FABRICATION OF PAPER-BASED MICROFLUIDICS BY MICROEMBOSSING

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Abstract - In recent years, microfluidic paper-based analytical device (μPAD) has been receiving attention because it is simple, inexpensive and power-free for low-cost chemical, biological and environmental detection. Moreover, paper is lightweight, easy to stack, store and transport, biodegradable, biocompatible, good for colorimetric tests, flammable for easy disposal of used paper-based diagnostic devices by incineration, and can be chemically modified. Different methods have been demonstrated to fabricate μPADs such as solid wax printing, craft cutting, photolithography, etc. In this study, we proposed and demonstrated microembossing to fabricate μPADs. The processing parameters like temperature, embossing pressure and embossing time were systematically studied. The results showed that the temperature and embossing time played important roles in fabrication of μPADs. Heavy metal detection was conducted to verify the performance of μPADs as fabricated. Fabrication of μPADs can be completed within 10 seconds.

Keywords - Embossing, Filter Paper, Heavy Metal Detection, Paper-Based Microfluidics, Wax

I. INTRODUCTION

Microfluidics have been gaining attention and been widely studied for more than two decades owing to its competitive advantages over conventional analytical methods such as fast response, high throughput, reduced waste product, high sensitivity and great portability. There are various substrates that can be used for microfluidics devices such as silicon, glass, polymer, elastomer, etc. [1], [2]. Paper is considered an attractive alternative because of its low cost and ubiquity [3]. Due to its hydrophilic and porous nature, utilization of paper provides several merits including power-free fluid transport via capillary, high surface to volume ratio, being easy for reagent storage and good to colorimetric tests. With the potential utility in point-of-care testing (POCT) and onsite analysis, microfluidic paper-based analytical devices (μPADs) has become a promising alternative to traditional laboratory experiments [4], [5].

Recently, many different fabrication methods for μPADs have been reported such as cutting, ink jet printing, plasma etching, photolithography, and wax printing [6], [7]. Each method has its pros and cons. Photolithographic technique involves filter paper being patterned by photoresist, which forms solid barriers that can achieve high resolution and small dimension. However, the cost to fabrication is not friendly and the chip is easily cracked [8]. Ink jet printing method makes barriers by printing special ink, but it may not be suitable for mass fabrication due to the speed [9]. Alternatively, wax printing is a low-cost fabrication method to manufacture paper-based devices [10]-[12]. By using wax, the total process duration can be 5-10 minutes and the fabrication method becomes simple. Despite of the advantages, the wax heating procedure is a concern since the heating temperature in most of the cases is 100°C above and it takes more than 10 minutes to complete. The embossing technique was also used to fabricate μPADs where a certain pressure is applied to the filter paper [13]. Paulo de Tarso Garcia, Thiago Miguel Garcia Cardoso, Carlos Diego Garcia, Emanuel Carrilhocd and Wendell Karlos Tomazelli Coltro used a hot metal stamp (150˚C) to contact the filter paper embedded with paraffin, which is on top of a pristine filter paper [14]. The wax was then melted and diffused in to the pristine filter paper to form the hydrophobic barriers. Although it is relatively efficient and possibly scalable, the concern of heating procedure still remains. In this study, we proposed a relatively fast, simple, reliable and in expensive process, i.e. microembossing to fabricate μPADs. Different processing conditions such as embossing temperature, pressure and time will be discussed.

II. MATERIAL AND METHODS

A. Materials and equipment

Paraffin wax was purchased from Sigma-Aldrich. Whatman filter papers #3 (diameter: 5.5 cm) was purchased from GE Healthcare. The embossing machine was purchased from Cometech (QC-601T). The fluorescent microscope, which is used for flow visualization, was purchased from Nikon (TE-2000S). Cupric sulfate ($CuSO_4$) and potassium ferrocyanide ($K_4Fe(CN)_6$) for demonstration of heavy metal detection were purchased from Sigma-Aldrich. Rhodamine Hydrazone Chemosensor for Cu(II) was synthesized from rhodamine B [15].

B. Method

A metallic mold was made by the computer numerical controlled (CNC) machine. In order to be used for multi-detection of different heavy metal ions, here we designed a pattern with 6 channels connected by a central reservoir and each channel has a reservoir at the end (Fig. 1). The length of the channel, radius of the central reservoir and radius of the end reservoir are

1.2cm, 0.2cm, and 0.1cm, respectively. The width of the channel is 0.2cm. Prior to fabrication, a plastic substrate coated with paraffin wax was made. The filter paper was then placed on top of the plastic substrate, followed by placing the mold on top of the filter paper inside the embossing machine under pressure as shown in Fig. 2. The operating temperature was set around 55°C and the embossing time ranged from 5 to 10 seconds. After embossing, the patterned paper was removed from the machine and ready for tests. For flow visualization and characterization, the □ PAD was placed under the microscope (SMZ-745T, Nikon) and the ink solution was dispensed in the central reservoir. The flow was recorded by the computer and analyzed by the software.



Fig. 1 The metallic mold for microembossing.

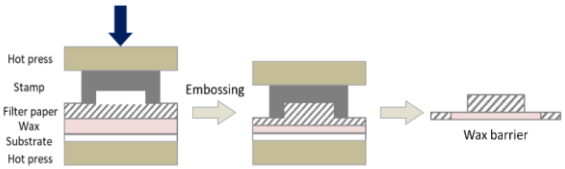

Fig. 2 The schematic of the microembossing process.

As to detection of heavy metal ions, first we dispensed 1 μl of Rhodamine Hydrazone solution in 2 of 6 reservoirs (reservoir "a" in Fig. 3), which is a copolymer for sensing Cu^{2+}, followed by 1.0×10^{-3} cm^3 of $K_4Fe(CN)_6$ solution in another 2 reservoirs (reservoir "b" in Fig. 3). After drying in the room temperature, either 2.0×10^{-2} cm^3 $CuSO_4 \cdot 5H_2O$ solution or $FeCl_3$ solution was dispensed in the central reservoir of the μPAD to carry out heavy metal detection.

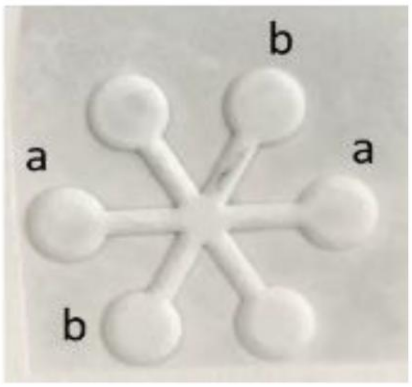

Fig. 3 Schematic diagram of heavy metal detection.

III. RESULTS AND DISCUSSION

A. Channel formation

A μPAD includes hydrophilic channels and hydrophobic barriers. Here we used paraffin wax to construct the hydrophobic barriers. After dispensing the ink solution, it can be seen that the ink was confined in the channel without leakage as shown in Fig. 4(a) The back side of the device was completely covered by the wax (Fig. 4 (b)).

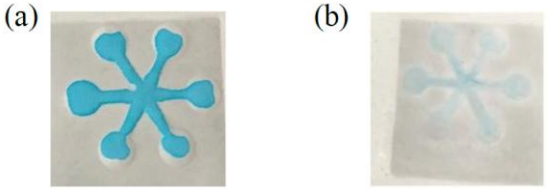

Fig. 4 (a) The front and (b) the back sides of the device after ink wicking through the channels.

B. Effect of microembossing temperature

The embossing temperature is the crucial variable among three main factors, i.e. temperature, pressure and time. If the temperature is too low, the barrier may not be completely formed and leakage will be observed as shown in Fig. 6(a). On the other hand, if the temperature is too high, the channels will be blocked by the wax as shown in Fig. 6(c). In order to find the appropriate temperature range for this process, DSC (Differential Scanning Calorimetry) was used to analyze the relationship between heat flow and temperature. (Fig. 5) Two peaks at 45 and 60 °C were observed in the chart, which represent fast transformation with larger enthalpy changes. Through trial and error, the suitable embossing temperature for the process was found to be approximately 55°C and the μPADs fabricated at this temperature show consistent and repetitive results without leakage as shown in Fig. 6(b).

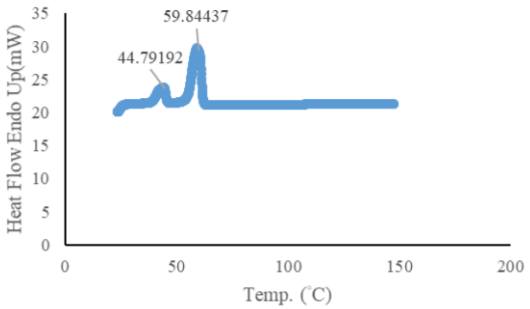

Fig. 5 DSC analysis of paraffin wax.

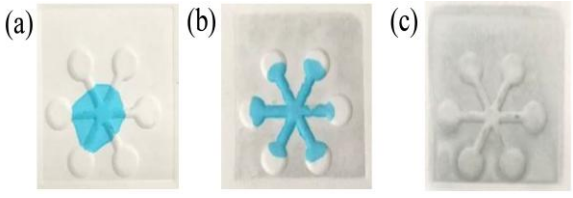

Fig. 6 Microembossing under the temperature of (a)50°C, (b)55°C, (c)60°C.

C. Effect of microembossing pressure

Different embossing pressure was applied to fabricate µPADs. It can be seen that µPADs without leakage were obtained under different pressure ranging from 1.00×10^5 to 1.40×10^5 g/cm^2 as shown in Fig. 7. Note that, at 1.00×10^5 g/cm^2, the boundaries are not as sharp as those made at higher pressure.

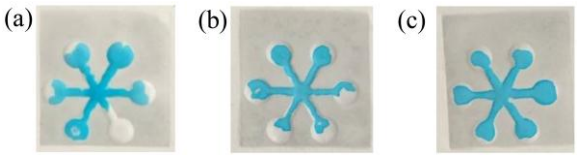

Fig. 7 Microembossing in 55°C, 5 seconds under the pressure of (a)1.00×10^5g/cm^2 (b) 1.20×10^5g/cm^2 (c) 1.40×10^5 g/cm^2.

Fig. 8 shows the thickness ratio at different pressure. The thickness ratio is defined as the thickness at the unembossed region divided by that at the embossed region. It can be seen that the ratio is approximately 1.2 at different pressure. We also measured the wicking time of the solution and it was found to be around 7 to 9 seconds after 1.0×10^{-2} cm^3 ink solution dispensed in the central reservoir (Fig. 9).

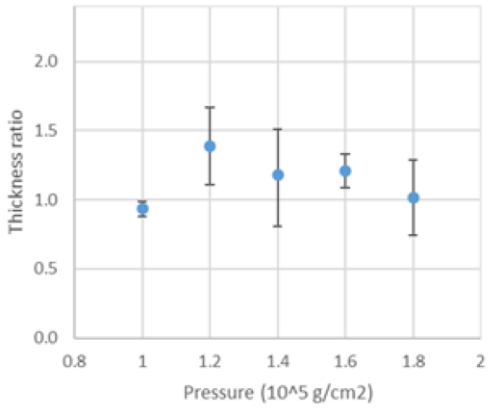

Fig. 8 Relationship between thickness ratio and pressure.

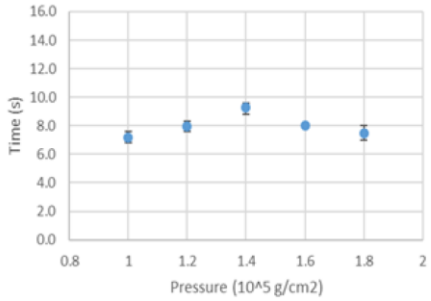

Fig. 9 Relationship between pressure and flowing time.

D. Effect of microembossing time

The embossing time is another crucial factor for successful fabrication of µPADs. If the embossing time is too long, channels will be blocked because of too much wax penetrating to the filter paper (Fig. 10). On the other hand, if the embossing time is not long enough, barriers cannot be formed completely and it may lead to the ink leakage.

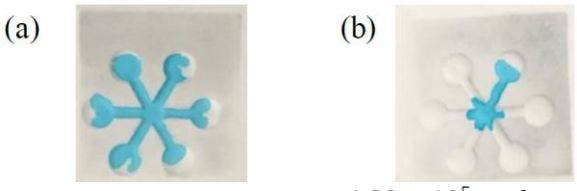

Fig. 10 Microembossing under 55°C, 1.00×10^5 g/cm^2 with the embossing time of (a) 5 sec. (b) 10 sec.

E. Detection of heavy metal ions

The µPADs as fabricated can be applied for detection of heavy metal ions. Fig. 12 shows the detection of copper ion. It can be seen that, in Fig. 12(a), the purple color was shown at reservoirs "a", as a result of the Cu(II)-induced ring opening of the spirolactam form of Rhodamine Hydrazone (Fig. 11)[15]. The deep red color was observed at reservoirs "b", indicating the coordination reaction of $Fe(CN)_6^{4-}$ and Cu^{2+} [16].

We dispensed the same volume of mixed solution which includes $CuSO_4 \cdot 5H_2O$ and $FeCl_3$. The reservoirs "b" turned into dark blue due to the reaction of $Fe(CN)_6^{4-}$ and Fe^{3+}, becoming a coordination complex, while reservoirs "a" still became purple as shown in Fig. 12(b). It is concluded that Rhodamine Hydrazone is specific to Cu^{2+} whereas $K_4Fe(CN)_6$ is not.

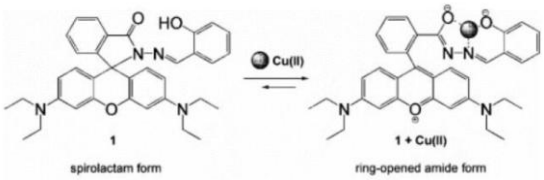

Fig. 11 Cu(II)-induced ring opening of the spirolactam form of Rhodamine Hydrazone[16].

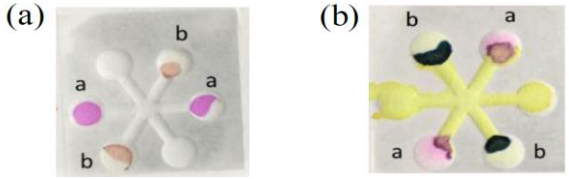

Fig. 12 Detection of heavy metal on the µPAD by (a)$CuSO_4 \cdot 5H_2O$ solution (b)Mixed solution of $CuSO_4 \cdot 5H_2O$ and $FeCl_3$.

CONCLUSIONS

We successfully demonstrated microembossing method to fabricate µPADs. This method is simple, fast, inexpensive, reliable, and potentially scalable. The embossing time and temperature play crucial roles in the process. With our proposed method, the µPADs can be constructed within 10 seconds.

REFERENCES

[1] W Xu Hou, Yu Shrike Zhang, Grissel Trujillo de Santiago, Mario Moisés Alvarez, João Ribas, Steven J. Jonas, Paul S. Weiss, Anne M. Andrews, Joanna Aizenberg and Ali Khademhosseini, "Interplay between materials and

microfluidics," Nature Reviews Materials, vol. 2, Article number: 17016, 2017
[2] Kangning Ren, Jianhua Zhou, and Hongkai Wu, "Materials for Microfluidic Chip Fabrication," Accounts of Chemical Research, vol. 46, pp. 2396–2406, 2013.
[3] Yuanyuan Yang, Eka Noviana, Michael P. Nguyen, Brian J. Geiss, David S. Dandy, and Charles S. Henry, "Paper-Based Microfluidic Devices: Emerging Themes and Applications," Analytical Chemistry, vol. 89, pp.71−91, 2017.
[4] Andres W. Martinez, Scott T. Phillips, Manish J. Butte, and George M. Whitesides, "Patterned Paper as a Platform for Inexpensive, Low-Volume, Portable Bioassay," Angewandte Chemie - International Edition, vol. 46, pp. 1318-1320, 2007.
[5] Lei Mou and Xingyu Jiang, "Materials for Microfluidic Immunoassays: A Review," Advanced Healthcare Materials, vol.6, DOI: 10.1002/adhm.201601403, 2017.
[6] Yanyan Xia, Jin Si, and Zhiyang Li, "Fabrication techniques for microfluidic paper-based analytical devices and their applications for biological testing: A review," Biosensors and Bioelectronics, vol. 77, pp. 774–789, 2016.
[7] David M. Cate, Jaclyn A. Adkins, Jaruwan Mettakoonpitak, and Charles S. Henry, "Recent Developments in Paper-Based Microfluidic Devices," Analytical Chemistry, vol. 87, pp.19−41, 2015.
[8] Tugce Akyazi, Lourdes Basabe-Desmonts, Fernando Benito-Lopez, "Review on microfluidic paper-based analytical devices towards commercialization," Analytica Chimica Acta, vol. 1001, pp. 1-17, 2018.
[9] Chunxiu Xu, Longfei Cai, Minghua Zhong and Shuyue Zheng, "Low-cost and rapid prototyping of microfluidic paper-based analytical devices by inkjet printing of permanent marker ink," RSC Adv., vol. 5, pp. 4770-4773, 2015.
[10] Yao Lu, Weiwei Shi, Lei Jiang, Jianhua Qin and Bingcheng Lin, "Rapid prototyping of paper-based microfluidics with wax for low-cost, portable bioassay," Electrophoresis, vol. 30, pp. 1497–1500, 2009.
[11] Wijitar Dungchai, Orawon Chailapakul, and Charles S. Henry, "A low-cost, simple, and rapid fabrication method for paper-based microfluidics using wax screen-printing," Analyst, vol. 136, pp.77–82, 2011.
[12] Wei Hong, Jing Zhou, Mandakini Kanungo, Nancy Jia, and Anthony D. Dinsmore, "Wax Spreading in Paper under Controlled Pressure and Temperature," Langmuir, vol. 34, pp. 432−441, 2018.
[13] Joong Ho Shin, Juhwan Park and Je-Kyun Park, "Organic Solvent and Surfactant Resistant Paper-Fluidic Devices Fabricated by One-Step Embossing of Nonwoven Polypropylene Sheet," Micromachines, vol. 8, doi:10.3390/mi8010030, 2017.
[14] Paulo de Tarso Garcia, Thiago Miguel Garcia Cardoso, Carlos Diego Garcia, Emanuel Carrilhocd and Wendell Karlos Tomazelli Coltro, "A handheld stamping process to fabricate microfluidic paper-based analytical devices with chemically modified surface for clinical assays," Royal Society of Chemistry, vol. 4, pp. 37637–37644, 2014.
[15] Yu Xiang, Aijun Tong,* Peiyuan Jin, and Yong Ju, "New Fluorescent Rhodamine Hydrazone Chemosensor for Cu(II) with High Selectivity and Sensitivity," Organic Letter, vol. 8, pp. 2863–2866, 2006.
[16] Jerry B. Ayers and William H. Waggoner, "Synthesis and properties of two series of heavy metal hexacyanoferrates," Journal of Inorganic and Nuclear Chemistry, Vol. 33, pp. 721-733, 1971.

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