Incorporation of prefabricated screw, pneumatic, and solenoid valves into microfluidic devices

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This paper describes a method for prefabricating screw, pneumatic, and solenoid valves and embedding them in microfluidic devices. This method of prefabrication and embedding is simple, requires no advanced fabrication, and is compatible with soft lithography. Because prefabrication allows many identical valves to be made at one time, the performance across different valves made in the same manner is reproducible. In addition, the performance of a single valve is reproducible over many cycles of opening and closing: an embedded solenoid valve opened and closed a microfluidic channel more than 100,000 times with no apparent deterioration in its function. It was possible to combine all three types of prefabricated valves in a single microfluidic device to control chemical gradients in a microfluidic channel temporally and spatially.

Introduction

This paper demonstrates a technique for creating standardized, prefabricated valves of three types—screw valves, pneumatic valves, and solenoid valves—for microfluidic applications. The valves are fabricated *en masse* before they are needed, and then embedded during the assembly of a microfluidic device. This method of prefabrication and embedding should be useful for introducing both valves, and other functional elements, into microfluidic devices.

As microfluidic technology becomes more sophisticated, there is a growing need for control components—such as valves—that can be integrated easily into microdevices. A variety of microfluidic valves are now available (reviewed in reference[1]), including valves using pneumatic actuation,[2–4] magnetic actuation,[5,6] the swelling of hydrogels,[7] the movement of ferrofluids,[8] and the thermal response of shape-memory alloys.[9] Takayama and coworkers have used the pins of a piezoelectric Braille display as valves in microfluidic systems.[10–15] Most of these valves require sophisticated fabrication or complex controllers, and are therefore not in common use.

Perhaps the most commonly used microfluidic valves in complex elastomeric devices are the pneumatic valves developed by Quake and coworkers.[3] In the Quake valve scheme, each valve is a three-layer microfluidic structure, consisting of a flow channel in one layer separated by a thin elastomeric membrane from a (usually perpendicular) control channel in the layer above. The application of pressurized air (or liquid) to the control channel (with actuation pressures of 5–30 psi, or 35–200 kPa, depending on the sizes of the flow and control channels, and the properties of the elastomeric membrane)[2] closes the flow channel. The Quake valves have made it possible to fabricate a number of complex systems because they are compatible with soft-lithographic technology, because they have a small footprint, and because they can be used in parallel at high densities. They also have two disadvantages: i) the off-chip infrastructure (computer-controlled pneumatic actuators, gas distribution system, computer) required to operate them is costly and bulky, and ii) these valves are "overkill" for simple devices. The Quake valves also share a common disadvantage with most other pneumatic valves: they require a continuing application of pressurized gas to maintain a valve in the "closed" state. Many microfluidic applications may need only one, or a small number, of valves. In these cases, inclusion of even a single Quake valve into the design requires the same three layers of microfabrication as do the much more complex systems for which these valves are best suited; this requirement unnecessarily complicates the fabrication of simple systems.

Quake valves are also inflexible in one sense: because the design of the control layer must be compatible with the design of the flow layer, any changes to the design of the flow layer may require changes in the design of the control layer. Similarly, if one needs to change the position of the valves in a device, the control layer may have to be redesigned. Although these steps are not prohibitively time-consuming, they do slow the iterative process of rapid prototyping. In addition, the fabrication of microfluidic systems incorporating any number of Quake valves requires registration of the flow layer and the control layer (although the use of perpendicular flow and control channels makes this process forgiving).

To meet the need for simple, single- or few-valve construction in microfluidic devices fabricated *via* soft lithography, our group developed what we call TWIST valves.[16] To construct a TWIST valve, a small machine screw is introduced directly above a microfluidic channel in a PDMS (elastomeric) device. The TWIST valve works by converting torque into downward compression. Rotation of the screw results in downward motion of the screw and compression of the underlying microfluidic channel; thus rotation closes the channel. Because this process is reversible (counterclockwise rotation of the screw reopens the microfluidic channel), the embedded screw can be used as a microfluidic valve. Because TWIST valves are fabricated individually and can be inserted into microfluidic systems as

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needed, they are useful in situations that require only small numbers of valves. One disadvantage in the fabrication of TWIST valves is that the valves are constructed directly in microfluidic devices; holes are bored manually in a PDMS device above the channels that are to be regulated.[16] For inexperienced users, the procedure may yield faulty valves—and a single faulty valve may result in a faulty device. Additionally, because the valves are constructed one-at-a-time, performance may vary significantly across valves, even in a single device.[16]

Here we describe pneumatic (Quake-like), screw (TWIST-like), and solenoid valves that can be fabricated, *en masse*, ahead of time, and then positioned and embedded in microfluidic devices as needed. We demonstrate the utility of the valves by incorporating all three types of valves into a single device: a microfluidic gradient generator.[17–19] The fabrication of the valves is very simple; it does not require photolithography or other complicated fabrication techniques. These valves are therefore convenient in systems in which they are needed only in small numbers, and in which fabrication of an integrated system is not required. One advantage of prefabrication is that a standardized procedure results in uniform operation: prefabricated pneumatic valves all require the same amount of pressure to close; prefabricated screw valves require the same number of turns; prefabricated solenoid valves require the same voltage. Thus, with prefabricated valves, one is able to achieve simplicity in fabrication without sacrificing performance (at the cost of component-level assembly and a relatively large footprint for each valve). Another advantage of the modular design is that there is a low risk of damaging the channels and other components of the final device when introducing the valve into a microfluidic system; the prefabricated valve is simply positioned on the silicon master, and uncured elastomer is poured around it.

This method of integrating valves into devices is highly flexible: one can change the configuration of valves without redesigning the device itself; the device is simply recast with the valves in different positions. This feature should allow the optimization and development of simple devices to proceed quickly. In addition, it will be possible to share the overhead costs of fabrication of the valves among users—a single facility can fabricate many valves, and then distribute those valves among multiple users—and so researchers will be able to add valves to their devices without needing to fabricate the valves themselves. The valves thus represent standardized components that can be used with standard design rules.

Results and discussion

Prefabrication of screw valves

Fig. 1a illustrates the prefabrication of screw valves. For the screw valves, we first fabricated a two-layer PDMS (Sylgard® 184, Dow Corning, Corning, NY) structure, which served as a physical support for the valves. For all devices, we prepared PDMS by combining the two components of Sylgard® 184 in a 10:1 (base:curing agent) ratio by weight, and cured the PDMS for at least 3 h at 65 °C. We punched an array of holes (3.5 mm in diameter) completely through a slab of PDMS, 2 mm in width. We exposed the slab containing the holes, and a second slab, also 2 mm in width, to an oxidizing plasma for one minute and placed them in contact to create covalent bonds between the two slabs. We then punched a second array of smaller holes, 1 mm in diameter, through the second slab of PDMS such that the smaller holes and the larger holes were positioned concentrically. Finally, we sealed a 1-mm membrane of PDMS to the bottom of the two-layer PDMS structure.

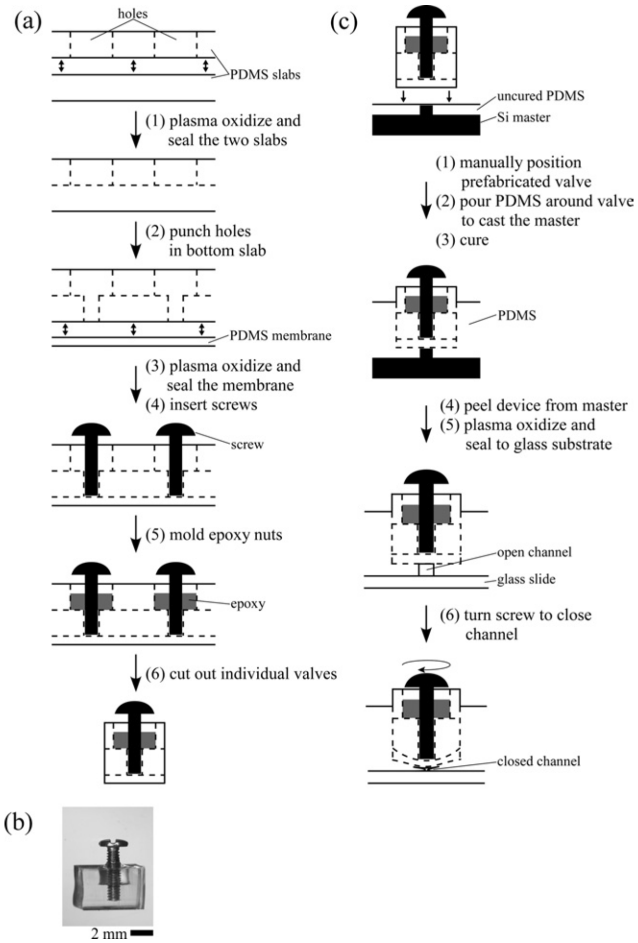

Fig. 1 Fabrication of screw valves. (**a**) Prefabrication of the screw valve. We started by punching 3.5-mm holes completely through a slab of PDMS, 2 mm in thickness. We sealed this slab to a second slab of PDMS of equal thickness, and punched smaller holes (1 mm in diameter) through this second slab. We then sealed this two-layer structure to a PDMS membrane, 1 mm in thickness. To assemble the valves, we inserted machine screws (shaft: 6 mm × 1 mm) into the 1-mm holes in the PDMS structure and added epoxy (NOA 81) to the 3.5-mm hole to create an improvised nut around each screw. The valves were fabricated in arrays; individual valves could be separated with a razor blade. (**b**) A photograph of a prefabricated screw valve. (**c**) Incorporation of the screw valve into a microfluidic device. The master for the microfluidic device consisted of SU-8 features on a silicon wafer. We spin-coated a layer of PDMS onto the master, manually positioned the prefabricated valve above an SU-8 channel on the PDMS-coated master, and pressed the valve down onto the channel. The device was then molded in PDMS and cured. The dashed lines indicate the fusion of different layers of PDMS. Once cured, the devices were removed from the master, plasma oxidized, and sealed to a glass slide. Twisting of the screw resulted in closure of the underlying channel.

To assemble the screw valve, we inserted small machine screws (part no. MX-0080-04B, Small Parts, Inc., Miami Lakes, FL) through the larger holes in the PDMS and into the smaller holes. The cylindrical shaft of each machine screw was approximately 1 mm in diameter and 6 mm in length. The smaller holes in the lower layer of PDMS anchored the screws in place. The purpose of the larger holes in the upper layer of PDMS was to create a small reservoir around each screw. We filled each reservoir with photocurable polyurethane (NOA 81, Norland Products, Inc., Cranbury, NJ), leaving the head of each screw exposed. The polyurethane molded to the shape of the threads of the screws and, once cured, created a "nut" against which the threads of the screws could turn. In order for NOA 81 to adhere to PDMS, it is necessary to expose PDMS to an air plasma for 1 min prior to filling the reservoirs with NOA 81. The chemical composition of NOA 81 is proprietary; nevertheless, we have observed that the contact angle of uncured NOA 81 with PDMS decreases from ~65° to ~15° following oxidation of the PDMS. It is likely that oxidation facilitates adhesion by enabling uncured NOA 81 to wet—and possibly penetrate—the surface of PDMS. (A number of compounds can partially dissolve PDMS.[20]) NOA 81 did not adhere to the stainless steel screws. When cured (for 10 min under a UV lamp), NOA 81 becomes hard, but not brittle; the bond between NOA 81 and PDMS therefore tolerated some bending of the PDMS device.

Prefabrication of pneumatic valves

Figs. 2a illustrates the prefabrication of pneumatic valves. For the pneumatic valves, we used a two-layer PDMS structure similar in shape to the structure used to support the screw valves (Fig. 1a), but inverted. For the pneumatic valves, the larger holes were 3.5 mm in diameter and the smaller holes were 1.5 mm in diameter. To create an inlet for air into the pneumatic valve, we used polyethylene tubing (PE190 Intramedic tubing, VWR International, Inc.). We melted the tips of several short (~5 cm) pieces of tubing by touching the tips of the tubing to a hot plate set at 130 °C for several seconds. Melting the tips created a rim of polyethylene (~3 mm in diameter) surrounding the opening of the tubing. We threaded each piece of tubing through the two-layer PDMS slab, starting on the side with larger holes. Because the outer diameter of the polyethylene rim was greater than 1.5 mm, the rim blocked the tubing from passing all of the way through the slab. The purpose of this rim was to prevent the tubing from detaching from the device of the valve during use. We added a small amount of photocurable polyurethane (NOA 81) around the tubing/PDMS interface and cured the polyurethane under UV light to ensure that there was an airtight seal. Finally, we sealed the bottom of the valves to a thin (100–200 μm) PDMS membrane, which had been made by spin coating PDMS onto a 3-inch silicon wafer (Silicon Sense, Inc., Nashua, NH).

Prefabrication of solenoid valves

Fig. 3a shows the prefabrication of solenoid valves. We created the valves using cylindrical, push-type solenoids (part no. S-69-38-H, Magnetic Sensor Systems, Van Nuys, CA). The solenoids were approximately 10 mm in diameter, and 24 mm in height, with external threads along the lower 6 mm of the casing of each solenoid. To fabricate a structural support for the solenoids, we molded nuts out of PDMS using headless bolts—with thread

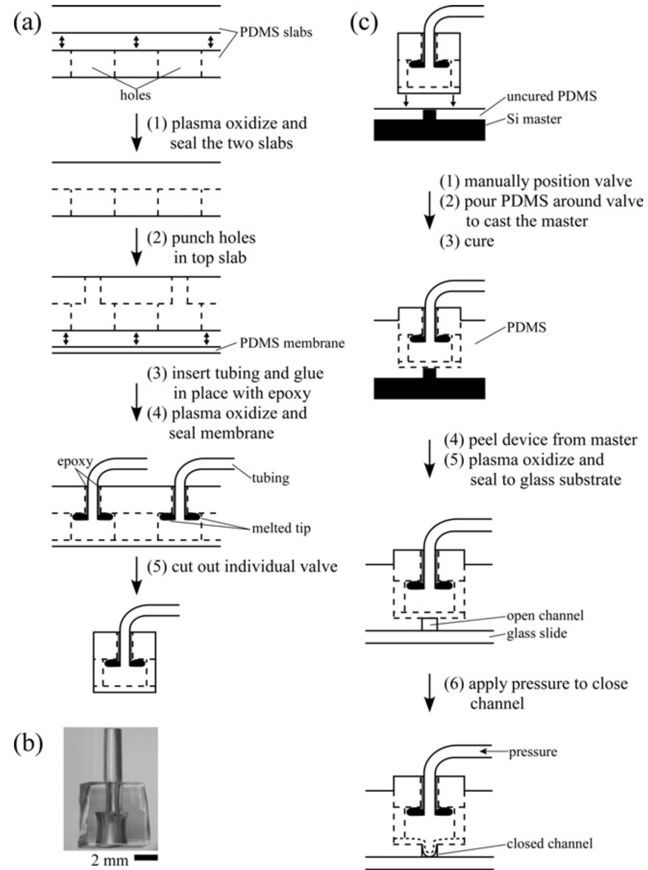

Fig. 2 Fabrication of pneumatic valves. (**a**) Prefabrication of the pneumatic valve. We started by punching a 3.5 mm hole completely through a slab of PDMS, 2 mm in thickness. We sealed this slab to a second slab of PDMS of equal thickness, and punched smaller holes (1.5 mm in diameter) through this second slab. We threaded polyethylene tubing (outer diameter: 1.5 mm) with a melted tip (diameter: ~ 3 mm) through the two-layer PDMS structure, applied epoxy (NOA 81) to the tubing/PDMS interface to create an airtight seal, and sealed a thin (100–200 μm) PDMS membrane to the bottom of the two-layer structure. The valves could be separated with a razor blade. (**b**) A photograph of a prefabricated pneumatic valve. (**c**) Incorporation of the pneumatic valve into a microfluidic device. We spin-coated a layer of PDMS onto the master, manually positioned the prefabricated valve above an SU-8 channel on the PDMS-coated master, and pressed the valve down onto the channel. The device was then molded in PDMS and cured. The dashed lines indicate the fusion of different layers of PDMS. Once cured, the devices were removed from the master, plasma oxidized, and sealed to a glass slide. Application of pressurized air resulted in closure of the underlying channel.

dimensions equal to those of the solenoids—as templates. These PDMS nuts would ultimately anchor the solenoids in place. We sealed the PDMS nuts to a thin (1 mm) PDMS membrane, and punched 1.5-mm holes through the membrane such that the PDMS nuts and the 1.5-mm holes were positioned concentrically. The holes would eventually enable us to position a small poly(methyl methacrylate) (PMMA) bead, as well as the armature of the solenoid, directly over a microchannel in a microfluidic device. It was possible to insert the threaded solenoid into the PDMS structure reversibly, simply by screwing the solenoid into the PDMS nut.

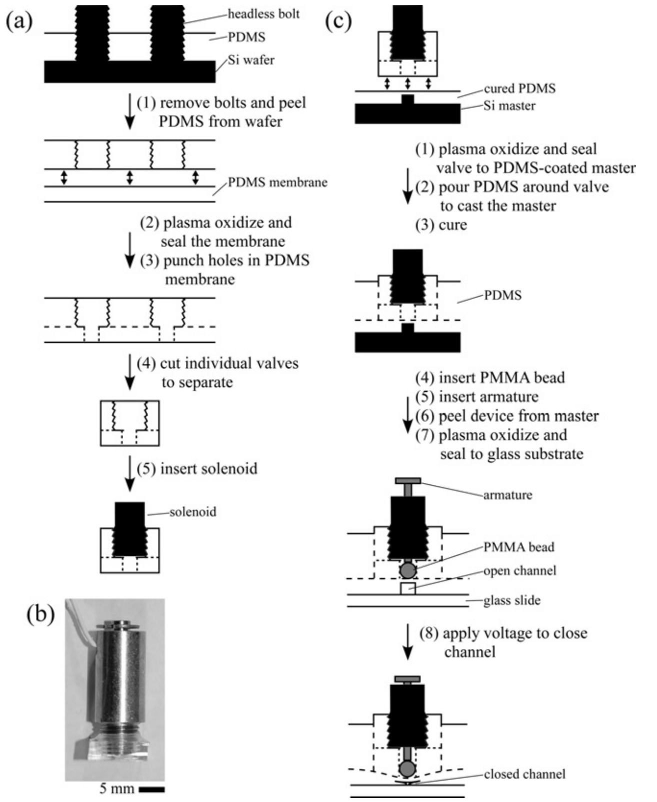

Fig. 3 Fabrication of solenoid valves. (**a**) Prefabrication of the solenoid valve. First, we created an array of PDMS nuts by molding PDMS around headless bolts. We sealed the array of PDMS nuts to a thin (1 mm) membrane of PDMS and punched 1.5-mm holes through the membrane, such that the holes were concentrically aligned with the PDMS nuts. Individual valves could be separated with a razor blade. It was possible to insert a cylindrical solenoid with into each PDMS structure. Solenoids were approximately 10 mm in diameter, and 24 mm in height, with external threads along the lower 6 mm of the casing of each solenoid. (**b**) A photograph of a prefabricated solenoid valve. (**c**) Incorporation of the prefabricated solenoid valve into a microfluidic device. We spin-coated a layer of PDMS onto the silicon master. After curing this layer of PDMS, we manually sealed the valve to the master directly above an SU-8 channel. The device was then molded in PDMS and cured. The dashed lines indicate the fusion of different layers of PDMS. Once cured, the devices were removed from the master, plasma oxidized, and sealed to a glass slide. A PMMA bead, 1.5 mm in diameter, was dropped through the center of the solenoid, and the armature of the solenoid was put in place. Application of voltage to the solenoid resulted in closure of the underlying channel.

For all types of valves, it was possible to construct a number of prefabricated valves together and then separate individual valves, as needed, using a razor blade. Fig. 4a shows a strip of prefabricated screw valves. Figs. 1b, 2b, and 3b show photographs of the three types of prefabricated valves.

The size of the valves limits the density of channels in the region of the device where valves are to be embedded. The footprint of each prefabricated valve determines the minimum valve-to-valve (and channel-to-channel) separation: the footprints of the prefabricated screw and pneumatic valves were approximately 3.5 × 3.5 mm; the footprint of the prefabricated solenoid valve was approximately 10 × 10 mm.

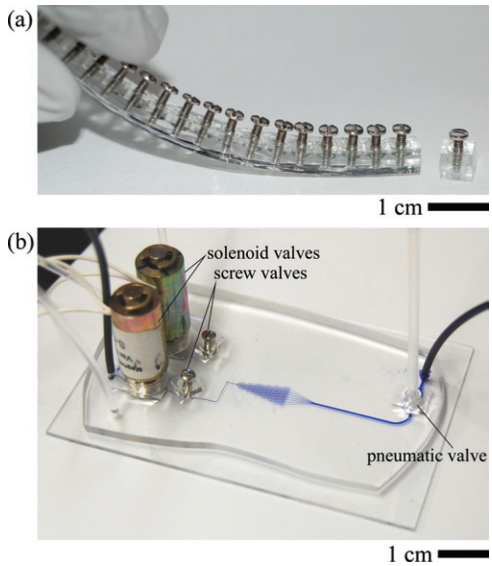

Fig. 4 (**a**) A photograph of a strip of prefabricated screw valves. A single valve has been separated from the strip using a razor blade. (**b**) A photograph of a microfluidic gradient generator containing two embedded solenoid valves, two embedded screw valves and one embedded pneumatic valve. The design of this device is shown in Fig. 8.

Embedding prefabricated valves into microfluidic devices

Figs. 1c, 2c, and 3c illustrate the incorporation of prefabricated valves into microfluidic devices. We fabricated masters for the microfluidic devices using conventional photolithography to produce raised features in SU-8 photoresist on a silicon wafer.[21] For both the screw valve (Fig. 1c) and the pneumatic valve (Fig. 2c), we first spin-coated a thin layer of PDMS onto the master. This layer was roughly the same height as the SU-8 features on the master. We then manually pressed the prefabricated valve directly onto an SU-8 channel on the PDMS-coated master. The initial coating of PDMS on the master served to balance the prefabricated valve on the SU-8 channel and to prevent the trapping of bubbles between the prefabricated valve and the master. Careful alignment of the valve and the channel was not necessary because the width of the features of the master were small compared to the diameter of the active region of the screw and pneumatic valves.

For the solenoid valve (Fig. 3c), we also used a spin-coater to coat the master with a thin layer of PDMS. In this case, however, we spun a layer of PDMS that was approximately 20 μm thicker than the features on the master: for example, if the features on the master were 30 μm in height, we spun a PDMS layer that was 50 μm in thickness. We cured this layer, and then, after exposing both the prefabricated solenoid valve and the PDMS-coated master to an oxidizing plasma, sealed the valve onto the master directly over an SU-8 channel. We sealed the prefabricated solenoid valve to the master (rather than simply pressing the prefabricated valve against an uncured coating of PDMS, as we did with the screw and pneumatic valves) because the solenoid valve required more accurate alignment of the valve over the channel than did the screw and pneumatic valves (we discuss the reason for this difference below). Sealing the valve directly onto the PDMS-coated master prevented any drift in the location of the valve with respect to the underlying channel. Despite the increased need for accuracy in alignment, it was still sufficient to position the solenoid valve manually (by hand, without the aid of a microscope).

The three valves differed in their abilities to close channels of different sizes. We tested all valves using channels that were 50 µm in width. For this specific width, pneumatic valves could successfully close channels that were 10 µm in height or less; the screw and solenoid valves could successfully close channels that were up to 100 µm tall.

For each of the three types of valves, once the valve was in place, we poured uncured PDMS onto the master around the valve in order to mold the device with the valve embedded directly above a microfluidic channel. Once cured, the PDMS device was removed from the master and sealed to a flat glass substrate to create a microfluidic device with a functional valve. Fig. 4b shows a photograph of a device—a microfluidic gradient generator[17–19]—containing embedded solenoid, screw and pneumatic valves. To actuate the screw valve, we used a screwdriver to rotate the screw manually. To actuate the pneumatic valve, we connected the tubing of the valve to a tank of pressurized air. To use the solenoid valve, we first inserted a PMMA bead into the bottom of the valve, through the center of the solenoid. We then inserted the armature of the solenoid into the solenoid (Fig. 2c). The length of the armature was such that the bottom of the armature rested on the top of the bead, and the top of the armature extended 1–2 mm out of the top of the solenoid. The purpose of the PMMA bead was to focus the force of the solenoid onto a small area. (Because the area of contact of the bead with the top of the channel was small, it was necessary to align the solenoid valve with more accuracy than for the screw and pneumatic valves). To actuate the valve, we connected the electrical leads of the solenoid to a source of voltage.

Performance and reproducibility of embedded valves

To test the operation of the prefabricated valves, we embedded each valve into a Y-shaped microfluidic device-a device in which two inlet channels, 50 µm in width, intersected at a Y-shaped junction to form a single outlet channel, also 50 µm in width. In each case, the location of the embedded valve was directly over one of the two inlet channels. To test the screw, pneumatic, and solenoid valves, we used microchannels that were 50 µm, 10 µm, and 40 µm in height, respectively. We created two laminar streams of equal width in the outlet channel of each device by connecting reservoirs of liquid to the two inlet channel, and raising the reservoirs approximately 30 cm above the level of the device. By adding dye to one of the inlet reservoirs, we were able to distinguish between the two streams of liquid. In order to characterize the performance of a valve, we closed the valve and simultaneously monitored the decrease in width of the associated laminar stream, which corresponded proportionally to the decrease in the rate of flow of liquid through the controlled channel. We closed the embedded screw valves by rotating the screws with a screwdriver in increments of 90°; we closed the embedded pneumatic valves by connecting the tubing of each valve to a tank of pressurized air and regulating the gauge pressure; we closed the embedded solenoid valves by connecting the electrical leads of the solenoid to a variable power supply and modulating the voltage.

For each type of valve, we compared (i) the performance of a single valve with itself over a number of cycles of closing and opening (Figs. 5a, 6a, and 7a), and (ii) the performance of different valves (of the same type) that were prefabricated using the same procedure (Figs. 5b, 6b, and 7b). For all three types of valves, performance changed only slightly over 20 cycles of closing and opening (Figs. 5a, 6a, and 7a). To completely close the underlying channel, screw valves required a clockwise rotation of 540° (1.5 turns). Pneumatic valves required the application of a gauge pressure of 45 psi (310 kPa) to close. This value is larger than the actuation pressure of 5–30 psi (35–200 kPa) required by Quake valves.[2] One difference between the pneumatic valves presented here and Quake valves is that in this study, the flow channels were rectangular in cross-section; for Quake valves, the flow channels are rounded to facilitate the closure of the valves at low pressure.[3] In the Quake scheme, the area of contact between the flow channel and the control channel—typically 50 µm × 50 µm—defines the area of the membrane that needs to deform (bulge) to close the flow channel. With the prefabricated pneumatic valves, the area of the membrane that closes the flow channel is much larger—3500 µm × 50 µm—and therefore the membrane deforms much more readily along the axis of the flow channel than the membrane in the Quake scheme. We believe that both the increased actuation pressure and the increased area of the pneumatic membrane enabled the successful closure of *rectangular* flow channels.

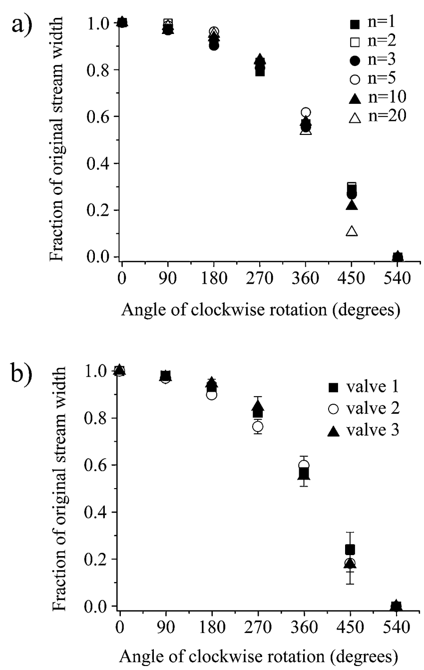

Fig. 5 Reproducibility in the behavior of screw valves. We tested the behavior of each valve by measuring the decrease in the width of a laminar stream in a Y-shaped microfluidic device as the valve was closed. We closed the screw valves by manually twisting the screws with a screwdriver in increments of 90°, and plotted the relative width of the laminar stream as a function of the angle of clockwise rotation. (**a**) We compared the behavior of a single embedded screw valve with itself over repeated cycles, n, of operation. (**b**) We compared the behavior of three different embedded screw valves that had been fabricated according to the same procedure.

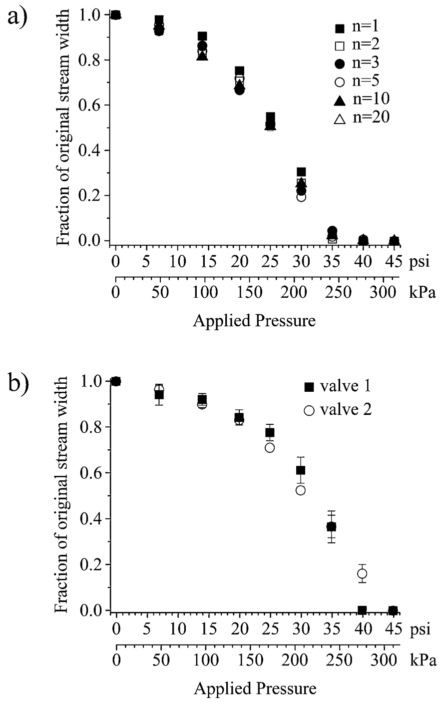

Fig. 6 Reproducibility in the behavior of pneumatic valves. We tested the behavior of each valve by measuring the decrease in the width of a laminar stream in a Y-shaped microfluidic device as the valve was closed. We closed the pneumatic valves by attaching the valve to a source of pressurized air (with a regulator), and plotted the relative width of the laminar stream as a function of applied pressure. (**a**) We compared the behavior of a single embedded pneumatic valve with itself over repeated cycles, n, of operation. The thickness of the PDMS membrane that separated the bottom of the valve from the underlying channel was 200 μm. (**b**) We compared the behavior of two different embedded pneumatic valves that had been fabricated according to the same procedure. For both of the valves, the thickness of the PDMS membrane that separated the bottom of the valve from the underlying channel was 150 μm.

The solenoid valves required the application of 10.5 V (this value would depend upon the electrical demands of the solenoid that is used). Because solenoid valves use electrical actuation, it was possible to operate the valves quickly: the response time of the valve was less than 1 s. In a separate experiment, we used a solenoid valve to open and close a 40 μm × 50 μm (height × width) channel more than 100,000 times with no apparent deterioration in its function.

Different valves of the same type that were prefabricated using the same procedure also performed reproducibly. Fig. 5b compares the performance of three different screw valves; Fig. 6b compares the performance of two different pneumatic valves; Fig. 7b compares the performance of two different solenoid valves. For each valve, the average of at least 7 cycles of closing and opening was plotted.

For the solenoids valves, the transition from "open" to "closed" was more abrupt than for the screw and pneumatic valves. Nevertheless, for all three valves, there was a continuous transition of the valve from "open" to "closed." This smooth transition suggests that the valves could be used as regulators of flow in microchannels.

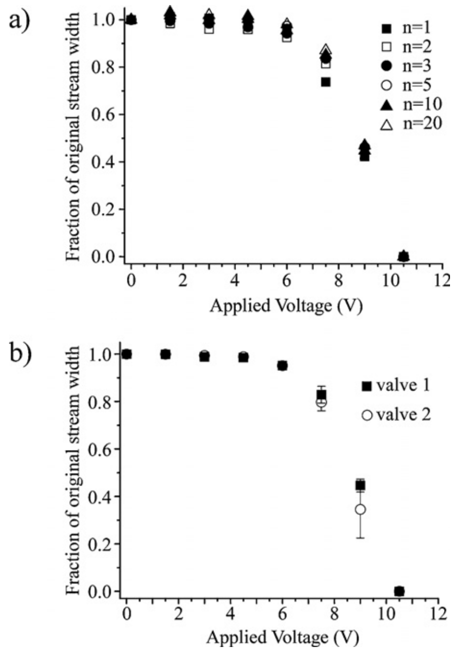

Fig. 7 Reproducibility in the behavior of solenoid valves. We tested the behavior of each valve by measuring the decrease in the width of a laminar stream in a Y-shaped microfluidic device as the valve was closed. We closed the solenoid valves by connecting the leads of the solenoid to a variable power supply, and plotted the relative width of the laminar stream as a function of applied voltage. (**a**) We compared the behavior of a single embedded solenoid valve with itself over repeated cycles, n, of operation. (**b**) We compared the behavior of two different embedded solenoid valves that had been fabricated according to the same procedure.

Generating spatial and temporal gradients in a device with embedded microfluidic valves

To demonstrate the utility of the prefabricated valves, we used the valves to control spatial and temporal gradients in a microfluidic gradient generator. A gradient generator is a network of microfluidic channels that uses the lateral diffusion of dissolved species across laminar streams to produce spatial concentration gradients perpendicular to the direction of flow in the device.[17–19] By adjusting the composition of the dissolved or suspended species at the inlets of the device, or by altering the design of the microfluidic network itself, it is possible to produce gradients with complex shapes.[17] We used embedded valves to produce gradients that were reconfigurable in time and space without needing to rearrange the connectivity of the feeding reservoirs off-chip, or to adjust the driving pressure. Fig. 4b shows a photograph of the device, and Fig. 8a shows the design. The two central inlets of the device—inlets 2 and 3—intersected at a T-shaped junction to form a single channel. The inset in Fig. 8a shows a magnified view of the network of channels in the gradient generator.

We incorporated valves into the device in a way that took advantage of the particular strengths of each type of valve. We embedded a pair of solenoid valves and screw valves over inlet channels 2 and 3. These valves enabled us to select which inlet would supply liquid to the gradient generator. Over each channel, we used a solenoid valve and a screw valve in tandem

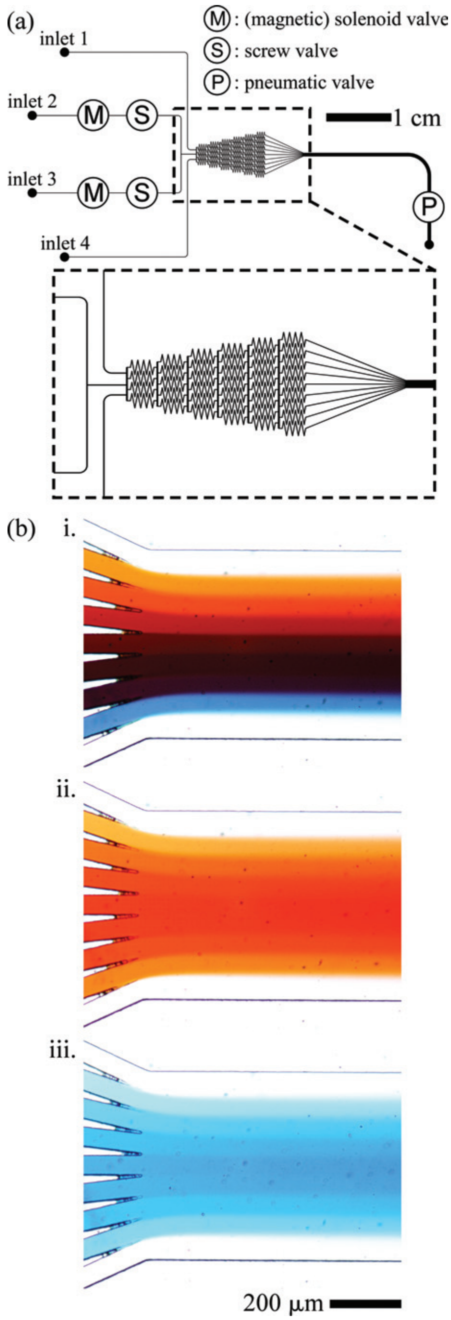

Fig. 8 Using embedded valves to create spatial and temporal gradients in a gradient mixer. (**a**) The design of the microfluidic gradient generator. The circled letters indicate the positions of the embedded valves. (**b**) Producing spatial gradients that fluctuate with time. We connected inlets 1 and 4 to reservoirs of clear water, connected inlet 2 to a reservoir of red dye, and connected inlet 3 to a reservoir of blue dye. (**i**) All valves in the device were open. (**ii**) The solenoid valve over inlet channel 3 was closed; all other valves were open. (**iii**) The solenoid valve over inlet channel 2 was closed; all other valves were open.

because of the complementary features of these two types of valves. Screw valves require no power to maintain a closed or open position, and were therefore useful for maintaining a particular concentration profile for an extended period. With the solenoid valves, it was possible to change the concentration profile rapidly, using electronic control. The concentration profile could be changed in less than 5 seconds; the rate of flow and the volume of liquid in the gradient generator ultimately limit this switching time. Because of the characteristically smooth transition of pneumatic valves from open to closed (Fig. 6), we embedded a single pneumatic valve over the outlet channel of the device to act as a regulator of flow in the gradient generator. The application of pressure to the pneumatic valve changed the resistance of the outlet channel, and therefore changed the volumetric rate of flow of liquid through the device. Although a screw valve is also capable of acting as a regulator of flow, the pneumatic valve could be controlled more precisely.

We connected inlet 2 to a reservoir containing red dye, connected inlet 3 to a reservoir containing blue dye, and connected inlets 1 and 4 to reservoirs of water without dye. To produce flow through the device, we raised the inlet reservoirs approximately 30 cm above the level of the outlet of the device. We closed the screw valves over inlet channels 2 and 3 to initiate the system with no gradient of dye in the outlet channel (image not shown). We then opened both screw valves; the resulting gradient in the outlet channel contained both red and blue dye (Fig. 8b,i). By alternatively closing and opening the solenoid valves over inlet channels 2 and 3, we were able to switch back and forth rapidly between two different spatial gradients: a red gradient when inlet channel 3 was closed (Fig. 8b,ii), and a blue gradient when inlet channel 2 was closed (Fig. 8b,iii).

Conclusions

The fabrication technique described here provides a simple, flexible method for integrating small numbers of valves into microfluidic devices. The valves operate uniformly, and perform reproducibly, with repeated use.

Because of the relatively large footprint of prefabricated valves, this method cannot compete with Quake valves for the fabrication of fully integrated, complex microfluidic systems. Nevertheless, the technique of prefabrication and embedding has many features that make it attractive for fabricating *simple* microfluidic systems. This simplicity and flexibility will enable researchers to produce prototypes for devices rapidly, and should thus decrease the amount of time it takes researchers to generate functional lab-on-a-chip devices. The valves are also inexpensive. The cost of materials for the screw and pneumatic valves is very low (less than US$1 per valve). The solenoids are slightly more expensive (US$10-20 per solenoid) than the screw and pneumatic valves, but are reusable and are significantly less expensive than the piezoelectric Braille displays (US$400-$1000) that have been adopted by Takayama and coworkers for use as valves in microfluidic systems.[11,12,22]

The procedure for prefabricating and embedding valves is compatible with multiple modes of actuation: manual actuation, actuation with pressurized air, and electrical actuation. This ability to use different modes of actuation will allow researchers to embed the type of valve that best suits their application. Certain types of valves offer unique advantages. For instance, screw valves do not consume power in the closed (or open) state. Solenoid valves, alternatively, offer the convenience of electrical actuation. Because valves are embedded into devices individually, it should be possible for researchers to exploit different modes of actuation in a single device.

Another useful feature of this technique is that one embeds the control elements for the valve directly above the channel that it controls (this is in contrast to Quake valves, in which the control channel crosses from the edge of the device to the channel of interest). This vertical placement allows a single channel to be controlled regardless of the spatial arrangement of the surrounding channels (provided that there is enough space for the valve).

We developed screw, pneumatic, and solenoid valves to demonstrate the concept of prefabrication and embedding; the technique we present here, however, should be applicable not only to other modes of actuation (such as piezoelectric actuation, or the thermal response of shape memory alloys), but also to other types of functional elements, including optical elements (light sources and photodetectors), heating elements, magnetic flux concentrators, electrochemical detectors, and microlens arrays.

Because the valves are easy to fabricate and require no specialized equipment, they should facilitate the development of simple microfluidic devices by lowering the "activation barrier" for the incorporation of valves in devices. The ability to prefabricate modular "valve units" should also promote dissemination of valve technology to a broad range of researchers: prefabrication allows valves to be distributed easily to multiple users from a single source.

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References

1 K. W. Oh and C. H. Ahn, *J. Micromech. Microeng.*, 2006, **16**, R13–R39.
2 V. Studer, G. Hang, A. Pandolfi, M. Ortiz, W. F. Anderson and S. R. Quake, *J. Appl. Phys.*, 2004, **95**, 393–398.
3 M. A. Unger, H.-P. Chou, T. Thorsen, A. Scherer and S. R. Quake, *Science*, 2000, **288**, 113–116.
4 W. H. Grover, R. H. C. Ivester, E. C. Jensen and R. A. Mathies, *Lab Chip*, 2006, **6**, 623–631.
5 W. C. Jackson, H. D. Tran, M. J. O'Brien, E. Rabinovich and G. P. Lopez, *J. Vac. Sci. Technol. B*, 2001, **19**, 596–599.
6 T. Pan, S. J. McDonald, E. M. Kai and B. Ziaie, *J. Micromech. Microeng.*, 2005, **15**, 1021–1026.
7 D. J. Beebe, J. S. Moore, J. M. Bauer, Q. Yu, R. H. Liu, C. Devadoss and B.-H. Jo, *Nature*, 2000, **404**, 588–590.
8 H. Hartshorne, C. J. Backhouse and W. E. Lee, *Sens. Actuators, B*, 2004, **99**, 592–600.
9 M. Kohl, D. Dittmann, E. Quandt and B. Winzek, *Sens. Actuators, A*, 2000, **83**, 214–219.
10 N. Futai, W. Gu, J. W. Song and S. Takayama, *Lab Chip*, 2006, **6**, 149–154.
11 W. Gu, H. Chen, Y.-C. Tung, J.-C. Meiners and S. Takayama, *Appl. Phys. Lett.*, 2007, **90**, 033505.
12 W. Gu, X. Zhu, N. Futai, B. S. Cho and S. Takayama, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 15861–15866.
13 Y. Kamotani, T. Bersano-Begey, N. Kato, Y. C. Tung, D. Huh, J. W. Song and S. Takayama, *Biomaterials*, 2008, **29**, 2646–2655.
14 J. W. Song, W. Gu, N. Futai, K. A. Warner, J. E. Nor and S. Takayama, *Anal. Chem.*, 2005, **77**, 3993–3999.
15 Y. C. Tung, Y. S. Torisawa, N. Futai and S. Takayama, *Lab Chip*, 2007, **7**, 1497–1503.
16 D. B. Weibel, M. Kruithof, S. Potenta, S. K. Sia, A. Lee and G. M. Whitesides, *Anal. Chem.*, 2005, **77**, 4726–4733.
17 S. K. W. Dertinger, D. T. Chiu, N. L. Jeon and G. M. Whitesides, *Anal. Chem.*, 2001, **73**, 1240–1246.
18 S. K. W. Dertinger, X. Jiang, Z. Li, V. N. Murthy and G. M. Whitesides, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 12542–12547.
19 N. L. Jeon, S. K. W. Dertinger, D. T. Chiu, I. S. Choi, A. D. Stroock and G. M. Whitesides, *Langmuir*, 2000, **16**, 8311–8316.
20 J. N. Lee, C. Park and G. M. Whitesides, *Anal. Chem.*, 2003, **75**, 6544–6554.
21 Y. Xia and G. M. Whitesides, *Angew. Chem. Int. Ed. Engl.*, 1998, **37**, 550–575.
22 W. Gu, X. Zhu, N. Futai, B. S. Cho and S. Takayama, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 15861–15866.