Electronic Supplementary Material (ESI) for Lab on a Chip.

A versatile valving toolkit for automating fluidic operations in paper microfluidic devices

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Running Title: Paper microfluidic valving and automation toolkit

Keywords: Point-of-care diagnostics, automation, actuators, system integration, 2DPN, wicking flow

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Supporting Information

I. Comparison of sodium polyacrylate and sponge actuators

Valves were also built using actuators made of a superabsorbent polymer, sodium polyacrylate (SP), which can absorb up to 100 times its weight of water. SP was purchased in powder form from Amazon.com (Superabsorbent diaper polymer; Seller: Eco-Novelty Inc). SP gel (0.05g SP / 1 ml deionized water) was cast in a square 4.5 mm x 4.5 mm cavity, 0.76 mm deep (3 layers of 10 mil thick Mylar), and dried overnight in a desiccator at room temperature. The cavity was built over one end of a 4.5 mm glass fiber GR 8975‡ (Ahlstrom, Helsinki, Finland) channel (Fig. S1A). During actuation, the other end of this channel was inserted into a fluid reservoir containing deionized water. Water flowed through the channel and reached the dried polymer, which absorbed water and expanded (Fig. S1A). The time at which deionized water reached the actuator cavity was designated as 0 seconds. The expansion of the SP actuator over 166 seconds is shown in Fig. S1A. A similar test was conducted with compressed sponge actuators. A 4.5 mm square sponge actuator was laser cut from a sheet of compressed cellulose sponge and placed on top of one end of a GR8975 channel in a cavity (Fig. S1B). The sponge actuator expanded considerably faster than the SP actuator. In 10 seconds, the sponge expanded to a height comparable to the height that the SP actuator expanded in 166 seconds (Fig. S1B). Sponge actuators, thus, provided more rapid actuation compared to SP actuators in this form. The rate of expansion of the SP actuators could be increased by increasing the SP concentration in the gel. However, higher SP concentration gels (cast in the same cavity size) also expanded to a larger final volume and tended to flow out of the cavity, which was a disadvantage. Engineered

‡ Note that this is a different glass fiber material than the one used for all experiments shown in the main body of this manuscript, which uses GR 8964. GR 8975 is more permeable than GR 8964 and increases the rate of fluid delivery to the actuators. However, GR 8964 has better flow reproducibility and produces more accurate timing channels.

actuators that contain a high concentration of SP, but limit expansion to a fixed final volume, could be used as effective actuators.

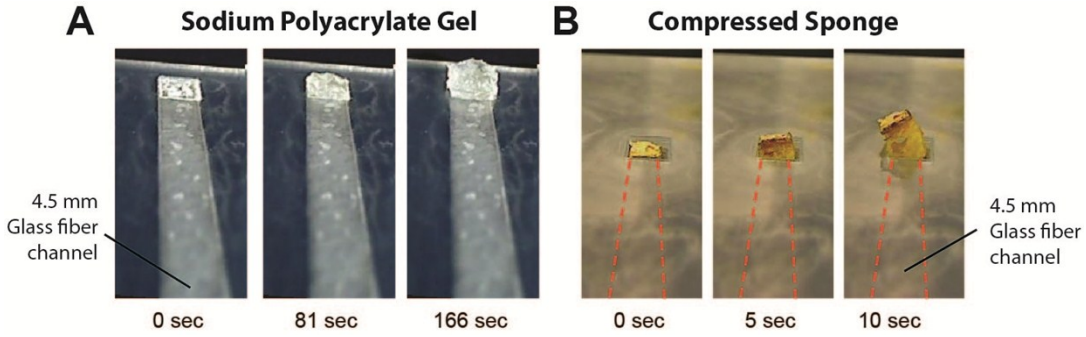

Figure S1. Expansion of sodium polyacrylate and sponge actuators. **A**. Sodium polyacrylate gel was cast on top of one end of a glass fiber actuation channel in a cavity built in Mylar. Deionized water was delivered through the other end of the channel and reached the actuator region at 0 seconds. The SP actuator expanded over 166 seconds. **B**. A similar test was conducted with sponge actuators. A compressed cellulose sponge actuator was laser cut and placed on top of one end of a glass fiber actuation channel, within a cavity in Mylar. Deionized water, delivered through the other end of the channel, caused expansion of the compressed sponge. The sponge expanded within 10 seconds to a height comparable to that of the SP actuator in 166 seconds.

II. On-switch with a SP actuator

We present a time-metered on-switch using a SP actuator (Fig. S2). The design of this valve is similar to the on-switch shown in Fig. 2B. The SP actuator was prepared as described above. A yellow fluid was introduced into channel A at time, $t = 0$. After channel A saturated, deionized water was introduced into the actuation channel at $t = 16.5$ min and reached the actuator at $t = 17$ min: 5 sec (Fig. S2). Fluid first appeared in channel B at $t = 19$ min: 10 sec. At t = 23 min, the yellow fluid front was in channel B (Fig. S2). Expanded SP polymer can be seen in the zoomed in image of the valve region at $t = 23$ min (inset; Fig. S2). It is separated from the flow channels by a rectangular fluidic barrier (dotted green rectangle; $t = 23$ min; inset; Fig. S2). In this valve, the time required for fluid to first appear in channel B after actuation fluid reached the actuator was 2 minutes and 5 seconds. For the similar set of on-switches constructed using sponge

actuators (Fig. 2B), this time was between 3 – 6 seconds (N=6), which is consistent with data shown in Fig. S1.

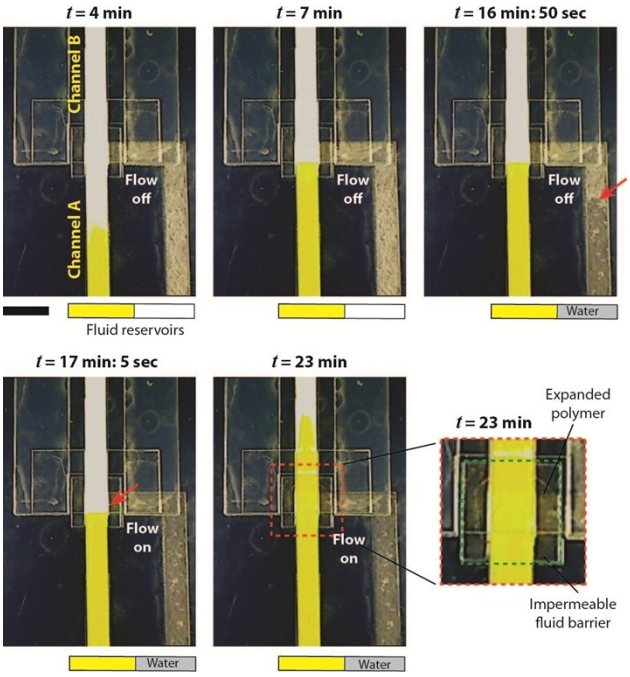

Figure S2. On-switch using a sodium polyacrylate actuator. Time-lapse images of the valve are shown. Yellow fluid was introduced into channel A at time, $t = 0$, and reached the end of the channel at $t = 7$ min. Deionized water was introduced in the actuation channel at $t = 16.5$ min. Red arrows shows the location of fluid front in the actuation channel. Expansion of the actuator connected channels A and B and yellow fluid flowed into channel B. Inset at $t = 23$ min shows the expanded SP under the fluid barrier (dotted green rectangle) after valve actuation. Scale bar is 1 cm.

III. Part-by-part assembly of a time-metered on-switch

Figure S3 shows how an on-switch is constructed from individual parts. Mylar layers were adhesive backed, either from one side (referred to as AC) or both sides (ACA). As received from the manufacturer, adhesive-backed Mylar surfaces were protected by a 1-mil thick laminate layer. The laminate layer had to be removed to expose the adhesive. On some parts of the device, the laminate was removed only from selected locations by using a laser cutter at low power to only cut through the laminate. The total part count for this device is 12 (Fig. S3).

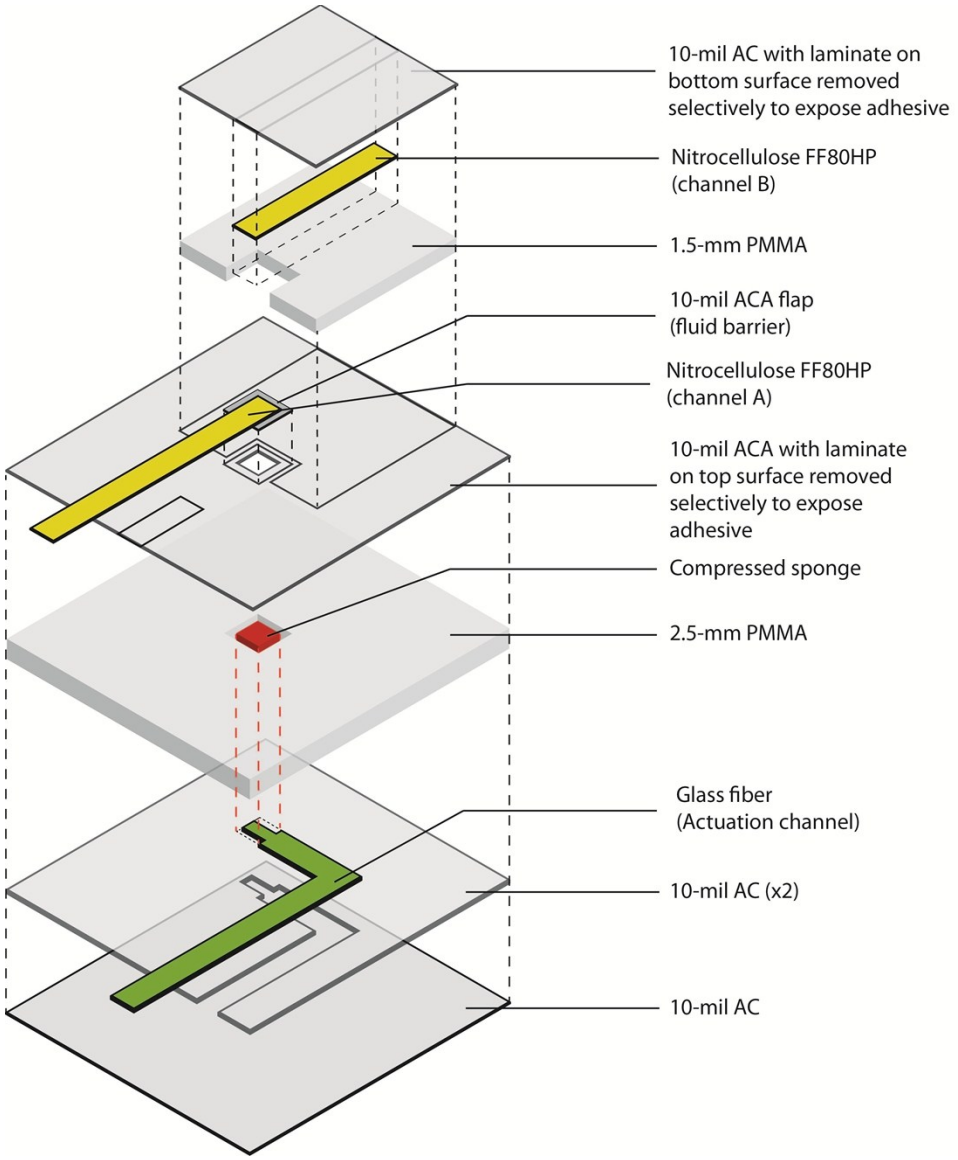

Figure S3. Part-by-part assembly of an on-switch with a compressed sponge actuator. AC and ACA refer to single and double sided adhesive-backed Mylar sheets, respectively.

Some critical requirements for proper operation of these valves are i) proper alignment of the end of the actuation channel, actuator, and the end of flow channels, ii) good contact between the end of the actuation channel and the actuator, and iii) securement of the fluid barrier (attached to the flow channel) on top of the actuator cavity in a way that it can be displaced after expansion of the actuator. Figure S4 shows details of the device design in the vicinity of the actuator that

ensures that these requirements are met. The width of the end of the actuation channel, just under the actuator, is reduced to half. Laminate from around this thin region is removed to expose adhesive (dark grey region; Fig. S4). This region aligns with the base of the square actuator. The adhesive ensures good contact between the end of the actuation channel and the actuator. The actuator resides in a square hole cut in the 2.5 mm thick PMMA slab. Because the PMMA slab is not adhesive-backed, an additional layer of 10-mil ACA is attached above it in order to continue stacking layers. This ACA layer has a square hole that aligns with that on the PMMA slab. On the top surface of this ACA layer, a rectangular rim is marked around the actuator cavity by laser etching (dashed region; Fig. S4). This rim aligns with the impermeable fluid barrier (made of ACA) that attaches directly on top of this. On top of the fluid barrier, laminate is removed selectively and nitrocellulose channel A is attached. The width of the rim where the fluid barrier attaches is small – for all time-metered switches, this rim was 0.75 mm wide. This thin rim creates high enough adhesion to hold the nitrocellulose channel in place before actuation, but low enough adhesion for the expanding actuators to detach the barrier and displace the channel.

IV. Tuning time delays using serpentine timing channels

This section refers to data presented in Fig. 3, which shows the time delays that were achieved using different lengths of actuation channels. Five different shapes of channels, 2.9 mm wide, with lengths increasing from 16.5 cm to 42 cm (A – E; inset; Fig. 3B), were used for comparison. t_{delay} increased proportional to L^2 ($R^2 > 0.999$; Fig. 3B), which is consistent with the Lucas-Washburn equation[1–4] for the flow rate of fluid through straight 1D channels. Channels A – E produced delays of 4.1, 8.3, 12.0, 17.4, and 23.8 minutes respectively (Fig. 3B). Except for design A, the CV's in t_{delay} for all other designs were under 8.5%. Reproducible time delays over

a wide time range can thus be obtained using this method. The delays can easily be increased further by increasing the lengths of actuators. However, because these CV's are material-dependent, reproducibility should be assessed before using alternate materials as timers.

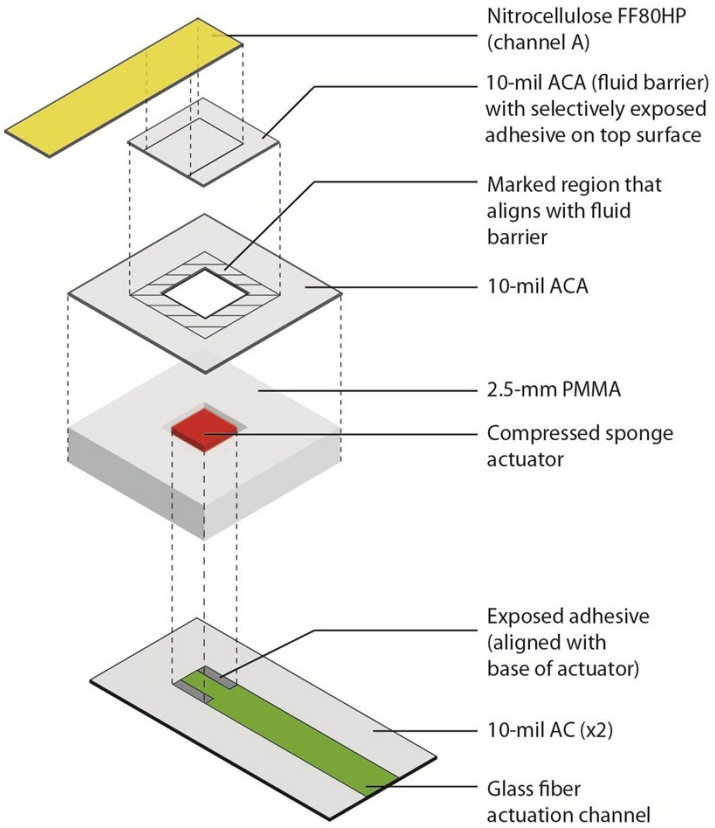

Figure S4. Details of design around the actuator region. The alignment of the actuation channel, actuator, impermeable fluid barrier, and the nitrocellulose flow channel is shown. The tip of the actuation channel is narrowed to expose adhesive to attach the actuator. The fluid barrier is adhered along a thin rim around the actuator cavity. The nitrocellulose flow strip is attached on top of the fluid barrier. AC and ACA refer to single and double sided adhesive-backed Mylar sheets, respectively.

V. The P*f*HRP2 Protein Detection Assay

A standard sandwich immunoassay for P*f*HRP2 as described in Fu et al[5] was performed. For the test spot, 1 µl of a 1 mg/ml solution of a murine antibody to P*f*HRP2 (Immunology Consultants Laboratory, Portland, OR) in PBS was hand spotted in the detection zone of the test strip. For the control spot, 1 µl of a 1 mg/ml solution of an antimouse antibody in PBS was hand spotted 4.5

mm downstream the test spot. A secondary murine antibody to P*f*HRP2 (Immunology Consultants Laboratory, Portland, OR) conjugated to gold nanoparticles (BBInternational, Cardiff, UK) was used as a labeling agent. For dry storage of the label, the conjugate solution at OD 10.2 was mixed with 0.1% BSA in TBS, 5 % sucrose, and 5% trehalose in water in volume ratios of 7:1:1:1. 30 µl of this mixture was pipetted on leg 1 of the device and dried in a 37°C incubator for 2 hours, followed by storage in a desiccator (Fig.S5A). 30 µl of TBST (tris-buffered saline with 0.1% tween 20) was used as a wash solution and dried on to leg 2 of the device (Fig. S5A). Leg 3 of the device was used to store commercially available gold enhancement reagents (Nanoprobes, Yaphank, NY). The reagent kit contained 4 components. 15 µl of each component was dried on a 6 mm x 6 mm glass fiber pad. During device assembly, the four pads were aligned with 2 mm overlaps (Fig. S5B) on to leg 3 of the device (Fig. S5A). The legs were stored in a desiccator for 2 days before conducting the test. For conducting the test, a mock sample was created by spiking a recombinant P*f*HRP2 protein into fetal bovine serum (Invitrogen, Carlsbad, CA) to a final concentration of 5 µg/ml.

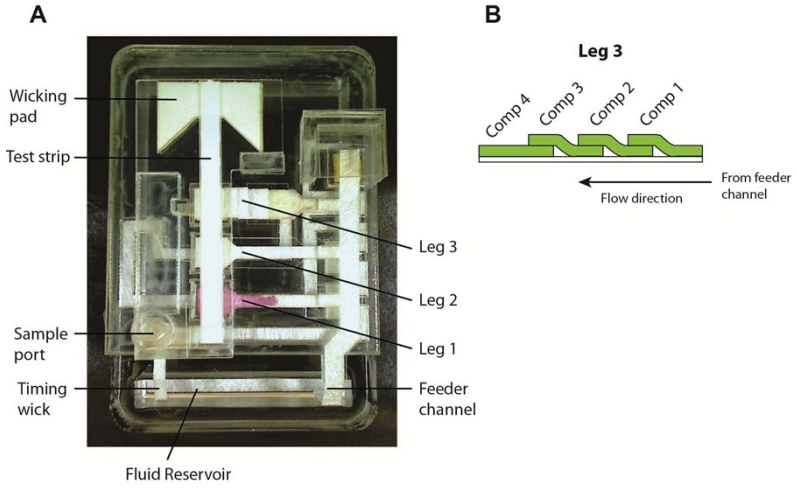

Figure S5. Protein detection device. **A**. Top view of the assembled device just before conducting the test. Gold conjugate, wash buffer, and gold enhancement reagents are dry stored on legs 1, 2, and 3, respectively. **B**. Schematic of the assembly of the 4 reagents pads in the gold enhancement leg of the device.

VI. Cost Analysis

The retail cost of all materials and reagents used for the P*f*HRP2 detection device is provided. All costs are in US Dollars.

MATERIALS

Material	Cost / Unit	Unit	Units Required	Cost
11.1 mm thick acrylic	30	6" x 12" sheet	7.5 cm x 5.2 cm	2.52
2 mm thick acrylic	2.57	6" x 12" sheet	7.5 cm x 5.2 cm	0.22
Mylar	5	8.5" x 11" sheet	6 x 7.5 cm x 5.2 cm	1.94
Nitrocellulose HF120	35	8.5" x 11" sheet	5 cm x 0.4 cm	0.12
Glass Fiber GR8964	0.75	8" x 10" sheet	10.75 sq cm*	0.02
Cellulose CFSP223000	2	8.5" x 11" sheet	2 cm x 1.4 cm	0.01
Compressed Sponge	1.05	4.75" x 3" sheet	4 x 0.6 cm x 0.6 cm	0.02
Total Materials Cost				**4.83**

REAGENTS

Reagent	Cost / Unit	Unit	Units Required	Cost
Capture Antibody	260	1 mg	1 µg	0.26
Secondary Antibody				0.26**
Gold and gold conjugation	5200	250 ml	30 µl	0.62
Gold enhancement	133	2 ml	15 µl	1.00
Capture Antibody	66	2 mg	1 µg	0.03
Buffer	10.8	1 litre	30 µl	0.00
Total Reagents Cost				**1.91**

TOTAL MATERIALS + REAGENTS	**6.75**

* Estimated total area of all glass fiber components on the device
** Because the detection antibody is conjugated with gold, it was difficult to determine the exact amount. A conservative estimate is used.

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