

Torque-Actuated Valves for Microfluidics

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This paper describes torque-actuated valves for controlling the flow of fluids in microfluidic channels. The valves consist of small machine screws ($\geq 500\ \mu\text{m}$) embedded in a layer of polyurethane cast above microfluidic channels fabricated in poly(dimethylsiloxane) (PDMS). The polyurethane is cured photochemically with the screws in place; on curing, it bonds to the surrounding layer of PDMS and forms a stiff layer that retains an impression of the threads of the screws. The valves were separated from the ceiling of microfluidic channels by a layer of PDMS and were integrated into channels using a simple procedure compatible with soft lithography and rapid prototyping. Turning the screws actuated the valves by collapsing the PDMS layer between the valve and channel, controlling the flow of fluids in the underlying channels. These valves have the useful characteristic that they do not require power to retain their setting (on/off). They also allow settings between “on” and “off” and can be integrated into portable, disposable microfluidic devices for carrying out sandwich immunoassays.

This work describes the fabrication of valves for microfluidic devices based on small machine screws embedded in a layer of polyurethane. While these valves should be widely useful in the field of microfluidics, we demonstrate their use in portable, disposable microfluidic devices.

Microfluidic-based platforms require methods for fabricating channels, pumps for actuating the flow of liquids, and valves and switches for controlling the flow of fluids and delivering specific amounts of reagents. A variety of methods and materials—especially polymers, glass, and silicon—are used for fabricating microfluidic channels. Elastomeric polymers—especially poly(dimethylsiloxane) (PDMS)—are used widely in microfluidics for several reasons: (i) ease of fabrication using soft lithography and rapid prototyping;^{1–3} (ii) ability to incorporate seals, membranes, valves, and switches;^{4–7} (iii) transparency and compatibility with

optical detection;^{8,9} and (iv) compatibility with water and biological molecules and cells.^{10,11}

A number of different pumps and valves have been reported, based on: (i) pneumatics;^{12–16} (ii) melting solids;^{17–21} (iii) bubbles;^{22–26} (iv) ferrofluids and polymers doped with ferromagnetic materials;^{27–29} (v) thermoresponsive polymers;^{30–32} (vi) pH-responsive hydrogels;^{33,34} (vii) electrokinetic effects;^{35–43} (viii) piezos;⁴⁴ (ix) colloids;⁴⁵ (x) surface energies of liquids;^{46,47} and (xi) microfabricated, mechanical actuators.^{48,49} Of these, the most

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widely used have arguably been the monolithic, pneumatic valves of Quake et al.^{4–6} Monolithic, pneumatic valves are fabricated in PDMS and are based on two sets of microfluidic channels that are bonded together, one on top of, and perpendicular to the other, with the two separated by a thin, elastomeric membrane ($\sim 30\ \mu\text{m}$ thick). One layer of microfluidic channels forms the valves. A pressure applied to the valve channels collapses the membrane of PDMS into the microfluidic channels and controls the flow of fluids. There are a number of advantages to these valves: they (i) are simple in design and materials; (ii) are easily integrated; (iii) have rapid response times; (iv) are based on common soft lithography techniques; (v) can be high density (≥ 30 valves mm^{-2}); (vi) resist back pressures up to 70 kPa; (vii) close channels with a range of aspect ratios; and (viii) are easily programmed and operated under computer control. There are also several disadvantages to these actuators: they (i) require constant pressure and thus do not have a “power-off” mode; (ii) require expensive external hardware for controlling pressure; (iii) are not portable; (iv) are not able to close channels with rectangular or square cross sections completely; (v) are not easily graded in response to the pressure that is applied; and (vi) require the user to optimize the fabrication.⁵⁰

We are interested in developing simple, inexpensive valves for microfluidic devices that are fabricated rapidly from readily available components, are compatible with rapid prototyping are portable, and have a “power-off” mode. This work describes torque-actuated valves (which we call “TWIST valves”) that can be used to control the flow of fluids in microfluidic channels with a variety of different aspect ratios. TWIST valves are based on small machine screws ($\geq 500\ \mu\text{m}$) that are fixed above microfluidic channels fabricated in PDMS. We cured urethane around screws to form a stiff layer of polyurethane that retained an impression of the threads of the screw. Using polyurethane as a fastener allowed us to avoid tapping holes for the screws. The layer of polyurethane bonded to the surrounding layer of PDMS and was capable of withstanding the restoring force of a screw pressing into the underlying PDMS layer without failure in all of our experiments. Using this strategy, we were able to fabricate valves using screws at angles between 45 and 90° and with any placement.

Operating valves was straightforward: we closed valves by turning screws clockwise (as viewed from above) with a screwdriver and opened them by turning screws counterclockwise. A virtue of TWIST valves is that they have a “power-off” mode and will remain closed indefinitely without any additional input.

TWIST valves are integrated easily into microfluidic devices fabricated in PDMS; the thickness of the PDMS layer between the valve and the ceiling of the channel can vary between 300 μm and 3 mm. Aside from the screw, TWIST valves are optically transparent; this characteristic makes it easy to analyze reactions inside of microfluidic channels.

These valves are simple to fabricate and operate, are compatible with rapid prototyping, are portable, and have a power-off mode; they allow for completely stand-alone microfluidic devices. Importantly, TWIST valves can control, or block, the flow of liquids in channels with semicircular, square, or rectangular cross sections. The tallest channels that have been previously reported with semicircular cross sections are $\sim 25\text{--}50\ \mu\text{m}$;^{14,51} TWIST valves allow channels that are $> 50\ \mu\text{m}$ to be closed completely. We have demonstrated that channels 100 μm tall ($\geq 100\ \mu\text{m}$ wide) can be closed completely with TWIST valves. TWIST valves withstand back pressures of > 500 kPa and can be used to valve channels > 150 times without failing.

Based on these characteristics, we believe that TWIST valves will be an indispensable component in microfluidic devices that require simple, manual operation, are portable, and include preloaded reagents.

EXPERIMENTAL SECTION

Materials. Attophos (Promega), Amplex red (Molecular Probes, A22188), urethane (NOA 81, Norland Optical), Shipley photoresist (Shipley 5740), SU8 photoresist (SU8-100, Microchem), PDMS (Sylgard 184, Dow Corning), (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (United Chemical Technologies), goat anti-human polyvalent immunoglobulins (Sigma, I1761) horseradish peroxidase conjugated to goat anti-human IgG (Sigma, A8775), alkaline phosphatase conjugated to goat anti-human IgA (Sigma, A9669), human serum (Golden West Biologicals), Trypan blue (Aldrich), Cibacron blue 3GA (Aldrich), Meldola's blue (Aldrich), metanil yellow (Aldrich), crystal violet (Aldrich), and methyl red (Aldrich) were used as received. Machine screws were purchased from Small Parts Inc. (Miami Lakes, FL) and cork borers from McMaster-Carr Inc.

Fabrication of Channels. Microfluidic channels were fabricated in PDMS using soft lithography.¹ As masters, we patterned photoresist in bas-relief (SU8-100 or Shipley 5740) on silicon wafers using conventional photolithography. Channels were typically 100 μm in diameter and the height varied between 15 and 100 μm . Channels with semicircular cross sections (15 μm tall and 100 μm wide) were fabricated out of photoresist (Shipley 5740) on silicon wafers, and the resist was reflowed using a previously reported procedure.⁴ Silicon wafers with photoresist patterned on their surface were silanized with a vapor of (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane for 3 h. PDMS prepolymer (300 μm –4 mm thick) was cast on silicon masters and cured thermally (65 °C). The PDMS layer containing microfluidic channels was

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(52) We have also used valves based on machine screws that are 500–800 μm in diameter.

peeled away from the silicon wafer, and inlet and outlet holes were drilled. The layer of PDMS containing microfluidic channels was plasma oxidized and sealed irreversibly to a glass slide.

Fabrication of Valves. Holes 4 mm in diameter, and aligned over channels, were drilled into the PDMS with a cork borer, to a depth of $\sim 2\text{--}3$ mm. The plugs of PDMS were removed with the cork borer; the holes served as a reservoir for the polyurethane. A second, smaller hole was drilled with a 16.5-gauge needle directly above the channel, and within the reservoir for the polyurethane, to hold the screw in place while the polyurethane cured; the depth was $\sim 1\text{--}2$ mm. Twisting the needle as it was pulled out of the PDMS typically dislodged the plug of PDMS; the plug could also be removed with a pair of tweezers. The diameter of the hole was approximately the same as that of the screws we used for valves, typically $\geq 1000\text{ }\mu\text{m}$. The microfluidic device was treated with an oxygen plasma for 60 s to render the PDMS hydrophilic. We pushed screws into the smaller of the two holes until they made contact with the underlying layer of PDMS and quickly filled the larger holes with urethane (NOA 81); the latter prevented the low-viscosity urethane (300 cP at 25°C) from flowing onto the device before it was cured. We cured the polyurethane with a UV lamp for 6–8 min (wavelength, 365 nm) and incubated the device at 60°C for 6 h to improve the adhesion between the polyurethane and PDMS. The urethane we used, NOA 81, is designed for adhesion to glass and contains an adhesion promoter that requires thermal curing after exposure to UV light.

Flow Rate versus Angle of Rotation Experiments. We fabricated a series of parallel microfluidic channels with square cross sections ($100\text{ }\mu\text{m}$ wide, $100\text{ }\mu\text{m}$ tall) in PDMS that were bonded to a glass slide. A TWIST valve (1-mm diameter) was fabricated above each channel; the bottom of the valve and the top of the channel were separated by a layer of PDMS between $750\text{ }\mu\text{m}$ and 1.7 mm thick. A vacuum (35 Torr) was applied to the outlet, and the inlet was immersed in food coloring. The vacuum was adjusted so that the flow rate was initially $\sim 60\text{--}80\text{ }\mu\text{L min}^{-1}$. We measured the volume of liquid that flowed from the outlet over 30 s. The valve was then rotated and the volume measured again (30 s). After four or five complete rotations (360°) of the valve, the flow of liquid in the underlying channel had stopped.

To study how the characteristics of these valves changed from their repeated use, we measured the flow rate as a function of the angle of valves that were used to open/close a channel >20 times. The distance between the bottom of the screws and the top of the microfluidic channels was 1.75 mm.

Laminar Flow Experiments. We fabricated PDMS devices consisting of six parallel microfluidic channels ($100\text{ }\mu\text{m}$ wide, and $15\text{--}100\text{ }\mu\text{m}$ tall) that converged to a single channel ($450\text{ }\mu\text{m}$ wide \times $15\text{--}100\text{ }\mu\text{m}$ tall). Channels that were $15\text{ }\mu\text{m}$ tall had a parabolic cross section; channels $100\text{ }\mu\text{m}$ tall were square. Each parallel channel contained a TWIST valve: for $15\text{ }\mu\text{m}$ tall channels, we used $500\text{-}\mu\text{m}$ -diameter screws (4.7 mm long, $150\text{ }\mu\text{m}$ pitch); for $100\text{ }\mu\text{m}$ tall channels, 1-mm-diameter screws (5 mm long, $0.3\text{ }\mu\text{m}$ pitch). We delivered solutions of different dyes to the six inlets using a syringe pump (flow rate $1\text{--}2\text{ mL/h}$ for $15\text{-}\mu\text{m}$ -tall channels, $3\text{--}10\text{ mL/h}$ for $100\text{-}\mu\text{m}$ -tall channels), or by connecting the outlet to vacuum (35 Torr).

Fabrication of Immunoassay Devices. We designed devices consisting of four pairs of reagent reservoirs (volume, $0.65\text{ }\mu\text{L}$) and two buffer reservoirs (volume, 0.65 and $0.44\text{ }\mu\text{L}$) that connected to a central channel ($500\text{-}\mu\text{m}$ diameter), and inlet and outlets ports. The reservoirs were connected to the central channel by channels that were $200\text{ }\mu\text{m}$ in diameter. The microfluidic channels and reservoirs ($15\text{ }\mu\text{m}$ tall) were fabricated in PDMS from masters containing photoresist (Shipley 5740) patterned—in bas-relief—on silicon wafers using photolithography. We reflowed the resist for 30 min at 200°C to obtain channels with parabolic cross sections. Wafers were silanized with a vapor of (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane for 3 h and then coated with a thin layer of PDMS prepolymer. The polymer was cured for 3 h at 65°C , cut out with a scalpel blade, and peeled away from the silicon master, and inlets and outlets were drilled.

A glass slide ($75 \times 50\text{ mm}$) and the layer of PDMS-containing channels were plasma oxidized and sealed together. Valves were fabricated as outlined above. Machine screws (1-mm diameter, 5 mm tall, 0.3-mm pitch) were used as valves; polyethylene tubing (PE 60, i.d. 0.76 mm , o.d. 1.22 mm , Becton Dickinson) was inserted into the holes for inlets/outlets.

Immunoassays. Two reservoirs were filled with horseradish peroxidase conjugated to goat anti-human IgG (HRP-aIgG) and alkaline phosphatase conjugated to goat anti-human IgA (AP-aIgA) in blocking buffer (0.05% Tween 20 in phosphate-buffered saline, pH 7.6) using a syringe, and the channels were closed using valves. We found that reagents could be delivered directly to the central channel by applying a vacuum at outlet A or B (see Figure 5), by opening the appropriate valves to reservoirs with a small screwdriver, or by connecting a syringe filled with air to the inlet leading to the reservoir containing the solution we wanted to deliver. TWIST valves kept the reagents isolated in the reservoirs and prevented them from contaminating the central channel out of sequence. A solution of goat anti-human polyvalent immunoglobulins (Sigma; $200\text{ }\mu\text{L}$) was introduced in the assay junction. Devices were incubated at 4°C for 10 h to allow the polyvalent immunoglobulins to adsorb onto glass and then purged by applying a vacuum to outlet A. We blocked devices by passing half of the volume of the reservoir containing blocking buffer through the central channel. The devices were blocked for 14 h at 4°C and then purged by applying a vacuum to outlet A.

To assay human serum for IgA, serum (Golden West Biologicals) ($200\text{ }\mu\text{L}$) was added to the inlet and passed through the assay junction. We washed the remaining serum out of the junction with a small amount of blocking buffer, opened the valve to the solution of AP-aIgA, and passed the contents of the reservoir through the assay junction by applying a vacuum at outlet B. This step caused the majority of the AP-aIgA to flow out the “side” of the junction and resulted in the uneven pattern of fluorescence shown in Figure 6A. Channels were washed with the remaining blocking buffer and filled with a solution of Attophos. After 15 min, we imaged the junction using epifluorescence microscopy. To check for nonspecific binding of the secondary antibody to the walls of the microchannel, we repeated the procedure above, but instead of flowing human serum through the assay junction, we used blocking buffer. Attophos was introduced, and the junction was imaged using epifluorescence microscopy.

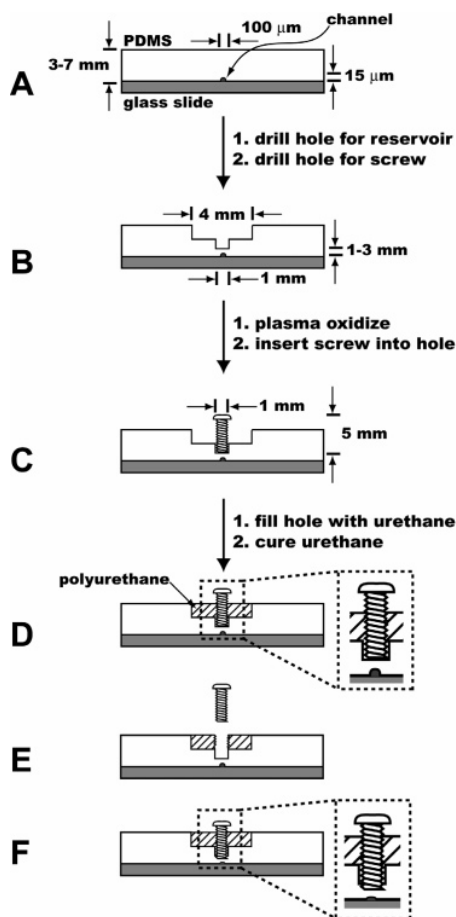


Figure 1. Schematic diagram depicting the fabrication of TWIST valves. (A) A microfluidic device consisting of a layer of PDMS containing a microfluidic channel bonded to a glass slide. The channel is 15 μm tall, 100 μm wide, and has a semicircular cross section. (B) A hole (4 mm wide, \sim 2 mm deep) centered over the microfluidic channel, was drilled with a cork borer as a reservoir for the urethane. A second, hole (1 mm wide, 1–2 mm deep) centered over the channel was drilled with a needle (16.5 gauge). (C) The device was treated with an oxygen plasma, and a screw (1-mm diameter, 5-mm length, 0.3-mm pitch) was pressed into the smaller of the two holes. (D) The reservoir hole was filled with urethane (NOA 81), cured with UV light, and incubated at 60 $^{\circ}\text{C}$ to completely cure the polyurethane (hatched lines). The cartoon inset shows the valve and channel at higher magnification. (E) The screw has been removed from the polyurethane, showing the threads of the screw cast in the polyurethane. (F) A cartoon showing how a valve compresses the underlying microfluidic channel. The cartoon inset shows the valve and channel at higher magnification.

Measurements of fluorescence in immunoassay experiments were made by carrying out a line scan analysis. We measured the intensity of fluorescence starting just outside one edge of the assay junction (where the fluorescence signal is zero), across the junction (where the fluorescence signal is zero), and ending just outside the other edge of the junction (where the fluorescence signal is also zero); the edges of the channel served as background. We calculated the average and standard deviation of fluorescence for the signal and background regions and subtracted the background from the signal region to obtain a corrected value of fluorescence. This analysis was repeated for experiments with IgA and IgG both with and without serum.

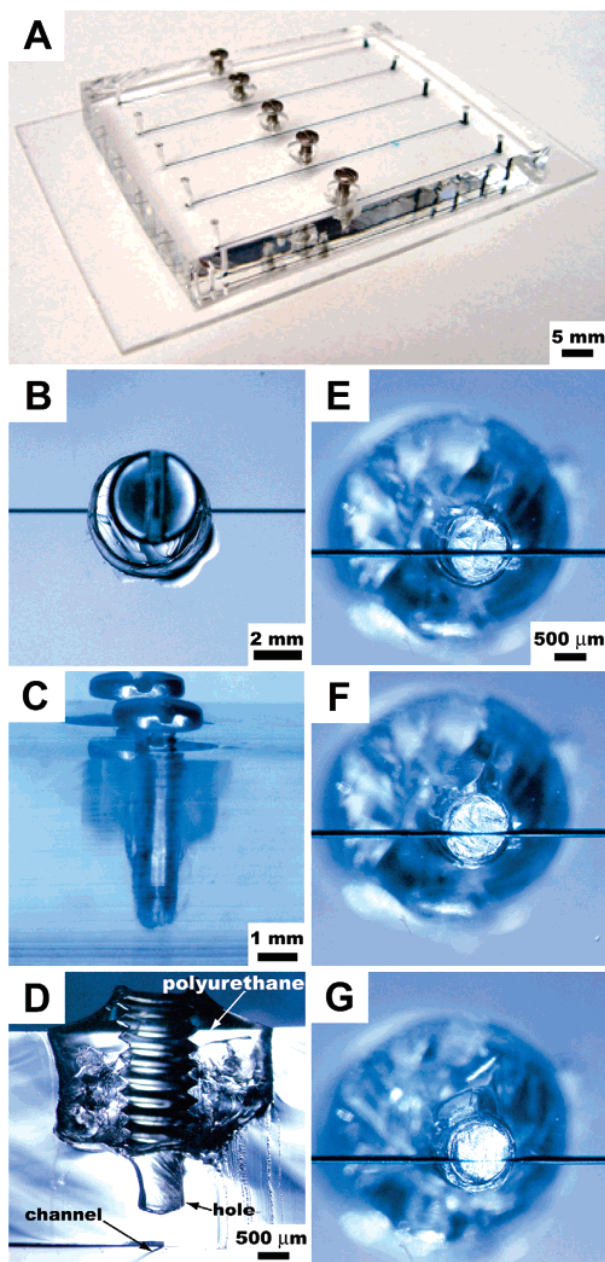


Figure 2. Images of TWIST valves. (A) A microfluidic device in PDMS containing five parallel channels (100 μm wide, 100 μm tall) with TWIST valves (1-mm diameter, 5-mm length, 0.3-mm pitch); the PDMS channels are bonded to a glass slide and have been filled with food coloring to make them easier to visualize. (B) Top view of a TWIST valve on the device. (C) Side view of a TWIST valve. (D) A cross section of a TWIST valve with the screw removed. (E) Bottom view of a channel (100 μm wide, 100 μm tall) filled with food coloring positioned above a TWIST valve (1-mm diameter); the valve is open. (F) Bottom view of a channel that has been partially closed by rotating the valve. (G) Bottom view of a channel that has been completely closed by rotating the valve (\sim 1440 $^{\circ}$).

To assay human serum for IgG, serum (200 μL) was added to the inlet and passed through the assay junction. We washed the remaining serum out of the junction with a small amount of blocking buffer, opened the valve to the solution of HRP-aIgG, and passed the contents of the reservoir through the assay

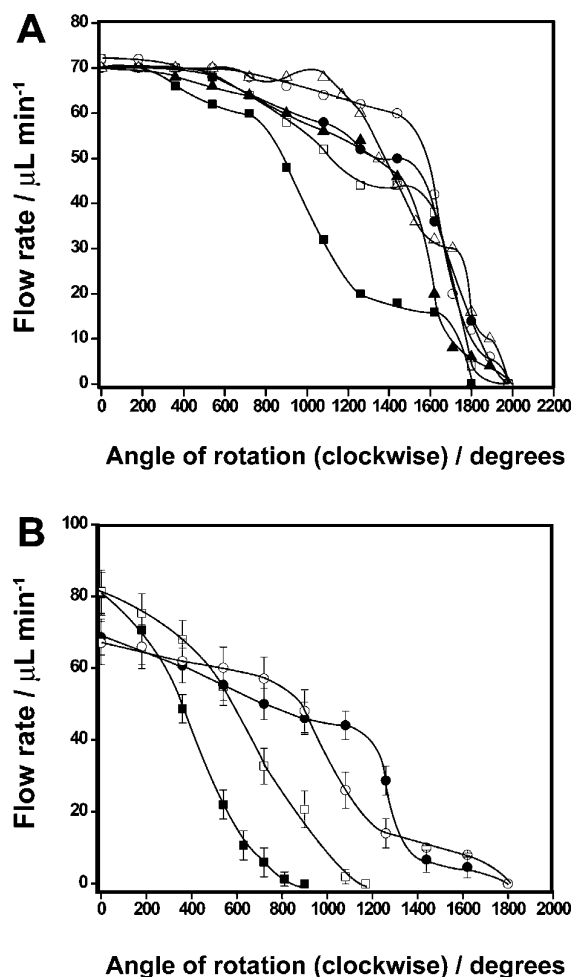


Figure 3. Flow rate as a function of the rotation of TWIST valves. A vacuum (35 Torr) was applied to the outlet of a microfluidic channel (100 μm tall, 100 μm diameter) with a TWIST valve (1-mm diameter, 5-mm length, 0.3-mm pitch). The tube connected to the inlet of the channel was immersed in a solution of dye. The flow rate was measured as the valve was rotated clockwise through $\sim 2000^\circ$ (as viewed from above). (A) A plot of the flow rate ($\mu\text{L min}^{-1}$) versus the angle of rotation for a valve repeatedly used to close a channel: $n = 1$ (\blacksquare); $n = 2$ (\square); $n = 5$ (\bullet); $n = 10$ (\circ); $n = 15$ (\blacktriangle); $n = 20$ (\triangle), where n refers to the number of times the valve has been used. (B) A plot of the flow rate ($\mu\text{L min}^{-1}$) versus the angle of rotation for valves in which the screws were separated from the channel by a layer of PDMS of the following initial thickness: 750 μm (\blacksquare); 1 mm (\square); 1.25 mm (\bullet); 1.75 mm (\circ).

junction by applying a vacuum applied at outlet A. The anti-human IgG was subsequently washed away with the remaining blocking buffer, and the junction was filled with a solution of Amplex red. After 90 min, we imaged the junction using epifluorescence microscopy. To check for nonspecific binding of the secondary antibody to the microfluidic channel, we repeated the conditions above, but instead of passing a solution of human serum through the assay junction, we used blocking buffer. Amplex red was introduced into the junction, and the channel was imaged using epifluorescence microscopy.

RESULTS AND DISCUSSION

Fabrication of TWIST Valves. Figure 1 illustrates the procedure for fabricating TWIST valves. Microfluidic channels

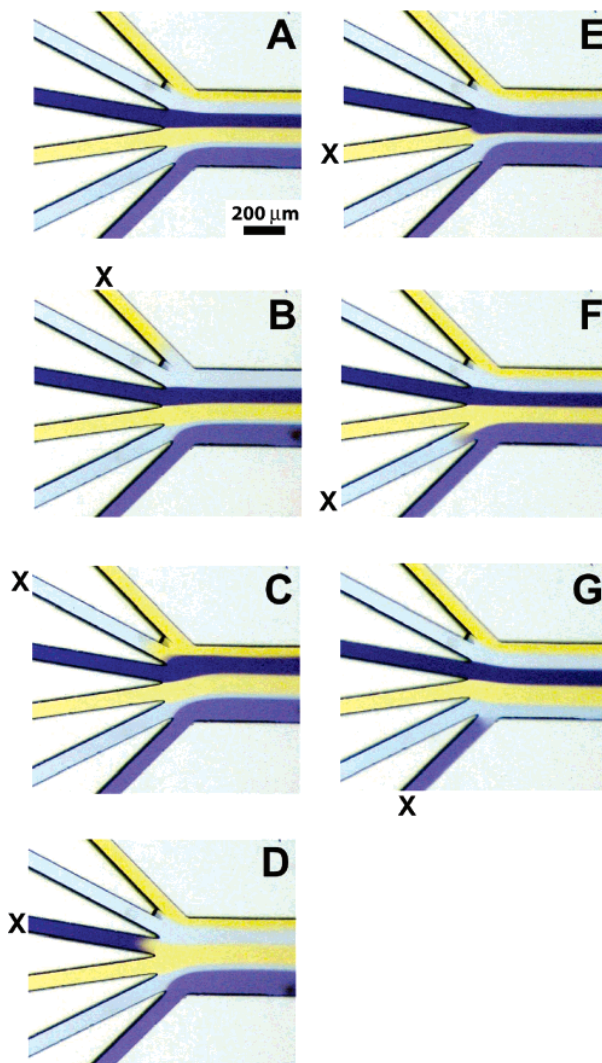


Figure 4. Operation of TWIST valves. A vacuum (35 Torr) was applied to the outlet channel (100 μm tall, 450- μm diameter). Tubing to the inlet channels (100 μm tall, 100- μm diameter) was filled with aqueous solutions of dyes; the channels had a square cross section. The flow of liquid in each inlet channel was controlled with a valve (1-mm diameter). The sequence of images show a single valve closed (denoted by an "X") while the rest of the valves are kept open. (A) All of the valves are open; (B) valve 1 (top valve) closed; (C) valve 2 closed; (D) valve 3 closed; (E) valve 4 closed; (F) valve 5 closed; (G) valve 6 closed.

with square, rectangular, and semicircular cross sections in PDMS were fabricated using soft lithography.¹ Inlet/outlet holes were drilled into the PDMS layer, and the channels were bonded to glass irreversibly. For a single valve, we used a cork borer to drill a hole (~ 4 mm in diameter) in the PDMS layer directly above a channel, as a reservoir for the urethane; the depth varied, but was typically ~ 2 mm (Figure 1B). A second, smaller concentric hole was drilled into the PDMS, 1–2 mm below the reservoir, for the purpose of holding the screw in place while the urethane cured. The diameter of the hole was the same as that of the screws we used as valves, typically ≥ 1000 μm .⁵² The transparency of PDMS allowed us to adjust the depth of the holes by monitoring the height of the drill through the PDMS layer (side view). Being

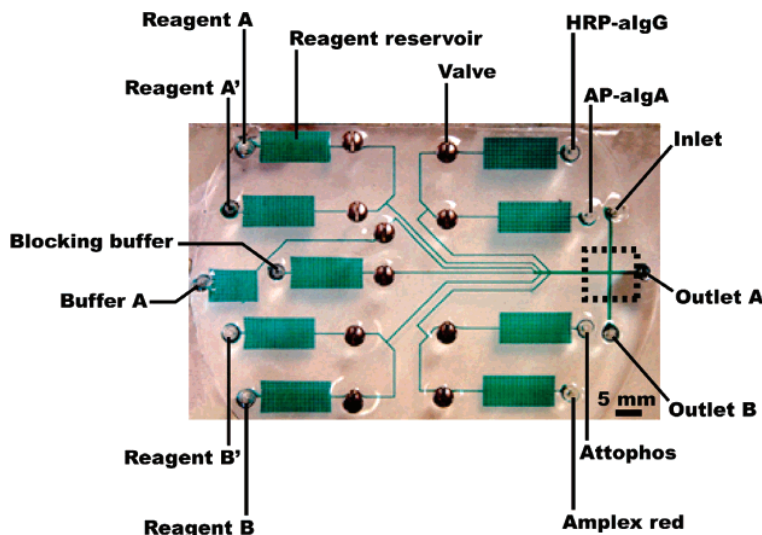


Figure 5. Device for sandwich immunoassays. An image of the device used for sandwich immunoassays. The channels were filled with food coloring to make them easier to visualize. Reservoirs A, A', B, and B' and buffer reservoir A were not used in these experiments. The dashed square shows the region of the microfluidic device—the assay junction—where the immunoassay was imaged.

able to monitor the position of the drill through the PDMS slab also made it possible to align the screws over the channel (particularly useful for screws set at angles). Microfluidic devices were treated with an oxygen plasma to render the PDMS hydrophilic and improve the adhesion between PDMS and the polyurethane (Figure 1C). We pushed a screw into the smaller of the two holes until it made contact with the underlying layer of PDMS and filled the larger hole with urethane; the reservoir prevented the low-viscosity urethane from flowing onto the device before it was polymerized (Figure 1D). We cured the urethane with a UV lamp and incubated the device at 60 °C for 6 h. Figure 2A shows five TWIST valves (1-mm diameter, 5-mm length, 0.3-mm pitch) fabricated above parallel microfluidic channels (100- μm diameter, 15 μm tall) in PDMS.

The optical adhesive we used hardened after curing (Shore D hardness 90) and left an impression of the threads of the screw imbedded in the adhesive. Screws could be backed out of the polyurethane fastener completely, leaving the threads intact (Figure 1E). The screws could be reinserted without damaging the pattern of the threads. We removed a screw from a TWIST valve and sectioned the PDMS. An image of the cross section is shown in Figure 2D.

Operating TWIST Valves. Channels with semicircular, square, or rectangular cross sections were closed completely with screws with a diameter of $\geq 500 \mu\text{m}$.⁵³ TWIST valves also have settings between on/off. Figure 3A illustrates how TWIST valves (1-mm diameter), separated from an underlying microfluidic channel (100 μm wide, 100 μm tall) can be used to control the rate of flow of fluid—in this case a solution of dye—as the valves are rotated. The screws were initially separated from the ceiling of the channel by a layer of PDMS that was varied between 750 μm and 1.75 mm thick. As the distance between a screw (in its initial position)

and the channel increased, the valve had to be rotated through greater angles to close the channel completely. Figure 3B illustrates how the profile of the flow of fluid as a function of the angle of a valve changes as the valve is used to open/close channels repetitively. A noticeable difference in the rate of flow of fluids versus angle of the valve occurs between the first and second time the valve is used to open/close channels. Repeated use of the valve does not change its characteristics significantly. We have found that valves can be used to open/close channels >150 times without failing.

To demonstrate the use of TWIST valves, we fabricated PDMS devices consisting of six parallel microfluidic channels (100 μm wide, and 15–100 μm tall) that converged to a single channel (450 μm wide \times 15–100 μm tall) (Figure 4). Channels that were 15 μm tall had a parabolic cross section; channels 100 μm tall were square. Each parallel channel contained a TWIST valve: for 15 μm tall channels, we used 500- μm -diameter screws (4.7 mm long, 150- μm pitch); for 100 μm tall channels, we used 1-mm-diameter screws (1-mm diameter, 5-mm length, 0.3-mm pitch).

We delivered solutions of different dyes to the six inlets using a syringe pump (flow rate 1–2 mL/h for 15 μm tall channels, 3–10 mL/h for 100 μm tall channels), or by connecting the outlet to vacuum (35 Torr). The six channels converged in the outlet channel; flow in the outlet channel was laminar (Figure 4). Each TWIST valve was sequentially operated (for example, the valve on one channel was closed while the others remained open), and images were collected.

We used screws that were ≥ 5 times wider than the diameter of channels and found that screws do not have to be aligned precisely above channels to produce functional valves—a degree of tolerance was permitted, and made it possible to fabricate devices in high yield (>98%).⁵⁴ Screws (1-mm diameter) that were offset 1 mm from the edge of channels with semicircular cross sections closed the channels completely.⁵⁵ We filled reservoirs

(53) Channels with semicircular cross sections that we used in these studies were 15 μm tall and 100 μm wide. Quake et al. have explored the relationship between the shape of the cross section of a channel and the amount of force required to close the channel. Channels with semicircular cross sections require ~25% less force to close than do rectangular channels.

(54) The yield was determined by fabricating 100 valves. Of the two that failed, both were due to drilling the holes for the screws too deep.

with aqueous solutions of dyes and blocked the channels leading to the reservoirs with TWIST valves. The solutions remained isolated in the reservoirs for weeks and did not leak past the valves.

Immunoassays in Microfluidic Devices with TWIST Valves.

A number of characteristics of TWIST valves indicate that they should be useful for controlling the flow of fluids in disposable microfluidic devices: they are portable, do not require external pressure or electrical power, and have a power-off mode. To explore this concept further, we fabricated microfluidic devices with valves and used these to carry out sandwich immunoassays (Figure 5). We designed devices that would be portable, disposable, and ready to use, with the capacity to assay a single sample for as many as four different immunoglobulins. This design allows the end user to add a sample and select which immunoassay will be performed without any additional pipetting or reagent-handling steps. We preloaded the reagent reservoirs in devices with reagents for detecting human IgA and IgG, prevented the contents in different reservoirs from mixing by using TWIST valves, and used these devices to detect either human IgA or IgG in human serum. The readout for these immunoassays was the fluorescent products of enzyme-catalyzed reactions that were detected by epifluorescence microscopy.

Sandwich Immunoassay for IgA and IgG. We prepared disposable devices by prefilling reservoirs with reagents and buffer using a syringe. The reservoirs were filled individually and isolated from each other by closing the TWIST valves on the connecting channels. The buffer reservoir was filled with blocking buffer (0.05% Tween 20 in phosphate-buffered saline, pH 7.6). One pair of reservoirs was filled with solutions of HRP-aIgG and AP-aIgA in blocking buffer. A second pair of reservoirs was filled with Amplex Red and Attophos, substrates for HRP and AP, respectively. A solution of goat anti-human polyvalent immunoglobulins (200 μ L) was introduced into the intersection of the inlet/outlet channels with the central channel, the "assay junction". The solution was then removed, and the channels were blocked with blocking buffer.

To assay human serum for the presence of IgA, we pulled serum (200 μ L) through the assay junction with a vacuum. After rinsing channels with blocking buffer, a solution of AP-aIgA was introduced and the assay junction rinsed again with remaining blocking buffer. The assay junction was filled with a solution of Attophos and, after 15 min, imaged using epifluorescence microscopy. The assay produced a strong fluorescence signal (Figure 6A). As a negative control, we repeated the procedure above, but instead of using human serum, we added blocking buffer. We detected a weak fluorescence signal arising from nonspecific binding (Figure 6B). Figure 6F shows a plot of the fluorescent intensity for the immunoassay of IgA and for the negative control.

To assay for the presence of IgG in the same device, we followed a procedure similar to that for IgA, but instead used HRP-aIgG and Amplex red. After incubating Amplex red in the assay junction for 30 min, we imaged the junction using epifluorescence

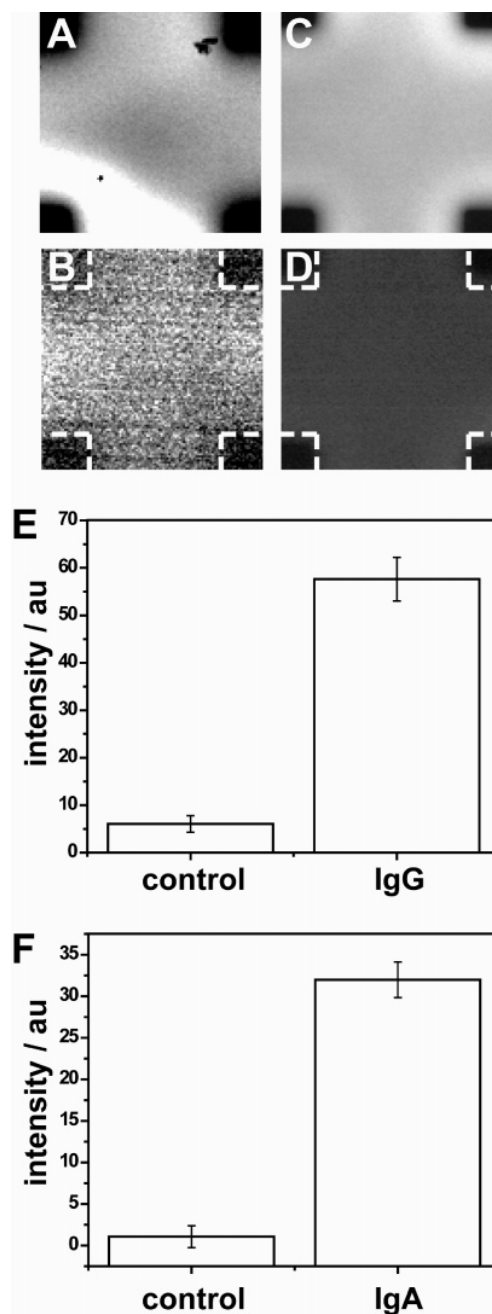


Figure 6. Immunoassays in microfluidic devices with TWIST valves. Using the microfluidic device depicted in Figure 5, we carried out sandwich immunoassays to detect the presence of IgA and IgG in human serum; see the Experimental Section for a description of the procedure. (A) Epifluorescence produced from the detection of IgA in human serum. (B) Epifluorescence in the background assay for IgA in the absence of human serum. (C) Epifluorescence produced from the detection of IgG in human serum. (D) Epifluorescence in the background assay for IgG in the absence of human serum. The fluorescence in each channel was quantified by line scan analysis (see Experimental Section). (E) A plot of the relative intensity ($\lambda = 590$ nm) of the fluorescence produced by the reaction of Amplex red and HRP in the background assay and in the immunoassay for IgG. (F) A plot of the relative intensity ($\lambda = 565$ nm) of the fluorescence produced by the reaction of Attophos and AP in the background assay and in the immunoassay for IgA.

(55) This characteristic of TWIST valves limits the density of valves that can be fabricated in a single device. Valves must be spaced from adjacent channels by at least 1 mm to avoid the valves from altering the flow of fluids in adjacent channels; this characteristic, however, can be useful when it is desirable to have a single valve controlling the control the flow of fluid in two parallel channels simultaneously.

microscopy. The assay produced a strong fluorescence signal (Figure 6C). As a negative control, we repeated the procedure above, but instead of human IgG, we filled the assay junction with blocking buffer. We detected a weak fluorescence signal arising from nonspecific binding (Figure 6D). Figure 6E shows a plot of the fluorescent intensity for the immunoassay of IgG and for the negative control.

Immunoassay Results. We were able to detect IgA and IgG in human serum with a portable, disposable microfluidic device containing TWIST valves.⁵⁶ This application demonstrates that TWIST valves have the useful virtue that they can remain closed indefinitely without any external equipment. We should, in principle, be able to detect other antigens, in addition to IgA and IgG, in human serum. It should also be possible to redesign the microfluidic elements in order to detect different antigens in *multiple samples* in the same device.

We have demonstrated microfluidic devices containing integrated TWIST valves that are simple to fabricate, disposable, portable, and can be configured to run as many as four different immunoassays. It should, in principle, be possible to design microfluidic devices that are capable of larger numbers of assays.

CONCLUSION

In this work, we demonstrate simple, mechanically actuated valves for microfluidic channels. This technique should be of interest to chemists, biologists, biochemists, and scientists working in the general areas of biotechnology and diagnostics. Valves were fabricated in a transparent layer of polyurethane that bonded to the surrounding layer of PDMS; the threads were turned around

the screw by molding, rather than by drilling/tapping. We actuated TWIST valves by turning screws that collapsed underlying microfluidic channels.

TWIST valves have several disadvantages: (i) they are serial; (ii) in the configuration illustrated here, they cannot be operated electronically; (iii) because they are manipulated manually, they are relatively slow—it takes ≥ 1 s to rotate a screw enough to close a channel completely; (iv) they can be configured in low density only (16 valves cm^{-2}); (v) the practical limit for the diameter of screws is 500 μm —smaller screws are difficult to operate.

The primary advantages of TWIST valves are that they are easy (and quick) to fabricate and operate. TWIST valves also have the following useful characteristics: they (i) have a power-off mode; (ii) are portable; (iii) do not depend on external instrumentation; and (iv) allow the user to close valves indefinitely without additional user intervention. These virtues suggest that TWIST valves may represent a better solution than pneumatic valves in particular applications. One such application may be as components in diagnostic devices for resource-poor settings.⁵⁷

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(56) We will report dose–response curves in a separate paper.

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