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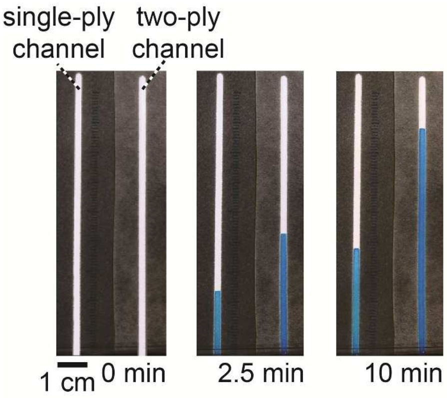

Two-ply Channels for Faster Wicking in Paper-based Microfluidic Devices

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Abstract

This article describes the development of porous two-ply channels for paper-based microfluidic devices that wick fluids significantly faster than conventional, porous, single-ply channels. The two-ply channels were made by stacking two single-ply channels on top of each other and were fabricated entirely out of paper, wax and toner using two commercially available printers, a convection oven and a thermal laminator. The wicking in paper-based channels was studied and modeled using a modified Lucas-Washburn equation to account for the effect of evaporation, and a paper-based titration device incorporating two-ply channels was demonstrated.

Introduction

Paper-based microfluidic devices, also known as microPADs, are a new class of fluidic devices that offer a promising platform for the development of low-cost point-of-care diagnostic devices.[1–3] MicroPADs can be made from inexpensive materials, they are inherently lightweight and portable, and they are simple to operate.[4] Unlike conventional microfluidic devices made from glass or plastic that typically require computer-controlled pumps to move fluids through their channels, microPADs contain channels patterned into a sheet of paper that wick fluids by capillary action.[2] With the appropriate design, a microPAD can automatically process a sample of fluid and combine it with reagents in specific sequences in order to detect analytes.[5–7]

One important characteristic of microPADs is the time required for the fluid to wick through the channels in a device. Under ambient conditions, typical porous, paper-based channels with cross-sectional dimensions of approximately 2 mm in width and 0.2 mm in height can wick liquids through short distances (<3 cm) in minutes, but can take hours to wick liquids through long distances (>5 cm). This slow wicking of fluids through paper-based channels places a practical limit on the length of channels that can be used in a microPAD, which in turn limits the complexity and applications of this class of devices.

Several research groups have described ways of fabricating faster-wicking paper channels. In the first example of fast channels that we are aware of, Jahanshahi-Anbuhi *et. al.* sandwiched paper channels between two plastic films to achieve faster flow.[8] Later on, Renault *et al.* fabricated hollow channels out of stacked paper to also achieve faster flows.[9] Then Giokas *et al.* cut grooves in the middle of a paper-based channel to increase the wicking rate.[10] Finally, da Silva *et al.* used triboelectrically charged poly(ethylene terephthalate) sheets to seal paper-based channels and control wicking.[11] In this article, we introduce two-ply channels as another option for overcoming the limitations of slow wicking in conventional paper-based channels.

The Lucas-Washburn equation describes capillary flow in a horizontal bundle of parallel capillary tubes and is often extended to model wicking in porous materials such as paper.[1,8,12,13] The Lucas-Washburn equation is given by:

$$l(t) = \sqrt{\frac{\gamma r \cos \theta t}{2\mu}} \tag{1}$$

where *l(t)* is the distance penetrated by a liquid flowing under capillary pressure into a channel, *t* is time, γ is the surface tension, *r* is the average radius of the capillary or is sometimes also described as the effective pore radius of the paper channel, θ is the contact angle, and μ is the dynamic viscosity of the liquid. Equation 1 was derived under several assumptions including that there are no effects of gravity on wicking and that there is no loss of fluid from the channel due to evaporation.[1,14,15]

Since the surface tension, contact angle and viscosity terms in Equation 1 are all constant for a given liquid wicking through paper, the only way to make a paper-based channel wick liquid faster is by increasing the effective pore radius. While the effective pore radius is assumed to be constant for a given type of paper, we found that we could increase the effective pore radius in a paper-based channel by stacking two paper-based channels on top of each other to create two-ply channels (Figure 1). Stacking two channels on top of each other generates a small gap between the layers of paper, which, we hypothesize, acts as a capillary with a much larger radius than the typical effective pore radius in a single sheet of paper and allows the two-ply channel to wick fluid more rapidly.

Fast channels have the potential to expand the capabilities and applications of microPADs since they will allow for longer channels to be incorporated into the devices and decrease the amount of time required for operation of the devices. One example of a new type of microPAD that would not be practical with conventional channels is a paper-based titration device with a large number of test zones.[16,17] Such paper-based titration devices, as we envision them, have a series of test zones containing an indicator and increasing amounts of a particular titrant dried in the test zone. When an aqueous sample is delivered to a test zone, the analyte will react with the titrant and, if the titrant is the limiting reactant, the color of the indicator will change. In contrast, if the analyte is the limiting reactant, the color of the indicator will remain unchanged. The concentration of analyte in the sample can then be estimated based on the number of test zones that change color. The more test zones on a particular device, the more precise the measurement will be, since more concentrations of titrant can be tested. Fast channels offer a convenient way of delivering liquid to a large number of test zones on a single device within a short amount of time. Here we demonstrate a prototype acid-base titration device for determining the concentration of hydroxide in solution.

Experimental

Fabrication of two-ply channels. Two-ply channels were fabricated in three steps. (1) Two sheets of Whatman No. 1 Chr chromatography paper were patterned by wax printing.[18] Wax lines were printed on the paper using a Xerox Phaser 8560 printer, and the paper was then heated for two minutes in a convection oven set to 195 °C to melt the wax into the paper. After allowing the paper to cool for five minutes, four layers of toner were printed onto one face of one of the sheets of patterned paper using a Samsung CLP-680ND printer. (3) The two sheets of paper were stacked on top of each other (with the layer of toner sandwiched between the two sheets of paper), and the stack was passed through a Purple Cows 3015c laminator set to the 5 mil setting in order to bond the two layers of paper together with the toner acting as a thermal adhesive.[19]

We imaged the cross-section of a two-ply channel by cutting the channel in half using a razor blade and placing the section under a dissecting microscope equipped with a Nikon D5100 digital camera. One layer of the channel was stained with yellow dye (10 mM Tartrazine in water) and the other layer of the channel was stained with blue dye (1 mM Erioglaucine in water) before the two-ply channel was assembled in order to facilitate the identification of the two plies in the image.

Evaluation and modelling of wicking in two-ply channels. Devices were prepared with six straight channels—three conventional, single-ply channels and three two-ply channels—each 9-cm long and 2-mm wide. The devices were dipped vertically to a depth of 2 mm into a fluid reservoir containing aqueous blue dye (1-mM Erioglaucine), and the fluid front of each channel was tracked until the fluid reached the end of the two-ply channels. The experiments were recorded with a Nikon D5100 digital camera. One set of experiments were conducted on a bench top under ambient conditions [21 °C, 35% relative humidity (RH)]. A second set of experiments was conducted in a glass chamber (a repurposed desiccator), with water on the bottom of the chamber and a wet paper towel around one side of the chamber to simulate 100% RH and minimize evaporation from the channels. For the experiments conducted in the glass chamber, we were only able to track the fluid front for 7 cm. A third version of the wicking experiments was conducted while suspending the two-ply channels horizontally and folding the bottom 2-mm portion of the channel at a 90° angle to dip it into the fluid reservoir. The horizontal wicking experiments were also conducted under ambient conditions (21 °C, 35% RH). The temperature and relative humidity were measured using an AcuRite 00613A1 Indoor Humidity Monitor.

The rate of evaporation of water from single-ply and two-ply channels was measured experimentally under ambient conditions (21 °C, 35% RH) by first wetting a 2-mm-wide by 9-cm-long channel with water and then supporting it vertically on an analytical balance using a binder clip. The doors of the balance were left open, and the mass of the channel was recorded every minute for 10 minutes. We observed random fluctuations in the mass during the first four minutes of the experiment, so we chose to only use the results from the 5th through 10th minute of the experiment to calculate the rate of evaporation from the channels.

In order to model the wicking properties of two-ply channels under conditions where evaporation of liquid from the channel occurred, we derived an equation to describe the distance wicked by the channel as a function of time starting from Darcy's law. We fit the experimental data with the resulting equation using Kaleidagraph. The porosity of Whatman No.1 chromatography paper was estimated by depositing 1 μL of a 1-mM solution of Erioglaucine on the paper, measuring the diameter of the resulting spot (5 mm) and assuming that the fluid had filled the pores in the paper completely within the area of the spot.

Titration device. Titration devices with 16 test zones for measuring the concentration of hydroxide in solution were constructed from three layers of paper using the same techniques that were described previously for fabricating two-ply channels. Toner was used to bond the three layers of paper together (Figure S1). Once assembled, the test zones on each device were spotted with 0.4 μL of hydrochloric acid in concentrations ranging from 0.001 M to 1 M. After drying the acid, 0.5 μL of a saturated solution of bromothymol blue in ethanol was added to each test zone and dried. Finally, in order to protect the device and minimize evaporation of fluid from the test zones, the devices were sealed by placing the device between two layers of Scotch® thermal lamination pouches and passing the assembly through an Apache AL13P thermal laminator set to 200 °C. To test the devices, 110 μL of sample solutions containing sodium hydroxide (NaOH) in concentrations of 0.001, 0.010, 0.050, 0.10 and 1.0 M were pipetted into the sample inlet zones. Each concentration of NaOH was tested four times. The concentration of NaOH in the sample solution was determined by recording the last test zone on the device in which a color change from yellow to blue was observed. For comparison, an analogous device made with a conventional single-ply channel was tested.

Results and Discussion

MicroPADs containing two-ply channels could be fabricated in approximately 30 minutes once the designs for the devices were complete. Most of this fabrication time was spent waiting for the paper to cool between print and bake cycles, so it is possible to fabricate large numbers of devices in a relatively short amount of time when the devices are fabricated in parallel. Once fabricated, the devices could be stored for months under ambient conditions with no changes in the wicking properties of the channels. When viewed from above, two-ply channels are indistinguishable from conventional single-ply channels (Figure 1C). When imaging a cross section of the two-ply channels, the two layers of paper and the gap between them are visible (Figure 1D).

Two-ply channels wicked aqueous solutions significantly faster than single-ply channels (Figure 2). This difference in wicking rate can lead to significant time savings for devices incorporating two-ply channels. For example, at 35% RH, two-ply channels wicked aqueous solutions across a 4.5-cm-long channel in 3.1 minutes while single-ply channels required 20 minutes to wick fluids across the same distance (Figure 2B). Another significant advantage of two-ply channels is that because the distance travelled by fluids via capillary wicking in vertical paper-based channels approaches an asymptotic height, two-ply channels were able to wick fluids to distances that could not be achieved with conventional channels.

We observed that the wicking in the two-ply channels was less precise than wicking in the single-ply channel, as can be seen by the size of the error bars in Figure 2. We attribute this difference in precision to the fact that the two-ply channels are more complex and thus are subject to more sources of indeterminate error. For example, the two layers of paper and the layers of toner in the two-ply channels are not aligned exactly the same way each time a two-ply channel is fabricated. In fact, the printers that we used to fabricate the devices are commercial printers designed to print a single layer of wax or toner on a piece of paper, and so their mechanisms for aligning sheets of paper is rudimentary. The alignment of the layers of paper and toner, and thus the precision of the wicking in two-ply channels, could probably be improved with additional optimization of the fabrication procedure and better equipment. If two-ply channels were to be manufactured on a large scale, the fabrication process could most likely be optimized to produce more consistent devices.

Compared to the previously published fast channels, two-ply channels do not wick fluids as fast as the channels described by Jahanshahi-Anbuhi *et al.* or Renault *et al.*,[8,9] but they do offer the advantage that they are simple to fabricate and operate, they can be made using conventional printing processes, and they do not comprise loose parts that need to be held in position. Having the option of selecting from and even combining different types of fast channels in a device will allow for greater control over the wicking of fluids in microPADs and will facilitate timed or sequenced events.

We found that both single-ply and two-ply channels wicked fluids faster at 100% RH than at 35% RH (Figure 2). The effect of relative humidity on wicking was expected since it had been observed previously for single-ply channels.[20] We hypothesize that at low humidity the channels lose a significant amount of water to evaporation,[21] which slows the wicking down. Gravity, on the other hand, did not have a significant effect on wicking in two-ply channels at least for the dimensions of the channels that we tested (Figure S2). This was a somewhat unexpected observation given that Jahanshahi-Anbuhi *et al.* observed a significant effect of the angle at which their fast channels were positioned on the wicking in the channels.[8] However, the two types of channels are completely different in their structure, so it is not surprising that they would have different wicking characteristics.

In an attempt to model the wicking in two-ply channels, we initially used the Lucas-Washburn equation and found that this equation described the wicking accurately for experiments performed at 100% RH for both the two-ply and single-ply channels (Figure 2C). For experiments performed at 35% RH, the Lucas-Washburn equation described the wicking accurately during the first five minutes, but the experimental results started to deviate significantly from the Lucas-Washburn equation after this time, with the Lucas-Washburn equation overestimating the distance wicked by the fluid (Figure S3). This result can be explained because the Lucas-Washburn equation does not account for loss of fluid to evaporation.

Since most microPADs will likely be used under ambient conditions and be affected by evaporation, we derived a new expression starting from Darcy's law to include a term for loss of fluid to evaporation:

$$l(t) = \sqrt{\frac{\gamma r \phi h cos\theta}{4\mu q_0}\left(1 - e^{-\frac{2q_0}{\phi h}t}\right)} \qquad (2)$$

where ϕ is the porosity of the paper, h is the thickness of the porous channel and q_0 is the rate of evaporation defined as the volume of evaporated liquid per unit area of wet channel and time (see supporting information for a complete derivation). In the limit where there is no evaporation from the channel ($q_0 \rightarrow 0$), equation 2 reduces to the Lucas-Washburn equation. A similar analytical approach for treating the influence of evaporation on imbibition in a porous medium was given by Fries *et al.*, but their model overestimated the distance wicked by the fluid.[15,22]

The experimental results obtained for wicking in the single-ply and two-ply channels at 35% RH were fit with Equation 2 in Kaleidagraph using the General curve fit function with t as the independent variable and r and q_0 as parameters. The magnitudes of all the other constants used for the curve fitting are described in the supporting information. From the curve fitting, we obtained values for r and q_0 for both types of channels (Table 1). For comparison, we fit the results at 100% RH using the Lucas-Washburn equation to obtain values for r, and we measured the magnitude of q_0 experimentally. All of the respective values are relatively close to each other, which suggests that Equation 2 is a useful model for describing wicking in porous paper-based channels under conditions where evaporation is significant.

In order to support our hypothesis that the gap between the two layers of paper is contributing to the larger magnitude for *r* in two-ply channels, we calculated the theoretical height of the gap assuming that *r* for the two-ply channels could be calculated as a cross-sectional-area-weighted average of the pore radius of the two layers of paper and the pore radius of the gap (Figure S4). This calculation gave us a value of 12 μm for the height of the gap, which based on our visual inspection of the gap appears to be a reasonable value (Figure 1D).

Using the titration devices, we were able to reliably differentiate between solutions of NaOH with concentrations of 0.001, 0.010, 0.050, 0.10 and 1.0 M by observing the position of the last test zone to change color from yellow to blue (Figure 3C). Of the 20 tests conducted with the titration devices, 16 tests produced the expected result shown in Figure 3C. In the remaining four tests, the position of the last test zone to change color deviated by one additional test zone from the expected result, thus slightly overestimating the concentration of hydroxide in the test solution. Tests for the highest concentrations of NaOH were completed within 35 minutes. For lower concentrations of NaOH, the results can be read as soon as a test zone fills with sample solution and does not change color. In analogous devices using conventional channels to carry the fluid to the test zones, the fluid took 1.5 hours to reach just the 11th test zone of 16. The fluid appeared to come to a stop after this point, so the experiments were stopped.

Our results support the conclusions of Karita and Kaneta that the titration devices could be used to determine the concentration of analytes semi-quantitatively by simply counting the number of test zones that change color and without relying on instruments such as digital cameras.[16] While the prototype was designed to differentiate between a large range of concentrations of NaOH with low precision, it may be possible to differentiate between a smaller range of concentrations of NaOH with better precision by changing the concentrations of HCl added to the test zones. This approach could likely be applied to other analytes for which titrations with colored indicators are possible.

Conclusions

Two-ply channels provide a new approach for achieving faster wicking in paper-based fluidic devices. These types of fast channels will enable the development of larger devices with more complex channel networks. Two-ply channels may also improve paper-based diagnostic devices by reducing run-times in order to provide results more quickly. The main disadvantage of two-ply channels is that, similar to all the other fast channels described in the literature, they require more involved fabrication procedures than those required to fabricate single-ply channels, and so we would only expect them to be used in situations where long channels are required for the particular application of the device.

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Table 1. Comparison of values for *r* and q_0 obtained by different methods.

	r (µm) (from fitting data at 100% RH with Lucas-Washburn)	*r* (µm) (from fitting data at 35% RH with equation 2)	q_0 (µm/s) (measured experimentally)	q_0 (µm/s) (from fitting data at 35% RH with equation 2)
Single-ply channel	0.113±0.001	0.11±0.02	0.057±0.007	0.046±0.002
Two-ply channel	0.30±0.01	0.34±0.02	0.073±0.002	0.076±0.001

Figure 1

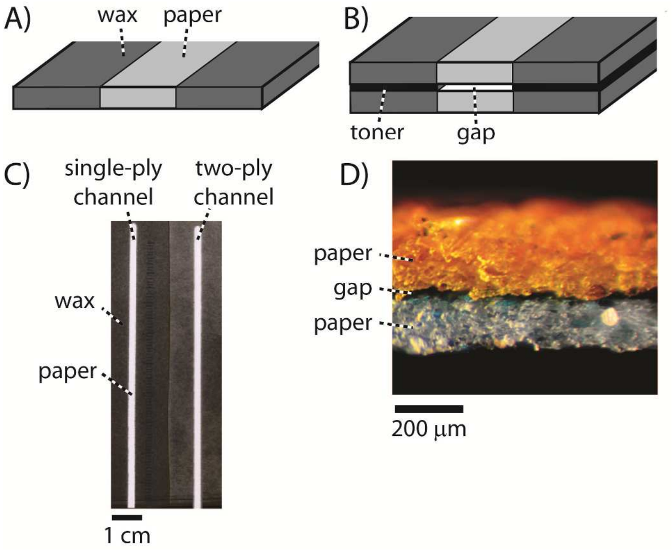

Figure 1. A) Schematic diagram representing a conventional, single-ply channel made from paper patterned by wax printing. B) Schematic diagram representing a two-ply channel made by stacking two conventional channels on top of each other and bonding them together using toner as a thermal adhesive. C) Top view of a single-ply channel and a two-ply channel. D) Cross-section of a two-ply channel illustrating the gap between the two layers of paper. The top layer of paper was stained yellow and the bottom layer of paper was stained blue before the two-ply channel was assembled. The luminosity of the image was adjusted in Photoshop for better visualization.

Figure 2

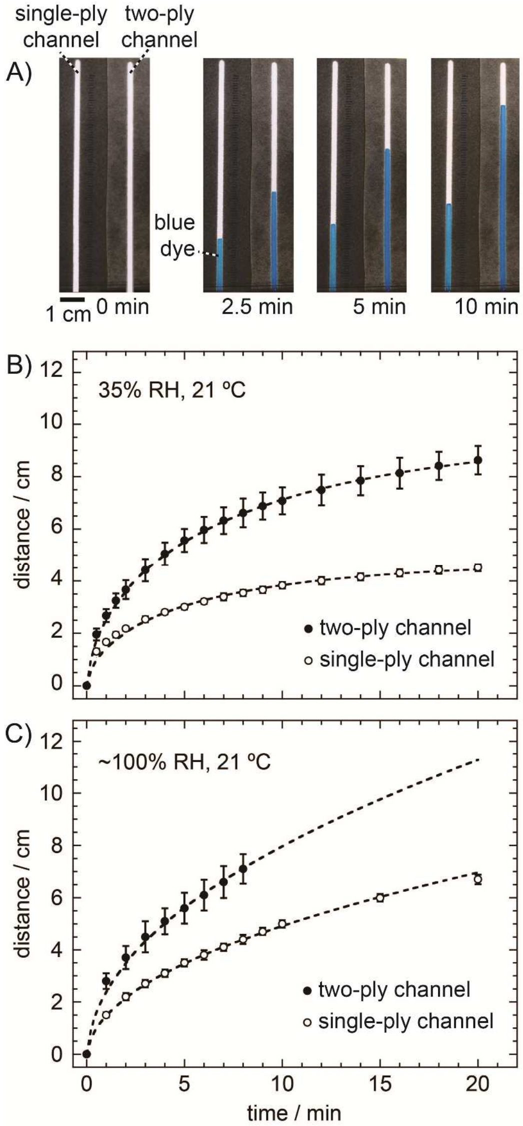

Figure 2. Wicking of fluids in single-ply and two-ply channels. A) A single-ply channel and a two-ply channel wicking an aqueous blue dye solution from a fluid reservoir over time at 35% RH. B) Plot of the distance wicked by the fluid versus time at 35% RH. Data points represent the mean of nine measurements, and the error bars represent one standard deviation from the mean. The dashed lines represent the wicking profile predicted by Equation 2. C) Plot of the distance wicked by the fluid versus time at 100% RH. Data points represent the mean of nine measurements, and the error bars represent one standard deviation from the mean. The dashed lines represent the wicking profile predicted by the Lucas-Washburn equation.

Figure 3

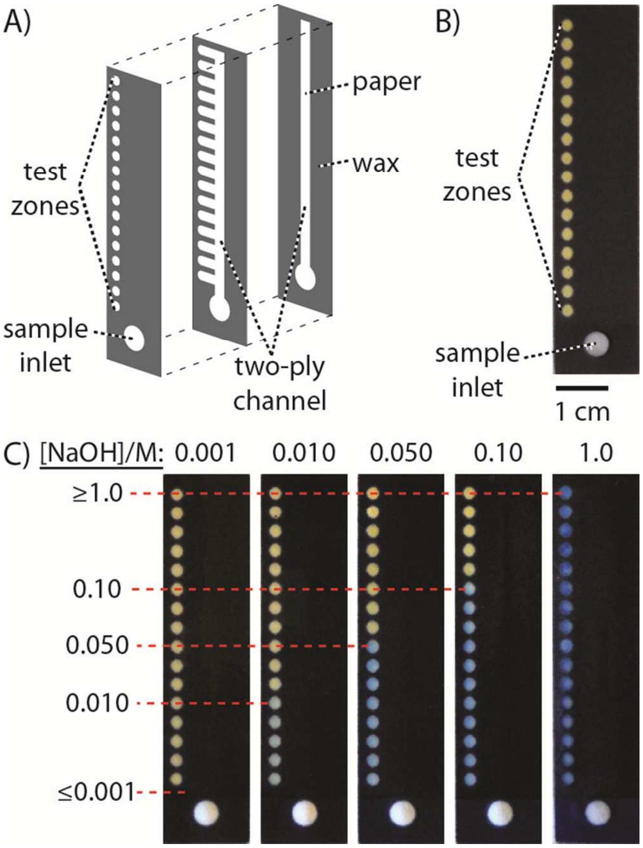

Figure 3. A) Schematic representation of the three layers of paper that were assembled to fabricate the titration devices. The layers of paper were bonded together using toner as a thermal adhesive. B) A completed titration device with a drop of sample solution added to the sample inlet. C) Images of titration devices that were run with various concentrations of NaOH. The position of the last test zone to change colors from yellow to blue can be used to determine the concentration of NaOH in the sample solution.

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