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Articles

Fabrication of a Configurable, Single-Use Microfluidic Device

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This paper describes microfluidic devices that contain connections that can be opened by the user after fabrication. The devices are fabricated in poly(dimethylsiloxane) (PDMS) and comprise disconnected fluidic channels that are separated by 20 μm of PDMS. Applying voltages above the breakdown voltage of PDMS (21 V/μm) opened pathways between disconnected channels. Fluids could then be pumped through the openings. The voltage used and the ionic strength of the buffer in the channels determined the size of the opening. Opening connections in a specific order provides the means to control complex reactions on the device. A device for ELISA was fabricated to demonstrate the ability to store and deliver fluids on demand.

We report the fabrication of a single-use microfluidic device in poly(dimethylsiloxane) (PDMS) in which connections between channels can be opened, at preset locations, after fabrication. Much of the initial work in microfluidics centered on the use of glass or silicon in fabrication,[1–5] but recently, there has been increasing use of PDMS[6–17] and other polymers.[18–27] Polymers are attractive materials for single-use disposable devices since they are inexpensive, can easily be cast or molded, and pose little hazard on disposal. PDMS has the additional characteristics that it is elastomeric, optically transparent, able to seal to smooth surfaces, and amenable to fabrication via rapid prototyping.[28]

Our devices consist of a network of initially disconnected channels molded in PDMS (Figure 1). Connections between these channels are made at locations where two channels are separated by 20 μm: a channel with cross-sectional area of 100 × 60 μm^2 is separated by a 100 × 60 × 20 μm^3 block of PDMS from the nearest channel. The user connects the channels by applying a voltage pulse sufficiently above the dielectric strength of PDMS[29] (420 V for 20 μm; 21 V/μm) to cause electrical breakdown. Electrical breakdown causes thermal and mechanical failure[30–33] and generates a hole (~2–50 μm in diameter) in the PDMS between the two channels through which fluids can move. We can control the size of the hole to some degree by changing the voltage used or the ionic strength of the buffer in the channels.

The discontinuous channels in these systems can *store* different fluids—reagents, analytes, or buffers—and the ability to open the connections as needed provides the means to control the flow of

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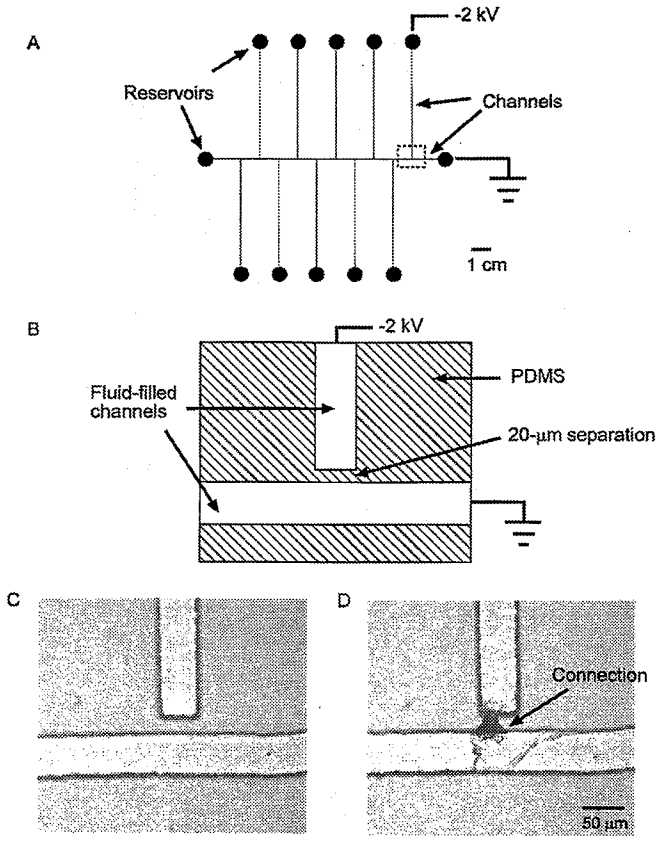

Figure 1. Scheme for opening a connection between channels. (A) Diagram of a device comprising a central channel and 10 side channels separated by 20 μm from the central channel. It was possible to connect each side channel to the central channel. (B) Applying a high voltage in one of the side channels and grounding the central channel opened a connection between the two channels through electrical breakdown of the PDMS. (C) Optical micrograph of a closed connection. (D) Optical micrograph of a connection opened by applying a 2-kV pulse for 1 s. The connection allowed the flow of fluids but was not as wide as the channels it connected.

these solutions from one channel to another and the access of the system to reagents or other supplies. The PDMS block thus acts as a closed valve that opens, but only once,[34–36] on application of high voltage. By opening connections in a particular sequence, the user can, in principle, carry out complex analyses or chemical reactions.

To demonstrate a use for a configurable device, we selected an ELISA experiment. In ELISA, antibodies are used to detect the presence of specific molecules such as proteins or drugs. The procedure requires several solutions, and these solutions must be added in a specific order. We developed the configurable device for ELISA[7,8] as a prototype to exploit this ability to control the storage and dispensing of fluids.

RESULTS AND DISCUSSION

A. Fabrication of Configurable Devices. Each device consisted of two layers, one layer with embedded channels and one flat layer. To fabricate the layer with embedded channels, we molded PDMS against a photolithographic master produced by rapid prototyping and comprising a positive relief of photoresist on a silicon wafer.[28] We cut holes in the PDMS using circular punches to form fluid reservoirs. We sealed the layer with channels against a flat piece of PDMS or a glass slide by oxidizing both layers in an air plasma and then bringing them into conformal contact. The plasma oxidation used in the sealing process rendered the channels hydrophilic, and they were readily filled with aqueous buffer. Dead-end channels filled by capillary action in less than 5 min.[37] Our devices consisted of at least two disconnected channels separated by 20 μm (Figure 1). This separation was limited by the resolution of the printing used in the first step of rapid prototyping. A higher resolution printer would allow thinner separations and lower voltages to operate. The channels were 50 or 100 μm wide and 60 μm tall.

B. Characterization of the Connection. To open a connection between two channels, we applied a pulse of voltage (>421 V) to an electrode in one channel with the second channel grounded. If one channel could make more than one connection, that channel was grounded to prevent a pulse of voltage from opening more than one of the connections (see Figure 1). We used negative polarity to open the channels, but positive polarity gave similar results. The applied voltage burned a pathway between the two channels and created a connection. We could pressure pump fluids through these connections.[38] Little debris formed on opening a connection, but the electrical breakdown caused scarring of the surfaces of the channels immediately adjacent to the connection. Depending on the magnitude and length of the pulse of voltage, both channels could fill with a bubble of gas after the connection opened. Low voltages or short pulse lengths minimized bubble formation. We believe that the high local temperature that accompanied electrical breakdown generated the bubble. The bubble dissipated after about 30 s or could be pumped out of the channels by applying pressure. The opening appeared only in the layer with the embedded channels: no breakdown occurred in the flat layer. The connection was not a single hole but a series of fissures in the PDMS caused by stresses encountered during breakdown—gas evolution and expansion, thermal stress, and bond breaking.[31–33]

Typically, we applied a programmed voltage of 1–5 kV for 1 s or less to open connections. Breakdown occurred at different times depending on the voltage used. In phosphate-buffered saline (PBS), breakdown was complete after 50 and 20 ms for 2- and 5-kV pulses. These times are within the ramp time of the power supply so the actual voltages at breakdown were 1.8 kV for 2 kV and 3.4 kV for 5 kV. The programmed voltage thus served only to set the ramp rate for the power supply. The steeper the ramp rate, the faster the connection opened. Figure 2 shows connections opened using three pulse lengths at a programmed voltage of 2 kV. The size of the connection was approximately the same for 50 ms and 1 s. A minimum pulse length was required to open a connection, and pulse lengths longer that the minimum did not affect the size of the connection. A longer voltage pulse did, however, form larger bubbles in the channels than a shorter voltage pulse.

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(37) The air in the dead-end channels dissolves into the buffer or the bulk PDMS.
(38) Pumping by electroosmotic flow is theoretically possible. In these devices, electroosmotic flow could not generate sufficient pressure to force fluid through the connections at an appreciable rate.

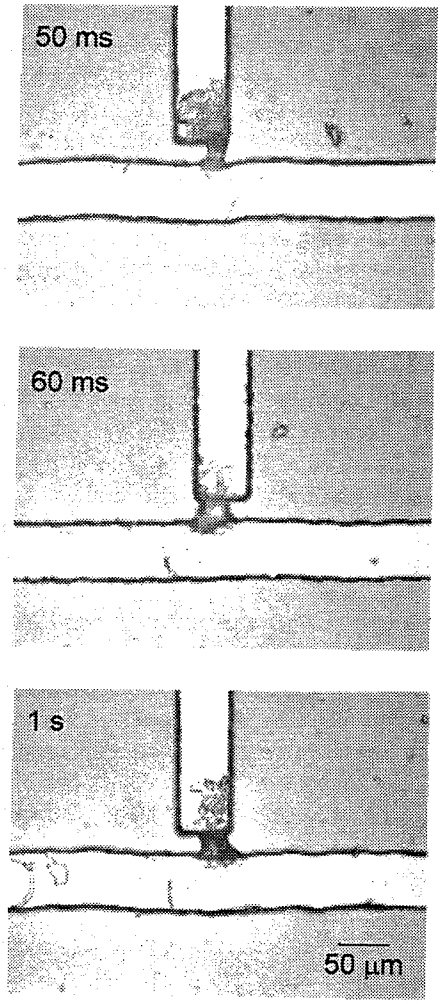

Figure 2. Optical micrographs of connections opened with 50-ms, 60-ms, and 1-s pulses of 2 kV in PBS. When a pulse of 40 ms was applied at 2 kV, the connection did not open. The connections are similar in size although the 50-ms connection is slightly narrower than the 60-ms and 1-s connections.

The pathway created when the connection opened was always smaller in cross-sectional area than the channels, but the absolute size depended heavily on the voltage used and the ionic strength of the buffer. Figure 3 shows fluorescent micrographs of openings as a function of the applied voltage. A higher applied voltage created a larger opening. At voltages of >2 kV, we achieved reproducible mechanical failure in the PDMS although the exact size and shape of the connection were variable. Reproducible opening of connections was also possible using a handheld voltage source—a gas igniter. The igniter produces a high voltage (>10 kV) from a hammer striking a piezoelectric material. Despite the higher voltage produced by the igniter, the connections were smaller (~5 μm in diameter) than those opened at 5 kV (~40 μm in diameter) with a commercial power supply. There are two likely explanations for the smaller hole from the igniter: (1) the very short duration of the pulse (20 μs) was insufficient to open a large hole, and (2) the power ($P = IV$) that the igniter could produce was limited and less than the power produced by the commercial power supply.

Figure 4 shows confocal micrographs of openings as a function of ionic strength. We studied two buffers 25 mM Tris−192 mM Gly ($I \sim 10$ mM) and PBS (10 mM phosphate, 138 mM NaCl, 2.7

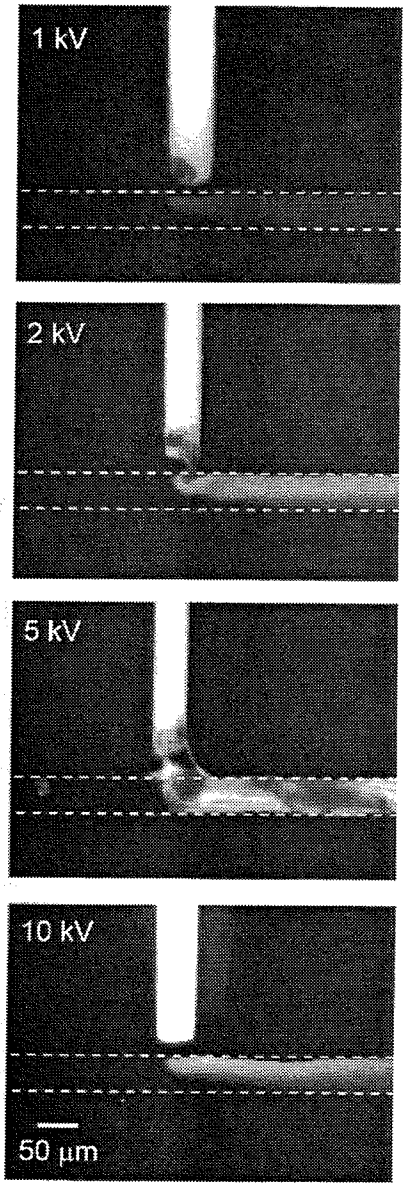

Figure 3. Fluorescent micrographs of connections opened in PBS at different voltages. Connections were opened with a 1-s pulse of 1 kV, a 50-ms pulse of 2 kV, and a 20-ms pulse of 5 kV from a power supply and a ~10-kV pulse from a gas igniter. The widths of the holes opened were ~5 μm for 1 kV, ~20 μm for 2 kV, and ~50 μm for 5 kV and 5 μm for the gas igniter. Once the channels were connected, fluorescein was pumped through the holes by hand using a syringe. The pressure required to pump the fluorescein was greatest for the 1-kV hole and least for the 5-kV hole.

mM KCl, pH 7.4, $I \sim 166$ mM) to determine the effect of ionic strength on the opening of connections. PBS with an ionic strength 17 times greater than Tris−Gly produced much larger holes.

Since the dielectric strength of PDMS is 21 V/μm, breakdown should occur at 420 V for a 20-μm section. We did observe current flow at 500 V, but the opening of connections at this voltage was not reproducible in either Tris−Gly or PBS. Connections opened more frequently as we increased the voltage to 600 V, 750 V, and 1 kV, but again the results were not reproducible. In some cases, it was possible to open a connection by repeatedly applying a given voltage or to open a larger connection by applying a higher voltage across a smaller opening. We believe these variations are most likely due to heterogeneities in the PDMS.

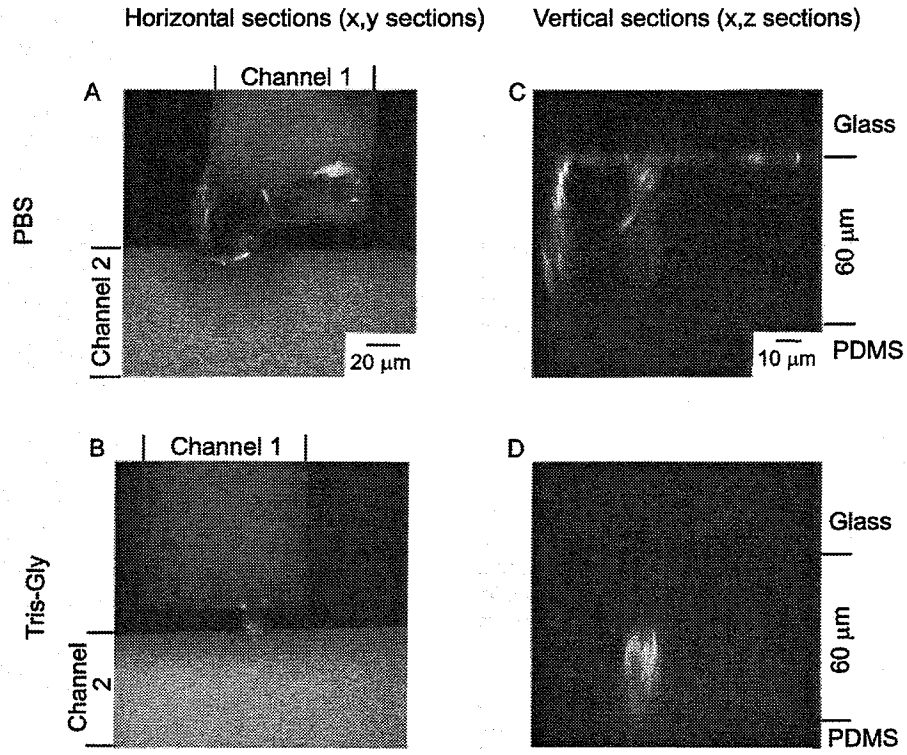

Figure 4. Confocal micrographs of connections opened in PBS and 25 mM Tris−192 mM Gly containing 0.1 μM fluorescein. The connection was made with channels (labeled 1 and 2) embedded in PDMS sealed to a glass cover slip to allow confocal imaging. The voltage used to open the connection was −5 kV in channel 1 with channel 2 grounded. (A) and (B) show horizontal sections (2.6 μm thick) of the connections (refer to Figure 1B). White regions indicate the presence of fluorescence and thus fluid. (A) shows a connection opened in PBS halfway between the PDMS−glass seam (top of the channel) and the bottom of the channel. (B) shows the middle of a connection opened in Tris−Gly (~5 μm from the PDMS bottom of the channel). (C) and (D) show vertical sections (2 μm thick) of connections halfway between channels 1 and 2 (10 μm from either channel). (C) The connection opened in PBS extended from the top to the bottom of the channel and mainly consisted of two large fissures that were each 10 μm wide. The connection also extended the full width of the channel (100 μm) at the glass−PDMS seam. (D) The connection opened in Tris−Gly was approximately 10 μm wide over its entire height (~30 μm).

The reproducible opening of a connection thus required a voltage several times greater than the breakdown voltage of the PDMS. In experiments where the voltage ramped up slowly (e.g., 500 V/50 ms), the opening was always smaller than when the voltage ramped up at the rate set by the power supply (exponential with τ = 16 ms). In our system, Pt electrodes made electrical contact with the buffer that in turn made electrical contact with the PDMS. The rate of the distribution of charge from the electrode to the PDMS is dependent on the resistivity of the buffer: increasing the ionic strength (decreasing the resistivity) of the buffer thus effectively shortens the ramp rate of the power supply. Reducing the ramp rate for a 5-kV pulse or, equivalently, lowering the ionic strength of the buffer, thus mimicked the application of a pulse of voltage lower than 5 kV.

C. Device for ELISA. We developed a device for ELISA as a model system for a configurable microfluidic chip to demonstrate the ability to store and deliver solutions on demand. ELISA provided a system in which solutions of capture antibody, blocking agent, sample, antibody−enzyme conjugate, and enzyme substrate must be added separately and in order. Microfluidic channels are especially suitable for ELISA since the high ratio of surface area to volume reduces mass transport limitations.[7] The capture antibodies used in this ELISA also readily adsorbed to the oxidized PDMS.

The device for ELISA consisted of three disconnected channels (Figure 5). Three reservoirs (labeled 3−5 in Figure 5) having diameters of 4 mm were connected to a T-shaped channel that served as the location for the assay. Reservoir 5 served as a reservoir for waste, and reagents, sample, and rinse solution were added in reservoirs 3 and 4. The remaining two channels were dead-end channels that acted as storage areas for reagents. Each of these channels had one reservoir (reservoirs 1 and 2, 1 mm in diameter) that was connected to a syringe containing a reagent. Coupling from a syringe to the device was accomplished by using polyethylene tubing (o.d. of 1.09 mm) that was pressure fit in the 1-mm reservoirs.

In our prototypical experiment, human hemoglobin (Hb) was the analyte. The Hb-specific capture antibody was added through reservoirs 3 and 4 (see Figure 5). A solution of bovine serum albumin (BSA) was introduced into the T channel to block the surface and to prevent nonspecific adsorption of proteins in subsequent steps. In contrast to Eteshola and Leckband,[8] we found this standard blocking step was effective in eliminating background interference. After the sample was introduced through the T channel, application of a 1-s pulse of 1 kV opened the connection between reservoir 1 and the T channel,[39] and anti-Hb antibody conjugated to alkaline phosphatase was pumped into the T channel using a syringe pump. Finally, the connection between reservoir 2 and the T channel was opened with a 1-s pulse of 1

(39) The polyethylene tubing was removed to provide access for the electrodes and replaced after the connection was opened. In principle, the electrodes could be molded into the PDMS and coexist with the tubing.