Perforated membrane method for fabricating three-dimensional polydimethylsiloxane microfluidic devices†

Yiqi Luo and Richard N. Zare

Received 7th May 2008, Accepted 25th June 2008
First published as an Advance Article on the web 19th August 2008
DOI: 10.1039/b807751g

A procedure is described for making layer-to-layer interconnections in polydimethylsiloxane (PDMS) microfluidic devices. Thin (~50 μm) perforated PDMS membranes are bonded to thicker (0.1 cm or more) PDMS slabs by means of thermally cured PDMS prepolymer to form a three-dimensional (3D) channel structure, which may contain channel or valve arrays that can pass over and under one another. Devices containing as many as two slabs and three perforated membranes are demonstrated. We also present 3D PDMS microfluidic devices for display and for liquid dispensing.

Introduction

Most lab-on-a-chip devices made from the silicone elastomer, PDMS, are fabricated using multilayer soft lithography.[1–3] Typically, one two-dimensional (2D) layer is for fluid flow and a second 2D layer, if present, is for pressure-actuated valves and pumps. As Whitesides and coworkers first showed,[4] however, it is possible to create three-dimensional (3D) channel structures, which allow for more complex functionality of the lab-on-a-chip device and better utilization of the device real estate. We present here another method for achieving this goal.

There are a few strategies for making 3D channel structures in PDMS microfluidic devices, most of which originated from the Whitesides research group:[5–9] the procedure we call the "mortise and tenon" method,[5,6] the "membrane sandwich" method,[7,8,10] the "solid-object printing" method,[9] and the "protruding mold feature" method.[11] The mortise and tenon method adds photoresist posts (tenons) on top of the normally used photoresist features, which can be inserted into pre-made 2D channel structures (mortises). The assembly is then used as a mold to cure PDMS prepolymer to form a 3D channel structure, in which the tenons fix the positions of the interconnections. However, because of the need for manual work to join tenons and mortises, the cross sections of the interconnections are restricted to be relatively large (>100 μm in one dimension) with a length in excess of a few hundred microns.[5]

The membrane sandwich method involves making a thin PDMS membrane having channel structures molded on both faces (with connections between the faces). In order to make such a membrane, a mold consisting of photoresist and silicon wafer is aligned under pressure with a plain sheet or a PDMS stamp, face to face, and PDMS prepolymer is present in between, which is later thermally cured. The resulting PDMS membrane is then sandwiched between two thicker PDMS slabs to form a 3D channel structure. This method can make short (typically 100 μm) interconnections with small cross sections (typically 70 μm in one dimension). However, this procedure may require careful manipulation to prevent forming thin PDMS films between the mold and the plain sheet (or the PDMS stamp), resulting in blocked holes.[7] The solid-object printing method employs thermoplastic features made by a solid-object printer, which are used for molding PDMS and then removed by heating. The application of this method has limitations arising from both the printer capability and the thermoplastic removal process.[9] The protruding mold feature method uses photoresist features to stick through a PDMS membrane formed on a mold. The PDMS membrane is bonded to a thicker PDMS slab while the protruding features are aligned in the channels of the PDMS slab. Thus, the protruding features result in through-holes (vias) which connect channels in different microfluidic layers.[11] This method is simple and easy to utilize, but the bonding between the PDMS membrane and slab must be tall enough and large enough to avoid the protruding features. Therefore, the diameters of the through-holes are usually smaller than the widths of the channels.

We present an alternative approach for fabricating interconnections using perforated membranes (hereafter called the "perforated membrane" method). Similar to the protruding mold feature method, a PDMS membrane is formed on a mold with photoresist features sticking through the PDMS membrane. However, the photoresist features are removed to result in a perforated membrane to eliminate the influence of protruding features in the following fabrication procedure. The perforated membrane is bonded to other PDMS membranes or slabs to form a 3D channel structure. The procedure for producing a perforated membrane involves making photoresist features such as posts on a silicon wafer, coating a PDMS membrane with a thickness less than the height of the posts, and removing the posts to generate perforations. The fabrication of such perforated membranes was first reported by Whitesides and coworkers, in which the membranes are directly peeled from the

Department of Chemistry, Stanford University, Stanford, California, 94305-5080, USA. E-mail: zare@stanford.edu; Fax: +1(650) 725-0259; Tel: +1(650) 723-3062

† Electronic supplementary information (ESI) available: Perforation fabrication by the membrane flap method; operation of a microfluidic display device; and operation of a microfluidic multichannel dispensing device. See DOI: 10.1039/b807751g

molds and used as metal deposition masks.[12–14] Another method reported is also capable of producing flat perforated membranes with tapered through-holes, which are used for patterning active proteins.[15] What we present is a variation of this procedure, which is able to produce short interconnections ~50 μm in our practice with flexible cross sections resulted from photolithography. In this manner, we have produced 3D connected channel structures having as many as five different layers. We believe that this alternative method is simpler than the membrane sandwich method and allows smaller interconnections than the mortise and tenon method.

Experimental

Chemicals and reagents

Photoresist SU-8 is purchased from Microchem (Sunnyvale, CA, USA). Methyltrichlorosilane (MTS) is purchased from Sigma-Aldrich (St Louis, MO, USA). Dodecyl-β-D-maltoside (DDM) is purchased from Anatrace (Maumee, OH, USA). PDMS RTV 615 is purchased from GE Silicones (Waterford, NY, USA). Photoresist SPR 220-7, hexamethyldisilazane (HMDS), and photoresist developing reagents SU-8 Developer and Shipley LDD-26W are common chemicals provided by Stanford Nanofabrication Facility (Stanford, CA, USA).

Fabrication of soft lithography molds

Photolithography is employed to form photoresist features on silicon wafers to produce soft lithography molds. Each PDMS member having a microfluidic layer is individually fabricated using a specific mold. The patterns are printed on transparency masks at high-resolution (40 640 dpi). There are two types of molds, high-feature molds (photoresist feature height 55 μm) and low-feature molds (photoresist feature height 10 μm). To make a perforated membrane, which is a particular PDMS member thinner than the photoresist features, a perforated membrane mold (PM-mold) is used. The PM-mold is a high-feature mold on which photoresist features are mainly photoresist posts, while other shaped features may also be present.

To make a PDMS member thicker than the photoresist features, either a high-feature mold or a low-feature mold can be used. The photoresist features print open channels to one face of the PDMS member formed on the mold. The open channels may seal with another PDMS member to produce closed channels, serving as microfluidic flow channels (channels for liquid flows) or valves (channels having expanding portions to close neighboring flow channels). Because of the thermal properties of photoresists, a low-feature mold may have photoresist features with round cross sections. By molding against such a low-feature mold, the PDMS member is able to produce flow channels with round cross sections, which can be closed by valves. A PDMS member is called a PDMS membrane or a PDMS slab depending on its thickness.

To fabricate a high-feature mold, photoresist SU-8 is spin-coated on an HMDS-primed silicon wafer at a thickness of 55 μm and baked twice (75 °C for 180 s and 105 °C for 360 s). The photoresist on the silicon wafer is exposed under UV-light through a transparency mask, baked twice (75 °C for 60 s and 105 °C for 300 s), and developed in SU-8 Developer. To fabricate a low-feature mold, photoresist SPR 220–7 is spin-coated on an HMDS-primed silicon wafer at a thickness of 10 μm and baked (110 °C for 200 s). The photoresist on the silicon wafer is exposed under UV light through a transparency mask and developed in Shipley LDD-26W. If needed, the mold is baked in an oven (110 °C for 1 h) to reflow the photoresist to form round cross sections for fabricating flow channels able to be closed by valves.[16] Before use, both types of molds are exposed to MTS vapor in a desiccator in order to prevent adhesion between cured PDMS members and molds.

Results and discussion

Making perforated membranes

PDMS microfluidic devices are fabricated through multilayer soft lithography, in which multiple PDMS members are laminated together. The PDMS members contain various microfluidic layers. A perforated membrane is an essential PDMS member facilitating channel interconnections. This perforated membrane method results in the creation of 3D channel structures. Fig. 1 and 2 outline two different methods for making a perforated membrane. Both methods follow the same concept: (1) making a pre-formed PDMS membrane on a PM-mold; and (2) removing the photoresist posts to expose through-holes. Thus, a perforated membrane is formed on the mold by sacrificing the photoresist posts, which may be a drawback of this procedure.

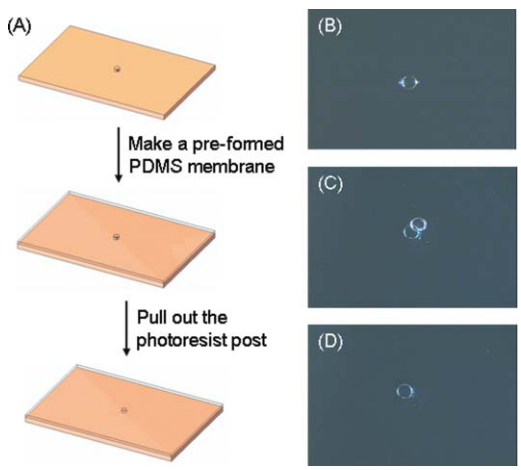

Fig. 1 (A) Schematic procedure of the first method of making a perforated membrane. (B)–(D) Pictured procedure of making a perforated membrane with a circular through-hole. (B) Picture of the through-hole region of the pre-formed PDMS membrane. (C) Picture of the through-hole region of the perforated membrane. The pulled out photoresist post remains close to the through-hole and is removed afterwards. (D) Picture of the through-hole region of the perforated membrane.

To make a perforated membrane by the first method (see Fig. 1), PDMS prepolymer (mixture of RTV 615A and 615B at a ratio of 10 : 1) is spin-coated (2.5 krpm for 60 s) on a PM-mold and cured in an oven at 80 °C for about 2 h to form a PDMS membrane. The thickness of the membrane (estimated to be between 40 μm and 50 μm) is less than the height of the photoresist posts. Then a scalpel (which is one arm of a pair of sharp tweezers) is used to directly remove the photoresist posts

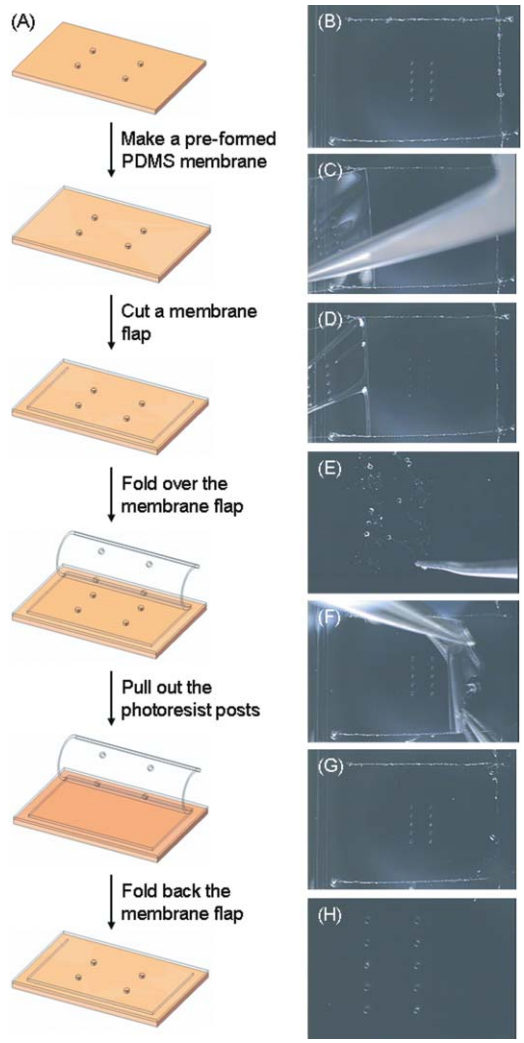

Fig. 2 (A) Schematic procedure of the second method of making a perforated membrane. (B)–(H) Pictures of the procedure for making a perforated membrane with an array of circular through-holes. (B) The pre-formed PDMS membrane cut along three sides to make a membrane flap. (C) Tweezers being used to fold over the membrane flap. (D) The folded membrane flap. (E) Zoomed-in view of the pulled out photoresist posts. The broken photoresist posts are removed afterwards. (F) Tweezers being used to fold back the membrane flap. (G) The perforated membrane. (H) Zoomed-in view of the through-hole region of the perforated membrane.

without moving the membrane. The broken photoresist posts are cleaned up using sticky tape. Thus, a perforated membrane is made on the silicon wafer, in which through-holes are present at the positions of the photoresist posts. The first method is simple and straightforward, although it has the risk of causing damage to the membrane while pulling out the photoresist posts. Therefore, it is well-suited for making a perforated membrane with a sparse number of through-holes.

To make a perforated membrane with an array of closely positioned through-holes, the second method is preferred (see Fig. 2). A PDMS membrane is formed by the same process described in the first method. The membrane is cut along three sides to make a membrane flap. The membrane flap is folded over by tweezers and reversibly attached to the PDMS surface next to it. The exposed photoresist posts are removed in the same way as before and the broken photoresist posts are removed once again using sticky tape. Then the membrane flap is folded back. Thus, a perforated membrane is made on the silicon wafer, in which through-holes are present at the positions of the photoresist posts. A movie showing the practice of this method is presented in ESI movie 1.† This method circumvents damage to the membrane while pulling out the photoresist posts. In some cases, a very thin PDMS film may be formed on top of a photoresist post owing to surface tension effects; therefore, an additional step of checking the through-holes is needed because these PDMS films may stick to the edges of the through-holes and remain with the perforated membrane.

Fabrication of microfluidic devices having 3D channel structures

(A) Fabrication of 3D channel structures. Fig. 3 shows the fabrication of simple 3D channel structures. According to the schematic procedure in Fig. 3A, a perforated membrane 2 and two PDMS slabs 1, 3 are laminated together. In order to create open ends of the channels, holes are made in appropriate steps.

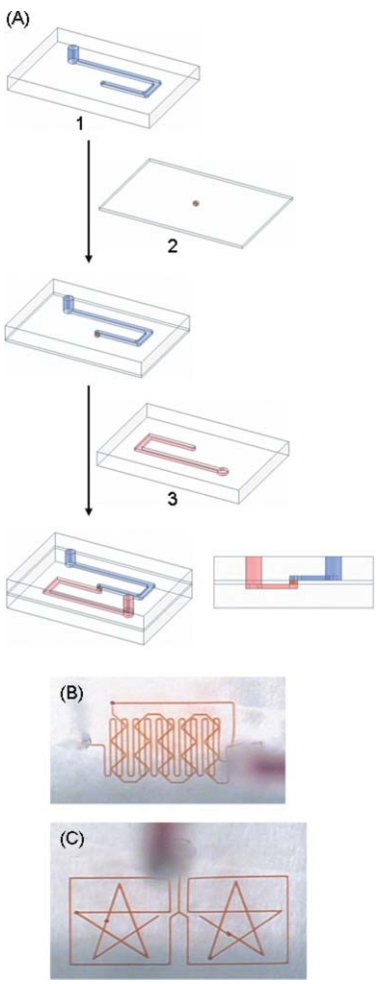

Fig. 3 **(A)** Schematic procedure of making a microfluidic device having a simple 3D channel structure. The cross sectional view is shown at right. Different microfluidic layers are labeled with different colors. (B) Picture of a fabricated microfluidic device having two zigzag channels. (C) Picture of a fabricated microfluidic device having two star-shaped channels. The channels are filled with a food coloring for visualization.

The perforated membrane 2 with circular through-holes (200 μm in diameter) is made by the method illustrated in Fig. 1. To make a PDMS slab, PDMS prepolymer is poured on either a high-feature or a low-feature mold and cured in an oven at 80 °C for about 2 h. Then the PDMS slab with open channels is cut and peeled from the mold. The PDMS slabs 1, 3 are made using low-feature molds. The open channels on the PDMS slabs 1, 3 are aligned properly to intersect the through-holes in the perforated membrane 2, as shown in Fig. 3A. In this manner the microfluidic layers in all the PDMS members constitute together a simple 3D channel structure.

PDMS members are bonded by a dipping-attaching method, as described elsewhere.[17,18] Briefly, a solution of 1/10 (w/w) PDMS prepolymer in cyclohexane is spin-coated on a glass slide at 3.3 krpm to form a thin film. A PDMS member is dipped into the thin film of PDMS prepolymer and attached to another PDMS member. Thus, between the two PDMS members a portion of the thin film of PDMS prepolymer is present, which is then cured in an oven at 80 °C for 2 h to become an adhesive PDMS layer.

Two microfluidic devices having simple 3D channel structures are shown in Fig. 3B and 3C. The first microfluidic device has one circular through-hole connecting two zigzag channels. The second has four circular through-holes facilitating two star-shaped channels. The channels are 100 μm in width and the through-holes are 200 μm in diameter.

For exploring the limit of applying the perforated membrane method, a double-helix channel structure is fabricated, as shown in Fig. 4. According to the schematic procedure in Fig. 4A, three perforated membranes 2, 3, 4 and two PDMS slabs 1, 5 are laminated together. In order to create open ends of the channels, holes are made in appropriate steps. All three perforated membranes are made by the method illustrated in Fig. 2. The normal perforated membrane 3 has circular through-holes, which are 200 μm in diameter. The two perforated membranes 2, 4 also have two-side open channels. Like normal open channels, the two-side open channels are able to seal with two other PDMS members to form closed channels. The PDMS slabs 1, 5 are made by molding against high-feature molds. The open channels in the perforated membranes 2, 4 and on the PDMS slabs 1, 5 are aligned properly to intersect the through-holes in the perforated membranes 2, 3, 4, as shown in Fig. 4A. Thus the microfluidic layers in all the PDMS members constitute together a channel structure consisting two entangled 3D channels, which mimics the geometry of a double helix.

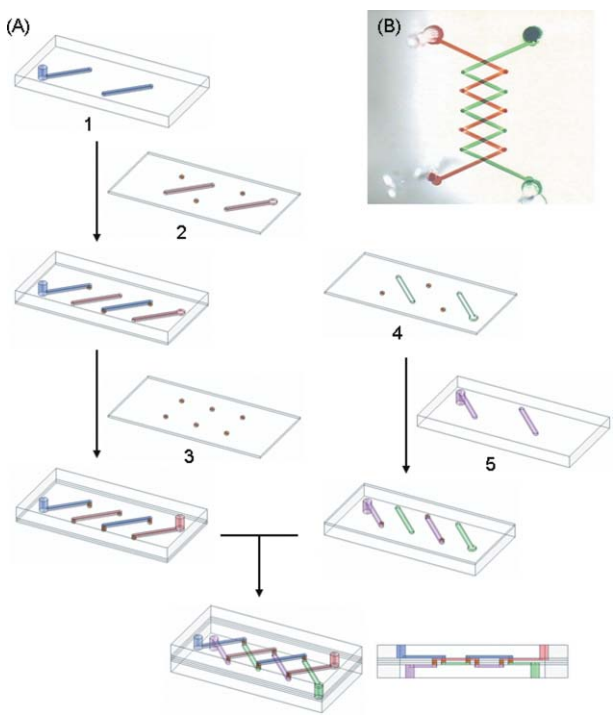

Fig. 4 (A) Schematic procedure of making a microfluidic device having a double-helix channel structure. The cross sectional view is shown at right. Different microfluidic layers are labeled with different colors. (B) Picture of a fabricated microfluidic device having a double-helix channel structure. The channels are filled with food coloring for visualization.

Fig. 4B shows a fabricated microfluidic device having a double-helix channel structure. Fabrication of this microfluidic device reaches the limit of applying the perforated membrane method. The limit is caused by accumulated deformation. Because of surface tension effects between photoresist posts and PDMS prepolymer, each perforated membrane has cone-shaped surfaces around through-holes. Therefore, a PDMS member must be slightly deformed to make complete attachment with a perforated membrane to form a combined PDMS structure. The deformation accumulates as additional perforated membranes are attached. We found that the limit of the perforated membrane method is three perforated membranes.

(B) Creation of a microfluidic display device. Fig. 5 shows the fabrication of a microfluidic display device, which involves two valve arrays for the purpose of microfluidic flow control. According to the schematic procedure in Fig. 5A, a perforated membrane 2, a PDMS membrane 3 and two PDMS slabs 1, 4 are laminated together. In order to create open ends of the flow channels or valves, holes are made in appropriate steps. Holes at the end of flow channels serve as reagent reservoirs. The perforated membrane 2 is made by the method illustrated in Fig. 2 and has rectangular through-holes, which are 100 μm in length and 75 μm in width. To make the PDMS membrane 3, PDMS prepolymer is spin-coated (1.2 krpm for 30 s) on a low-feature mold and cured in an oven at 80 °C for about 2 h. The thickness of the PDMS membrane 3 is more than the height of the photoresist features. The PDMS membrane 3 has open channels on one face printed from the features as well as the PDMS slabs. The PDMS slab 1 is made by using a high-feature mold while the PDMS slab 3 is made by using a low-feature mold, where the photoresist features have round cross sections. The PDMS slab 1 is aligned and attached to the perforated membrane 2 to form a combined PDMS structure. In the same manner, the PDMS slab 4 is aligned and attached to the PDMS membrane 3 to form another combined PDMS structure. Because of geometric restrictions, L-shaped openings are made in the second combined PDMS structure to access the channels from sidewalls of the device.[10] The two combined PDMS structures are aligned and attached to form a microfluidic display device. The open channels on the PDMS slab 1 and PDMS membrane 3 are aligned properly to intersect the through-holes in the perforated membrane 2, as shown in

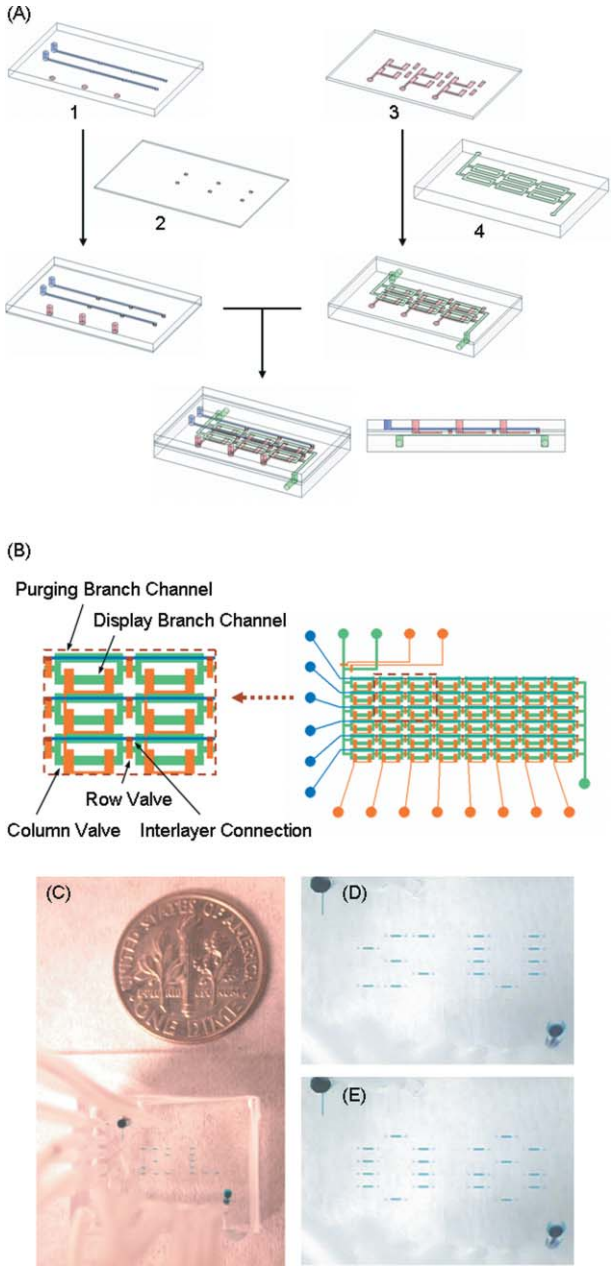

Fig. 5 (A) Schematic procedure of making a microfluidic display device. The cross sectional view is shown at right. Different microfluidic layers are labeled with different colors. (B) Layout of the microfluidic display device. (C) Picture of a fabricated microfluidic display device displaying a pattern "ZL". A dime is used as a size marker. (D) (E) Zoomed-in views of the microfluidic display device displaying patterns "SU" and "08". The colored solution is prepared from a food coloring.

Fig. 5A. The channels in the PDMS membrane 3 are valves and valve-connecting channels while the channels in the PDMS slab 4 are flow channels. A portion of the valves are linked to form a valve array by the valve-connecting channels in the PDMS slab 1 and the through-holes in the perforated membrane 2. This particular 3D valve array contains valves and valve-connecting channels located in different microfluidic layers; thus, it is possible to place the connections for actuating the valves over or under another 2D valve array.

Fig. 5B shows the layout of the microfluidic display device. The design is based on the concept of orthogonal control, which is used in liquid crystal displays (LCD) and dynamic random access memories. For instance, an LCD has two orthogonal arrays of electrodes connected to the pixels. Each pixel is controlled by a row electrode and a column electrode. The pixel will only be activated when voltages are applied to both electrodes. Therefore, a large number of pixels can be individually addressed from row to row by a small number of electrodes. The application of this concept to microfluidic devices has been reported by Quake and coworkers, in which a microfluidic memory storage device is established.[19] In this work, the device contains flow channels either for information display or for purging. Once a display channel is filled with a colored solution, multiplexers and multiplexer-controlled valves are utilized to regulate flow paths to purge the display channel with water while keeping particular portions of the display channel closed as compartments. Thus the colored solution is held in the selected portions of the display channel while the rest is filled with water. By this means, memory storage is achieved.

As shown in Fig. 5B, the flow channels in our microfluidic display device also contain display branch channels and purging branch channels. The orthogonal control is more straightforward. By using the perforated membrane method, two orthogonal valve arrays are constructed to provide row valves and column valves, respectively. The display function is carried out by the following steps: (1) while the row valves of a flow channel and all the column valves are open, a colored solution is injected into both display and purging branch channels; (2) while certain column valves are closed to form compartments in corresponding display branch channels, a washing buffer is injected into the flow channel to clean all purging branch channels as well as the open display branch channels; (3) the first two steps are repeated row by row until all flow channels are "updated", and then all row and column valves are closed to form compartments in all display branch channels; and (4) by opening each row valve, a washing buffer is injected row by row to further clean the purging branch channels in the flow channels. Therefore, a pattern is displayed in which the not purged compartments serve as "activated" pixels and the purged ones as "inactivated" pixels. While updating the flow channels, the row valves are able to block diffusion between two neighboring display branch channels in a flow channel. Because the column valves may be closed for updating this flow channel but open for other flow channels, such a blocking effect is an advantage of the crossed valve arrays. Although the row valves do not prevent the diffusion between a display branch and its neighboring purging branch, this diffusion effect can be cleaned in the last step. Fig. 5C shows a fabricated microfluidic display device, and Fig. 5D and 5E show two patterns displayed in the device. A movie of the operation of this microfluidic display device is presented in ESI movie 2.† The manipulation of the microfluidic display device is controlled by a computer program written in LabVIEW.

(C) Creation of a microfluidic multichannel dispensing device. Fig. 6 shows the fabrication of a microfluidic multichannel dispensing device, which involves an entangled flow channel array for the purpose of dispensing multiple liquids into multiple

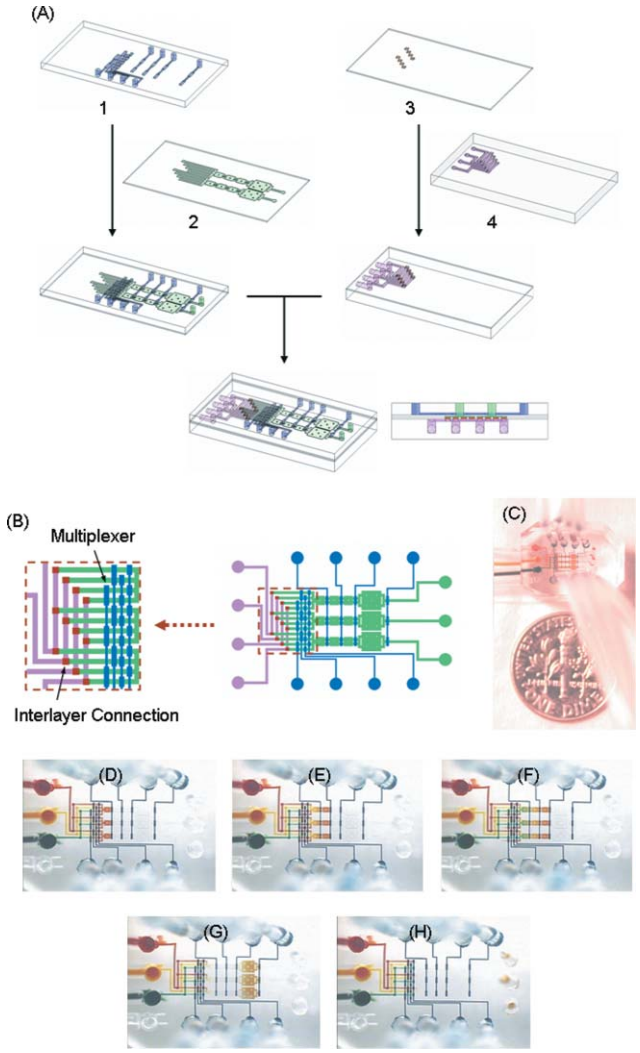

Fig. 6 (A) Schematic procedure of making a microfluidic multichannel dispensing device. The cross sectional view of the device is shown at right. Different microfluidic layers are labeled with different colors. (B) Layout of the microfluidic multichannel dispensing device. The entangled flow channel array serving as the multichannel dispensing module is enlarged. (C) Picture of a fabricated microfluidic multichannel dispensing device. A dime is used as a size marker. (D)–(H) Pictures of dispensing different colored solutions to the parallel chamber chains in the microfluidic multichannel dispensing device. The sequence of injection is red, yellow, green, and clear solutions. The colored solutions are prepared from food colorings.

staggered flow channels. According to the schematic procedure in Fig. 6A, a perforated membrane 3, a PDMS membrane 2 and two PDMS slabs 1, 4 are laminated together. In order to create open ends of the flow channels or valves, holes are made in appropriate steps. Holes at the end of flow channels serve as reagent reservoirs. The perforated membrane 3 is made by the method illustrated in Fig. 2 and has square through-holes, which are 130 μm in side-length. To make the PDMS membrane 2, PDMS prepolymer is spin-coated (2.5 krpm for 60 s) on a low-feature mold and cured in an oven at 80 °C for about 2 h. The thickness of the PDMS membrane 2 is more than the height of the photoresist features, which have round cross sections. The PDMS membrane 2 has open channels on one face printed from the features as well as the PDMS slabs. The PDMS slabs 1, 4 are made by using high-feature molds. The PDMS slab 1 is aligned and attached to the PDMS membrane 2 to form a combined PDMS structure. In the same manner, the PDMS slab 4 is aligned and attached to the PDMS membrane 3 to form another combined PDMS structure. Because of geometric restrictions, L-shaped openings are made in the second combined PDMS structure to access the channels from a sidewall of the device. The two combined PDMS structures are aligned and attached to form a microfluidic multichannel dispensing device. The open channels on the PDMS membrane 2 and PDMS slab 4 are aligned properly to intersect the through-holes in the perforated membrane 3, as shown in Fig. 6A. The channels in the PDMS slabs 2, 4 are flow channels while the channels in the PDMS slab 1 are valves and valve-connecting channels. The through-holes in the perforated membrane 3 connect flow channels in different microfluidic layers to form an entangled flow channel array serving as the multichannel dispensing module.

Fig. 6B shows the layout of the microfluidic multichannel dispensing device. This particular device is designed analogous to the multiple displacement amplification (MDA) device reported by Quake and coworkers,[20] but only for the purpose of showing the multichannel dispensing of liquids. In this device, the 3D channel interconnections in the multichannel dispensing module allow all-to-all connections between the multiple reagent reservoirs and the multiple chamber chains. Such all-to-all connections have to be realized by entangled flow channels. By using multiplexers to control flow pathways,[19] the multichannel dispensing of liquids is achieved by injecting one reagent into one empty chamber each time. Because PDMS is gas-permeable, the filling is realized by simply driving the air out of the device. Fig. 6C shows a fabricated microfluidic parallel-dispensing device, and Fig. 6D–6H show the sequential injection of reagents into the chambers. A movie of the operation of this microfluidic multichannel dispensing device is presented in ESI movie 3.† The manipulation of the microfluidic display device is controlled by a computer program written in LabVIEW.

Conclusions

From the microfluidic devices reported, we know that the perforated membrane method provides short and small interlayer connections for fabricating 3D channel structures. Because the properties of a perforated membrane are flexible, including the length, size, and density of the through-holes, and the size of the entire membrane, the method is well-suited to be applied in a wide range of high-throughput microfluidic devices. The further application may include single cell analysis,[20–24] microscale biochemical assays,[25–30] cell culture and sorting,[31–34] and more.

The perforated membrane method has three advantages: (1) it only requires normal alignment in fabrication without adding complicated steps to the protocols of multilayer soft lithography; (2) it creates smooth connections between the channels in different microfluidic layers; and (3) it accurately controls the size and shape of connection through-holes by employing photolithography for sacrificial photoresist posts formation. The dimensions of perforated membranes are reported in centimetres and may be made even larger, which provides

flexibility of constructing microfluidic devices with various sizes. In this article, the smallest through-holes are 100 μm in length and 75 μm in width in the perforated membrane used in the fabrication of the microfluidic display device. We believe that the size of through-holes can be made even smaller, and experiments are underway to determine what limits its size.

The method has the disadvantage that it requires a certain amount of manual work to produce each perforated membrane. It also requires the destruction of photoresist features in molds. Thus its application to industrial manufacturing is limited. Moreover, when a perforated membrane is made by the membrane-flap method illustrated in Fig. 2, the shrinking nature of cured PDMS may result in slight nonuniform shortening between the through-holes in the flap, which could make the large-scale integration of microfluidic functional modules difficult. However, the method is currently one of the simplest for prototyping 3D PDMS channel structures.

Acknowledgements

The authors thank National Science Foundation grant NSF BES-0508531 for supporting this work.

References

1 Y. Xia and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 1998, **37**, 550–575.
2 M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer and S. R. Quake, *Science*, 2000, **288**, 113–116.
3 T. M. Squires and S. R. Quake, *Rev. Mod. Phys.*, 2005, **77**, 977–1026.
4 J. C. McDonald and G. M. Whitesides, *Acc. Chem. Res.*, 2002, **35**, 491–499.
5 D. T. Chiu, E. Pezzoli, H. Wu, A. D. Stroock and G. M. Whitesides, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 2961–2966.
6 H. Wu, T. W. Odom, D. T. Chiu and G. M. Whitesides, *J. Am. Chem. Soc.*, 2003, **125**, 554–559.
7 D. T. Chiu, N. L. Jeon, S. Huang, R. S. Kane, C. J. Wargo, I. S. Choi, D. E. Ingber and G. M. Whitesides, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **97**, 2408–2413.
8 J. R. Anderson, D. T. Chiu, R. J. Jackman, O. Cherniavskaya, J. C. McDonald, H. Wu, S. H. Whitesides and G. M. Whitesides, *Anal. Chem.*, 2000, **72**, 3158–3164.
9 J. C. McDonald, M. L. Chabinyc, S. J. Metallo, J. R. Anderson, A. D. Stroock and G. M. Whitesides, *Anal. Chem.*, 2002, **74**, 1537–1545.
10 B. Jo, L. M. Van Lerberghe, K. M. Motsegood and D. J. Beebe, *J. Microelectromech. Syst.*, 2000, **9**, 76–81.
11 E. P. Kartalov, C. Walker, C. R. Taylor, W. F. Anderson and A. Scherer, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 12280–12284.
12 R. J. Jackman, D. C. Duffy, O. Cherniavskaya and G. M. Whitesides, *Langmuir*, 1999, **15**, 2973–2984.
13 D. C. Duffy, R. J. Jackman, K. M. Vaeth, K. F. Jensen and G. M. Whitesides, *Adv. Mater.*, 1999, **11**, 546–552.
14 T. R. Sodunke, K. K. Turner, S. A. Caldwell, K. W. McBride, M. J. Reginato and H. Noh, *Biomaterials*, 2007, **28**, 4006–4016.
15 K. Atsuta, H. Noji and S. Takeuchi, *Lab Chip*, 2004, **4**, 333–336.
16 V. Studer, G. Hang, A. Pandolfi, M. Ortiz, W. F. Anderson and S. R. Quake, *J. Appl. Phys.*, 2004, **95**, 393–398.
17 H. Wu, B. Huang and R. N. Zare, *Lab Chip*, 2005, **5**, 1393–1398.
18 Y. Luo, F. Yu and R. N. Zare, *Lab Chip*, 2008, **8**, 694–700.
19 T. Thorsen, S. J. Maerkl and S. R. Quake, *Science*, 2002, **298**, 580–584.
20 Y. Marcy, T. Ishoey, R. S. Lasken, T. B. Stockwell, B. P. Walenz, A. L. Halpern, K. Y. Beeson, S. M. D. Goldberg and S. R. Quake, *PLoS Genet.*, 2007, **3**, 1–7.
21 A. R. Wheeler, W. R. Throndset, R. J. Whelan, A. M. Leach, R. N. Zare, Y. H. Liao, K. Farrell, I. D. Manger and A. Daridon, *Anal. Chem.*, 2003, **75**, 3581–3586.
22 J. S. Marcus, W. F. Anderson and S. R. Quake, *Anal. Chem.*, 2006, **78**, 3084–3089.
23 B. Huang, H. Wu, D. Bhaya, A. Grossman, S. Granier, B. K. Kobilka and R. N. Zare, *Science*, 2007, **315**, 81–84.
24 J. F. Zhong, Y. Chen, J. S. Marcus, A. Scherer, S. R. Quake, C. R. Taylor and L. P. Weiner, *Lab Chip*, 2008, **8**, 68–74.
25 M. A. Burns, B. N. Johnson, S. N. Brahmasandra, K. Handique, J. R. Webster, M. Krishnan, T. S. Sammarco, P. M. Man, D. Jones, D. Heldsinger, C. H. Mastrangelo and D. T. Burke, *Science*, 1998, **282**, 484–487.
26 B. M. Paegel, C. A. Emrich, G. J. Wedemayer, J. R. Scherer and R. A. Mathies, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 574–579.
27 J. W. Hong, V. Studer, G. Hang, W. F. Anderson and S. R. Quake, *Nat. Biotechnol.*, 2004, **22**, 435–439.
28 L. Warren, D. Bryder, I. L. Weissman and S. R. Quake, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 17807–17812.
29 S. J. Maerkl and S. R. Quake, *Science*, 2007, **315**, 233–237.
30 Braslavsky, M. Causey, J. Colonell, J. DiMeo, J. W. Efcavitch, E. Giladi, J. Gill, J. Healy, M. Jarosz, D. Lapen, K. Moulton, S. R. Quake, K. Steinmann, E. Thayer, A. Tyurina, R. Ward, H. Weiss and Z. Xie, *Science*, 2008, **320**, 106–109.
31 H. P. Chou, C. Spence, A. Scherer and S. R. Quake, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 11–13.
32 B. Chueh, D. Huh, C. R. Kyrtsos, T. Houssin, N. Futai and S. Takayama, *Anal. Chem.*, 2007, **79**, 3504–3508.
33 S. Thorslund, O. Klett, F. Nikolajeff, K. Markides and J. Bergquist, *Biomed. Microdevices*, 2006, **8**, 73–79.
34 F. K. Balagadde, L. You, C. L. Hansen, F. H. Arnold and S. R. Quake, *Science*, 2005, **309**, 137–140.

