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Novel, simple and low-cost alternative method for fabrication of paper-based microfluidics by wax dipping

Temsiri Songjaroen[a], Wijitar Dungchai[b], Orawon Chailapakul[c,d], Wanida Laiwattanapaisal[e,*]

[a] Graduate Program in Clinical Biochemistry and Molecular Medicine, Faculty of Allied Health Sciences, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand
[b] Department of Chemistry, Faculty of Science, King Mongkut's University of Technology Thonburi, 91 Prachautid Road, Thungkru, Bangkok 10140, Thailand
[c] Electrochemical Research Group, Department of Chemistry, Faculty of Science, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand
[d] National Center of Excellence for Petroleum, Petrochemicals, and Advanced Materials, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand
[e] Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand

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ABSTRACT

Paper-based microfluidic devices are an alternative technology for fabricating simple, low-cost, portable and disposable platforms for clinical diagnosis. Hereby, a novel wax dipping method for fabricating paper-based microfluidic devices (μPADs) is reported. The iron mould for wax dipping was created by a laser cutting technique. The designed pattern was transferred onto paper by dipping an assembly mould into melted wax. The optimal melting temperature and dipping time were investigated. The optimal melting temperature was in the range of 120–130 °C, and the optimal dipping time was 1 s. The whole fabrication process could be finished within 1 min without the use of complicated instruments or organic solvents. The smallest hydrophilic channel that could be created by the wax dipping method was 639 ± 7 μm in size. The reproducibility of the μPAD fabrication for hydrophilic channel width of the test zone and sample zone was 1.48% and 6.30%, respectively. To verify the performance of the μPAD, multiple colorimetric assays for simultaneous detection of glucose and protein in real samples were performed. An enzymatic assay and the bromocresol green (BCG) method were conducted on the paper device to determine the presence of glucose and protein in a test solution. The results of the assays were not significantly different from those of the conventional methods ($p > 0.05$, pair *t*-test and one-way ANOVA method). The wax dipping provides a new alternative method for fabricating lab-on-paper devices for multiple clinical diagnostics and will be very beneficial for developing countries.

1. Introduction

Lab-on-a-chip (LOC) devices have been developed to minimise the scale of laboratory tests. These LOC devices only need a small volume of the reagents and samples, therefore providing portable and disposable diagnostic devices [1,2]. However, the fabrication processes of LOC devices are quite complicated, as demonstrated by the need for mechanical components, such as pumps or valves, to control the flow of the solution within the microfluidic device.

Currently, paper tests or strip tests are widely used in clinical laboratories for diagnosing various diseases. The strip tests are utilised in several areas of healthcare, such as screening tests, self-monitoring by patients, treatment monitoring or preventive medicine. Recently, Whitesides's group has developed microfluidic paper-based analytical devices (μPADs) [3], also known as a lab-on-paper technology. The concept of a μPAD is to perform an experiment on a small piece of paper. Unlike the conventional strip test, the lab-on-paper devices can be configured for multiple tests or detection of several analytes simultaneously on one device [4]. Furthermore, quantitative measurement using a μPAD is feasible based on a variety of detection methods. Colorimetric assays on paper [5,6] are widely used to quantify the colour intensity of the test zone because it is easy to actualise and only requires simple equipment such as a digital camera, cell phone or scanner [4,7]. Moreover, a μPAD is able to perform several types of measurements, including electrochemical [8–11], transmittance [12], fluorescence and absorbance measurements [12,13]. According to WHO guidance, lab-on-paper devices are very promising for use as diagnostic tools in developing countries [4].

Currently, lab-on-paper devices have become an attractive technology for a number of research groups, resulting in the development of numerous methods for their fabrication. Various methods for fabrication of the μPAD have been proposed in the literature, including the following: photolithography [3,14], polydimethylsiloxane (PDMS) plotting [15], inkjet printing [16,17], cutting [18], plasma etching [19], wax printing [20–22] and wax screen-printing [23]. Photolithography was the first reported fabrication method, which involved the use of hydrophobic

* Corresponding author. Tel.: +66 02 218 1081x307; fax: +66 02 218 1082.
E-mail address: wanida.k@chula.ac.th (W. Laiwattanapaisal).

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SU-8 photoresist and UV light to construct the hydrophobic and hydrophilic barriers on the paper [3]. This method can create a small barrier (200 μm width) and yield sharp resolution between the hydrophilic and the hydrophobic channels. However, the photolithography technique requires several organic solvents, which can damage the flexibility of the paper. In addition, photolithography requires expensive instrumentation and the fabrication process involves many complicated steps. The PDMS plotting method uses a desktop plotter and a hydrophobic polymer, namely PDMS, to create hydrophilic patterns on paper [15]. Although PDMS plotting does not destroy the flexibility of the paper, this method requires special preparation of PDMS diluted in hexanes [15]. The inkjet printing method involves removing a hydrophobic coating from the paper by using a modified an inkjet printer to print a solvent onto paper that has been coated with a hydrophobic polymer. The solvent melts the hydrophobic polymer, resulting in the formation of hydrophilic areas on the paper [16,17]. This method can create direct patterning on paper, which is a benefit for high mass production. Plasma etching is a method to remove a hydrophobic coating on paper by using plasma treatment [19]. However, the hydrophilic areas generated by both the inkjet printing and plasma etching methods are still exposed to solvents and polymers during the fabrication processes. In cutting method, a knife plotter is used to cut paper into designed microfluidic channels [18]. Nevertheless, the paper devices have to use tape to help support the paper structures, limiting the ability to produce variety of free-standing hydrophilic patterns [4]. Wax printing has several advantages such as fast and easy to produce, use commercially available printer and hotplate, and preserve native paper chemistry [20,22]. However, it is difficult to produce the exact designed patterns with high resolution due to the spread of the wax. Careful determination of wax spreading must be considered before production of the channels [4,22].

Common obstacles to fabricating lab-on-paper devices for most developing countries are the cost of the instruments used in the fabrication process, such as a spin coater, UV lithography system, and plasma cleaner. Although wax screen-printing, which requires only a hot plate for patterning wax onto paper, is economical and therefore promising for developing countries, it suffers from poor reproducibility [23]. Hence, a simple, rapid and cheap fabrication technique that also provides good resolution and repeatability needs to be developed.

This paper proposes a novel method for the fabrication of paper-based microfluidic devices by wax dipping. Wax is a material generally used worldwide because it is inexpensive and non-toxic. The wax dipping procedure requires only a hot plate for patterning hydrophobic and hydrophilic areas on Whatman No.1 paper. The fabrication of the μPAD is simple and only involves a single step. Moreover, the wax dipping method can create patterns on paper without using any chemical compounds, so that the hydrophilic area is not exposed to any solvents or polymers. To demonstrate its applicability to real world situations, we also employ the paper device for colorimetric assays for simultaneous detection of glucose and protein in real human samples.

2. Materials and methods

2.1. Materials and chemicals

Whatman No.1 filter paper was purchased from Whatman International, Ltd. (Maidstone, England). White Beeswax pellets were purchased from a stationary shop in Bangkok, Thailand. Glass slides were obtained from Sail Brand (Jiangsu, China). Iron moulds (1 mm thick) were made-to-order by a laser cutting shop in Bangkok. Permanent magnets were purchased from a local area shop. Other equipment that was purchased included a Canon digital camera (7.1 megapixels, Powershot A570 IS), an IKA® hotplate (C-MAG HS7, Wilmington, USA), and an Olympus Microscope (Olympus BX50, Tokyo, Japan).

D-(+)-Glucose, glucose oxidase (from *Aspergillus niger*-Type II), peroxidase (Type I from horseradish), potassium iodide, ethylenediaminetetraacetic acid disodium salt (EDTA), Brij™ 35 and bovine serum albumin were purchased from Sigma–Aldrich. Sodium hydroxide and succinic acid were purchased from Merck. Bromocresol green was supplied by BDH Chemicals. Glucose reagent (GLUCOSE liquicolor) and human control serum (Humatrol N and Humatrol P) were obtained from HUMAN (Wiesbaden, Germany). Accu-Chek for blood glucose monitoring was obtained from Roche Diagnostics. All chemicals were prepared in MilliQ water.

2.2. Wax dipping fabrication method

To create a mould for wax dipping, a local laser cutting shop cut an iron bar into the desired shape and size using a laser cutting technique. The price for cutting an iron mould was about $0.35 US per piece. An iron mould can be repeatedly used to produce numerous pieces of μPAD. In particular, based on our experience so far, more than 1000 pieces of μPAD have been fabricated from the same iron mould without affecting the resolution. For the wax dipping method, white Beeswax pellets were put in a beaker and heated until they melted using a hotplate. To ensure that the temperature was kept in the range of 120–130 °C, the temperature was monitored throughout the experiment by means of an electronic contact thermometer (IKA® ETS-D5). Whatman No.1 paper was cut into a 1.5 cm × 2.5 cm piece and placed onto a glass slide. Then, the iron mould was put onto the paper, and it was temporarily attached by means of magnetic force using a permanent magnet placed on the backside of the glass slide. Next, the assembly was dipped into a chamber of melted wax for 1 s. After the paper was cooled to room temperature, it was peeled off of the glass slide, and the iron mould was removed from the paper. The wax-dipping fabrication process for the μPAD is shown in Fig. 1. Then, the hydrophobic and hydrophilic areas of the μPAD were observed under a microscope (Fig. 2).

2.3. Applicability of the μPAD for clinical analysis

To evaluate the colorimetric assays on the μPAD, the paper device was designed to be a Y shape, which was composed of two test zones (circular shape, 3 mm width) for the simultaneous detection of glucose and protein. For the glucose assay, the reagent ratio was adopted from Self-Stik reagent strips (Chungdo Pharm. Co., LTD, Korea). A volume of 0.5 μL of a 10 mg mL^{-1} potassium iodide solution was spotted onto the paper test zone and allowed to dry for 5 min. Then, a 5 μL mixture of 451 U mL^{-1} glucose oxidase and 186 U mL^{-1} peroxidase was dropped on the same test zone. For protein detection, 0.5 μL of 10× bromocresol green (BCG) working reagent [24] was dropped on the other test zone. Then, the paper was allowed to dry at room temperature. To detect glucose and protein, the bottom side of the paper devices was dipped in sample solutions until colour developed at both test zones and could be observed by the naked eye. The colour of the test zones on the μPADs were captured by a digital camera, and then, the colour intensities were analysed using Adobe Photoshop CS2.

3. Results and discussions

3.1. μPAD made with the wax dipping method

The wax dipping method uses melted wax to coat a hydrophobic barrier onto paper while the hydrophilic channel is protected by an