Three-Dimensional Paper Microfluidic Devices Assembled Using the Principles of Origami

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ABSTRACT: We report a method, based on the principles of origami (paper folding), for fabricating three-dimensional (3-D) paper microfluidic devices. The entire 3-D device is fabricated on a single sheet of flat paper in a single photolithographic step. It is assembled by simply folding the paper by hand. Following analysis, the device can be unfolded to reveal each layer. The applicability of the device to chemical analysis is demonstrated by colorimetric and fluorescence assays using multilayer microfluidic networks.

Here we report a method for fabricating three-dimensional (3-D) paper microfluidic devices that is based on the principles of origami (paper folding). The concept is illustrated in Scheme 1. Using this method, the entire device is fabricated on a single sheet of flat paper, and then it is assembled by simple paper folding. This method is important for several reasons. First, instead of sequential layer-by-layer fabrication, which is the usual approach for preparing 3-D microfluidic systems, the entire device is fabricated on one piece of paper in a single photolithographic step. This speeds the fabrication process and reduces cost. Second, the multilayer device is assembled by simple paper folding, which can be completed in less than 1 min without tools or special alignment techniques. Third, the device can be easily unfolded so that all layers, rather than just the surface, can be used for parallel analysis. Fourth, incorporation of additional intermediate layers should not result in much additional fabrication overhead.

The principles of 2-D and 3-D microfluidic paper analytical devices (μPADs) have been described by Whitesides and co-workers, and a number of interesting applications have been reported. Briefly, for 2-D μPADs, microfluidic channels and reservoirs are fabricated by patterning channel walls on chromatography paper using a hydrophobic material, such as photoresist (PR) or wax. Aqueous solutions are then driven along the hydrophilic paper channels by capillary action. For 3-D μPADs, individual layers are patterned sequentially by photolithography and then stacked using double-sided tape. Holes are punched in the tape using a laser cutter, and the resulting holes are filled with cellulose powders or are compressed to provide vertical connections between adjacent layers. The results of an analysis are determined using colorimetric detection on one of the two surface layers. The 3-D μPADs show great promise for applications such as power-free, point-of-care detection and diagnosis, particularly in underdeveloped or remote areas. However, as presently practiced, device fabrication requires a photolithographic step for each layer and then laser cutting of vias to establish fluidic connections between layers. Moreover, assembly of the device using double-sided tape is irreversible so that only the surface layer can be used for colorimetric detection. The approach we describe addresses these points.

As previously discussed, 3-D μPADs are fabricated by stacking 2-D layers. An alternative approach is based on the principles of origami. Origami is the traditional Japanese art of paper folding, and it has been in use for ~400 years to construct 3-D geometries starting with a single piece of flat paper. Within the context of modern science and engineering, there has not been much interest in origami. However, there is one report in which it was used to fabricate 3-D printed circuit boards in the shapes of airplanes and cranes. With that as a starting point, we show here that origami can be used to fabricate simple and functional microfluidic devices, which we call origami paper analytical devices (oPADs), having several highly desirable characteristics.

Figure 1a shows a piece of chromatography paper that has been patterned with channels, reservoirs, and a frame (to provide a template for subsequent folding) fabricated in a single photolithographic step. The fabrication process is based on previously reported procedures and is described in the Supporting Information (SI). As reported previously, the entire photolithographic process can be performed without a cleanroom, using just a hot plate, UV lamp, and a mask produced on a printer. Following photolithography, the 3-D device was assembled by folding the paper along the lithographically defined frame. The frame ensures that the channels and reservoirs are properly aligned after folding into the 3-D assembly. The folding sequence is provided in Figure S1 of the SI. The four corners of the folded paper were trimmed, as shown in parts b and c of Figure 1, to accommodate an aluminum clamp (Figure 1d). Solutions could then be injected into the four holes drilled into the top aluminum plate of the clamp (Figure 1d).

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Importantly, this origami assembly method does not require adhesive tape, which can lead to contamination and nonspecific adsorption. Avoiding tape also speeds the assembly of the device and eliminates the need for laser cutting. The photosensitive pattern serves as the channel wall to separate solutions into different channels in all three dimensions. As described previously, the vertical connections are made by direct contact of paper channels or reservoirs on adjacent layers, and this avoids the use of cellulose powders.

The nine-layer device shown in Figure 1 was used to demonstrate the ability of the origami device to direct the flow of fluids in three dimensions. Specifically, 10.0 μL of the following four 1.0 mM aqueous solutions were injected through the openings in the top plate of the clamp: rhodamine 6G (red), erioglaucine (blue), tatrazine (yellow), and a mixture of erioglaucine and tatrazine (1:10, green) through the four injection ports in the aluminum clamp. The colored solutions passed through their designated channels and reservoirs without mixing.

Second, four 5.0 μL aliquots containing different amounts of glucose and BSA were injected into the four inlets at the top of the device (Figure 2b). The samples flowed toward the detection reservoirs, and a portion of these samples was allowed to react with the preloaded reagents for 10 min. Finally, the paper was unfolded so that both layers having detection reservoirs were accessible for colorimetric analysis. The degree of color change is directly related to the concentration of glucose or protein in the samples.

A comparison of parts c and d of Figure 2 indicates that the assay was successful and that there was no mixing between channels or reservoirs. Specifically, the color of the solution in the detection reservoirs exposed to glucose (samples s2 and s4, Figure 2b) or BSA (samples s3 and s4) changed from colorless to brown or from brown to blue, respectively. Although only two layers on the device were required for this very simple colorimetric assay, it is obvious that more complex analysis could be performed. To scale up the device for analyzing more analytes or more samples, additional layers might be required. However, since all layers of the multilayer network are fabricated simultaneously, the addition of more layers or more complex structures does not present much of a practical barrier.

Fluorescence detection usually provides substantially higher sensitivity and lower detection limits than simple colorimetric measurements. However, to the best of our knowledge, fluorescence detection has not thus far been used for 3-D μPAD-based assays. Accordingly, we fabricated three-layer μPADs (similar to the device illustrated in Figure 2b, but with just three layers) that could be used to carry out four simultaneous BSA assays using fluorescence detection. The assay is based on the dye epicocconone, which exhibits enhanced fluorescence in the presence of more layers or more complex structures does not present much of a practical barrier.

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of BSA (Figure S3, SI). The assay for BSA using the paper device was carried out as follows. First, 1.0 μL of a buffered epococcone solution was spotted onto each detection reservoir and then dried at 20 °C for 5 min. Second, 3.0 μL aliquots of buffered BSA solutions were injected into the four inlets at the top of the device. Third, the oPAD was placed in a humidity chamber for 30 min, during which time the BSA solutions passed to the detection reservoirs and reacted with the preloaded fluorescent dye. Finally, the bottom layer of the device was scanned using a fluorescence imager. Each scan was performed at 100 μm resolution and was complete within 1 min.

Figure 3a shows the result of an assay in which all four BSA aliquots were of the same concentration (3.0 μM), while in Figure 3b the concentrations of BSA were different (0, 0.75, 1.50, and 3.00 μM). Qualitatively, Figure 3b shows that the color of the detection reservoirs becomes darker as the concentration of BSA increases. To quantify these results, the images were imported into Adobe Photoshop CS2 and transferred to gray-scale mode. The mean fluorescence intensity was determined from the image histogram for each detection reservoir, and then it was background-corrected by subtracting the average intensity measured at the center of the paper where no BSA was present. These data constitute a calibration curve, which is shown in Figure 3c. The error bars represent the standard deviation of at least three independent measurements. The detection limit, defined as 3 times the standard deviation of the sample containing no BSA (0 μM) divided by the slope of the calibration curve, is 0.14 μM BSA. Because the fluorescence intensity, rather than the color change, is directly proportional to protein concentration, quantification by fluorescence is more straightforward than colorimetric detection.

To summarize, we have reported an origami-based method for fabricating 3-D paper microfluidic devices. This method provides a number of key advantages compared to previously reported approaches that rely on stacking individual layers and holding them in place with double-sided tape. First, origami fabrication only requires one photolithographic patterning step, regardless of the number of layers. Therefore, the devices can be made arbitrarily complex without much additional fabrication overhead. Second, oPADs can be produced by automated printing techniques and subsequently assembled without tools. Third, detection points can be placed on any layer of oPADs, because the paper can be easily unfolded to reveal them. Fourth, the resulting permanent record of an assay can be qualitatively analyzed using the naked eye, or the results can be quantified using a high-throughput automated scanner. We believe oPADs will prove promising for applications that involve low cost and simplicity.

**REFERENCES**