One-step patterning of hollow microstructures in paper by laser cutting to create microfluidic analytical devices†

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In this paper, we report a simple, low-cost method for rapid, highly reproductive fabrication of paper-based microfluidics by using a commercially available, minitype CO2 laser cutting/engraving machine. This method involves only one operation of cutting a piece of paper by laser according to a predesigned pattern. The hollow microstructures formed in the paper are used as the 'hydrophobic barriers' to define the hydrophilic flowing paths. A typical paper device on a 4 cm × 4 cm piece of paper can be fabricated within ~7–20 s; it is ready for use once the cutting process is finished. The main fabrication parameters such as the applied current and cutting rate of the laser were optimized. The fabrication resolution and multiplexed analytical capability of the hollow microstructure-patterned paper were also characterized.

Introduction

The development of simple, inexpensive and portable bioassays is a crucial need in less-industrialized countries, remote regions, and home health-care settings where the detection of disease and the monitoring of health are becoming increasingly important.1–4 Modern laboratory analytical instruments often are difficult to use in these situations, since they are expensive and large, and require complicated skilled-labour instrumentation. Microfluidic paper-based analytical devices (µPADs) (or lab-on-a-paper analytical systems) which are generally created by patterning hydrophobic materials (e.g., polymer and wax) in hydrophilic paper or other porous membrane (e.g., nitrocellulose), have recently received considerable interest.5–27 The µPADs are low-cost, portable, easy to use (to carry and store) and disposable, only require a low sample volume, and can be readily disposed of in an environmentally friendly way via incineration. In particular, they can enable the multiplex analysis and complex microfluidic function of the conventional lab-on-a-chip devices without the need for any external power supplies. These attractive features make them ideally suitable for use as a new class of point-of-care diagnostic devices in the forenamed remote and resource-limited settings. So far, µPADs can be fabricated by photolithographic,28 wax printing,7–15 toner printing,16 inkjet printing,17 flexographic printing,18,19 plasma etching,20 knife-based cutting,21 laser treating of hydrophobic paper22 and plotting.23,24 Further comparison information on these previous methods can be found in recently published review articles.25,26 Although each method has its own advantages, the need still remains for more simple fabrication techniques that can be widely implemented in a wide variety of laboratories for large-scale, highly reproducible production of µPADs at minimized cost.

This article describes a simple laser cutting method for the one-step fabrication of µPADs by patterning hollow microstructures in paper with the use of a commercially available, minitype CO2 laser cutting/engraving machine. The fabrication process involves only a single operation of paper cutting by laser. When the laser cuts the paper according to a pattern that is predesigned on a personal computer (PC) with the aid of drawing software, the heat produced by the laser will burn the paper across its thickness. The formed complete hollow microstructures can act as the 'hydrophobic barriers' to produce microfluidic paths that are capable of distributing...
fluids in the resulting patterned paper via capillary action. Fig. 1 shows a schematic illustrating the principle behind the proposed one-step laser cutting method.

The laser cutting method is valuable for μPAD fabrication for several reasons: (i) the one-step fabrication process is quite rapid, and a typical paper device on a 4 cm × 4 cm piece of paper could be fabricated within ~7–20 s (depending on the complexity of the device’s pattern to be created); (ii) each device is fabricated separately and is ready for further use once the cutting process is accomplished, while large sheets of patterned paper fabricated by any other non-cutting method must be cut into small pieces of single device before use; (iii) since no additional materials are required to construct the hydrophobic barriers in paper in most cases, the material cost of the devices depends almost entirely on the type of paper that is used; (iv) the inexpensive minitype CO2 laser cutting/engraving machine used in this work costs ~$450, so it is accessible to a broad range of laboratories; (v) excellent fabrication reproducibility can be achieved due to the laser’s high stability; and (vi) it should be further extended to be compatible with flow-line processes for manufacturing in bulk.

Herein, we optimized the applied current and cutting rate of the laser for cutting paper. We also defined the fabrication resolution of this method. The multiplexed assay capability of the resultant air barriers-patterned μPADs was additionally demonstrated by using a set of three-path devices for simultaneously detecting glucose and bovine serum albumin (BSA) as two model analytes.

**Experimental**

**Reagents and apparatus**

Glucose, BSA, glucose oxidase and horseradish peroxidase were purchased from Sangon Biotech Co., Ltd (Shanghai, China). Potassium iodide, tetrabromophenol blue (TBPB), citrate acid, and sodium citrate were the products of Shanghai Jingchun Reagent Co., Ltd (Shanghai, China). Rosin was from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All other reagents were of analytical grade. TBPB solution (3.3 mM) was prepared in 95% ethanol solution. Citrate buffer solution (0.25 M, pH 1.8) was prepared with citric acid and sodium citrate. Each artificial urine sample (pH 6) contained 1.1 mM lactic acid, 2 mM citric acid, 25 mM sodium bicarbonate, 170 mM urea, 2.5 mM calcium chloride, 90 mM sodium chloride, 2 mM magnesium sulfate, 10 mM sodium sulfate, 7 mM potassium dihydrogen phosphate, 7 mM dipotassium hydrogen phosphate, and 25 mM ammonium chloride. All solutions were prepared with deionized water (specific resistivity >18 MΩ cm) from an ultrapure water system (UPS-II-20L) that was provided by Chengdu Yue Chun Technology Co. Ltd (Chengdu, China), unless otherwise specified.

Shuangquan filter paper (9 cm in diameter) was obtained from Hangzhou Xinhua Paper Industry Co., Ltd (Hangzhou, China). Hydrophilic red ink was the product of Guilin Fuxuan Chemical Product Co., Ltd (Guilin, China). The CO2 laser cutting/engraving machine (XB-3020) was provided by Shandong Xibang Laser Equipment Co., Ltd (Shandong, China).

The actual widths of the paper paths were measured using an optical microscope (XYH-06) that was from Shanghai Optical Instrument Factory (Shanghai, China). All images were captured using a Sony H50 digital camera (Japan).

**Patterning hollow microstructures in paper by laser cutting**

The patterns of the hollow microstructures and the sizes of the devices to be fabricated as black lines on a white background were predesigned on a PC using Coreldraw X5 drawing software. For the μPAD fabrication, a piece of paper was placed flat onto the working platform in the laser cutting/engraving machine. Then the laser with a spot size of ~0.3 mm cut the paper according to the designed pattern at the applied current of 5 mA, cutting rate of 20 mm s⁻¹, laser wavelength of 10.64 μm and laser power of 40 W to produce the hollow microstructure-patterned paper device.

**Bioassays of glucose and BSA**

Shape-coded three-path μPADs were fabricated and used for the simultaneous qualitative and semiquantitative bioassays of glucose and BSA based on the well-established principle of color reactions²⁷,²⁸ and reported general procedures. For each device, the square region was spotted with 0.5 μL of a potassium iodide solution (0.6 M) and 0.5 μL of an enzyme solution consisting of a 1:5 (v/v) mixture of glucose oxidase and horseradish peroxidase solution sequentially for the glucose assay. The circular region was spotted with the potassium iodide solution but not with the enzyme solution and served as the control zone for the glucose assay. In addition, 0.5 μL of a citrate buffer solution (0.3 M, pH 1.8) and 0.5 μL of TBPB solution (3.3 mM) were added to the diamond region for the BSA assay. The spotted reagents were allowed to air dry at room temperature (25 °C). Then the obtained paper devices were used for the analysis of artificial urine samples containing varying concentrations of glucose and BSA.

**Results and discussion**

**One-step fabrication of μPADs with hollow microstructures by laser cutting**

Fig. 2 shows the figures of the hollow microstructure-patterned two-path μPAD that was created by the proposed laser cutting method (according to the corresponding pattern shown in Fig. 1) before and after adsorbing hydrophilic red ink by capillary action. One can find from Fig. 2A that the one-step laser cutting could allow for the formation of well-defined hollow microstructures in paper. The patterned hollow microstructures across the thickness of the paper acted well as the ‘hydrophobic barriers’ to direct the flowing of the red solutions in the device (Fig. 2B–D). In other words, the hydrophilic paper micropaths were defined by the patterned regions of hollow microstructures. Four more examples of μPADs, i.e., three-, four-, eight- and twelve-path devices were also fabricated (see Fig. S1 in ESI†).

In a previous complementary study by Fenton et al.²¹ the multiplex lateral-flow test strips that were cut off the paper body...
by a knife were so flexible that they must be attached onto additional supporting substrates such as glass plates by using adhesive tape before use. The advantage of the pattern design of joining the two paths to their circumjacent paper body presented herein is that the resulting hollow microstructure-patterned device can be directly handled without the need for any extra supporting substrates.

**Optimization of the main experimental conditions**

To optimize the main experimental conditions for the fabrication of µPADs, a set of circular paper microzones of approximately 5 mm in diameter were fabricated by the laser cutting at varying applied currents and cutting rates (Fig. 3). As the results manifested in Fig. 3, both the applied current and the cutting rate played a vital role in generating complete hollow microstructures in the paper. At the currents ≥5 mA, complete hollow microstructures across the paper’s thickness could be obtained at all the tested cutting rates except for the rate of 25 mm s⁻¹; they could effectively localize the spreading of the red ink solution in the resulting paper microzones (Fig. 3d, g, h, l and p). Moreover, when the relatively small applied currents (i.e., 4.5 and 4.8 mA) and low cutting rates (i.e., 15 and 18 mm s⁻¹) were adopted synergistically, they could also enable the production of complete hollow structures in the paper (Fig. 3k, n and o). In contrast, if the applied current was too small (i.e., 4 mA), only the cutting marks slightly visible to the naked eye were formed on the surface of the paper at almost all the tested cutting rates, across which the red ink solution could easily spread (Fig. 3a, b, e and i). Interestingly, the insufficient heat per unit area of the paper (as a result of the inappropriately synergistic applications of the cutting rates and currents of the laser) and the non-homogeneous structures of the chosen paper might contribute to the formation of the paper microzones that suffered from partial hollow microstructures (Fig. 3c, f, j and m). In order to realize the rapid patterning of complete hollow microstructures in the type of paper that was used in this work at relatively low energy consumption, the applied current of 5 mA and the cutting rate of 20 mm s⁻¹ were thus recommended for all of the following experiments.

In general, the fabrication time for the hollow microstructure-patterned µPADs (without taking into account the pattern design time) mainly depends on the complexity of the device to be created. It was experimentally found that a typical device on a 4 cm × 4 cm piece of paper could be fabricated within ~7–20 s under the optimized experimental conditions. For instance, it took about 7, 11, 15, 17, 19 and 14 s to accomplish the production of one paper microzone shown in Fig. 3g, the two-path device shown in Fig. 2, and the three-, four-, eight- and twelve-path devices shown in Fig. S1 in ESI† respectively.

**Characterization of the fabrication resolution**

The fabrication resolution of the hollow microstructure-patterned paper was further characterized, with results shown

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Fig. 2 The pictures of a hollow microstructure-patterned two-path µPAD (4 cm × 4 cm) before (A) and after (B–D) absorbing hydrophilic red ink by capillary action. The scale bar is 8 mm. The applied current and cutting rate of laser were filled to be 5 mA and 20 mm s⁻¹, respectively.

Fig. 3 The figures of circular paper microzones of approximately 5 mm in diameter fabricated at different applied currents and cutting rates of laser after absorbing hydrophilic red ink by capillary action.

Fig. 4 A hollow microstructure-patterned eight-path µPAD (4 cm × 4 cm) with different predesigned path widths before (A) and after (C) absorbing hydrophilic red ink by capillary action: (a) 0.2, (b) 0.4, (c) 0.6, (d) 0.8, (e) 1.0, (f) 1.2, (g) 1.4, and (h) 1.6 mm. The scale bar is 5 mm. The magnified partial image of part (A) was show in part (B), in which the parts indicated by the arrows were hollow. The fabrication conditions were the same as described in Fig. 2.
in Fig. 4. In the design, eight straight paper paths with pre-designed widths changing from 0.2 to 1.6 mm were branched out of a circle of ~5 mm in diameter (Fig. 4A). Hydrophilic red ink was added into the center circular reservoir in the resultant paper device; it would wick outward by capillary action. Fig. 4C suggests that the smallest hydrophilic path (strip) the red solution can pass is 0.8 mm. The actual width of the paper path is estimated to be ~0.5 mm, which is thus considered as the highest fabrication resolution that this proposed laser cutting method can achieve in the chosen type of paper. On the other hand, too narrow a pre-designed path width (i.e., 0.2–0.6 mm) would only lead to the production of hollow microstructures in the paper (Fig. 4B, the parts indicated by the arrows), because the paper paths bent had been burned by the laser. Moreover, the average width of the hollow microstructures that functioned as the ‘hydrophobic barriers’ obtained at the filled experimental conditions, namely the applied current of 5 mA and the laser cutting rate of 20 mm s⁻¹, was estimated to be ~0.4 mm (Fig. 4).

A quantitative comparison between the actual widths of the paper paths that were shown in Fig. 4 (i.e., paths d–h) and the corresponding pre-designed widths was further made. As can be seen from Fig. 5, the actual path width is in a very nice linear relation with the designed width. A regression equation of \( y = 0.7783x - 0.1429 \) (\( x \geq 0.8 \)) and a correlation coefficient of 0.9993 were obtained.

**Paper microzone array**

No free-standing hydrophilic paper microzone has been reported in the previous study of the knife-based cutting method.²¹ Herein the laser cutting was successfully utilized to fabricate an array (8 × 8) of free-standing circular paper test microzones (Fig. 6A). Although potential cross-contamination would take place between the adjacent paper microzones, it was demonstrated herein that this could be easily achieved by additionally hydrophobizing of the corresponding paper path that joined two microzones by using a very low amount of hydrophobic rosin (Fig. 6B, the parts indicated by the arrows). Other hydrophobic polymers such as polycarbonate might also be available, but the rosin solution (600 mg mL⁻¹) prepared with ethanol was chosen for the reason that it could be used in an open environment without an external fume cupboard. Moreover, the average diameter of the 64 circular microzones was estimated to be ~5.25 mm, which was in good agreement with the extrapolated value (~5.31 mm) predicted from the above regression equation assuming the designed diameter of 7 mm for each paper microzone. In addition, a relative standard deviation as low as ~0.2% of the measured diameters of these microzones was achieved, indicating the good fabrication reproducibility of the proposed laser cutting method.

**Simultaneous colorimetric detection of glucose and BSA**

The simultaneous qualitative and semiquantitative detection of glucose and BSA followed with the well-established principle of color reactions,²⁷,²⁸ by using a set of shape-coded three-path microarrays (µPADs) (Fig. 7). Each paper device was coded with square, circular and diamond shapes to minimize the operator or reading error (Fig. 7A). The square test region designed for the glucose assay (Fig. 7A,a) was spotted with a potassium iodide solution and a mixture of glucose oxidase and horseradish peroxidase solution. The circular region as a control zone for the glucose assay (Fig. 7A,b) was spotted with the potassium iodide solution but not with the enzyme solution. The final square and circular regions after spotting with the reagents would have clear surfaces. The diamond test region for the BSA assay (Fig. 7A,c) was spotted with a citrate buffer solution and a TBPB solution that would produce a yellow surface.

As obviously shown in Fig. 7B, the presence of glucose and BSA in artificial urine samples resulted in the color changes
from clear to brown (as a result of the enzymatic oxidation of iodide to iodine\(^27\)) in the test circular regions and from yellow to blue (caused by the binding of TBPB with BSA\(^28\)) in the test diamond regions, respectively (Fig. 7B,2–6). The varied concentrations of the two analytes in different samples presumably contributed to the color variations developed in the corresponding test regions. On the other hand, no color changes were observed in the square and circular regions in the absence of glucose and BSA (Fig. 7B,1). Moreover, in all of these experiments (1–6), the surfaces of the circular (control) regions always remained clear as original (data not shown). These results demonstrated the use of the hollow microstructure-patterned \(\mu\)PADs in multiplexed bioassays. The used paper devices could be readily disposed of safely via incineration (Fig. 7C). The colorimetric readout was chosen so that any untrained personnel could provide reliable results of the multiplex assays without a specialized detector\(^{29,30}\) and it might be further compatible with visualization by a cell phone.\(^{31}\)

**Conclusions**

We have developed an efficient cutting method for patterning hollow microstructures in paper to create \(\mu\)PADs with the use of a commercially available minitype laser cutting/engraving machine. The one-step fabrication process for a typical single paper device could be finished in \(~7–20\) s and the device was ready for use once the laser cutting operation was completed. The material cost of the resulting \(\mu\)PADs with patterned hollow microstructures as the hydrophobic barriers depended almost entirely on the type of the paper used. We foresee laser cutting as a simple, rapid and low-cost paper micropatterning method for highly reproducible, large-scale production of \(\mu\)PADs in a wide variety of laboratories for point-of-care diagnostic applications. In addition, this method could also hold the potential of being adapted to flow-line processes for manufacturing in bulk.

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