Review Article

Int Neurourol J 2013;17:155-161
http://dx.doi.org/10.5213/inj.2013.17.4.155
pISSN 2093-4777 · eISSN 2093-6931

International Neurourology Journal
INJ

Paper-Based Analytical Device for Quantitative Urinalysis

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Paper-based analytical devices are fluidic chips fabricated with extremely inexpensive materials, namely paper, thereby allowing their use as a zero-cost analytical device in third-world countries that lack access to expensive diagnostic infrastructures. The aim of this review is to discuss: (1) microfluidic paper-based analytical devices (μPADs) for quantitative analysis, (2) fabrication of two- or three-dimensional μPADs, (3) analytical methods of μPADs, and (4) our opinions regarding the future applications of μPADs for quantitative urinalysis.

Keywords: Paper-based analytical device; Zero-cost analytical device; Urinalysis; Quantitative detection

INTRODUCTION

Limitations of Conventional Analytical Devices

The conventional analytical device for urinalysis is a dipstick. The first dipstick was introduced in 1957 to detect glucose [1]. Today, urinalysis dipsticks can detect a number of analytes [2-7]. The measurements with dipsticks are typically carried out using the naked eye to evaluate the color change of the indicator when analytes present in urine are captured. However, dipsticks based on colorimetric detection for quantitative analysis require an expensive electrical reader, which is not appropriate for resource-limited environments such as third-world countries that lack access to electrical sources.

An Ideal Analytical Device

The World Health Organization has listed seven guidelines for ideal analytical tests. Specifically, tests must be affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users [8]. According to these criteria, the conventional analytical device is not adequate as an ideal analytical tool, because it is neither equipment-free, nor affordable.

Microfluidic Analytical Devices for Quantitative Analysis

A microfluidic paper-based analytical device (μPAD) is fabricated with an extremely inexpensive material, paper. This allows it to be used as a zero-cost analytical device [9]. μPADs, first introduced by Martinez et al. [10] in 2007, are microfluidic platforms that use cellulose paper to generate flow by the capillary action. Unlike the conventional dipstick, μPADs can enable fluid control including injection, transportation, and flow rate on the paper. These characteristics are key for the use of μPADs and their potential applications in zero-cost quantitative urinalysis.

TWO-DIMENSIONAL μPADs

μPADs can be fabricated in two-dimensions (2D) or three-dimensions (3D) to transport fluids in the vertical or horizontal dimensions depending on the diagnostic application.

Fabrication of 2D μPADs

Fabrication of μPADs can be divided into two types of patterning: chemical patterning [11-19] and physical shaping [20-23]. In this review, we focus on chemical patterning. The chemical treatment changes specific areas (or lines) of cellulose paper from hydrophilic to hydrophobic. The two parallel hydrophobic lines act as a channel, because the hydrophilic sample solution cannot penetrate the hydrophobic line or barrier. Consequently,

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Submitted: November 14, 2013 / Accepted after revision: November 26, 2013

the liquid flows in the channels owing to capillary action.

Photolithography

The chemical fabrication of μPADs using photolithography was first introduced by Martinez et al. [10,13]. They demonstrated a simple fabrication of patterned paper to create channels comprising hydrophilic cellulose paper combined by the hydrophobic polymer, SU-8 2010 photoresist (Fig. 1A). They soaked a piece of chromatography paper in SU-8 2010 photoresist, spun it, and then baked it to remove the cyclopentanone in the SU-8 formula. After removing cyclopentanone, they exposed the photoresist and paper to UV light through a photomask. After the exposure, the paper was baked to cross-link the exposed portions of the resist. To remove unpolymerized photoresist from the paper, it was soaked in propylene glycol monomethyl ether acetate and the pattern was washed with propan-2-ol. After it was patterned, the paper was more hydrophobic, including the area which was supposed to be the hydrophilic channel, because of residual resist bound to the paper. Accordingly, the hydrophilicity of the paper was increased by exposing the entire surface to oxygen plasma. The patterned paper could transport biological liquids to a specific position. For the glucose and protein assay, the patterned paper was functionalized by the addition of reagents, which changed color upon reaction with glucose or albumin (Fig. 1B).

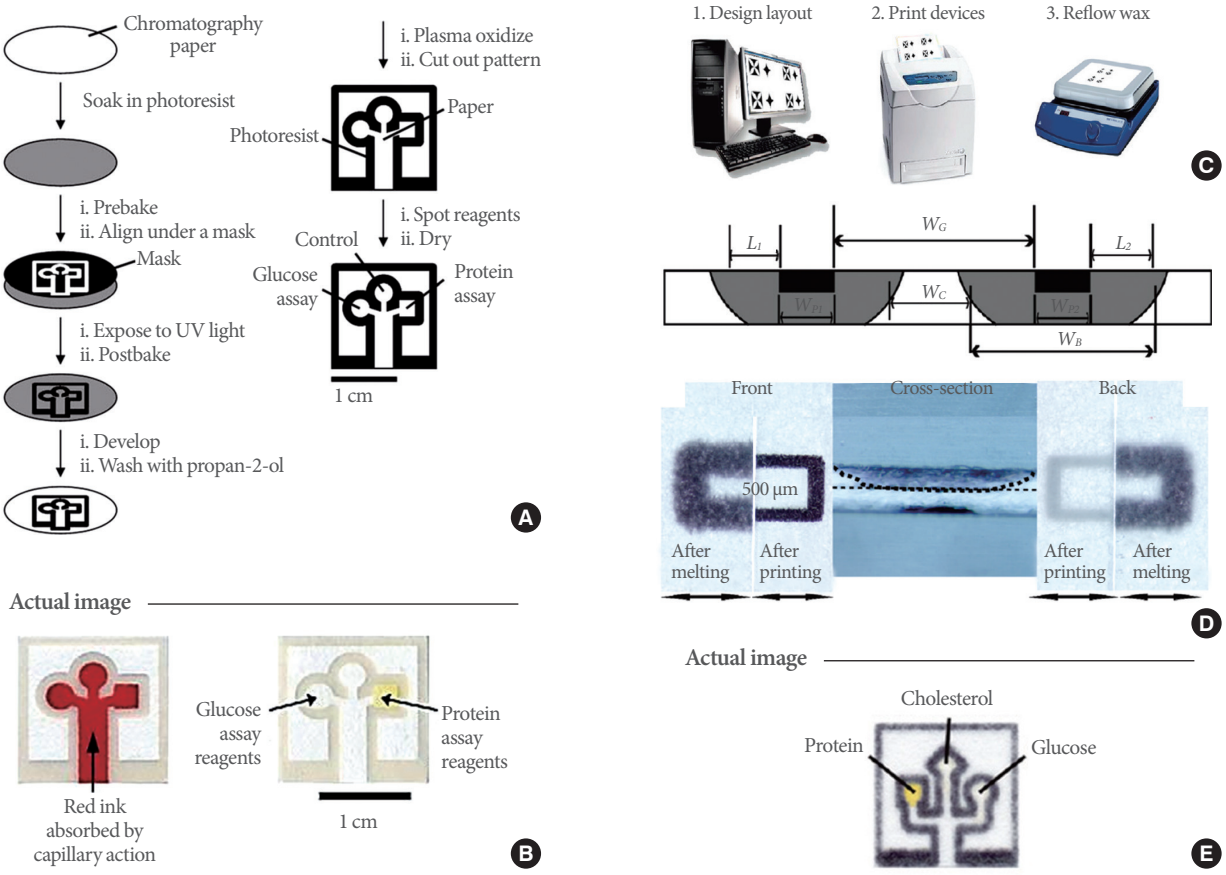

Fig. 1. Fabrication of two-dimensional microfluidic paper-based analytical devices (μPADs). (A) Schematic diagram of the fabrication of μPADs by patterning hydrophobic photoresist onto paper by photolithography. (B) Actual image of μPAD functionalized by wicking two reagents, which are sensitive to glucose and proteins, respectively. (C) The three steps for fabricating μPADs by wax patterning. (D) Schematic illustration of the spreading of molten wax during printing or the thermal process on paper, and the variables for the design of μPADs: W_P is the printed width of the wax, W_G is the gap between the edge of the parallel printed wax lines before melting, W_B is the thickness of the hydrophobic barrier, W_C is the width of the channel after thermal treatment, L is the length of the spread wax. (E) Actual image of μPAD functionalized by individually wicking three different reagents including albumin, glucose, and cholesterol.

 http://dx.doi.org/10.5213/inj.2013.17.4.155

Wax Patterning

One simple chemical patterning is wax pattering on the paper (Fig. 1C). The process comprises three steps including (1) designing the shape of the pattern, (2) printing the wax-pattern on the paper using a wax printer, and (3) heating the patterned paper on hot plate [16]. The first step is to design patterns as hydrophobic barriers on the chromatography paper using softwares such as Clewin or Adobe Illustrator. The designed patterns are printed using the solid-wax printer. After printing patterns on the paper, the printed paper is placed on a hot plate and the wax on the paper melts and spreads through the thickness of the paper, thereby forming hydrophobic barriers (Fig. 1D). The distance of the spread molten wax on paper can be described by Washburn's equation [24]. Carrilho et al. [16] suggested the following equation to predict the width of the hydrophobic barriers of wax after the thermal process:

$$W_B = W_P + 2L$$

Where W_B is the width of the wax barrier, W_P is the width of the printed line, and L is the distance of the width of the spread wax after heating ($2L = L_1 + L_2$) from the edge of the printed line in the direction vertical to the line. To predict the width of the hydrophilic path that acts as a channel determined by two parallel hydrophobic barriers of the wax, Carrilho et al. [16] also suggested equation:

$$W_C = W_G - 2L$$

Where W_C is the width of the channel between two parallel hydrophobic barriers and W_G is the gap between the two printed lines. It is easy to predict the width of the designed pattern on the paper after printing and the thermal process with these two equations. Therefore, the fabrication of μPADs using wax patterning is relatively simple to design and the formation of the channels on the paper is relatively facile. After wax patterning, the device was functionalized by depositing three reagents to detect albumin, glucose, and cholesterol (Fig. 1E).

Quantitative Analysis With 2D μPADs

The measurements of conventional analytical devices, such as dipsticks, are carried out using the naked eye and a calibration chart to determine the color change of the dye absorbed in the device when it binds to analytes. This colorimetric determination is required for quantitative measurements. However, these measurements can be expensive and might pose an issue for use in developing, third world countries. Recently, Cate et al. [25] demonstrated the ability of a μPAD to detect glucose quantitatively by measuring the color change of an absorbed dye on the paper upon reaction between glucose and the dye. The length of the color change was determined by the concentration of glucose in the sample solution. They called this concept distance-based detection. The distance-based detection represents a simple quantitative detection using μPADs without the requirement of an electric reader. However, there were issues with the reproducibility of assay, because the distance could be changed depending on the volume of the sample solution. Therefore, a relatively expensive micropipette was required.

We demonstrated the quantitative detection of albumin using μPADs and retention factors [26]. A retention factor (R_f) is the ratio of the distance traveled by albumin to that by the solvent in the sample solution in the sample solution. The μPAD was fabricated by printing wax-patterns on chromatography paper using a wax-printer. The shape of the wax-pattern was designed using Adobe illustrator CS5. The design of the pattern comprised two components: the sample pad to absorb the sample solution and the assay site to measure the distances traveled by the albumin and solvent in the sample (Fig. 2A). We spotted a 0–8 mg/mL sample solution of albumin on the sample pad. The assay method is very simple; the level of albumin was

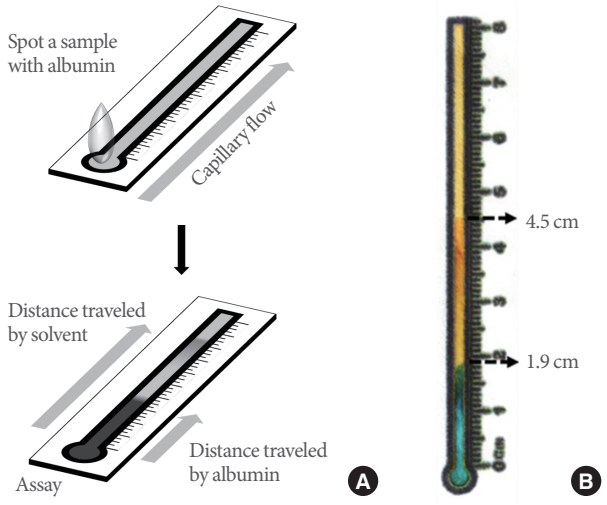

Fig. 2. Two-dimensional microfluidic paper-based analytical devices (μPADs) for quantitative urinalysis without requiring an external electrical equipment. (A) Schematic diagram of the assay. The assay is carried out by an analysis of the retention factor (R_f). R_f is the ratio of the distances traveled by albumin and the solvent in the sample. The R_f indicates the concentration of albumin in the sample. (B) Actual image of the device after the injection of the sample. In the image, the distance traveled by the solvent is 4.5 cm and the distance traveled by albumin (green) is 1.9 cm.

quantified by measuring the ratio of the distance traveled by the sample solvent versus the distance traveled by the albumin developed by the transfer of the solvent. The ratio indicates the concentration of albumin in the sample.

$$R_f = \frac{\text{The distance traveled by albumin}}{\text{The distance traveled by solvent}} = \text{The concentration of albumin}$$

The distance traveled by albumin in the sample strongly depends on the interactions among albumin, solvent, and the adsorbent (tetrabromophenol blue, TBPB) in the paper. There is a difference between the distance traveled by albumin and water because albumin interacts more with TBPB which is absorbed in the assay site within the channel, as compared to water. Additionally, the color of TBPB changes from yellow to blue when bound to albumins. The assay results can be quantified by calculating the ratio of the length of the color change of TBPB (from yellow to blue; distance traveled by albumin) versus the distance traveled by water (Fig. 2B).

In conclusion, we confirmed that the ratio was constant in samples with the same concentration of albumin regardless of the volume of the sample. This μPAD could be utilized without an external electronic reader. This μPAD also provided an equipment-free and inexpensive urinalysis to third world countries that lack access to external electronic equipment.

THREE-DIMENSIONAL μPADs

A 3D μPAD can run fluid horizontally and vertically; therefore, it has potential application in quantitative analysis and multiple assays within a compact space [27,28].

Fabrication of 3D μPADs

The original fabrication involves stacking papers to form vertical channels in the bundle of papers.

Stacking Papers With Tape

The 3D μPADs are fabricated by stacking layers of patterned paper with double-sided tape [27]. After stacking the layers of paper and tape, there are gaps between the layers of paper because of the thickness of the tape. Therefore, fluid cannot flow vertically across the hydrophobic tape and the gap between the layers of paper. The gap is closed by punching holes in the tape and filling it with hydrophilic materials such as cellulose powder [29], or by compressing the two layers of paper via a hole in

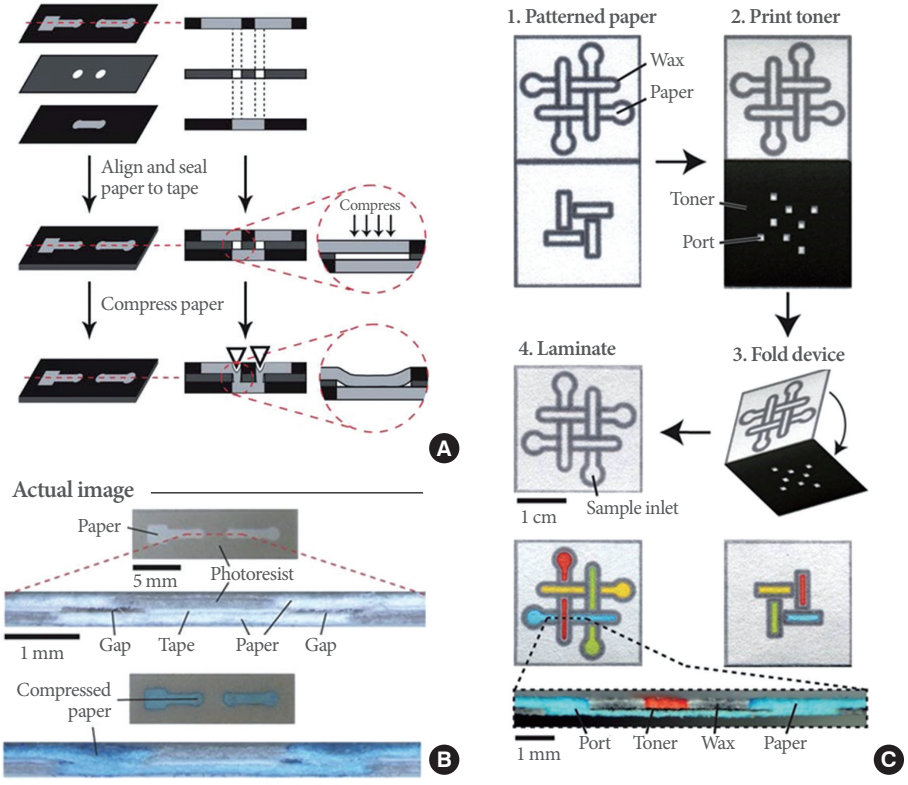

Fig. 3. Fabrication of three-dimensional (3D) microfluidic paper-based analytical devices (μPADs). (A) Schematic illustration of the fabrication of 3D μPADs by stacking layers of papers with tape. (B) A cross-section of the device after compression to close the gap between individual layers. (C) 3D μPAD fabricated by lamination with toner as a thermal adhesive. Ports act as vertical flow paths.

http://dx.doi.org/10.5213/inj.2013.17.4.155

the tape between the two layers (Fig. 3). The gaps filled with hydrophilic materials play a similar role to vertical channels.

Lamination With Toner

The fabrication of 3D μPADs uses toner as a thermal adhesive to bind multiple layers of patterned paper together. The toner can be used to print patterns with hydrophilic areas that act as ports, designed by an imaging software. Therefore, this method can be viewed as an alternative to the fabrication of 3D μPADs using tape that requires punching holes in the tape to form the vertical channels [30]. The fabrication of 3D μPADs using toner starts with printing wax-patterns on layers of paper followed by printing one of the layers with toner. The printed paper with wax and toner is folded to assemble the layered 3D device and the folded paper is passed through a laminator to melt the toner, thereby binding each layer together with toner that serves as a thermal adhesive. This method is a simplified version of the original method for preparing 3D μPADs using toner.

Quantitative Analysis With 3D μPADs

Lewis et al. [31] introduced 3D μPADs for quantitative detection based on counting the number of bars dependent on the concentration of analytes. The 3D μPAD was fabricated by assembling patterned chromatography papers and tapes (Fig. 4). Samples were injected onto the layer 1 on the top of the device; then, the liquid flowed through the device vertically to layer 7 in the bottom of the device. In layer 7, the sample separated into the 16 surrounding conduits (Fig. 4A). These conduits transported the sample to the bars of layer 1 that were visible on the top of the device. Layer 8 was a single-sided tape to prevent contact with other surfaces when the user placed the device on a surface. The 16 conduits, surrounding the central conduit of this device, were composed of paper disks containing hydroxyethyl-piperazineethane-sulfonic acid (HEPES) buffer salts, followed by two layers containing hydrophobic compound 1 and a paper disk containing a dried green dye. An assay with samples containing different amounts of hydrogen peroxide was performed. After the injection of the sample, the liquid was separated in layer 7 into the 16 conduits and moved through layers 6, buffering the sample with the HEPES. At the time, the sample arrived in layer 5 and hydrogen peroxide in the sample reacted with compound 1 to yield hydrophilic products [31]. The reaction changed the wetting properties of the paper to let the sample flow through the device to layer 2, where it dissolved the green dye in the paper disk (Fig. 4B, C). The liquid and the dye flowed into layer 1, where the green-colored liquid was revealed as green bars.

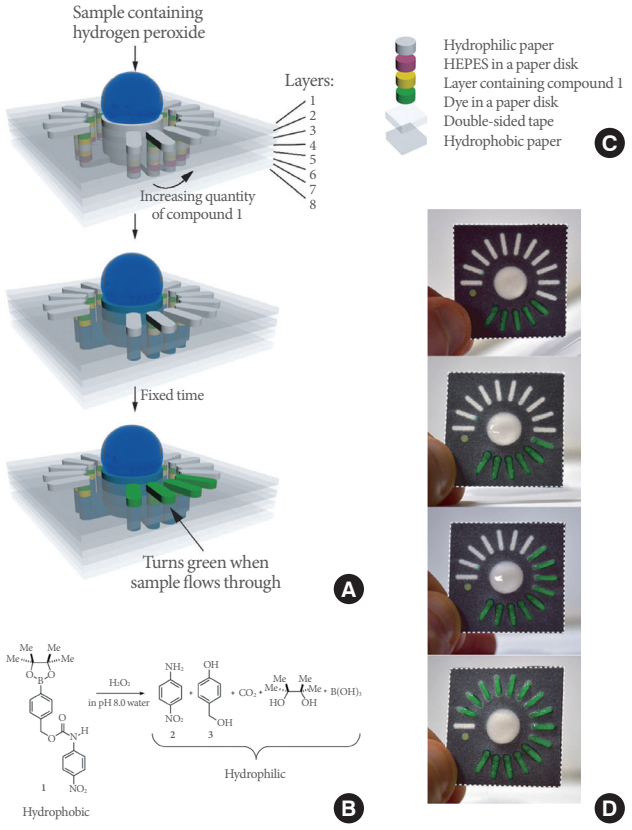

Fig. 4. Three-dimensional (3D) microfluidic paper-based analytical device (μPAD) for quantitative assays. (A) 3D illustration of the movement of sample flow. (B) The reaction mechanism between component 1 and hydrogen peroxide. (C) The layers assembled in a conduit. (D) Actual image of device after assay. The number of green colored bars vary depending on the amount of hydrogen peroxide in the sample at a fixed time during the assay. HEPES, hydroxyethyl-piperazineethane-sulfonic acid.

With an increase in the amount of hydrogen peroxide in the sample, the speed of the liquid flow through the disks containing compound 1 increased. Consequently, the number of green-colored bars resulting from the flow could differ depending on the amount of hydrogen peroxide in the sample at a fixed time during the assay. Therefore, the number of green-colored bars indicated the concentration of hydrogen peroxide in the sample solution (Fig. 4D).

In conclusion, Lewis et al. [31] described a simple quantitative assay by measuring the number of colored bars at a fixed time during the time. This assay did not require external equipment, and therefore, this 3D μPAD is a useful analytical device in the developing world.

CONCLUSIONS

In this article, μPADs for quantitative analysis were reviewed. μPADs fabricated using extremely inexpensive paper can serve as zero-cost analytical devices for urinalysis. Three-dimensional μPADs can transport liquid vertically and horizontally within the device. Accordingly, this renders the μPADs viable as novel analytical devices for the quantitative detection of target materials in urine without external electrical equipment, a commodity which is difficult to access by users in resource-limited environments such as third world countries. Additionally, 3D μPADs generate multiple flows in a small space to detect multiple targets in urine for the diagnosis of various diseases. Therefore, we assume that 3D μPADs will be developed for quantitative urinalysis. However, the fabrication of 3D μPADs is still time-consuming. Therefore, fabrication of 3D μPADs must be simplified to render zero-cost analytical devices for quantitative urinalysis.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

The research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT & Future Planning (MSIP) (No. NRF-2011-0017322).

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