Supplementary Material (ESI) for Lab on a Chip

Supplementary Information for:

Programmable Diagnostic Devices Made from Paper and Tape

Andres W. Martinez,[a] Scott T. Phillips,[a] Zhihong Nie,[a] Chao-Min Cheng,[a] Emanuel Carrilho,[b] Benjamin J. Wiley,[a] and George M. Whitesides[a]*

[a]Department of Chemistry & Chemical Biology, Harvard University, Cambridge, MA 02138

[b]Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP, 13566-590, Brazil

* Corresponding author E-mail: gwhitesides@gmwgroup.harvard.edu

Fabrication of Programmable μPADs

Paper-based microfluidic channels. We patterned paper by photolithography as described previously.[1, 2] Paper was impregnated with SU-8 photoresist, baked on a hot plate (10 min, 110 °C), cooled to room temperature and exposed to UV light (14 s, 100 mW/cm^2, IntelliRay 600, UVitron International, Inc.) through a transparency mask. The paper was then baked a second time (5 min, 110 °C) and cooled to room temperature. The patterns were developed in a bath of acetone (1 min) followed by a rinse in acetone and a rinse in 70% isopropyl alcohol. The paper was blotted between two paper towels, rinsed a second time with 70% isopropyl alcohol, blotted, and allowed to dry under ambient conditions. We used Whatman No. 1 chromatography paper and ITW Technicloth (TX 609) to assemble the devices. Chromatography paper was used for the top layer of the devices and also for the layer of paper between the two gaps in the buttons. Technicloth was used for all the layers with channels because it wicks fluids quickly.[2] The patterned Technicloth was exposed to an oxygen plasma (Harrick plasma cleaner) for 3 s at 700 millitorr.[2]

Laser cutting of tapes. Double-sided adhesive tape (ACE plastic carpet tape 50106) was patterned with holes using a laser cutter (Universal Laser VL-300 50 Watt Versa Laser). The tape was placed on a sheet of parchment paper to protect the adhesive during the cutting process.

Assembly of μPADs. We assembled the devices by stacking alternating layers of paper and double-sided tape from the bottom up.[2] The alignment of holes and channels between layers was achieved by a manual process with an accuracy of ~500 μm. The bottom face of the double-sided tape was first attached to the bottom layer of the device. The top face

of the double-sided tape remained protected by the plastic backing supplied with the tape. The holes in the tape not used for 'on' buttons were filled with a paste made from a mixture of cellulose powder and water (1:3 w/w cellulose powder–water). The holes in the tape that were part of the 'on' buttons were left empty. Excess paste was scraped off of the plastic backing using a metal spatula, and the plastic backing was peeled from the tape. The top layer of paper was attached to the tape, and the entire device was compressed with a manual press (61401 AP40 Arbor Press, Palmgren, Inc.) under pressure of approximately 20 kg/cm^2 to achieve conformal contact between paste and paper. Additional layers of paper and tape were then added using the same process to complete the device.

Urinalysis Assays

The reagents for the glucose, protein, ketones and nitrite assays were spotted and dried in their respective test zones on the top layer of the device before assembling the device. All of the reagents were purchased from Sigma-Aldrich.

Glucose assay. A reagent solution [0.3 μL, 5:1 solution of glucose oxidase–horseradish peroxidase (120 units of glucose oxidase enzyme activity and 30 units of horseradish peroxidase enzyme activity per mL of solution), 0.6-M potassium iodide, and 0.3-M trehalose in a pH 6.0 phosphate buffer prepared in Millipore-purified water was spotted in the glucose test zone using a micro-pipette; the paper was air-dried for 10 min at 23 °C.[3]

Protein assay. A priming solution (0.3 μL, 92% water, 8% ethanol by volume, and 250-mM citrate buffer, pH 1.8) was spotted in the test zone using a micro-pipette; this solution was allowed to air-dry for 10 min at 23 °C. A reagent solution (0.3 μL, 95%

ethanol, 5% water by volume, 9-mM tetrabromophenol blue) was spotted on top of the priming solution and dried for 10 min at 23 °C.[3]

Nitrite assay. A reagent solution (0.3 μL, 14-mM *p*-arsanilic acid, 35 mM 1,2,3,4-tetrahydrobenzo[h]quinoline-3-ol and 0.1-mM citric acid dissolved in propanol) was spotted in the test zone with a micro-pipette and allowed to air-dry for 10 min at 23 °C.[4]

Ketone assay. A reagent solution (0.3 μL, 0.3-M sodium nitroprusside and 38-mM nickel chloride) was spotted in the test zone using a micro-pipette and allowed to air-dry for 10 min at 23 °C.[5, 6]

Preparation of Standard Solutions in Artificial Urine

We prepared an artificial urine solution as previously reported by Brooks and Keevil.[7] The artificial urine solution contained 1.1 mM lactic acid, 2.0 mM citric acid, 25 mM sodium bicarbonate, 170 mM urea, 2.5 mM calcium chloride, 90 mM sodium chloride, 2.0 mM magnesium sulfate, 10 mM sodium sulfate, 7.0 mM potassium dihydrogen phosphate, 7.0 mM dipotassium hydrogen phosphate, and 25 mM ammonium chloride all mixed in Millipore-purified water. The pH of the solution was adjusted to 6.0 by addition of 1.0 M hydrochloric acid. All reagents were purchased from Sigma-Aldrich. Solutions containing the desired concentrations of glucose, bovine serum albumin (BSA), acetoacetate and sodium nitrite were prepared using this artificial urine.

Using Programmable μPADs

The μPADs were programmed by pressing the 'on' buttons with firm pressure for ~ 2-3 seconds using the tip of a ballpoint pen. The compression of the paper channel does not obviously affect the rate of wicking of fluids through the compressed area. Depending on the design of the device, the sample inlet was then either manually dipped

into a drop (30 μL) of sample, or the sample was added to the fluid inlet with a pipette. The sample was allowed to wick through the device and into the test zones. Once the test zones were wet completely, the device was removed from the sample and the color for each assay developed for 30 min.

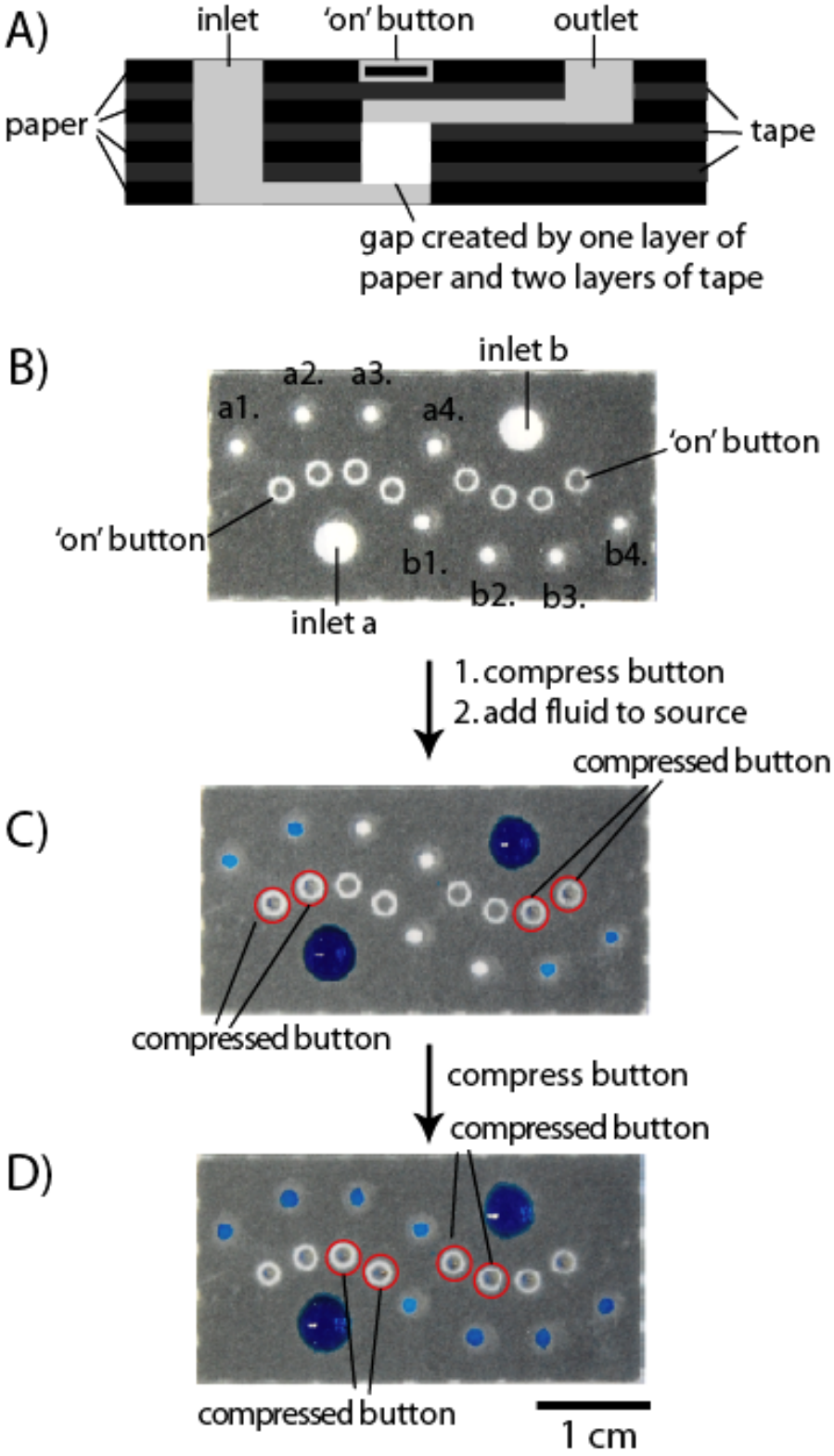
'On' Buttons in 3-D μPADs with Increased Spacing of Gaps

In order to enhance the robustness and reproducibility of the 'on' buttons in 3-D μPADs, we optimized the design of the gaps for the function of 'on' buttons. Instead of using two (or more) consecutive gaps for one button, we used one single gap created by one layer of patterned paper and two layers of tape; all three layers comprise designed perforations (Figure SI1). The spacing of the gaps that separate the inlet channel and outlet channel significantly increased to ~ 400 μm, compared to ~100 μm of each gaps in Figure 3A,B.

The paper channels used in the 3-D μPADs (Figure SI1) were patterned using wax-printing reported previously.[8] Briefly, we printed a piece of chromatography paper (Whatman 1 Chr) with printer (Xerox Phaser 8560), baked it at 150 ^{o}C for 2 min in oven. The printed wax melted and diffused into paper to form the hybrophobic barriers for paper channels. Wax-patterned paper was patterned with holes by laser-cutting. We assembled the devices by stacking alternating layers of paper and double-sided tape as described in the main text.

Figure SI1. Fabrication of 'on' buttons in 3-D microfluidic devices. A) Schematic of the cross-section of an 'on' button where the inlet channel is below the outlet channel. The inlet channel and outlet channel are separated by a gap created by one layer of paper with designed perforations and two layers of tapes patterned with holes. B) Top of the assembled device with two inlets and eight 'on' buttons. Four buttons (labeled as a1-a4) share the same inlet, and four buttons (labeled as b1-b4) share another inlet. C) A 50 μL aqueous solution of 1 mM Erioglaucine was added to the two inlets, and buttons a1, a2 (circled by red line) and b3, b4 (circled by red line) of the device were compressed, while others were not compressed. The fluid reached the outlet within 10 seconds only when the buttons were compressed. D) Buttons a3, a4 (circled by red line) and b1, b2 (circled by red line) of the device were compressed.

Figure SI1.



REFERENCES

1. A. W. Martinez, S. T. Phillips, B. J. Wiley, M. Gupta and G. M. Whitesides, *Lab Chip*, 2008, **8**, 2146-2150.
2. A. W. Martinez, S. T. Phillips and G. M. Whitesides, *Proc. Natl. Acad. Sci. U.S.A.*, 2008, **105**, 19606-19611.
3. A. W. Martinez, S. T. Phillips, E. Carrilho, S. W. Thomas, 3rd, H. Sindi and G. M. Whitesides, *Anal. Chem.*, 2008, **80**, 3699-3707.
4. *United States Pat.,* 6537823, 2003.
5. M. Cheesbrough, *District Laboratory Practice in Tropical Countries*, Tropical Health Technology, Cambridgeshire, UK, 1998.
6. *United States Pat.,* 3880590, 1975.
7. T. Brooks and C. W. Keevil, *Lett. Appl. Microbiol.*, 1997, **24**, 203-206.
8. E. Carrilho, A. W. Martinez and G. M. Whitesides, *Anal. Chem.*, 2009, **81**, 7091-7095.