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ARTICLE

Fabrication of Microfluidic Paper-Based Analytical Device by Silanization of Filter Cellulose Using a Paper Mask for Glucose Assay

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We developed a novel, low-cost and simple method for fabrication of microfluidic paper-based analytical devices (μPADs) by silanization of filter cellulose using a paper mask having a specific pattern. The paper mask was penetrated with trimethoxyoctadecylsilane (TMOS) by immersing into TMOS-heptane solution. By heating the filter paper sandwiched with the paper mask and glass slides, TMOS was immobilized onto the filter cellulose via the reaction between cellulose OH and TMOS, while the hydrophilic area was not silanized because the hydrophilic area was not in contact with the paper mask penetrated with TMOS. The effects of some factors including TMOS concentration, heating temperature and time on the fabrication of μPADs were studied. This method is free of any expensive equipment and metal masks, and could be performed by the untrained personnel. These features are very attractive for the fabrication and applications of μPADs in the developing world or resource-limited settings. A flower-shaped μPAD was fabricated and used to determine glucose in human serum samples. The contents determined by this method agreed well with those determined by a standard method.

Introduction

The paper-based microfluidics is a newly developed technology which utilizes the filter paper as the substrate for patterning microstructure on it to achieve complex functions.[1] In comparison with the microfluidic devices fabricated on glass, silicon and polymers, the microfluidic paper-based analytical devices (μPADS) possess a number of advantages including low cost, easy and fast fabrication, ease of use, portability and disposability. Furthermore, the aqueous solutions could wick through a porous matrix of hydrophilic cellulose fibers delimited by hydrophobic barriers, thus the external pump is not needed. These features make the μPADS very attractive for point of care diagnostics,[2,3] environmental testing[4,5] and food analysis,[6] especially in those less-industrialized regions and resource-limited settings. As a result, the past few years have witnessed the fast development of μPADS in those above-mentioned fields since Whitesides and coworkers first introduced this concept in 2007.[7]

A number of methods including photolithography,[7-9] wax printing,[10,11] plasma treating[12] and laser etching[13] have been reported for the fabrication of μPADs. By photolithography, various materials like SU-8, poly(*o*-nitrobenzylmethacrylate) (PoNBMA) and octadecyltrichlorosilane (OTS) have been used to fabricate μPADs on filter paper. However, this method suffered the limitation of time-consuming fabrication process. Additionally, expensive equipment such as lithographic equipment is required. Other methods like wax printing, plasma treating and laser etching could offer fast speed, easy process and high resolution in fabrication of μPADs, and could be used for mass production of μPADS. Unfortunately, these methods are also limited by the expensive equipments such as wax printers, plasma oxidizer and CO_2 lasers required for fabrication of μPADs, which pose challenges for fundamental researches and applications of μPADs in common laboratories, especially in those less-industrialized and resource-limited regions. Moreover, trained personnel are required to operate and maintain these expensive equipments. Thus, cost-effective and simple methods free of expensive external equipments are highly desirable. Recently, an inkjet printing method has become a simple and cost-effective alternative to those expensive methods for patterning the microstructure on filter paper.[14-18] Shen's group[14,15] developed a method for fabrication of μPADs by directly printing alkenyl ketene dimer (AKD) solution onto the filter paper. Alternatively, Abe et al.[16] printed toluene solvent on the filter paper to dissolve the hydrophobic poly(styrene) layer which was obtained by soaking the filter paper into a 1.8% (wt.) poly(styrene) solution prepared in toluene. These methods allow mass production of μPADs with simple and fast fabrication process. However, the commercial

inkjet printers are required. Additionally, the printers have to be modified by replacing the ink in cartridge with the patterning reagents. Furthermore, the cartridges and printers may be damaged by the organic solvents in the printing solutions. Recently, much effort has been dedicated to the development of simple and cost effective fabrication methods free of expensive equipments and inkjet printers. For instance, Songjaroen et al.[19,20] described a wax dipping method for prototyping of μPADs by dipping the paper that was sandwiched with a metal mask and magnet into the molten wax. Recently, Nurak et al.[21] described a similar method for fabrication of μPADs by spraying acrylic lacquer on the filter paper sandwiched with a metal mask and magnet. Acrylic lacquer was sprayed on the filter paper to generate a hydrophobic area while the hydrophilic area was protected with the metal mask, thus the hydrophilic-hydrophobic contrast was generated on the filter paper. Alternatively, Dungchai et al.[22] reported a low-cost, simple and rapid method for fabricating μPADs by wax screen-printing method. In their work, solid wax was firstly rubbed through a screen onto the filter paper, the printed wax was then melted into the paper to form hydrophobic barriers using a hot plate. Nie et al.[23] described a facile one-step plotting method to generate hydrophilic-hydrophobic contrast on paper by simply using a permanent ink and metal masks with specific patterns. More recently, Mu et al.[24] developed a method to generate multiple test zones by craft punch patterning (CPP) on the paper substrate. These methods offer advantages of low cost, simplicity, easy and fast fabrication speed for fabrication of μPADs, and the relative expensive equipments are not required. Nevertheless, a common limitation suffered by these methods is that each type of μPAD has to be fabricated using a customized metal mask or mould which are usually cut with expensive equipment such as linear cutting machine or laser cutting machine.

In most of the above-mentioned methods, the hydrophobic barriers are formed by physical deposition of hydrophobic materials such as wax, SU-8, polydimethylsiloxane (PDMS) and so on. It was reported that the hydrophobic barriers formed by physical deposition might be attacked by the organic solvents, and the patterned hydrophobic barriers may be damaged by folding and bending.[8] Wang et al.[25] reported that both wax and AKD based hydrophobic barriers could be breached by the cell lysing detergents, while the μPADs patterned with siloxane could be used for cell analysis.

In this work, we developed a novel, simple and low-cost method for fabrication of μPADs based on chemical patterning of alkyltrimethoxysilane on the filter paper. A home-made paper mask was first immersed into alkyltrimethoxysilane-heptane solution, the paper mask was then picked up and a native filter paper was aligned onto the mask. After being heated for 35 min, the native filter paper aligning onto the paper mask was silanized to be hydrophobic by alkyltrimethoxysilane via reaction taking place between alkyltrimethoxysilane and cellulose OH, while the hydrophilic area was not penetrated and silanized by alkyltrimethoxysilane because the hydrophilic area was not in contact with the paper mask penetrated with alkyltrimethoxysilane. Thus the hydrophobic-hydrophilic contrast was generated on the filter paper. Being free of any expensive equipment, inkjet printers and metal masks, this method utilizes only a paper mask having a specific pattern and alkyltrimethoxysilane-heptane solution for the prototyping of μPADs. The μPADs fabricated by this method was used to determine glucose in human serum, the analytical results compared well with that determined by the standard method, demonstrating its great potential in analytical applications.

Experimental

Chemicals and apparatus

All chemicals used were of analytical grade unless mentioned otherwise, and demineralized water was used throughout. Trimethoxyoctadecylsilane (TMOS) was purchased from Aladdin Industrial Co. (Shanghai, China). 2.0% (v/v) TMOS-heptane (containing 5% ethyl acetate) was used as the patterning agent. A 200 mM phosphate buffer solution was prepared by combining 21.85 g $Na_2HPO_4 \cdot 12H_2O$ (Xilong Chemical Co., Ltd., Shantou, China) and 6.08 g $NaH_2PO_4 \cdot 2H_2O$ (Xilong Chemical Co., Ltd., Shantou, China) in 300 mL of H_2O and pH was adjusted to 7.0 with 1.0 mol L^{-1} NaOH or HCl solution and then was diluted to 500 mL. 6.0 mol L^{-1} potassium iodide solution was prepared by dissolving 4.980 g potassium iodide (Xilong Chemical Co., Ltd., Shantou, China) in 5 mL of water. Glucose oxidase solution was prepared by dissolving 20 mg of glucose oxidase (Biological grade, Shanghai Jinsui Bio-Technology Co., Ltd. Shanghai, China) in 50 mL of buffer solution. Horseradish peroxidase solution was prepared by dissolving 13.4 mg of Horseradish peroxidase (Biological grade, Shanghai Jinsui Bio-Technology Co., Ltd. Shanghai, China) in 50 mL of buffer solution. The enzyme solutions were mixed by a ratio of 1:1 before use. A glucose stock standard solution (100 mmol L^{-1}) was prepared by dissolving 1.9820 g glucose in 50 mL of H_2O and diluted to 100 mL. The glucose working standard solutions were prepared by appropriate dilution of the stock standard solution. For the standard method, the mixed enzyme solutions were prepared by dissolving 10 mg of glucose oxidase, 10 mg of Horseradish peroxidase, 10 mg of 4-aminoantipyrine and 100 mg of sodium azide in 80 mL of buffer solution and pH was adjusted to 7.0 with 1.0 mol L^{-1} NaOH or HCl solution, followed by being diluted to 100 mL with buffer solution. Phenol solution was prepared by dissolving 100 mg of phenol in 100 mL of H_2O. The mixed enzyme-phenol solution was prepared by a ratio of 1:1 before use. 5.0 mmol L^{-1} of glucose working standard solution was prepared by appropriate dilution of the glucose stock standard solution (100 mmol L^{-1}) with 12 mmol L^{-1} benzoic acid. Human serum samples were provided by Chaozhou Central Hospital. The samples were collected and prepared from patients' blood. Briefly, the blood samples were collected and placed static for 20 min followed by being centrifuged at 3000 rpm for 20 min. The serum samples were then collected for further analysis. A knife was used to fabricate the mask on filter paper (102, Hangzhou Xinhua Paper Limited, Hangzhou, China). A digital camera (Canon IXUS9515, Japan) or a mobile phone was used to capture the images of colorimetric assays performed on μPADs. A contact angle meter (JC20001, Shanghai Zhongchen Digital Technic Apparatus Co., Ltd, Shanghai, China) was used to measure the contact angles based on the sessile drop method, using a water drop of 6 μL.

Fabrication of μPAD

The fabrication principle and process are schematically shown in Figure 1. A pattern designed with Coredrawl software was printed onto a native filter paper with a laserJet printer (HP LaserJet 1020 plus, USA). The paper mask was fabricated with a knife cutting along the printed pattern on the filter paper (Figure 1B). The paper mask was then immersed into a 2.0% TMOS solution for 10 s (Figure 1C). The mask was picked up and air dried for 1 min followed by putting onto a glass slide, then a native filter paper and another glass slide were covered onto the paper mask sequentially (Figure 1D). The integrated part was then heated on a heating plate at a temperature of 100°C for 35 min (Figure 1E). The TMOS adsorbed on the

paper mask was evaporated and penetrated into the native filter paper aligning onto the mask, allowing the silanization of filter cellulose by TMOS. Other parts that were not directly in contact with the paper mask, however, were not silanized and remained hydrophilic. As a result, the hydrophilic-hydrophobic contrast was generated on the filter paper (Figure 1F). After 2 h from withdrawing from the heater, the μPAD was ready for use.

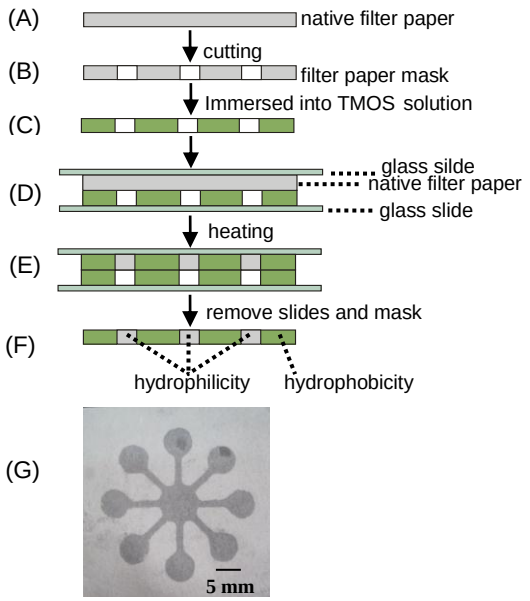


Figure 1 Schematic diagram of fabrication of μPAD. Cross section of (A) the native filter paper, (B) filter paper mask obtained by cutting on the native filter paper, (C) the paper mask penetrated with TMOS solution, (D) the native filter paper sandwiched with two glass slides and a paper mask, (e) the assembly after being heated, and (F) the fabricated μPAD with hydrophilic-hydrophobic contrast. (G) Photograph obtained by spraying water on the fabricated μPAD.

Glucose assay on μPAD

A flower-shaped μPAD used for glucose assay consists of 8 channels, 8 detection zones and 1 central unit. The principle of glucose assay was based on that reported by Whitesides and Culbertson group.[7,9] To detect glucose in human serum, 60 μL of potassium iodide was spotted onto the central unit of the μPAD. The potassium iodide spotted onto the central unit would flow along the 8 channels into the detection zones. After the device was allowed to air dry for 10 min, 0.6 μL of 1:1 glucose oxidase/horseradish peroxidase reagent was spotted onto the 8 detection zones, respectively. The device was allowed to air dry for 8 min, followed by spotting 0.6 μL of the standard solutions and samples onto the detection zones. The images of the colorimetric assay was captured with a camera or a mobile phone, the gray values of the detection zones were measured with the ImageJ software for quantitative analysis of glucose contents.

Glucose assay by a standard method

20 μL of H_2O, 5.0 mmol L^{-1} of glucose solution, and the serum sample were added into three cuvettes respectively, then 3.0 mL of enzyme-phenol solution was added into these three solutions. After the solutions were incubated at 37°C for 15min, the absorbance of these solutions at a wavelength of 505 nm were measured with respect to the blank solution. The glucose concentration in serum samples was calculated using the equation of $C(mmol\ L^{-1}) = \frac{A_{sample}}{A_{standard}} \times 5$, where A_{sample} is the absorbance of solution having serum sample and $A_{standard}$ is the absorbance of solution containing glucose standard solution (5.0 mmol L^{-1}).

Safety considerations

TMOS is irritating to eyes, respiratory system and skin. The experiments of using and preparing TMOS solution should be performed with caution, while wearing protective gloves, goggles and long-sleeve lab coat.

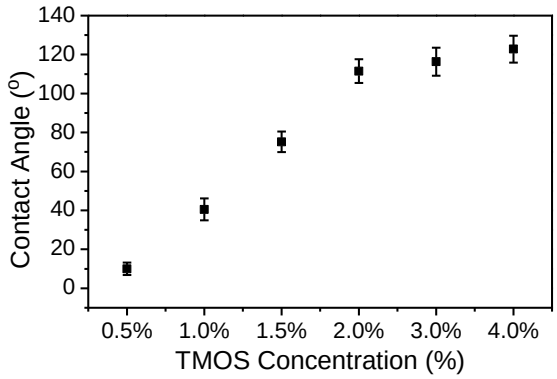
Results and discussions

Hydrophobization of filter paper

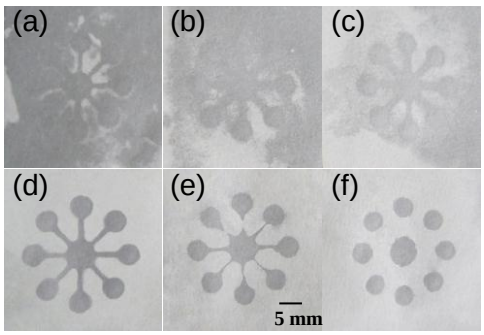
Si-OR does not react with hydroxyl group of filter cellulose. But the addition of moisture induces partial hydrolysis of the siloxane, the ensuing silanol groups then react with the cellulose OH.[26] As the paper mask was immersed into the TMOS-heptane solution, TMOS-heptane solution was adsorbed onto the cellulose of the paper mask. The TMOS solution on the paper mask was evaporated and penetrated into the native filter paper aligning onto the mask by heating. Si-OH groups were produced by hydrolysis of TMOS under ambient water vapour. The TMOS was then immobilized onto filter cellulose via the reaction taking place between the silanol groups and cellulose OH. Meanwhile, the hydrolyzed TMOS may interconnect via the self-condensation of silanol groups.[27] As a result, TMOS was immobilized onto the filter fiber and covered by the hydrophobic groups.

Effect of TMOS concentration

The native filter paper was patterned to be hydrophobic by TMOS-heptane solution. TMOS-heptane solutions in a range of 0.50-4.0% were prepared to study the effect of TMOS concentration on the water contact angles on the filter paper. The experiment was performed with the heating temperature and heating time fixed at 100°C and 35 min, respectively. As shown in Figure 2A, the water contact angle increased with the TMOS concentration. The filter paper remained super-hydrophilic and the water drop could penetrate into the patterned filter paper rapidly by using 0.5% TMOS as the patterning agent. The filter paper was silanized to be super-hydrophobic and a water contact angle of 111.5° was observed with the 2.0% TMOS as the patterning agent. The water contact angle increased slowly with TMOS concentration in a range of 2.0-4.0% TMOS, and a water contact angle of 122.75° was observed with 4.0% TMOS as the patterning agent. Larger contact angle may be obtained by further increasing the TMOS concentration. However, the width of hydrophilic channel and area of detection zones decreased with the increased TMOS concentration and the channels may disconnect when the TMOS concentration is higher than 3.0% (Figure 2B). Therefore, 2.0% TMOS prepared in heptane was used as the patterning agent in this work.



(A)



(B)

Figure 2(A) Effect of TMOS concentration on the water contact angle on the patterned filter paper. (B) Images obtained by spraying water on the μPAD fabricated with 0.5% (a), 1.0% (b), 1.5% (c), 2.0% (d), 3.0% (e) and 4.0% (f) TMOS-heptane solution. Heating temperature, 100°C; Heating time, 35 min.

Effect of heating temperature

The effect of heating temperature in a range of 80-120°C on the water contact angle was studied by keeping the heating time and TMOS concentration constant at 35 min and 2.0%, respectively. Figure 3 shows that the water contact angle increased rapidly with the temperature in a range of 80-100°C, while the water contact angle increased slowly with the temperature in a range of 100-120°C. This may be due to the higher reaction velocities taking place between cellulose OH and TMOS obtained at higher heating temperatures. However, the width of hydrophilic channel decreased dramatically and the channel may disconnect at a temperature higher than 110°C. This phenomenon may be due to the enhanced lateral diffusion and penetration of TMOS in filter paper at a higher heating temperature. Thus, 100°C was selected as the heating temperature in this work.

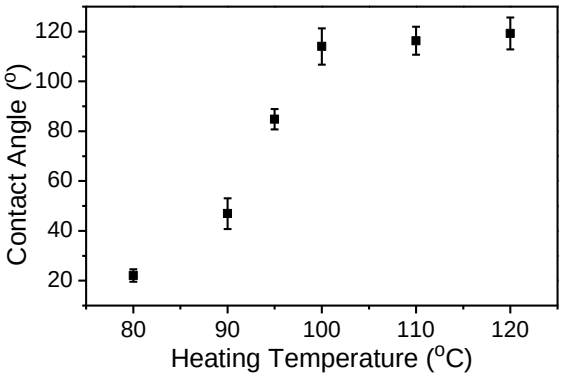


Figure 3 Effect of heating temperature on the water contact angle on the filter paper. TMOS concentration: 2.0%; Other conditions were the same as Figure 2.

Effect of heating time

The effect of heating time in a range of 10-50 min on the water contact angle was studied by keeping the heating temperature and TMOS concentration fixed at 100°C and 2.0% respectively. As shown in Figure 4, the filter paper remained super-hydrophilic and the water contact angle is zero at a heating time of 10 min. The water contact angle increased to 75.3° at a heating time of 20 min, and the water being sprayed onto the paper could eventually penetrate into the filter cellulose. The water contact angle increased slowly with the heating time in a range of 30-50 min. On the other hand, the increased heating time may enhance the lateral diffusion and penetration of TMOS in the filter paper, which may reduce the channel width or the channel may disconnect. Thus, a heating time of 35 min was selected to fabricate the device in this work. We also compared the hydrophobicity of different sides of the device fabricated with the optimum conditions described above. The water contact angle on the front side is 114.3° while the contact angle on the back side is 110.5°. This result indicated that TMOS completely penetrated into the filter cellulose and the hydrophobic barrier was generated in the filter paper successfully.

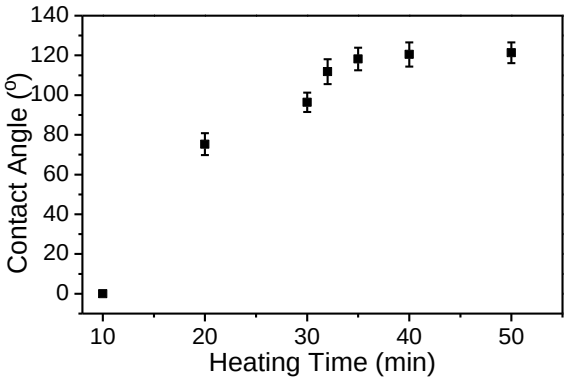


Figure 4 Effect of heating time on the water contact angle on the patterned filter paper. TMOS concentration: 2.0%; Other conditions were the same as Figure 2.

Glucose assay

To demonstrate the feasibility of the devices fabricated by this presented method as a quantitative analysis device, a flower-shaped μPAD was fabricated and used to detect glucose in human serums. In this reaction, hydrogen peroxide was produced via the oxidation of glucose by using glucose oxidase as the catalyst. The hydrogen peroxide oxidized the potassium

iodide to generate iodine with the Horseradish peroxidase as the catalyst. Figure 5A shows the image of glucose assay on μPAD where 6 detection zones for standard samples and the last two for two human serum samples. The gray intensity in each detection zone was obtained with the ImagJ software. Data were imported into Origin (version 7.5) to obtain a linear correlation between gray intensity, GI, and glucose concentration, *C*. The linear equation between gray intensity and glucose concentration in a range of 0-20 mmol^{-1} was GI = 3.50 *C* (mmol L^{-1}) +8.1, with a correlation coefficient of 0.971 (Figure 5B). The glucose contents in two human serum samples determined by this proposed method were 7.4±0.4 and 15.1±0.6 mmol L^{-1} (n=3), respectively, which compared favorably with those (8.5±0.4 and 14.4±0.5 mmol L^{-1}) (n=3) determined by a standard method.[28] This result indicates that the μPAD fabricated by this proposed method could be applied to glucose assay in biological samples.

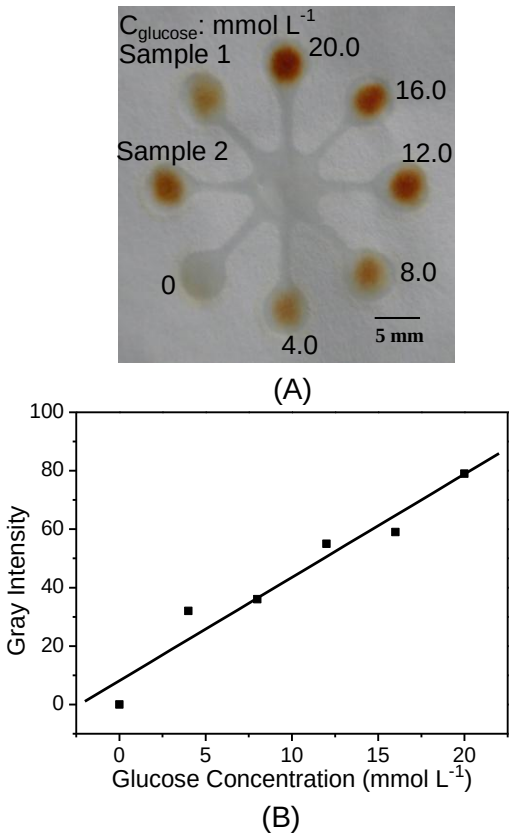


Figure 5(A) Image of glucose assay on the μPAD with varied glucose concentrations and two human serum samples. (B) The gray value data varies as a function of glucose concentration, obtained from (A). The gray value was obtained by the ImageJ software after subtraction of the blank value.

Conclusions

We developed a simple, cost-effective and novel method for fabrication of μPAD by chemical patterning of filter cellulose using a paper mask. The successful application in glucose assay demonstrates its potential in medical testing. Compared with those fabrication methods reported previously, this proposed method is free of any expensive equipment, inkjet printer or metal mask. Thus it could be used for fabrication of μPADs in any common lab, especially those in the developing world and resource-limited regions by untrained operators at minimum cost. The μPADs fabricated with this method can be not only applied to glucose assay, but also in environmental testing, food analysis and biological assays. Furthermore, compared with μPADs patterned with other agents, siloxane patterned μPADs could resist organic solvents and surfactants,[25] demonstrating the potential of this method in analytical applications especially when the surfactants or organic solvents are required, for example cell analysis. Additionally, the channels on the fabricated μPADs shrinks (*ca.* 30%) comparing with the channels on the paper mask. This may be due to the lateral diffusion of TMOS in the filter paper while heating the filter paper sandwiched with paper mask and glass slides. One limitation of this method is the relative low resolution and reproducibility in fabrication of μPADs, this could be attributed to the low resolution and reproducibility in fabrication of paper mask with a common knife. The resolution and reproducibility could be improved by using some automated cutting machine or skillful operators.

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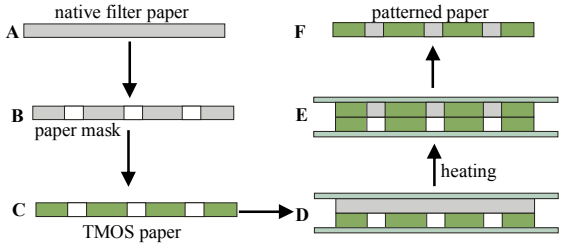
Colour graphic



The μPAD was fabricated based on chemical patterning of filter paper by using a paper mask and TMOS solution.