Analytical Methods

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Preparation of paper micro-fluidic devices used in Bio-assay based on drop-on-demand wax droplet generating

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Keywords: paper-based microfliudic device, drop-on-demand generating, wax droplet, bio-assay

Abstract. Paper microfluidic devices are a promising technology in developing analytical devices for point-of-care diagnosis in developing world. This article described a simple method for paper microfluidic devices based on a PZT drop-on-demand droplet generator. Wax was jetted in the form of droplet, linked with each other and formed into wax pattern on filter paper with a PZT actuator and a glass nozzle. The heated wax pattern became hydrophobic barrier for reagent used in bio-assay. The glass nozzle fabricated by a home-made micronozzle puller without complicated fabrication technology was low cost, simple and easy-made. Coefficient of variation of the jetted wax droplet diameter was 4.0% which showed well reproductivity. The width of wax line was experimentally studied by changing the driving voltage, nozzle diameters and degree of overlapping. Wax line with width of 700-1700 μm was prepared for paper based microfluidic devices. Multi-assay of glucose, protein and PH and 3×3 arrays of glucose, protein and PH assay were realized with the prepared paper microfluidic devices. The wax droplet generating system supplied a low-cost, simple, easy-to-use and fast fabrication method for paper microfluidic device.

1 Introduction

The measurements of biological fluids the analysis of which is necessary for monitoring the health of populations are difficult to implement in developing world[1]. Although conventional laboratory instruments provide quantitative measurements of biological samples, they are unsuitable for less-industrialized countries, emergency situations, or home health-care settings as these instruments are large, expensive, uneasy to use and biological sample consuming[2]. The platform that uses small volumes of sample and that is sufficiently inexpensive to be widely used is needed, particularly in

less-industrialized countries. Paper-based microfluidic devices are emerging as a new technology for applications in diagnostics for the developing world, where low cost and simplicity are essential[3-6]. Patterned paper diagnostic devices have advantages of easy to use, inexpensive, low volume, easily adaptable, and are capable of rapid on-site detection. Paper is made of naturally abundant materials and is biodegradable. The most important is that fibrous nature of paper provides intrinsic (capillary) pumping, thus they can lead to multiplexed and miniaturized assays that run by capillary action on a piece of paper and realize inexpensive on-site analysis.

There exist several methods for fabrication of paper based microfluidic device, which include photolithography[7-8], plotting[9-10], inkjet etching[11-13], plasma etching[14-15], flexographic printing[16-17], cutting[18], Polydimethylsiloxane (PDMS) stamp[19] and wax printing[20-22]. Photolithography method provides 200 μm resolution of functional channel. However phtoresist is very expensive and photolithography equipment is needed. Inkjet based technique needs two steps of which the first is soaking the paper with 1.0%(mass ratio) solution of polystyrene in toluene and then forming hydrophilic channel by inkjet printing of toluene on the desire regions. Flexographic printing is suitable for large scale production; however it requires two prints of polystyrene and different printing plates. Although plasma etching method creates patterns without affecting their flexibility or surface topography, an oxygen plasma treatment is required to create hydrophilic areas. Water resist ink plotting requirs laser cutting metal templates with specific patterns. The boundary of PDMS plotting is rough, as its resolution is 1 mm. Paper devices by knife or laser cutting are not easy to be folded and stored. Compared with photoresist, PDMS, AKD-heptane or polystyrene in paper, patterning paper with wax printing technology has obvious advantages such as fast and easy fabrication, cheap, and no use of expensive organic solvent. The edge of wax line formed by a wax pen is rough and the resolution is no better than plotting method. Commercial wax printer is very expensive and only accompanying wax could be used for printing. Screen wax printing method has low resolution of microfluidic channels (rough barrier) and requires different printing screens for creating different patterns. Further comparison information on these previous methods can be found in recently published review articles [23-27]. Although each method has its own advantages, the need still remains for gentle and simple fabrication techniques of paper microfluidic devices that can be widely implemented in developing world at minimized cost.

The aim of this study was to develop low-cost, simple, easy-to-use and fast fabrication method for paper microfluidic device. In this paper we reported a new method for paper microfluidic device based on wax droplet generating. The melted wax liquid in glass nozzle could be jetted on the filter paper in form of droplet by a PZT actuator and the droplets linked with each other to form into wax pattern. Compared with other two dimensional methods, first this method introduced here used cheap hydrophobic material-wax, second the wax could form directly without any PDMS or metal templates which made the patterning process fast and easy, and finally the glass nozzle used here was easy made, cheap , good chemical resistance, low friction and simple. Firstly we studied the liquid height in the glass nozzle in the steady wax jetting notion, and then determined the sizes of wax droplet changed with the nozzle diameter and amplitude of driving signal. Next the spread of wax line on filter paper influenced by the temperature, heating time and the degree of overlapping was determined, and finally the paper device were fabricated and tested with de-Ionized water. A multi bio-assay of glucose, protein, and PH, and 3×3 arrays of glucose, protein and PH assay were presented.

2. Experiment

2.1 Materials

Paraffin wax 56-58 was purchased from Shanghai Specimen and Model Factory. Ethanol AR was purchased from Sinopharm Chemical Regent Co., Ltd. Qualitative filter paper 102 Ø7 cm and Ø10 cm were purchased from Hangzhou Xinhua Paper Industry Co., Ltd. Glass nozzle of 6.5 mm×5.0 mm was from Nanjing Lupu Chemical Co., Ltd. Disodium hydrogen phosphate dodecahydrate and potassium dihydrogen phosphate were purchased from Tianjin Kaixin Chemical Industry Co., Ltd. Glucose oxidase (Amresco 0243) and horseradish peroxidase (JS10540, Cas 9003-99-0, RZ≥ 1.5) were purchased from Shanghai Jinsui Bio-tec Co., Ltd. Potassium iodide was from Nanjing Chemical Reagent Co.,Ltd. Citrate and trisodium citrate dehydrate were supplied by ChengDu KeLong Chemical Co. Ltd. Tetrabromophenol blue (TBPB) (MW 691.9) was from Beijing Dingguo Changsheng Biotechnology Co. Ltd. Bovine serum albumin (BSA) was from Beijing Solarbio Science & Technology Co., Ltd. Glucose was purchased from Tianjin Damao Chemical Reagent Factory.

2.2 Platform of the wax printing system

In the drop-on-demand wax droplet generating system, as shown in Fig. 1, the pulse influencing driven force was

supplied by a PZT actuator P-844.10, produced by Polytec PI, Inc. and the schematic of which with a connector and a glass micronozzle was shown in Fig. 1. The drop-on-demand wax droplet generating was proposed on the friction coupling effect between the microfluidic boundary layer and solid wall of the micro channel [28-30]. As shown in Fig. 2(a), the piezoelectric actuator connected to the solid wall of the micro nozzle exerts a periodicity disturbing on the solid wall and the boundary flow obtains a movement along with the solid wall. Then the viscous force F_1 within the fluids transfers the movement and the microfluidic body obtains a velocity v_1. When the applied pulse voltage decreases rapidly to zero in magnitude, the PZT actuator contracts and the liquid inside the micro-nozzle obtains a pulse inertia force F_2 relative to the micro-nozzle. When the inertia force F_2 is large enough in magnitude, the inertia force F_2 exceeds the viscous force F_1, a droplet of the liquids will be thrown out of the micro-nozzle drop by drop in the direction of the inertia force F_2.

Various kinds of wax patterns could be formed on the filter paper when the *xy* worktable moved along the pattern. Two Nickel-cadmium alloy (Cr20Ni80) resistance coils were fixed outside of the nozzle to melt the wax. The one with 1.5 cm diameter was used to heat the reservoir part of the nozzle and the Ø0.5 cm one was used to heat the nozzle tip. With the drop-on-demand wax droplet generating system, the melted wax was jetted on the filter paper in the form of wax droplet, and the droplets linked with each other to form a wax pattern.

2.3 Fabrication of the glass nozzle

Glass is a non-crystalline solid and thermal viscoelastic material. Glasses can be optically transparent and abundant in natural. Compared with silicon and PDMS, glass is cheap and easy to be processed. In this work, the glass nozzle was made of borosilicate glass tube with 6.5 mm out-diameter and 5.0 mm inner-diameter, and an improved micro nozzle fabrication apparatus was proposed. The schematic of the apparatus was shown in Fig. S1. The pull force is supplied by the gravity of the weight blocks, and the glass tube is heated by cadmium-nickel alloy coil. Both ends of the glass tube are fixed to floating clamps which move along the guide and a pre-load fore is applied by the weight blocks through a group of pulley sets. To make the glass tube heated sufficiently, a time delay structure composed of a drawable electromagnet bracket and a step motor driven screw was designed and the photo of the apparatus is shown in Fig. S2.

The process of the whole micro nozzle pulling was shown as follows:

Step 1: fix the glass tube on the clamps, then the glass tube was in the center of the heating coil and the weight blocks were hanged by steel wire rope through the pulley sets.

Step 2: draw the electromagnet bracket to carry the down side clamp.

Step 3: turn on the power to make the glass tube heated, and when the set time (t_1) was reached, the glass tube was melted, and the DC motor would move to make the weight blocks drop slowly with the guide screw with time(t_2). When the defined distance (L_1) of the down side clamp was reached, another time delay (t_3) was set. When t_3 was reached the electromagnet drew the bracket and the blocks dropped as their gravity. With the force supplied by the blocks, the glass tube was deformed into two micro nozzles. The time of the whole process cost t_0 was an addition of t_1, t_2 and t_3 .

t_1 was set as 20 s, and L_1 was 2 mm, so the pre pulled length of the glass tube was 4 mm. t_3 increased from 10 s to 40 s, and the tape length of the glass nozzle ranged from 7.5 cm to 20 cm, and the tip was quite thin. To distinguish the nozzle, we cut them from 1.5 cm of tape length part, and measured the inner diameter of the cross section as shown in Fig. S3. The relation between the diameter and the delay heating time t_3 was shown in Fig. S4, and the diameter ranged from 200 μm to 750 μm.

Then we forged the nozzle with platinum wire heater to constrict the tip using Sutter MF-900, as shown in Fig S5. The final micro nozzle filled with wax was shown in Fig S6.

2.4 Preparations of chemical sensing inks

For glucose assay, a 1:5 horseradish peroxidase/glucose oxidase solution (100 units of protein per ml of solution) was obtained with de-ionized water, glucose oxidase and horseradish peroxidase (150 unite/mg) and stored at 4 ℃. A 0.6 mM potassium iodide (KI) solution was obtained by adding 0.498 g KI into 5 ml de-ionized water. For protein assay, a 3.3 Mm tetrabromophenol blue (TBPB) in ethanol solution was prepared, and a 250 mM citrate buffer solution (pH 1.8) was obtained. 7.5 mM albumin from bovine serum (BSA) solution was obtained by adding 0.84 g BSA into 16.8 ml de-ionized water, and 50 mM glucose solution was obtained by adding 4.5 g glucose into 50 ml de-ionized water, and then it was respectively diluted into 40 mM, 35 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM, 1 mM glucose solution.

2.5 Fabrication of paper based microfluidic devices

2 ml melted wax was drew with a 5 ml plastic syringe, and then fill the glass micro nozzle through a 400 mesh screen to remove the solid particles which may block the nozzle. Block structure of wax which fitted the size of nozzle was prepared, and the nozzle was replenished at regular intervals during wax droplet generating. Filter paper (Ø7 cm) was placed on the stage of the wax droplet generating system. Wax droplets were jetted on the paper and as the *xy* worktable moved along a patterned set before, the droplets linked with each other to form a pattern. Then the filter paper with wax pattern was allowed in 75 ℃ for a period of time(1-2 min), the melted wax penetrated into the paper to form a hydrophobic wall. The width of the wax line dispensed on the filter paper was allowed to be controlled by changing the system parameters such as the driving voltage (V), nozzle diameter (D), and the degree of overlapping (k).

As shown in Fig. 2(b), two assumptions were taken, of which one was that the wax droplet was a piece of cylinder, and the other was that the overlapping area of two neighbor droplets was determined only by the overlap distance. k refered to the ratio of overlapping area between two neighbor droplets and the area of a whole wax droplet.

Thus *k* could be written as equation (1), where *L* was the length of the overlap area (μm); D_b was the diameter of a wax droplet (μm); *a* was the distance of each jetting step of the work stage (μm).

$$k = \frac{L}{D_b} = 1 - \frac{a}{D_b} \quad (1)$$

3 Result and discussion

3.1 Influence of system parameters on wax droplet jetted

When the glass micro nozzle was overloaded with wax, static pressure on the nozzle tip would cause exuding. Melted wax kept flowing out of the reservoir and gathering around the nozzle tip, then formed into big droplet, and finally fell on filter paper as its own gravity. Whereas the liquid in the glass micro nozzle was not enough, the hydrodynamic pressure caused by inertia force was not powerful enough to jet out the wax droplets. There was a range for steady jetting. 1.5 ml melted wax was respectively drew into four types of micro nozzle fabricated with the homemade apparatus varies in tip diameters as shown in table s1. Steady jetting was defined as jetting without exuding and scattering. The amplitude of driving signal was 40 V which was the minimum amplitude to jet out wax droplet. The maximum length of wax liquid in the micro nozzle was shown in Fig. 3(a), and the maximum length ranged from 2.1 cm to 3.8 cm varies with inner

diameters of the nozzle tip.

The sizes of the wax droplet were measured after being jetted on glass slide with each kind of micro nozzle in different voltage *V*. Result showed that the wax could not be jetted with *V* less than 40 V. Diameters of the wax droplets jetted by 150 μm nozzle formed on the slide glass increased from 180 μm to 350 μm, and the photo of wax droplets jetted with *V* of 50 V, 60 V, 70 V, and 80 V was shown in Fig. 3(b). The distance between each droplet was set as 600 μm, and the jetting frequency was 3 Hz. The amount of wax increased with amplitude of electrical driving signal *V*. The shapes of wax droplets were almost circular unless prepared by 80 V driving signal as shown in Fig 3(b-4) which was resulted by that the amount of per droplet was too much and contact speed with glass slide was high. We treated this kind of droplets approximating a circle which equaled in the area. After linking with each other, the shape of the droplets would have little influence on the wax line. Some droplets with diameter less than 10 μm resulted by scattering were accompanied which would not affect the wax line quality on filter paper as they were too small to penetrate filter paper. The coefficient of variation (CV) of the jetted wax droplet diameter were determined by the 10×10 wax droplet arrays, and CV of wax droplets was 4.0% which showed well uniformity of the wax droplets. The sizes of the wax droplets jetted with other types of nozzles on driving voltage from 40 V to 80 V were measured with the same method, and result was shown in Fig. 3(c). The smallest droplet prepared was 125 μm and the droplet sizes ranged from 125 μm to 450 μm.

3.2 Spread of wax line on filter paper

We tested the 2 mm flow channel with four circular reaction zones prepared with randomly chosen parameters by dipping 20 μL de-ionized water on one area, and with capillary force, the water slowly reached the other three areas. There existed water leakage and water blocking. The leakage included side leakage and back leakage. The reason of side leakage was that the degree of overlapping was too small, and back side leakage was that the amount of wax was not sufficient enough to penetrate the whole filter paper. When the wax lines linked with each other, the microfluidic channel would be blocked. To avoid leakage and blocking, the influence of system parameters on the wax line was studied.

Width of wax line and penetration of filter paper were two character factors for wax line formed on filter paper for bio-assay. Both of the two factors were influenced by the original sizes of the droplets, degree of overlapping *k* and

melting conditions which include the temperature and lasting time. The width of the melted wax line could be measured directly and the wax penetration was assessed by the color and dimensional homogeneity of the wax line on the back side. Wax line of 200 μm with k=50% was jetted on the filter paper. The melting point of wax was 59 ℃, and with more than 100 ℃ as mention in paper [22], the filter papers needed to be manipulated very fast. So, these filter papers with wax line samples were put in 75 ℃ thermostatic well for 20-120 s to determine the width of wax line. As shown in Fig. 4(b), the width increased with the heating time after 20 s. The wax line width changed slightly with time surpassed 80 s. The surface topography of the wax line was shown in Fig. 4(a), 20 s could not melt the wax line, whereas 40 s made some wax penetrate the paper, but the edge of wax line on back side was rough and the color was not consisting. 60 s made more consisting and regular of the line edge and some solid wax stayed. 100 s melted almost the whole wax and the wax on the back side filter paper spread adequately enough to be a hydrophobic barrier. Considering the wax line width and spreading of wax on the back side, lasting 80 s in 75 ℃ was chosen for wax line heating.

Four wax line formed by 60 wax droplets with diameter of 250 μm on filter paper were prepared as shown in Fig. 4(c-1), *k* were respectively 10%, 30%, 50% and 70%. The width of wax lines formed on filter were all around 500 μm, whereas the thickness of the wax line increased with *k*, as there was more wax in per unite length. The filter papers were put in 75 ℃ thermostatic well for 80 s, and then the wax melted into and penetrated the whole filter paper as shown in Fig. 4(c-2). The widths of wax line increased slightly from 900 μm to 1100 μm, and the color of *k*=70% was the deepest, because there still existed solid wax on the surface. Too many wax droplets(*k*>50%) would result a waste and too wide wax line, whereas when *k* was too small, it may lead to leakage of liquid out of the micro channel. With this kind of method, other widths of wax line were studied, result was shown in Fig. 4(d), and widths of wax line barrier ranged from 700 μm to 1700 μm. 1 mm resolution was adequate for rapid prototyping of hand-held, visually read, diagnostic assays based on paper [8].

3.3 Paper-based microfluidic devices

The glass micro nozzle was prepared by three steps, first pulled, then cut to 300 μm of inner diameter on the tip, and finally forged to 150 μm of diameter on the tip. Amplitude of the driving signal (*V*) was set as 50 V, frequency was 3

Hz, and the distance between each droplet was 100 μm (*k*=50%). The droplets were jetted on filter paper and linked with each other to form into a wax pattern as shown in Fig. 5(a). The pattern was designed with four 4 mm circular reaction areas and fluid channel of 1100 μm. This kind of pattern could also be realized by paper strips fabricated by two-dimensional shaping, but for micro channel ranged to micro meter; it was not convenient to be stored. Compared with *x, y*-plotter [8] and flexographic printing [22], the glass nozzle need not to contact the filter paper which kept the paper clean and dry.

The paper microfluidic device fabricated was then put in 75 ℃ thermostatic well for 80 s to melt the wax droplets. Wax line spread and the width of final flow channel was around 1mm. The paper microfluidic device used for multi assay was tested by dipping 50 μL of de-ionized water into one of the four reaction zones. The water flow into three branches in the cross part, and reached the other three parts in 10 minutes with capillary fore. The reaction zones were filled with water in another 12 minutes without any leakage. The micro reaction zone was shown in Fig. 5(b, c). Slight difference on line width along the flow channel would affect the speed of capillary flow when the flow channel was quite thin, as one reaction zone was filled a little earlier than the other two.

3.4 Bio-sensing paper for glucose, enzyme and PH

For the glucose assay, a cross section microfluidic device with four flow channels (2 mm), four reaction zones and a test agent adding zone was prepared as shown in Fig. 5(d). 3 μL potassium iodide solution was dipped in zone A, and followed by 3 μL 1:5 horseradish peroxidase/glucose oxidase solution (100 units of protein per mL of solution). For the protein assay, 3 μL of a 250 mM citrate buffer solution (pH 1.8) in a well separate from the glucose assay was spotted in zone B and then layered a 3.3 mM solution (3 μL) of TBPB in 95% ethanol over the citrate buffer solution. For PH assay, 3μL PH reagent was dipped in zone C. The paper microfluidic device was dried in room temperature, and then stored in 4 ℃ environment. The test reagent was made by mixing 50mM of glucose solution and 7.5 mM of BSA solution with 1:1 ratio. 20 μL test reagent was dipped in the middle of the cross area, and the solution reached the glucose, protein, PH assay, and contrast area with capillarity driving. The paper was laid in room temperature for 10 minutes, and after that the test paper dried, the protein assay turned blue, the glucose assay turn yellow, and PH assay turned red(PH7.0) as shown in Fig.

5(e). Photographs are taken with Nikon D2X camera in a controlled intensity light environment and there existed coffee ring effect in the reaction area.

3.5 Array of micro reaction zone for glucose ,protein and PH

For different concentration glucose solution assay, an array of circle reaction zone with each diameter of 4.5 mm was prepared.3 μL of potassium iodide solution was dipped in each zone from 1 to 9 as shown in Fig. 6(a), and followed by 3 μL of 1:5 horseradish peroxidase/glucose oxidase solution (100 units of protein per mL of solution) on each zone. Finally the paper device was dried in room temperature for 20 minutes, and stored in 4 ℃ environment. 3 μL of 50 mM, 40 mM, 35 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM, 1 mM glucose solution were dipped in zone 1-9 separately, and dried for 20 minutes. The darkness of the yellow color resulted by the glucose reaction changed from deep to pale, which showed that the glucose concentration gradient decreased obviously. The gray scale of the circular area measured by the mean value of gray image matrix resulted with MATLAB was shown in Fig. 6(b), and the gray value increased from 173 to 192 as the yellow color of 50 mM was the deepest. For different concentration protein solution assay and PH testing, arrays of circle reaction zone with each diameter of 3 mm were prepared. For the protein assay, 3μL of a 250 mM citrate buffer solution (pH 1.8) was spotted in zones from 1 to 9 respectively and then layered a 3.3 mM solution (3 μL) of TBPB in 95% ethanol over the citrate buffer solution in each zone, and finally the device was dried in room temperature. 4 μL of BSA solution with the concentration of 0, 1, 4, 10, 25, 66, 90, 130, 200 mg/ml were dipped in 1-9 zones separately. Once the test reagent was dipped, there emerged blue color as the reagents with BSA, and as shown in Fig. 6(c), the color changed from aquamarine blue to cobalt blue as the concentration of BSA solution increased. For PH testing the 9 kinds of test reagents were obtained by mixing 100 mM of disodium hydrogen phosphate dodecahydrate solution and 100 mM potassium dihydrogen phosphate solution respectively with rations from 1:9 to 9:1. 20 μL of test reagents was dipped in 1 to 9 separately and then followed by 0.5 μL of PH reagent. As shown in Fig. 6(d), the color changed with the PH value from yellow to green and to blue as the test reagents changed from acidity to alkalinity, and the PH value could be read with PH color chip.

4 Conclusions

Here we demonstrated a new method for paper based microfluidic device based on wax droplet generating. A PZT stack actuator was used as driving source to provide enough pulse inertia force for wax droplet generating. A novel glass nozzle puller for micro nozzle 7.0 mm reservoir tube was designed and the glass nozzle fabricated with borosilicate glass tube had advantages of easy made, cheap , good chemical resistance, low friction and simple. The wax pattern is formed directly without multiple printing steps or templates and the filter paper does not need to contact with anything but the wax droplets. Almost all kinds of paraffin wax commercial available were suitable for the wax droplet generator. The wax droplet size could be easily changed by changing the micro-nozzle outlet diameter and the amplitude of the applied pulse voltage. The widths of wax line melted into filter paper increased with the wax droplet sizes, degree of over lapping (*k*). The melted wax line width on filter paper could be controlled easily for paper microfluidic device fabrication, and this method fitted filter paper with almost all kinds of thickness. Multi-assay of glucose, protein, and PH were realized on paper microfluidic device with cross-shaped wax pattern, and concentration gradient of glucose, protein solution, and PH were tested with circular micro reaction array. In further work, two or more nozzles could be fixed on one PZT actuator and more than one wax patterns could be produced at the same time for large scale production. The wax pattern could be overlaid or folded to prepare three-dimensional (3-D) device for point of care diagnosis [31].

Acknowledgements

This paper is supported by the National Natural Science Foundation of China (NO.51175268, NO.11102090) and the Specialized Research Fund for the Doctoral Program of Ministry of Education (NO.20113219110004).

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Figure legend

Fig. 1 Schematic of the drop-on-demand wax droplet generating system for paper-based microfliudic device

Fig. 2 (a) Principle of microfluidic driving (b) Schematic diagram of the wax droplets

Fig. 3 (a) Maximum length of wax liquid in micro nozzle on steady jetting motion (b) Photo of 3×3 wax droplet arrays jetted by micro nozzle of 150 μm outlet diameter (c)Relationship between droplet sizes and driving voltage for nozzles with outlet diameters from 100 μm to 200 μm

Fig. 4 (a) Photo of melted wax line on filter paper for wax line of 200 μm with k=50% in 75 ℃, (1) the original wax line, (2) wax line heated for 40 s, (3) wax line heated for 60 s, (4) wax line heated for 100 s (b) Relation between wax line width and heating time for wax line of 200 μm with *k*=50% (c) Photo of wax line formed on filter paper (1) wax line formed by 60 droplets of 250 μm with *k*=10%, 30%, 50%, 70%, (2) melted wax line formed by 60 droplets of 250 μm (d) Relation between the degree of overlapping *k* and the width of wax line

Fig. 5 (a)Photo of wax pattern jetted on filter paper (b) Device run with de-ionized water capillary flowing to reaction zone, one of the reaction zones were dipped with 50 μL de-ionized water, (c) Reaction zones filled with water (d) Device run with 20 μL of mixture of 25 mM glucose and 3.75 mM of BSA solution, 5 minutes after dipping of the test agent (e) 20 minutes after dipping of the test agent

Fig. 6 Photo of micro reaction array, (a) Glucose assay, 20 minutes after respectively dipping of 3 μL of 50 mM, 40 mM, 35 mM, 25 mM, 20 mM, 15 mM, 10 mM, 5 mM, 1 mM glucose solution in zone 1-9, (b) Numerical fitting curve of gray scale for glucose assay, each point was mean of 10 measurements, and error bars represent uncertainty of measurement data. A linear model was used to fit the changing trend of gray scale. (c) Protein assay, 30 seconds after respectively dipping of 4 μL of BSA solution with different concentrations in zone 2-9 and 4 μL of deionized water in zone 1, (d) PH testing, 30 second after that 20 μL of test reagents was dipped in 1 to 9 separately and then followed by 0.5 μL of PH reagent

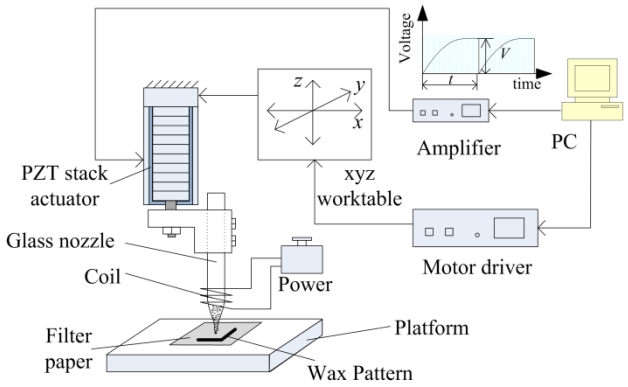


Fig. 1 Schematic of the drop-on-demand wax droplet generating system for paper-based microfliudic device

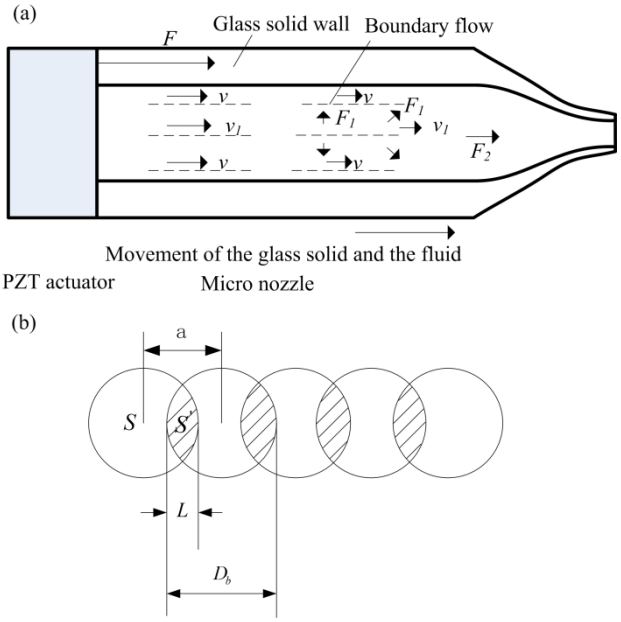


Fig. 2 (a) Principle of microfluidic driving (b) Schematic diagram of the wax droplets

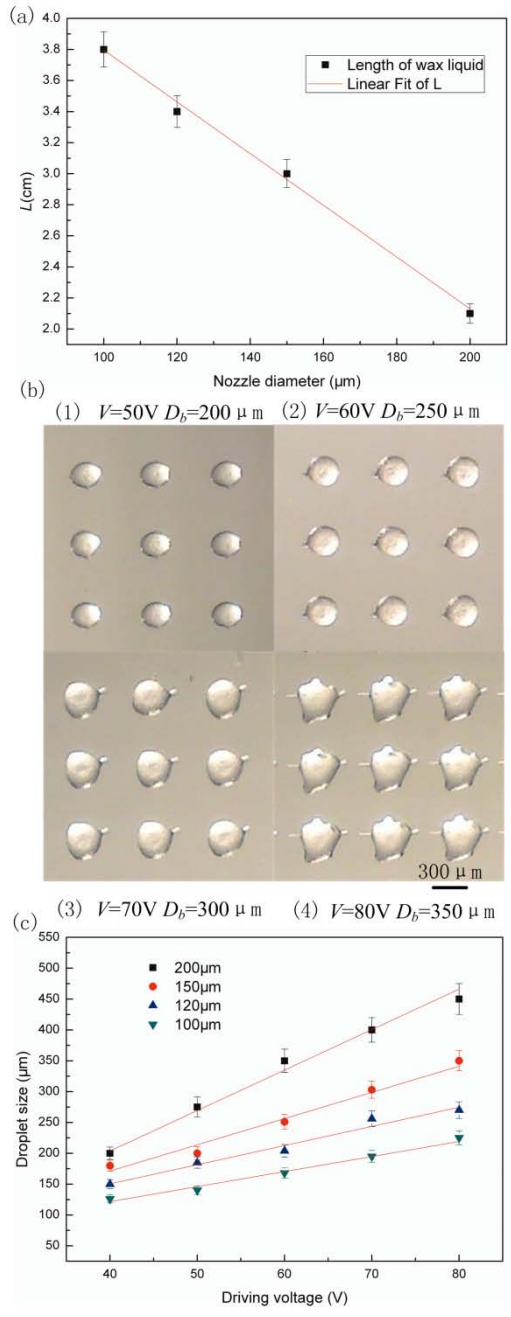
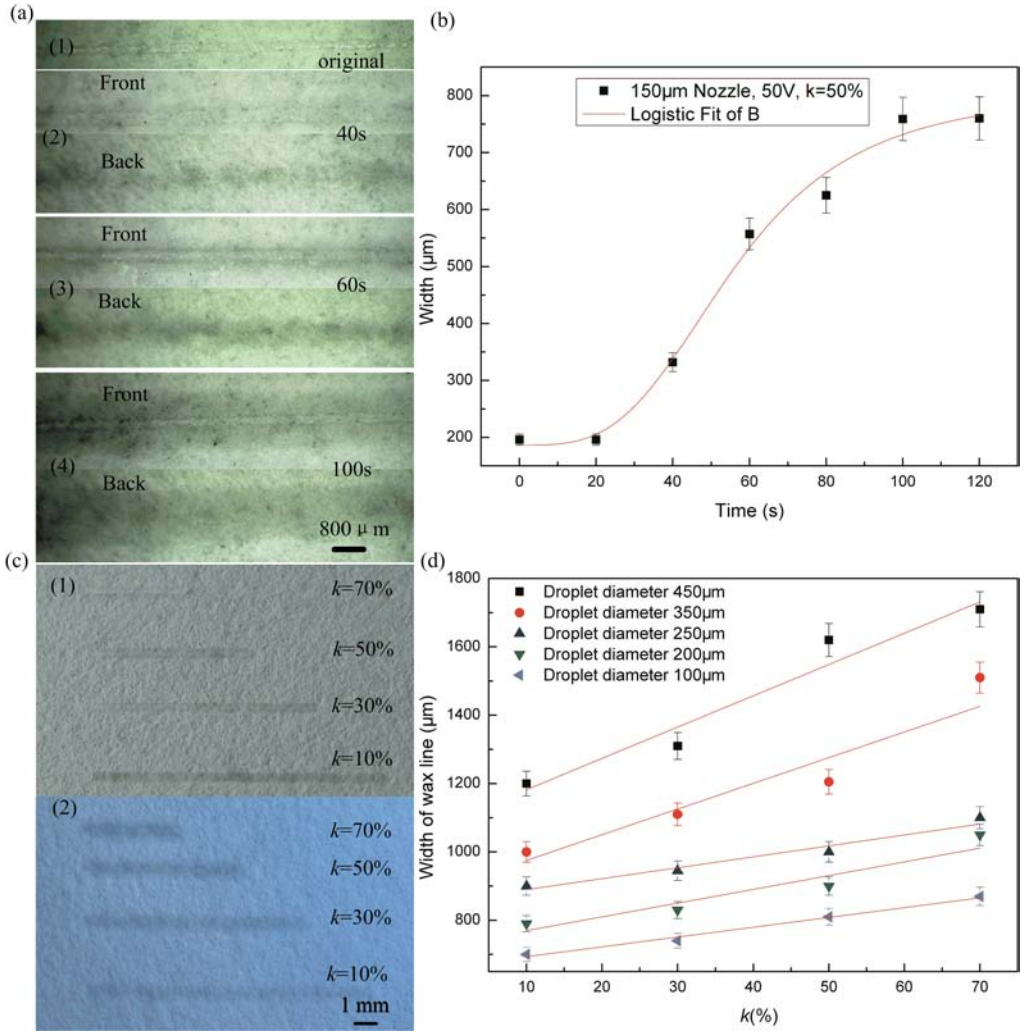


Fig. 3 (a) Maximum length of wax liquid in micro nozzle on steady jetting motion (b) Photo of 3×3 wax droplet arrays

jetted by micro nozzle of 150 μm outlet diameter (c)Relationship between droplet sizes and driving voltage for nozzles with outlet diameters from 100 μm to 200 μm



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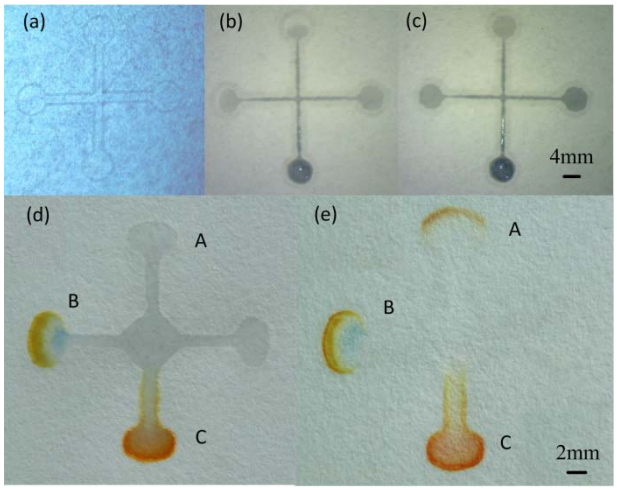

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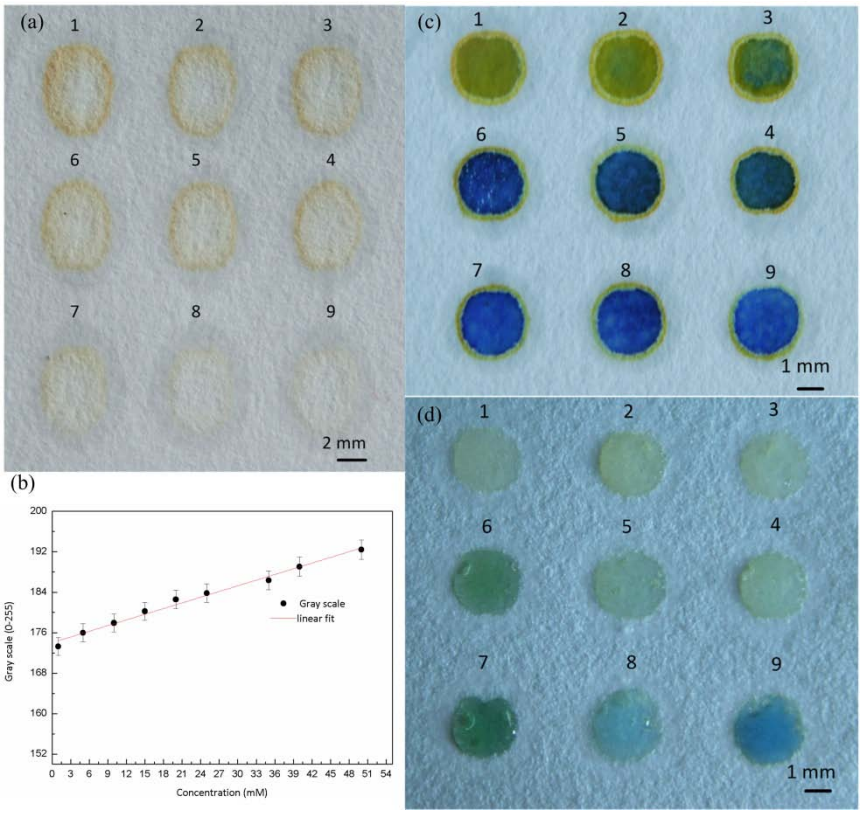

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This paper describes a method for the paper based microfluidic devices used in Bio-assay based on wax droplet generating method.