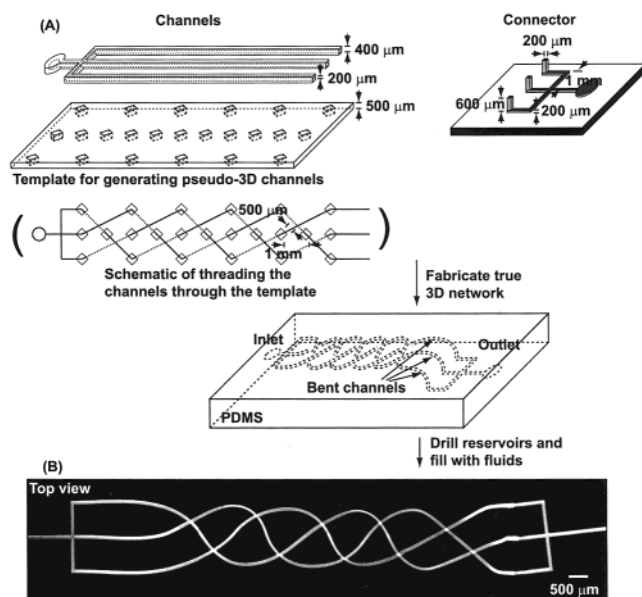


Figure 8. A braided structure comprising three channels. (A) Schematic sketch of the structure and its substructures. Note that the channels close to the outlet were bent (rotated in the plane on the page) in order to make connections to the connector. (B) Photograph of the structure filled with an aqueous solution of fluorescein and illuminated with UV light. The channel had a cross-section of $200 \times 200 \mu\text{m}^2$. The portion of the structure on the right side (part II) was not in focus because it is in a plane 5–10 mm below the rest of the system, which was in focus.

flat slab of PDMS. The structure was completed by drilling inlet and outlet reservoirs to allow fluids to flow into the channels. We filled the network with fluorescein to demonstrate its continuity (Figure 8B).

(2) a 3D Grid of Channels. A more complex example of a 3D microchannel system is a 3D grid of channels (Figure 9A). A grid having n layers of planar structures can be decomposed into n identical substructures; each substructure comprises a planar grid of channels with an array of vertical channels (perpendicular to the plane) that connects the 2D channels to those on its top layer. Its fabrication is described in detail in the Supporting Information. We fabricated a 3D grid of channels with eight layers; each layer consisted of a grid of 12 channels crossing with another 12 orthogonal channels (Figure 9B). At each crossing point, a short vertical channel connected adjacent layers. The grid occupied a volume of approximately $5 \times 5 \times 3 \text{ mm}^3$.

After cutting openings at one side of the grid, we filled the channels with a UV-curable epoxy. Exposure to UV-light and dissolution of the PDMS matrix in a tetrahydrofuran (1.0 M) solution of tetrabutylammonium fluoride converted the structure of channels into a grid of the epoxy resin (Figure 9C).

(C) a Microfluidic System with Large Area. An integrated micrototal analysis system (μTAS)⁵ usually requires multiple components on one chip for different functions such as injection of samples, mixing of reactants, separation and detection of samples and products, and storage of waste. A chip from photolithography commonly has a limited area (e.g., 3 in. across), which can be inadequate to accommodate all the necessary components for an integrated system. The plug-in-and-mold method here can be used to build a microfluidic system over an area much larger than that allowed by photolithography alone (Figure 10A). Masters of components that

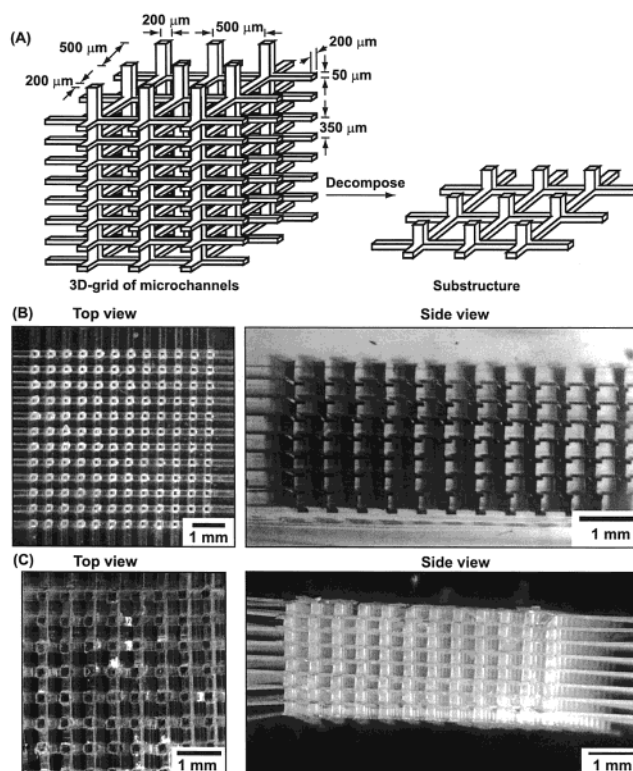


Figure 9. (A) Schematic outlining the decomposition of a 3D grid of microchannels. (B) Optical micrographs of a 3D grid of empty microchannels; this grid was made up of stacks of eight layers of planar grids with each having 12×12 channels. The dimensions of the channels are shown in part A. (C) Optical micrographs of a 3D grid of epoxy that were molded from the grid of microchannels shown in part B.

had complex planar patterns over small areas of the desired system were fabricated with vertical posts on separate silicon wafers and used as the connectors. Simple channels having a “C” shape in PDMS with open ends were fit onto the posts of the masters to join all the components on different wafers. After embedding the system in PDMS, and removing the masters, we enclosed the channel network by sealing its bottom to flat slabs of PDMS and drilled holes in the PDMS for inlets and outlets. This procedure yields a system comprising three serpentine channels, joined by a “Y”-shaped channel (Figure 10B); the system occupied an area of $20 \times 19 \text{ cm}^2$. This system was filled with fluorescein to demonstrate its continuity.

Conclusions

This work demonstrates a very flexible (figuratively and literally) procedure to fabricate a range of topologically and geometrically complex, 3D microfluidic systems in PDMS. Using planar channels in PDMS as a starting point, this technique takes advantage of the flexibility of PDMS and bends the 2D channels into out-of-plane structures (pseudo-3D). These structures are fixed in place by molding them into PDMS. Templates provide a way of controlling bending, weaving, and braiding. This procedure also uses a joining methodology to resolve crossings of channels so that a true 3D channel system can be decomposed into substructures that are amenable to planar fabrication. The convenience of molding and sealing PDMS facilitates the fixation of the 3D shapes of the PDMS channels, the connection of separate substructures (no fractures and no differences between materials at the interface), and the

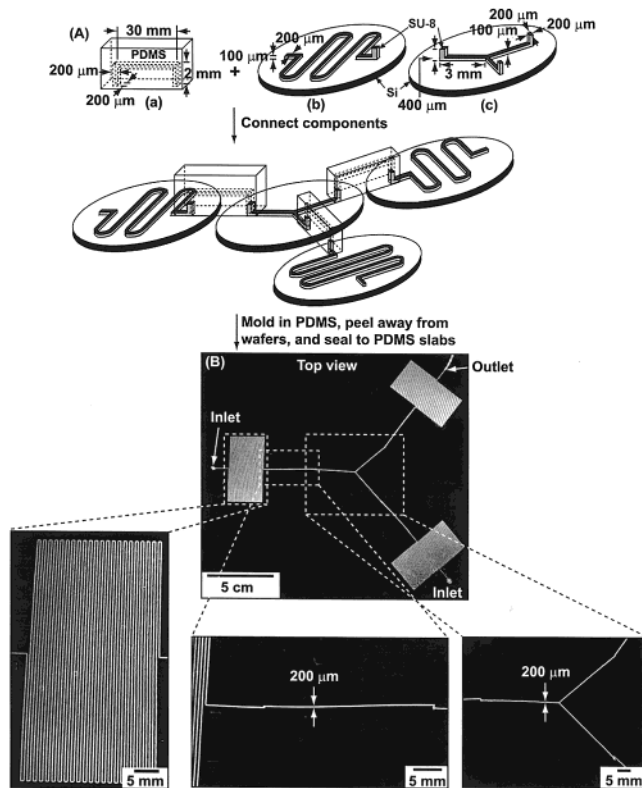


Figure 10. (A) Schematic of the fabrication of a microfluidic system by connecting several substructures on separate wafers. (B) Photograph of the microfluidic system, which covers an area of $\sim 20 \times 19 \text{ cm}^2$, formed by connecting three serpentine separation channels with a “Y”-shaped channel. The master for each of the four channels (serpentine channels and the “Y”-shaped channel) was on a 3 in. wafer before connection. The details of the system are shown in the photographs on the bottom line. All the photographs were taken with the channel system filled with an aqueous solution of fluorescein and illuminated with UV light.

embedding of the entire system in the same material. After the embedding process, the embedded pieces and the embedding material become one object.

This technique has five advantages: (i) It only requires casual alignment in connections (plugging posts into PDMS channels is a procedure that is very tolerant of differences in geometry). (ii) It creates smooth connections between separate channels. (iii) It can generate structures over large areas (that is, it is not restricted to structures that can be produced on one wafer). (iv) It can create both sharp (90° angle) corners and corners consisting of smooth arcs. (v) It allows the manipulation of *networks* of small channels conveniently and in parallel (e.g., Figure 4B). PDMS has the advantage as the matrix material of flexibility, optical transparency to visible and UV light, and water impermeability.

This methodology is currently most useful for prototyping structures. It has the disadvantage that it requires a certain amount of manual work; steps such as joining channels must be done by hand and thus are best done with relatively large channels ($> 100 \mu\text{m}$). Structures with small channels can be fabricated by replacing the manual work with using micromanipulators. It is not suitable for opaque materials or materials that cannot be easily bent, molded and sealed. The properties of PDMS make the channels inappropriate for use with nonpolar organic solvents or with an aqueous solution containing nonpolar solutes.

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Supporting Information Available: Procedures for two-layer photolithography and fabrication of a 3D grid of channels. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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