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Microfluidic paper-based analytical devices (µPADs) attract tremendous attention as an economical tool for in-field diagnosis, food safety and environment supervision. We innovatively fabricated 2D and 3D µPADs by photolithography patterning microchannels on Parafilm® and subsequently embossing to paper. This truly low-cost, wax printer and cutter plotter independent approach offers opportunity for researchers from resource-limited labs to work on paper-based analytical devices.

The requirement of low-cost, portable, disposable analytical tool for point-of-care diagnosis, daily food safety supervision and environmental monitoring, especially for resource-limited region drive the research on micro total analysis system (µTAS). Microfluidic is evidenced as a prominent candidate of µTAS. Since the pionered work presented by Dr. Whiteside’s group at 2007, microfluidic paper-based analytical devices (µPADs) have arouse tremendous attention due to its simplicity, low-cost comparing with microstructures fabricated from silicon, glass, polymethylsiloxane (PDMS) or poly(methyl methacrylate) (PMMA). In addition, liquid flows in paper matrix by capillary action endowing paper-based microfluidic chips a great advantage over traditional microfluidics, which heavily rely on pump to drive liquid moving within channels. The advantages and potential application of µPADs have been well-reviewed elsewhere. The fundament of fabricating µPADs is to building hydrophobic barriers on paper that constituting walls of micrometer-size to guild the flow. To achieve this goal, several smart approaches have been proposed and they can be categorized as wax printing, photolithography patterning and others. (a) Wax printing on paper, desired structures are printed on a piece of chromatography paper using special wax printer, normal using the model of Xerox Phaser 8560N. Then the wax embedded paper is placed on a hot plate to melt the wax, which can penetrate into the paper matrix to build hydrophobic region. This well-established method demonstrates its potential in several works. Later on, wax material became a popular ink to draw structures on paper by using injecting printing, wax screen-printing and paper stamp-based printing. (b) Photolithography patterning on paper; hydrophobic photosresist SU-8 is coated on paper; following a standard photolithography protocol, structures of channels and wells can be patterned on paper. (c) Laser ablation, paper cutting and flexography printing were also reported. Most recently, Cai et al fabricated µPADs using trimethoxysiladecyl-silane (TMOS) and NaOH plus 30% glycerol. Those fabrication strategies broaden the application and especially lowering the threshold to this research area. But, as reviewed by Dr. Shen et al, involvement of expensive wax-printer might shut out researches from resource-limited labs. Wet-chemical processing using organic solvent, such as acetone, SU-8 and TMOS, likely destroys the integrity of paper. Enthusiasm will be strength if an approach can get rid of both expensive equipment and wet-chemical processing on paper, while meets low-cost and feasibility to generate complex 2-D or even 3-D µTAS.

Parafilm® is a thermoplastic film extensively used in laboratories for sealing or protecting flasks or cuvettes. The paraffin wax property enables the film be solid at room temperature and begin to melt above approximately 60°C. Dunfield et al post a rapid approach to fabricate paper microfluidic utilizing Parafilm® on Chips and Tips forum of Lab Chip. The channel mask is a polycarbonate (PC) film, and being sandwiched between the paper and the Parafilm®. The PC film mask prevents the melted Parafilm® from penetrating into the paper in the channel region, and therefore defines the hydrophobic boundaries of the paper channel. This smart method indeed exempt from organic solvent, avoid damage to the delicate paper during fabrication. But the PC film has to be processed by a cutting plotter. And it is difficult to obtain free-stand complex structures, particularly in dealing with structures down to micrometer-size.

Inspired by this post, we innovate a truly low cost, highly processability and easy way to fabricate paper microfluidics by marrying of photolithography and embossing of Parafilm®. Instead of directly photolithography on paper, microstructures were patterned on a Parafilm®. As illustrated in Fig. 1, a layer of printing circle broad (PCB) UV photosensitive inks with a thickness around 0.1 mm was painted on a piece of Parafilm® and allow dry at room temperature (~30 min). It should be note, if the photosensitive layer is thicker than 0.5 mm, the patterned structure would be peeling off from Parafilm® during embossing process. The target structure designed by Corel Draw was printed on a transparent film and covered on the photosensitive ink-painted Parafilm® (Fig.1 a). The structure embedded transparent film and photosensitive ink-painted
Parafilm® was then exposed to a 12W ultraviolet (UV) lamp for 4 seconds (Fig.1 b). Then, the UV light-exposed Parafilm® was immersed into alkaline solution to reveal target structure on Parafilm® (Fig.1 c). To transfer the structure from Parafilm® to paper, the photolithography patterned Parafilm® and paper was assembled face to face and a piece of aluminum foil was laminated on the backside of the Parafilm® to make a paper-Parafilm®-aluminum foil sandwich (Fig.1 d). Then the sandwich was placed between two glass slides and fastened with clamps. The fully assembled material was placed in an oven or on a hot plate and incubated at 120°C for ~2-5 minutes for Whatman #1 filter paper and ~ 5-10 minutes for polyvinylidene difluoride (PVDF) membrane because of the different pore size of the paper. Finally, the paper was disassembled from the sandwich structure. It is expected that the melted Parafilm® will penetrate into the paper forming hydrophobic barriers, while the photosensitive patterned structure on Parafilm® can prevent the melted Parafilm® from penetrating into the paper (Fig.1 f). As shown in Fig.1g, the entire fabrication process can be performed only with a hot plate or an oven, UV lamp, and a mask produced from an office printer. In addition, the paper was not directly immersed in photosensitizer, avoiding the harsh chemical condition induced corrodng of paper.

Fig.1 A low-cost strategy for fabricating of microfluidic paper-based analytical devices (µPADs). Step 1. Photolithography-patterned Parafilm®: Photosensitive blue ink painted Parafilm® and a transparent film with desired structures are assembled and exposed to UV lamp (a, b); the UV exposed Parafilm® is developed in alkaline solution to reveal target structure (c); Step 2 Embossing of Parafilm® to transfer microstructures: filter paper, lithography-patterned Parafilm® and a piece of aluminum foil are assembled then sandwiched between two glass slides (d) the sandwich is fasten by clamps and heated at 120°C for few minutes (e, f); disassemble the filter paper from the sandwich and obtained Parafilm®-patterned paper; materials used in the fabrication process (g).

Fig.2A shows the images of photolithography-patterned structures on Parafilm®. Encouragingly, structures as small as 100 µm can be successfully revealed on Parafilm® with low-cost PCB photosensitive ink and UV lamp, demonstrating an economical way for fast prototyping. In addition, the easy of design and printing structure on transparent film with ordinary office tools gives the flexibility to generate desiring pattern on Parafilm®.

We explored the processability for fabricating microstructures with different types of paper through the method illustrated in Fig.1. Representing material include Whatman #1 filter paper, PVDF membrane (pore size 0.2 µm) and organic filter membrane (nylon, pore size 0.45µm) because they have different phydrophilicity and pore size. In the experiment, it was found that the minimum size (width of the microchannel) can be realized on them is vary because the melted Parafilm® penetrate not only vertically into the paper, but also horizontally across the confined region. In our test, it is reproducible to obtain channels on Whatman #1 filter paper from line with width of 1mm on Parafilm®. While 0.1 mm and 0.5 mm line patterned on Parafilm® can safely emboss integral channel on PVDF and nylon filter membrane, respectively, because smaller pore-size of them effectively restrict the horizontally diffusion of the melted Parafilm®. As clearly shown in Fig. 2B, fine microstructures can be transferred from Parafilm® (Fig.2A) to filter paper (a), PVDF membrane (b) and nylon filter membrane (c). The Parafilm® does not completely melt during the heating process. The residual Parafilm® will stick to the aluminum foil and can be easily separated from the paper chip.

The fluidic characters were demonstrated with the assistant of water based liquid green food color. To facilitate the observation of the liquid on the Whatman #1 filter paper, the concentrated food coloring was diluted with water. While for the assay on nylon membrane, the green dye was diluted by ethanol. Floating the Parafilm®-patterned paper on the solution can only wet the non-patterned area (Fig. 3A). A drop of water-diluted dye can fast flow-through Whatman #1 filter paper-based µPADs (Fig.3B). While ethanol-diluted green dye quickly travel through a microchannel fabricated from an organic membrane (Fig.3C). As far as know, this is the first microfluidic structure generated from an organic membrane. We believe it has potential in separation, mixing or reaction that involving organic solution. Importantly, multi-layer of Parafilm®-patterned paper can be vertically assembled by the melting and bonding of Parafilm® to realize complex 3-D microfluidic system (supplementary sFig.1). As shown in Fig.3D, drop of green water on left eye of the panda can go through the underneath channel and reach the right eye. Comparing with previous 3-D paper fluidic system, this Parafilm®-patterned paper assembling does not involve of adhesion tape to sequential assemble multi-layer paper. And the vertical directly contacting of paper channels avoids using of cellulose powders to guide the flow.
Low-cost and portable µPADs are excellent candidates in health diagnostics, biochemical analysis, forensic and food quality control. In this study, colorimetric assays of methomyl, a kind of pesticide, and uric acid, an biomarker of obesity, hypertension and metabolic syndrome, were carried out using µPADs fabricated from Parafilm®-patterned paper to demonstrate their potential as an economical, disposable analytical tool. The detection principle of methomyl roots in that cholinesterase (ChE) catalyzes the hydrolysis of acetylcholine (ACh) into acetic acid and thiocholine. And thiocholine reacts with dihydropropionic nitrobenzene acid (DTNB) showing yellow color. Methomyl as a kind of pesticide will inhibit the catalytic function of ChE, reducing the production of thiocholine. Consequently, less DTNB will be converted to yellow (Fig.4A). By monitoring the colorimetric changes, the amount of methomyl in sample could be determined. In our trial, 2 µL (10U/mL) ChE was dropped at the one-end of the channel, while 1.5 µL (20mg/mL) ACh and 3µL (5mg/mL) DTNB were casted at the well on the other end of the µPADs. During the assay, 10 µL analyte was placed on the ChE pre-loaded region and automatically migrated through the channel to the rear. Without methomyl in an analyte, a clear yellow color can be observed at the spot pre-loaded with ACh and DTNB. While, 2µg/mL methomyl can clearly inhibit the appearance of yellow color (Fig.4B).

Another enzymatically colorimetric reaction was demonstrated on a three-channel µPAD for uric acid (UA) detection. As we know uricase catalyzes the oxidation of UA to 5-hydroxyisourate and hydrogen peroxide (H₂O₂). The later participates in horseradish peroxidase (HRP) mediated color change of tetra-methyl benzidine (TMB) (Fig.4C). In this test, 0.5 µL HRP (10mg/mL) and 1µL TMB (2mg/mL) were pre-loaded at the reservoir a, 1µL TMB (2mg/mL) was in reservoir b (negative control), and 2 µL uricase (12U/mL) was pre-loaded in reservoir c at the centre of the three-channel system (supplementary- uric acid detection). During the assay, 10 µL sample with different concentration of UA was dropped on the top wells (reservoir d), and the solution flow through the channel to point c where pre-loaded uricase reacts with UA and the subsequently produced H₂O₂ can further flow-through the channels to point a and b. As shown in Fig.4D, with increased concentration of UA in sample, a dark blue can be observed at reservoir a. Since every three-channel system has a negative control, the UA induced color change can be calculated as signal (reservoir a)/noise (reservoir b) ratio. Concentrations of UA in the range of 0-1.0 mM were assayed with Parafilm®-patterned µPAD because the range of uric acid currently used in clinic lab is 0.25–0.45 mM for male and 0.18-0.36 mM for female. The corresponding signal/noise ratio of 0 mM (1), 0.25 mM (2), 0.5 mM (3) and 1.0 mM UA (4) are 0.98±0.02, 1.21±0.01, 1.37±0.08 and 1.64±0.01 (n=5), respectively, demonstrating the quantitative analysis potential.

Conclusions
To summarize, the beauty of this Parafilm® photolithography and embossing-assistant µPADs fabrication lines in (a) exempting from expensive wax printer, laser cutting etc. equipment, (b) avoiding direct photolithography on paper caused corrosion of paper, (c) capability for easily fabricating complex structures by patterning mold structures on Parafilm®, (d) potential for constructing 3D-µPADs without using of adhesive tape and cellulose powders, (e) suitable for transferring Parafilm®-depicted structures to different materials, such as filter paper, PVDF membrane, organic film and even silk cloth, to meet the requirement of a specific application. We believe this approach can open the door of µPADs to those researchers who is struggling with limited lab resources but has strong interest to develop low-cost, convenient and sensitive analytical tools.

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