FABRICATION OF THREE-DIMENSIONAL MICROFLUIDIC CHANNELS IN A SINGLE LAYER OF CELLULOSE PAPER

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ABSTRACT

This paper reports a novel technique for fabricating three-dimensional (3D) microfluidic channels in a single sheet of paper, which greatly simplifies the fabrication process of 3D microfluidic paper-based analytical devices (3D-μPADs). This technique, derived from the popular wax printing technique for paper channel patterning, is capable of controlling the penetration depth of wax upon heating, which is printed on both sides of a paper substrate, and thus forming multi-layers of patterned channel in the substrate (up to four layers of channels were fabricated in a 315 μm thick paper sheet). Heating time and density of printed wax (i.e., grayscale level for wax printing) were used as parameters to control the penetration depth of melted wax. This technique holds a great potential to further popularize the use of 3D-μPADs and enhance the mass-production quality of these devices.

KEYWORDS: Paper-based microfluidics, Three-dimensional paper channels, Wax printing, Disease diagnostics

INTRODUCTION

Microfluidic paper-based analytical devices (μPADs) are highly promising for low-cost bioanalysis in remote and/or resource-limited settings [1, 2]. Three-dimensional (3D) μPADs have been invented since the emergence of paper-based microfluidics, which allows the construction of 3D paper channel networks and the manipulation of fluidic flows in a 3D, complex fashion [3]. However, the application of 3D-PADs is relatively limited, largely due to their multi-layer fabrication processes which raise technical challenges in device manufacturing and quality control. Although new methods have been proposed recently for parallel fabrication of 3D-PADs with a higher throughput [4, 5], they still require stacking and alignment of multiple layers of patterned paper and are thus tedious and less controllable in quality. In this paper, we report a novel technique for fabricating 3D channels in a single sheet of paper through solid wax printing, which significantly simplifies the fabrication process of 3D-PADs.

EXPREIMENTAL

The technique is derived from the popular wax printing approach for patterning hydrophobic wax barriers in paper substrates [6, 7]. The underlying mechanism of this technique is to control the penetration depth of wax printed on a paper substrate upon heating/melting. Interconnected layers of paper channels were patterned at different levels along the thickness of a paper substrate, by controlling the penetration depth of melted wax on both sides of the paper and thus forming 3D hydrophobic barriers. We adjusted the grayscale level of wax printing to deposit wax to the paper surface with different densities on the top and bottom surfaces of the paper. Upon being heated, high-density wax wicks faster than low-density wax, resulting in hydrophobic barriers with different thickness values formed in the paper.

We controlled the printing density of wax by setting different grayscale levels for the pattern to be printed. In the 8-bit RGB color system we used in AutoCAD®, a grayscale color was displayed when the RGB values are equal. This RGB color system was converted to the CMYK color system of the wax printer (Xerox ColorCube 8570) in printing. Fig. 1(A) shows microscopic photographs of solid wax printed on top of a paper substrate with different RGB values. The grayscale intensity changed from pure black RGB = (0, 0, 0) to pure white RGB = (255, 255, 255).

We chose Whatman® 3 MM chromatography paper as the substrate material. Its thickness was measured to be 315.4 ± 6.9 μm (n = 5), greater than that of Whatman No. 1 chromatography paper (~180 μm) normally used in μPAD fabrication, thus providing more space for fabricating 3D channels. Figs. 1(B) and 1(C) schematically show the formation of 3D channels in single-time printing and heating. In the first kind of designs (Fig. 1(B)), channel layers were formed

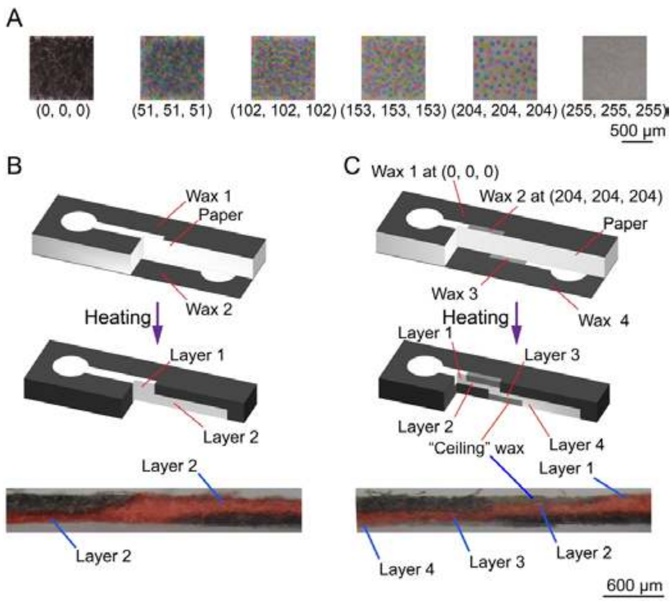

Figure 1. Formation of 3D microfluidic channels inside a single sheet of paper. (A) Microscopic photographs of wax printed at different RGB levels. The printing intensity changes from pure black RGB=(0, 0, 0) to pure white RGB=(255, 255, 255). (B)(C) Schematic illustrations of the formation of (B) double-layer and (C) four-layer channels. In (B), both sides of the paper were printed at the same RGB level.

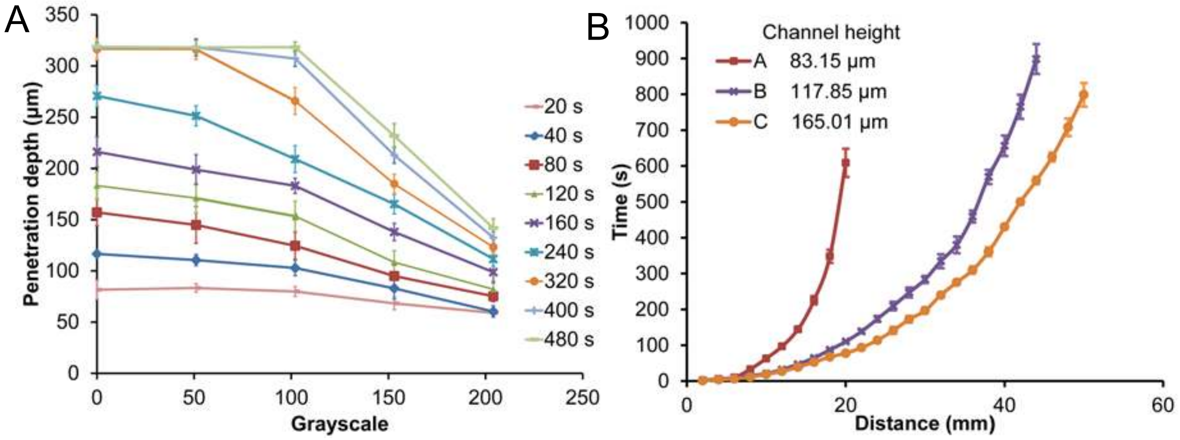

Figure 2. (A) Experimental results of wax penetration depth vs. RGB level and heating time (n=4). (B) Experimental results of wicking time vs. distance inside 2mm-wide enclosed channels with different channel heights (n=3).

through alignment of un-patterned paper areas on both sides of the paper substrate. Both sides of the paper were printed using the same RGB level. Once heated, the wax from both sides reflowed into the paper and formed hydrophobic walls of double layers of channels. The heating time was controlled to adjust the penetration depth of the melted wax. The overlap of the un-patterned paper areas formed an vertical interconnect between the two channel layers.

In another kind of designs (Fig. 1(C)), different areas on each side of the paper were printed with wax at different densities. High-density wax was found to wick faster than low-density wax upon heating; thus, two layers of open channels (layers 1 and 4) and two layers of enclosed channels (layers 2 and 3) can be formed. Although we demonstrated fabrication of up to four layers of channels in Whatman® 3 MM paper, one can achieve, in principle, more than four layers of channels using this technique.

RESULTS AND DISCUSSIONS

The control of penetration depth of melted wax is critical in this technique. We examined two fabrication parameters for penetration depth control. (i) *Heating Time*: With the same amount of wax printed to the paper surface, longer heating time led to deeper penetration into the paper. (ii) *Density of Printed Wax*: With the same period of heating time, wax printed at a higher density wicked faster than that printed at a lower density. Fig. 2(A) illustrates the experimental results of the penetration depth as a function of the heating time (at 100 °C) and the density of printed wax.

As shown in Fig. 1(C), this technique can also form enclosed paper channels inside a paper substrate, which is potentially useful for reducing evaporation and preventing contamination. By controlling the penetration depth of the "ceiling" wax layers (Fig. 1(C)), the height of the enclosed channel can be controlled. We characterized the wicking speed of aqueous solutions (PBS was used in experiments) in enclosed channels with different heights (Fig. 2(B)). We also investigated the wax barrier quality by checking the relationship of the barrier's capability of preventing leakage and its width, and found that wax walls with a size down to 220 μm after heating, formed by printing wax at RGB = (0, 0, 0) on both surfaces of the paper and heating at 100°C for 80 s, stably held solutions without leakage (Fig. 3).

Finally, we fabricated a three-layer device (Figs. 4(A)(B)) on a single paper substrate to demonstrate multiplexed colorimetric detection of glucose, lactate, and uric acid. We stored corresponding enzymes/oxidizers for the three analytes in nine test zones on the backside of the device, and added a 10-μL drop of artificial urine (AU) containing the three analytes to an inlet on the front side of the device. The AU sample was then distributed through the three-layer channels to the test zones and reacted with the stored reagents. Linear calibration curves of the colorimetric signal vs. the analyte

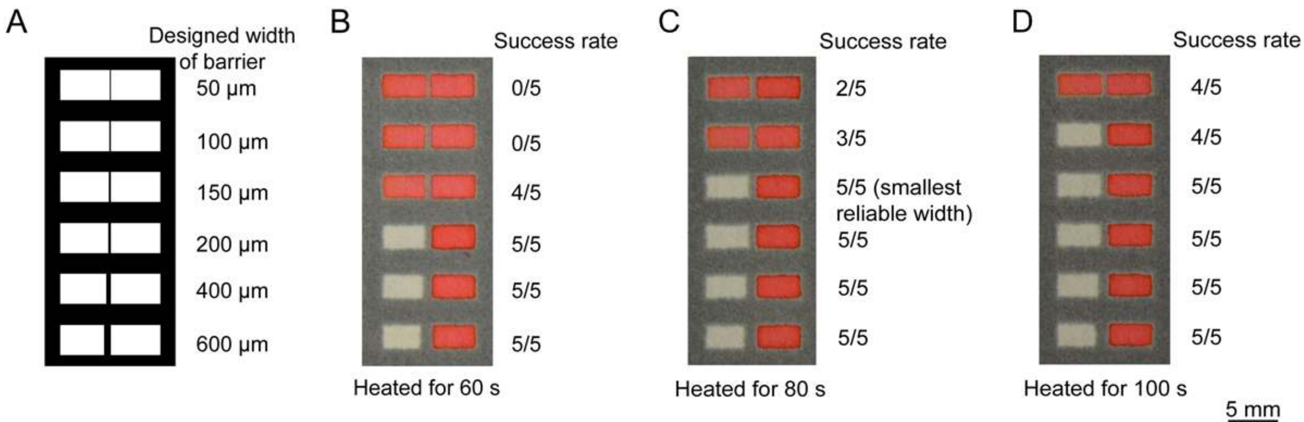

Figure 3. Experimental results of barrier-quality testing. Wax was printed both surfaces of the paper at RGB = (0, 0, 0) and then heated for different periods of time. Red dye was added to the right paper area to test the leakage.

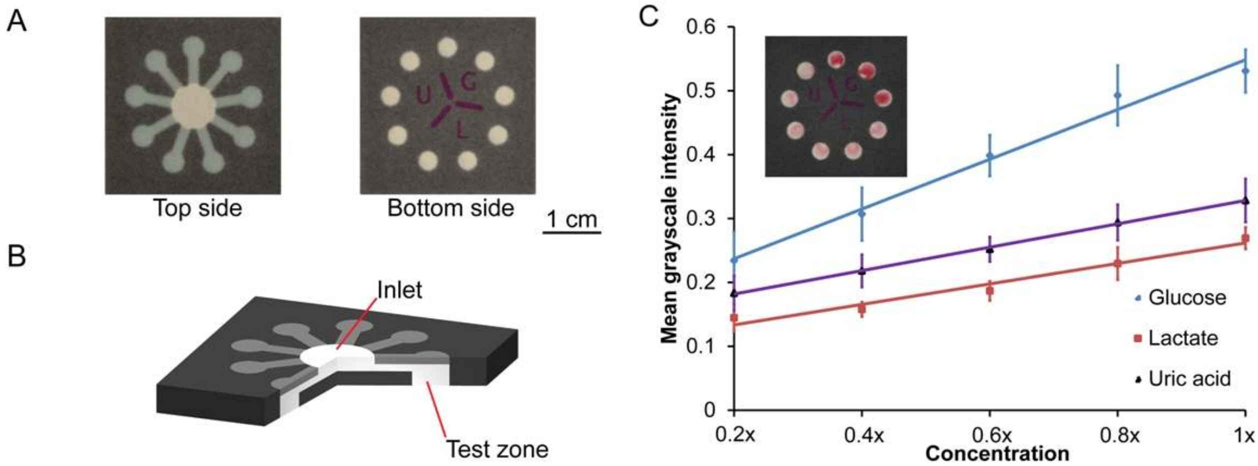

Figure 4. A three-layer μPAD for multiplexed colorimetric detection. (A) Photographs of top and bottom surfaces of the μPAD. Letters wax printed on the bottom side to indicate a group of three test zones for the same marker (G: glucose; L: lactate; U: uric acid). (B) Cross-sectional schematic of the three-layer channel structure of the μPAD. (C) Experimental results of the colorimetric intensity vs. the marker concentration (n=5). The analyte concentrations in 1× sample are 20 mM, 25 mM, and 15 mM for glucose, lactate, and uric acid, respectively. The inset shows a photograph of the test zones after colorimetric reaction.

concentration were obtained (Fig. 4(C)).

CONCLUSION

A wax-printing-based technique was presented for fabricating 3D microfluidic channels in a single paper substrate. By controlling penetration depth of melted wax on both sides of the paper substrate, multiple layers of paper channels were formed at different levels along the paper thickness. Major fabrication parameters for controlling the penetration depth were characterized, providing guidelines for designing this type of paper-based devices. It was demonstrated that the technique allows easy fabrication of up to four layers of paper channels in a 315 µm thick paper substrate, without any process optimization. Using this simple technique, we created a µPAD with a three-layer channel network and demonstrated multiplexed colorimetric detection of three biomarkers (glucose, lactate, and uric acid).

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