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A one-step polymer screen-printing method for fabrication of microfluidic cloth-based analytical devices

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ABSTRACT

We report the first use of a one-step polymer screen-printing method for fabrication of microfluidic cloth-based analytical devices (μCADs). The method involves a simple, low-cost and one-step fabrication process and requires only polystyrene and a patterned screen. Firstly, polystyrene solution is poured through the patterned screen and allowed to penetrate through the cotton fabrics to create a three-dimensional hydrophobic barrier which defined the test zones. Different types of cotton fabric and polystyrene concentrations were first investigated. By adjusting polystyrene concentration, various types of cotton cloth can be successfully fabricated. Under optimal condition, the smallest hydrophilic channel and hydrophobic barrier that the fabrication method can provide were 678 ± 59 μm and 329 ± 27 μm, respectively. High device-to-device repeatability was achieved with a relative standard deviation (%RSD) in the range of 1.10–2.03% (n = 64) obtained from the measured diameter of the circular-shaped fabricated test zones with a designed diameter of 5 and 7 mm. The cloth treatments using hot water, Na_2CO_3 and NaOH solutions were found to affect the wicking ability of the cloths where the highest wicking rate was found in the Na_2CO_3 treated-cloth followed by NaOH, hot water treated-cloths and untreated-cloth. To demonstrate the significance of the fabricated μCAD, the analysis of glucose and albumin in control human serum was carried out. The results showed no significant difference at 95% confident intervals of the glucose and albumin levels obtained from the μCAD analysis and the certified values verifying that the polymer-screen printing method can be alternatively used as a method for μCAD fabrication.

1. Introduction

Microfluidic analysis systems are an effective and alternative technology to the traditional analytical methods because they provide for a rapid, portable and sensitive analysis which is much needed in resource-poor countries and remote regions [1,2]. Over the past decade, microfluidic devices have been developed and applied in several areas including food sciences, medical diagnostics, environmental monitoring, clinical chemistry as well as biological analysis [3–6]. Early microfluidic devices were made from glass, silicon and polymer materials such as poly(methyl methacrylate) (PMMA), an epoxy based negative photoresist polymer (SU-8) and poly(dimethyl siloxane) (PDMS) [7]. These devices have been widely used for various biochemical assays for decades due to their small size, low reagent and sample consumption, rapid analysis, high integration and easy automation. However, the devices are expensive, non-disposable and complicated to fabricate in developing countries.

Recently, both paper and cloth have been used as materials to create microfluidic devices called microfluidic paper- or cloth-based analytical devices (μPADs or μCADs) since they are affordable, low-cost, worldwide available and have wicking properties by capillary forces allowing the solution to flow during the analysis without the need of an external pump [8–11]. μCAD was first introduced in 2011 by Bhandari and coworkers who reported the use of silk yarn to fabricate the fabric microfluidic chips using a weaving method [12]. It has since been shown to have the potential for the development of simple and low-cost chemical and biological assays and has been applied for the analysis of many compounds including glucose, protein, nitrite and lactate [13,14]. Major advantages of using cloth as a microfluidic substrate over existing alternative materials such as paper are (i) cloth has better mechanical properties such as greater tensile strength, more flexibility and higher durability (ii) more choices of fibers can be used to make

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cloth and (iii) cloth has more interstitial space to allow the solution flow faster [15,16]. Like μPADs, the μCADs are obtained by patterning their hydrophilic cellulose area with impermeable materials serving as barriers to define flow channels or test zones.

Several methods have been developed for μCAD fabrication including weaving [12,17–20], cutting [21], wax-patterning [22–24], wax screen-printing [25] and photolithography [26]. The weaving method was first reported for μCAD fabrication using hydrophilic and hydrophobic yarns to systematically construct woven textile fabric to create the patterned test zones [12,17–20]. This method could offer mass production of devices but well-defined detection zones and complicated patterned structures, such as two- and three-dimensional μCADs, are still difficult to create. Cutting by hand is a simple and cheap method [21], however, the major disadvantages are the inability to convert to mass production and to create small mechanically stable structures. Wax patterning offers an inexpensive fabrication method as it employs patterned wax-impregnated paper fixed to the cloth and the wax from the paper is transferred to the cloth by heat treatment to create patterned μCADs [22–24]. This fabrication approach, however, suffers from slow fabrication speed, low throughput and low accuracy because of the difficulty of creating patterns on the wax-impregnated paper by the hand carving process. The wax screen-printing method could also provide a low-cost, rapid, simple and low power consumption fabrication as it requires only an inexpensive patterned screen and a hotplate. However, it suffers from low feature resolution due to wax spreading and the inability to use it with organic solvents during chemical analysis applications [25]. Photolithography could be used to create high resolution channels. However, it involves long and multiple steps and also requires expensive instruments to accommodate all the fabrication processes including oven, hot plate, UV-light, patterned transparency film as well as exposure system [26].

An ideal fabrication method for cloth-based analytical devices should offer fast, low-cost and simple fabrication using worldwide available materials to allow for massive device production in developing countries. In an effort to reach this goal, this work proposed a novel fabrication method for a μCAD using polymer screen-printing method. The fabrication process is similar to the μPAD fabrication method previously reported by our group [27]. To the best of our knowledge, however, no work on patterning μCAD by polymer screen-printing has been reported. The obtained μCAD fabricated using the developed fabrication method can be more flexible μPAD hence, can be further embedded into daily textile products that are in direct contact to human fluids to do real time chemical and biochemical assays. This fabrication method is simple, fast and inexpensive and is able to fabricate the μCAD in one-step without the use of any further heating step and complicated instruments. The fabrication process is based on screen-printing technology where the polystyrene was used as a hydrophobic material to create a hydrophobic barrier on the hydrophilic cloth substrate. Polystyrene polymer as well as screen-printing equipment are inexpensive and widely available in developing countries. The fabrication process involves squeezing solution of polystyrene dissolved in toluene through the pattern screen so it penetrates through the cloth underneath the screen creating a hydrophobic barrier and leaving unexposed areas as detection zones. The optimization of parameters affecting the device structures has been evaluated, including the polystyrene concentration and types of cotton cloth. The performance of the fabricated devices was tested for several features including the resolution, wicking properties, organic solvent compatibility as well as hydrophilic-hydrophobic contrast. The device was then used for the analysis of glucose and protein in chemistry control serum to determine the performance of the fabricated devices for real world application.

2. Experimental

2.1. Chemicals and materials

The three different types of white plain 100% cotton cloth that have different properties (Table S1, Supplementary information) were employed as substrate materials for fabricating μCADs and obtained from a local market in Chon Buri, Thailand. Polystyrene, a squeegee and clear packing tape were from a stationery shop (Chon Buri, Thailand). The patterned screen was obtained from a local screen-printing shop (Chon Buri, Thailand). The chemicals where obtained as follows: Horseradish peroxidase (HRP), glucose oxidase (GOx) and 3,3′,5,5′-tetramethylbenzidine (TMB) (Sigma Aldrich, Saint Louis, Missouri, USA), bromocresol green (BCG) (Fisher Scientific, UK), D(+)-glucose ($C_6H_{12}O_6$), 30% hydrogen peroxide (H_2O_2), sodium carbonate (Na_2CO_3) and sodium hydroxide (NaOH) (Merck KGaA, Germany), sodium phosphate monobasic ($NaH_2PO_4{\cdot}2H_2O$) and sodium phosphate dibasic (Na_2HPO_4) (Ajax Finechem, Australia), bovine serum albumin (BSA) (Calbiochem, USA), sodium acetate anhydrous (CH_3COONa) (QRëC, New Zealand), acetic acid (CH_3COOH), acetonitrile (CH_3CN), dimethyl sulfoxide ($(CH_3)_2SO$), ethanol (C_2H_5OH), hydrochloric acid (HCl), methanol (CH_3OH) and toluene ($C_6H_5CH_3$) (RCI Labscan, Bangkok, Thailand). All reagents were of analytical grade and used as received without any further purification.

Enzyme solutions including GOx and HRP were prepared in 1 mg/mL BSA solution in 0.1 M phosphate buffer pH 7. GOx solution (150 U/mL) and HRP solution (50 U/mL) were mixed in a 5:1 ratio and used for glucose analysis. The TMB chromogenic agent (20 mM) was prepared in methanol and kept in a vial covered in aluminum foil to protect it from light. BCG solution was prepared in 0.1 M acetate buffer pH 4.

2.2. Fabrication of μCADs using the polymer screen-printing method

The fabrication process of the developed method is shown in Fig. 1. The patterns on the screen were generated using the Adobe Illustrator program consisting of white and black areas to generate a solution flow-

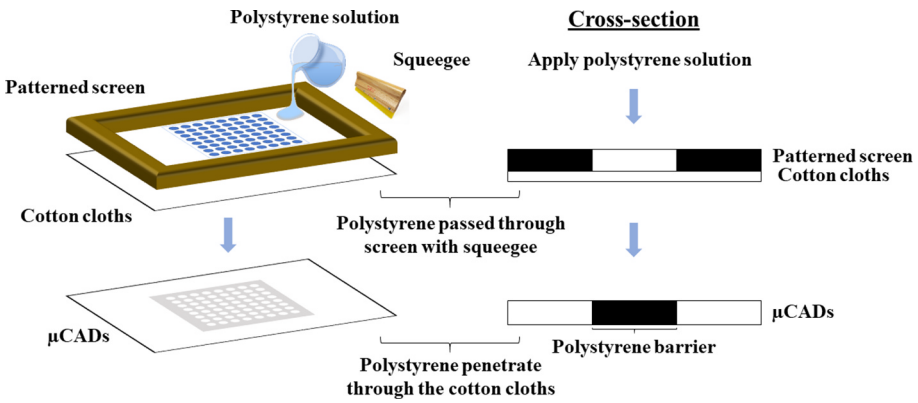

Fig. 1. Schematic diagram showing the one-step polymer screen-printing method for fabrication of microfluidic cloth-based analytical devices (μCADs).

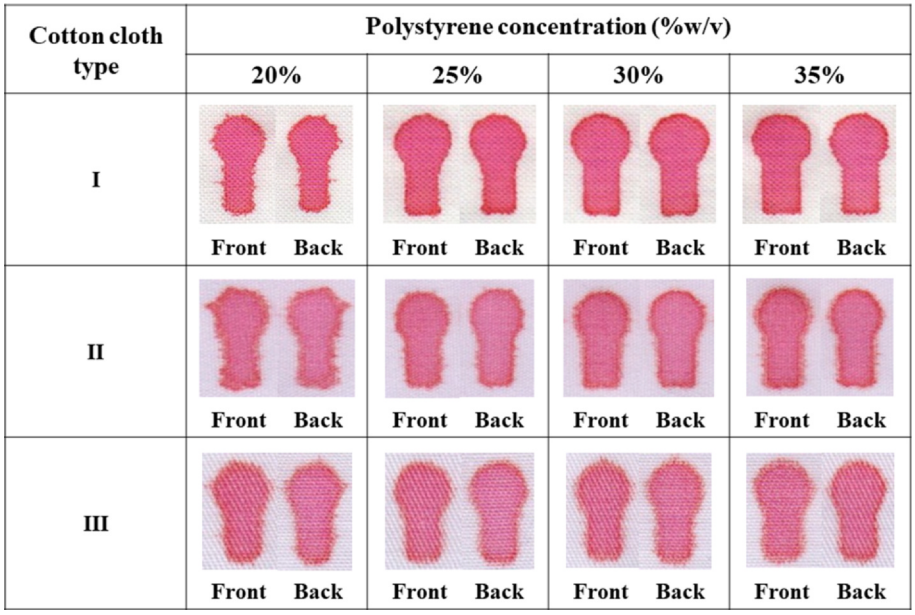

Fig. 2. Structures of μCADs fabricated using the polymer screen-printing method with different concentrations of polystyrene in toluene (20–35 %w/v) and different types of cotton cloth. Red food dye was added to the areas not filled with the polymer to indicate the hydrophilic regions.

through and non-flow through regions on the screen, respectively (See Supplementary Information Fig. S1). The screens were made from 800 mesh polyester fabric on a wooden frame. The polymer solution was prepared by dissolving polystyrene in toluene and degassed to remove air bubbles. The polymer screen-printing method for μCAD fabrication was carried out as follows. Firstly, the patterned screen was tightly placed over the cotton cloth. The polystyrene solution was then applied over the screen and squeezed to pass through the patterned screen and then penetrate to the bottom of the cotton cloth to form 3D hydrophobic barriers. The polymer-patterned cloth was ready to use after drying at room temperature (25 °C). The screen was washed and cleaned using paper towel soaked with toluene between each fabrication. All the steps were performed in a fume hood. Prior to use for any chemical assay, clear packing tape was added to backside of the fabricated μCAD to prevent the solution leaking out of the devices during analysis.

2.3. Pretreatment of cotton cloths and wicking property test

Pretreatment of the cotton cloth was studied to determine whether non-cellulosic impurities such as wax and pectin needed to be removed prior to use for μCAD fabrication and to perform chemical analyses. Scouring using hot water, 10 mg/mL NaOH or 10 mg/mL Na_2CO_3 were investigated following the method reported previously [28]. The cotton cloth was cut into small pieces and boiled at 100 °C for 5 min in solutions prepared in Millipore purified water. After each treatment, the cotton cloths were rinsed with water until the pH of the wash water was approximately neutral. The cloths were then dried at room temperature. Hydrophilic channels (2 mm wide) were fabricated in each type of treated and untreated cotton cloths using the polymer screen-printing method described above. Red food dye solution was used to test the wicking properties of each material when placed either horizontally or vertically. In the horizontal test, the red dye was dropped onto one end and the length that the dye moved due to capillary action was measured over time using a ruler. For the vertical test, the channels were placed vertically with the lower end dipping into the dye solution. The wicking height that the dye moved was also measured over time.

2.4. Colorimetric detection of glucose and albumin

The fabricated μCADs were employed for the analysis of glucose and albumin using colorimetric detection to demonstrate their applicability for real world analysis. The device was designed and fabricated to have a well-shaped format with a circular diameter 5 mm. For glucose analysis, 2 μL GOx/HRP mixture solution was dropped on to the detection zone followed by 1 μL TMB solution. The devices were then dried at room temperature for 5 min. The glucose standard or sample solution (1 μL) was then added and allowed to react for 5 min in the dark at room temperature (25 °C). The device was allowed to dry and the image was captured using a scanner and analyzed for the blue color intensity using Image J software (See Supplementary Information for color intensity measurement, Fig. S2).

For albumin analysis, 2 μL of 1 mM BCG solution in 0.1 M acetate buffer pH 4 was dropped into the detection zones and allowed to dry at room temperature for 5 min. The albumin standard or sample (1 μL) was then added to the detection zones and allowed to react for 5 min. The device was allowed to dry and the image taken and measured for hue degree of the color that changed from yellowish-green to blue using ImageJ software (See Supplementary Information, Fig. S3).

3. Results and discussion

3.1. One-step fabrication of μCADs using a polymer screen-printing method

Here, inexpensive and worldwide available polystyrene and a patterned screen were employed for rapid and simple fabrication of μCADs. Although, this method was created for the microfluidic paper-based analytical device (μPAD) fabrication by our group [27], it is the first time that the polymer screen-printing method was used for μCADs. Moreover, unlike the wax screen-printing method which has been widely used for μCAD fabrication, the polymer screen-printing method does not require any extra heating step that caused the wax spreading creating poor resolution structure on the devices.

The polystyrene concentrations and types of cotton cloth were first evaluated to study the effect of spreading and penetration of the polystyrene solution into the cotton cloth. Three different types of cotton cloth with different properties (*i.e.* mass density of cloth and linear density of thread, see Supplementary Information) were

employed. As shown in Fig. 2, both polystyrene concentrations and cotton cloth types affected the structures of the fabricated devices. Lower polystyrene concentrations gave lower solution viscosity and provided better penetration though the cotton cloth. However, a too low polystyrene concentrations can cause solution spreading resulting in low resolution structures and irreproducible dimensions. Higher polystyrene concentrations, on the other hand, can result in a higher viscosity and hence lower penetration ability into the cloth to form complete three-dimensional hydrophobic barriers. Cotton cloth types with different mass density also had a significant impact on the final structure of the fabricated devices and was found to relate with optimal concentration of polystyrene. Cotton cloth type I has lower mass density (~7.8 mg/cm^2) than type II (~12.0 mg/cm^2) and type III (~12.5 mg/cm^2) providing more space for better penetration of polystyrene solution. For cotton cloth type I (Fig. 2, row 1), all of the polystyrene concentrations investigated penetrated through the cloth forming complete 3D hydrophobic barriers. Low polystyrene concentrations (20–30% w/v), however, resulted in the solution spreading into the cloth creating low resolution hydrophobic barriers due to the low viscosity solution and the low mass density fabric as shown by the spreading of the red dye. Higher concentrations (35% w/v) gave a well-defined pattern both at the front and back of the cloth indicating that high resolution structures and complete hydrophobic barriers were obtained. For cotton cloths type II and III (row 2 and 3, Fig. 2), the optimal polystyrene concentration that gave well-defined structures were 30% and 25% w/v, respectively. Higher or lower polystyrene concentrations gave incomplete 3D hydrophobic barriers and/or low resolution structures. These results demonstrate that the fabrication method can be applied to various types type of cotton cloth that have different mass density. By adjusting the polystyrene concentrations, well-defined hydrophobic barriers on the μCADs were obtained using cotton cloth with various properties. In the subsequent experiments, cotton cloth type I with 35% polystyrene was used for device fabrication.

The fabricated devices were further characterized for their hydrophobic/hydrophilic surface and the chemical change within the two regions using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX). As shown in Fig. 3, the hydrophobic and hydrophilic contrast was clearly observed as the gaps in the weave of the cotton cloth was filled with polystyrene to create hydrophobic regions and the thread of the cloth was also seen in the intact hydrophilic substrate. From the EDX results, the percent elemental composition of carbon (C) and oxygen (O) present in the hydrophilic and hydrophobic regions were also different. The percent C in hydrophobic regions was much higher than in the hydrophilic zones as a result of the polystyrene filling in the hydrophobic zone of the cotton cloth.

The compatibility of the μCADs with organic solvents commonly used in chemical assays was evaluated whether the polystyrene barrier can prevent leaking of the solvents. The organic solvents, methanol, ethanol, acetonitrile, and DMSO, with dissolved blue food dye were tested on the devices with circular test zones 5 mm in diameter. As shown in Fig. S4, well-defined test zones highlighted with the blue color were observed indicating that the polystyrene barrier can prevent leaking of the organic solvents tested. This is attributed to the pores of the cloth gets blocked by the organic solvent insoluble polystyrene, and hence, preventing capillary action wicking of the solvents to penetrate the polystyrene barrier. Therefore, the μCADs fabricated using the polymer screen-printing method can be used in various chemical/biological assays that require the use of these organic solvents. Comparing this to the wax-based fabrication methods shows that the method developed here is superior since wax is soluble in all of these organic solvents. The μCADs fabricated using polystyrene barrier can be potentially used as a platform for metal ion sensors using ion-selective optodes where the ion-selective ionophores acting as key reagents for colorimetric detection process are only soluble in organic solvent such as tetrahydrofuran (THF) [29,30].

Various patterns including simple and complex designs can be created on the μCADs using this fabrication method (Fig. S5). By adjusting the screen designs, all μCAD patterns exhibited well-defined hydrophobic borders that wick uniformly throughout the hydrophilic cotton cloth networks. This result indicated that it is possible to use the polymer screen-printing method to create a variety of device designs that suit any specific assays and applications.

3.2. Resolution, repeatability and stability

The resolution of the μCADs fabricated using the optimized parameters mentioned above was evaluated. The actual dimension of the investigated hydrophilic channel and hydrophobic barrier was measured using ImageJ software with the process described in Fig. S6 (Supplementary Information). To evaluate the obtained narrowest hydrophilic channels, the device was designed having a circular fluid reservoir (5 mm in diameter) at one end connected by a long straight hydrophilic channel to a triangle reservoir at the other end (Fig. 4A). The channel width was varied from 500 to 3000 μm. Red food dye solution was dropped into the circular fluid reservoir, and the fluid flowed along the channel (Fig. 4B). The narrowest hydrophilic channel that the proposed fabrication method could produce had a design width of 700 μm and a measured width of 678 ± 59 μm (n = 5). The reduction in size of the actual width is due to spreading of the polystyrene solution during fabrication. This resolution, however, is better than that obtained using the wax-printing method since the spreading of polymer solution is easier to control by one-step fabrication process than wax spreading which has additional heating step [25]. The thinnest hydrophobic barrier that can prevent fluid flow was investigated using a hydrophilic channel separated from a circular fluid reservoir (5 mm

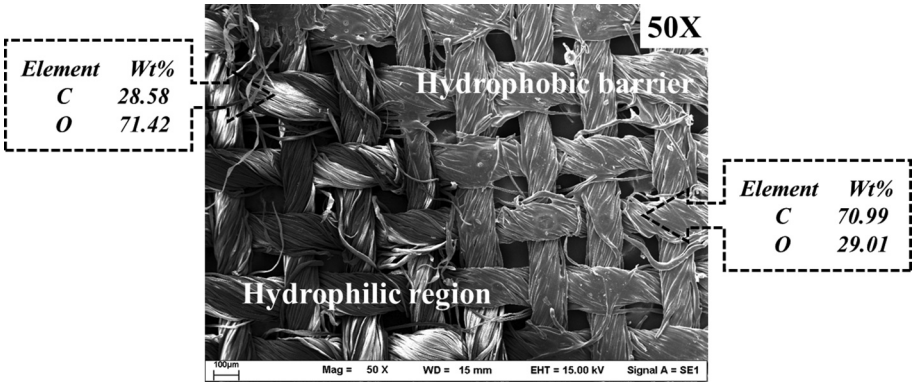

Fig. 3. SEM images of the surface structure between the hydrophilic regions and hydrophobic barriers and the elemental compositions of each area of μCADs fabricated using the polymer screen-printing method.

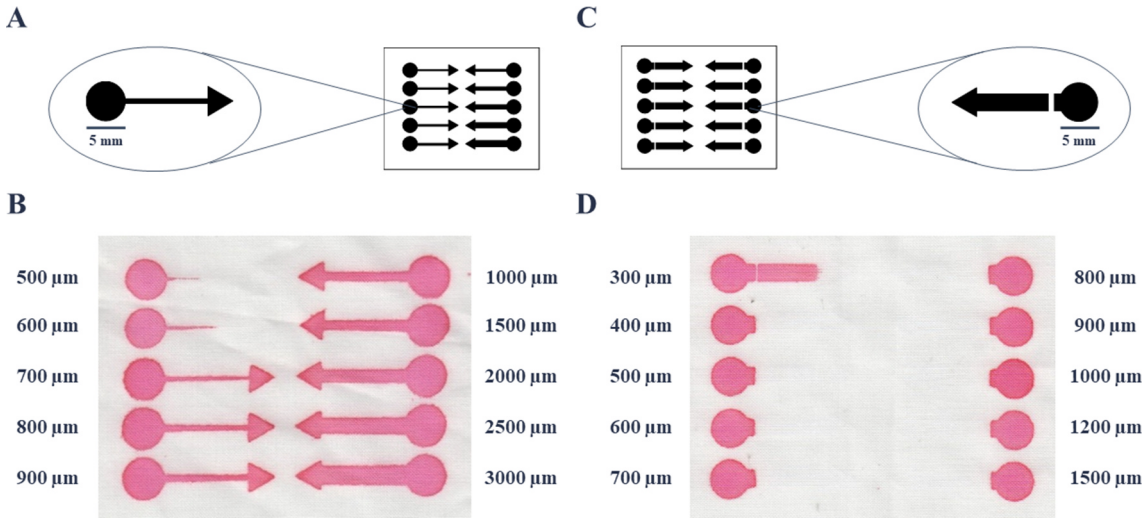

Fig. 4. (A) The design patterns used to evaluate the effect of the hydrophilic channel width in the range 500–3000 µm and (B) the resolution of the hydrophilic channel that allows flow along the channel. (C) The µCAD design used to evaluate the smallest hydrophobic barrier in the range of 300–1500 µm and (D) the resolution of the hydrophobic barrier that could prevent the flow of red food dye solution.

diameter) by a polystyrene barrier (Fig. 4C). The designed widths of a hydrophobic barrier were varied from 300 to 1500 µm. The smallest designed barrier that could prevent fluid flow was 400 µm and the actual width was 329 ± 27 µm (n = 5) (Fig. 4D). Both hydrophobic and hydrophilic resolutions obtained here are similar to those reported on the paper-based devices fabricated using polymer screen-printing method indicating that the developed fabrication method is applicable to various types of fiber materials [27].

The fabrication performance was further evaluated by comparing the designed width and actual width of the hydrophilic channel. The actual hydrophilic region on the fabricated µCADs corresponding to the designed width can be predicted using a linear equation, as follows: $W_a = 1.02W_d + 22.00$ ($R^2 = 0.9974$) (n = 5), where W_a and W_d are the actual width of the hydrophilic channel on the cotton cloth and the designed width on the patterned screen, respectively (Fig. S7).

The reproducibility of the developed fabrication method was investigated by constructing the µCADs containing circular hydrophilic zones of 5 and 7 mm in diameter for ten different fabrication times. The actual diameter of the zones of the µCADs obtained from each fabrication time was measured and calculated to determine the reproducibility. High fabrication reproducibility was obtained with the relative standard deviations (RSD) of 1.26 and 1.83% (n = 10) for the device containing 5 and 7 microzones, respectively. The repeatability of the microzones obtained from each fabrication was also investigated by creating 64 circular hydrophilic zones with a diameter of 5 or 7 mm (Fig. S8). The actual diameters of the microzones were 5.20 ± 0.11 mm and 7.09 ± 0.08 mm which agree well with the extrapolated values of ~5.12 mm and ~7.16 mm, calculated using the equation in Fig. S7. The relative standard deviations (RSD) for the measured diameter were 2.03% and 1.10%, respectively (n = 64). These results demonstrated the good repeatability obtained using the developed fabrication method. The method provided structures with higher repeatability than those obtained from other µCAD fabrication methods such as photolithography (RSD of 6.9–8.8%) [26] and wax screen-printing (RSD of 13–15%) [25]. This is a result of the greater ability to control the spreading the hydrophobic polystyrene solution as it involves only one step for fabrication compared to the multiple steps of coating, baking and UV-exposure required for photolithography and the additional heating step needed for wax screen-printing.

The µCADs produced were found to be stable for more than 6 months with the hydrophobic barrier maintaining its initial ability to prevent leaking of the solution from the channel. The stability of the devices is a result of the inertness of polystyrene polymer which is a relatively non-biodegradable material and is also difficult to photo-oxidize [31].

3.3. Wicking properties of the treated-cotton cloth

Non-cellulosic impurities such as cuticle, wax, protein and fat present on the surface of natural cotton fibers can make the cotton fiber hydrophobic and hence, reduce its fluid wicking ability for polar liquids. To increase wicking, a scouring treatment has been employed to remove these hydrophobic impurities. Scouring treatments using hot water (100 °C), NaOH and Na_2CO_3 were evaluated for their effect on the wicking rate in the microchannels of the cloth-based devices held in both the horizontal and vertical directions. As shown in Fig. 5, all types of cotton cloths evaluated have the ability to wick polar liquids through the microfluidic channels. Even without any prior treatment, a natural cotton cloth can wick the liquids because it is hydrophilic and thus wettable through the interstitial spaces between the woven threads and by the capillary force within a single thread. However, a slight increase in the wicking ability was observed in both the horizontal and vertical experiments for the treated cotton cloths compared to the untreated one as shown by the average wicking height recorded in Fig. 5. For both horizontal and vertical directions, the Na_2CO_3 treated-cloth showed the highest wicking ability, followed by the NaOH and hot water treated-cloths. NaOH and hot water treatment may only break aliphatic chains of the natural wax layer by hydrolysis and oxidation, while Na_2CO_3 also adds carbonyl (CdbndO) functional groups onto the surface of the cotton cloth [11]. These results demonstrated that the wicking ability of cotton cloth can be altered by selecting appropriate scouring treatments. In this work, the cotton cloth without any treatment was chosen for further experiments since a higher wicking rate was not required for the chemical assays investigated.

3.4. Applications of µCADs for glucose and albumin analysis

To demonstrate the applicability of the polymer screen-printing method, the analysis of glucose and albumin was carried out using µCADs with circular shaped hydrophilic test zones. Glucose determination in body fluids such as blood and urine is important for the diagnosis and treatment of diabetes [32]. Various biosensors have been, therefore, developed as effective diagnostic tools for monitoring glucose. In this work, a colorimetric biosensor for glucose analysis has been demonstrated on the µCAD. The analysis is based on the catalytic oxidation of glucose by glucose oxidase enzyme to generate gluconic

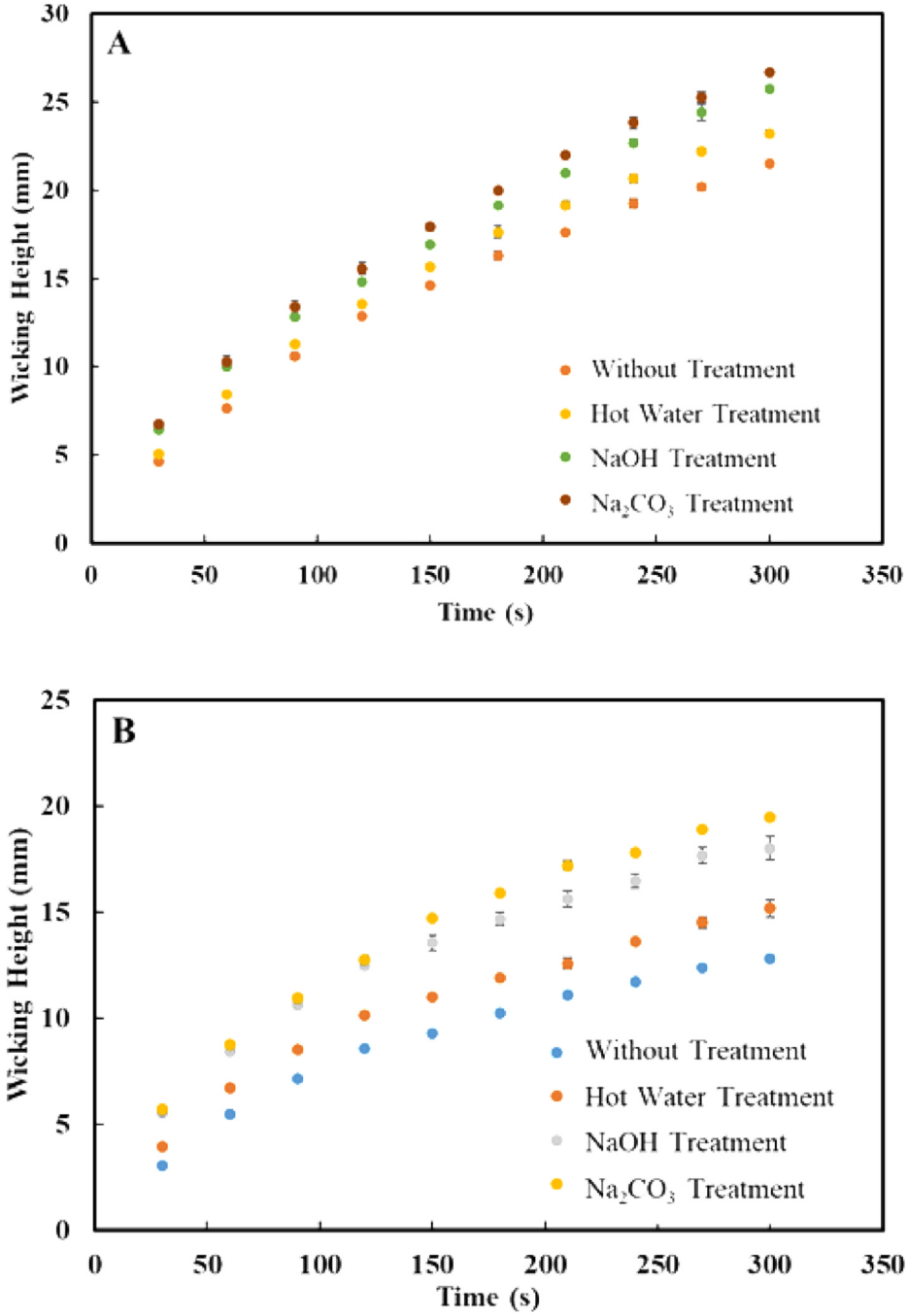

Fig. 5. Capillary rise capability of cotton cloths treated by different solutions measured in (A) horizontal and (B) vertical directions. Wicking height in the channels is recorded as a function of time (0–300 s) (n = 3).

acid and hydrogen peroxide (H_2O_2) which is then catalytically oxidized by horseradish peroxidase (HRP) in the presence of a colorimetric substrate, 3,3′,5,5′-tetramethylbenzidine (TMB) to produce the blue color of oxidized TMB (Fig. S9). The intensity of the blue color is directly proportional to the amount of glucose in the samples. The color was quantitatively analyzed using imageJ software. The analysis of H_2O_2 was first evaluated since it is the key to quantify glucose. As expected, the blue color intensity increased when concentration of H_2O_2 increased indicating that the catalytic oxidation of H_2O_2 and TMB in the presence of HRP could occur on the μCAD. The linear calibration curve (a plot of mean gray intensity as a function of H_2O_2 concentration) in the H_2O_2 concentration range 0.3–10 mM was $y = 53.8x + 60.6$, $R^2 = 0.999$ (Fig. S10). The assay reproducibility was found to be lower than 7%RSD (n = 5) for H_2O_2 analysis across all concentrations tested. This error of the analysis might be attributed to the heterogeneity of color distribution in the detection zones causing the reproducibility problem in colorimetric detection on the μPAD or μCAD. To improve the reproducibility, controlling the volume of the reagent and wicking velocity of the sample as well as the surface modification of cellulose to retain the flow of the color products have been suggested and will be employed in our future works [33,34]. The limit of detection (LOD) defined as the concentration giving a signal three-times higher than the standard deviation of a blank (DI water) was 0.013 mM. These results clearly demonstrated that the μCAD can be effectively used to analyze H_2O_2 making it suitable for glucose analysis. Therefore, glucose analysis was further investigated using the fabricated μCAD. As shown in

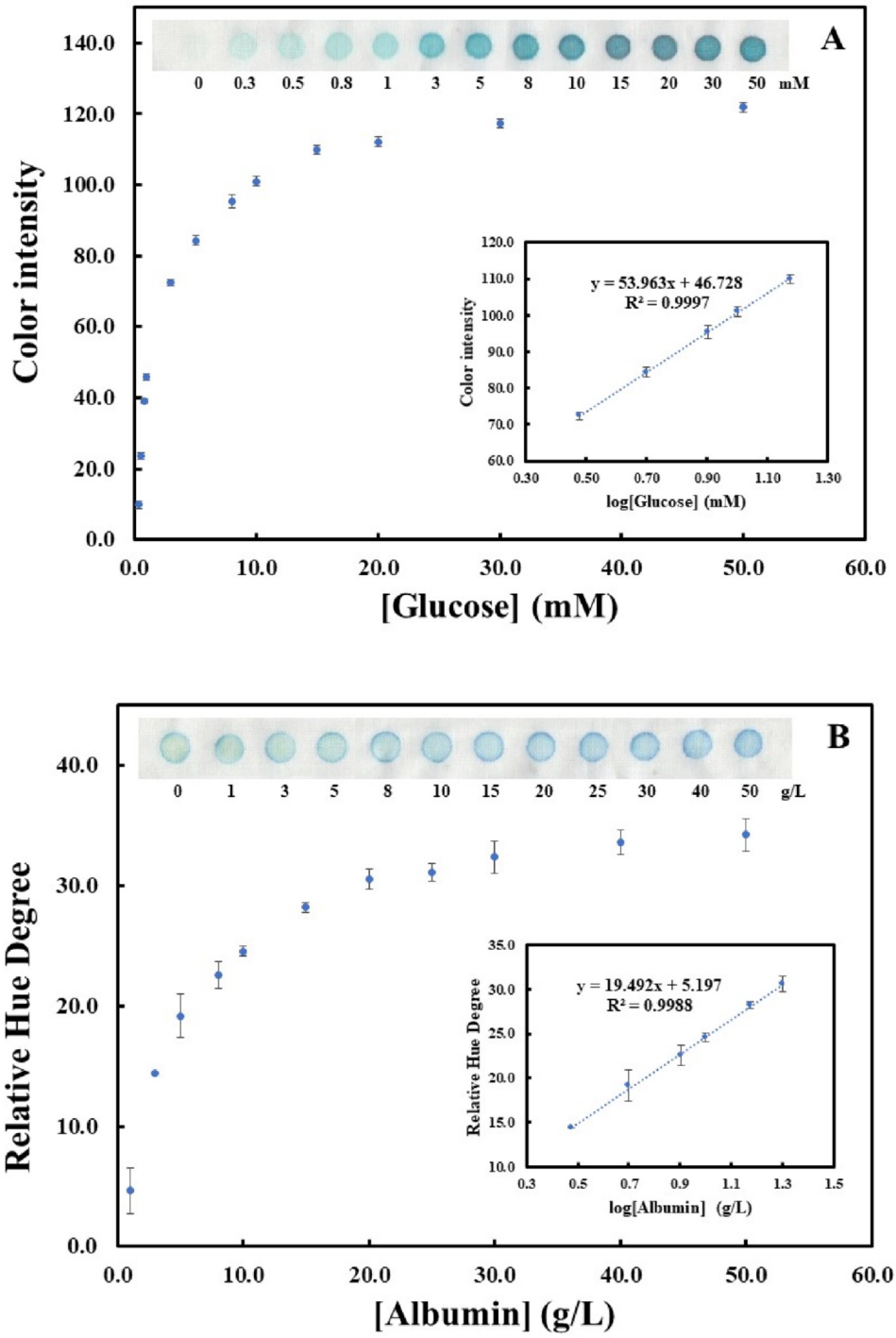

Fig. 6. Analysis of glucose and albumin using μCADs fabricated using the polymer screen-printing method. Calibration curve for (A) glucose and (B) albumin analysis. Inserts show the linear concentration ranges between 3 = 15 mM for glucose (n = 5) and 3–20 g/L for albumin (n = 5).

Fig. 6A, the blue color intensity increased as the glucose concentration increased. The plot of gray intensity as a function of glucose concentration was linear in the range 3–15 mM (y = 54.0x + 46.7, R^2 = 0.999). This range is suitable for the analysis of glucose in whole blood and serum with the normal levels of 3.5–5.3 mM and 2.5–5.3 mM, respectively [35]. The reproducibility obtained from the analysis of glucose with the concentration in the linear range was in the range 1.30–1.91% (n = 5). The LOD using the developed μCAD for glucose analysis was 1.01 mM which is comparable in performance to conventional blood glucometers that can detect levels as low as 1.7 mM [36]. Therefore, our μCAD developed here should be suitable for the determination of glucose in various biological fluids such as whole blood and serum.

The μCAD was further applied for albumin analysis using bovine serum albumin (BSA) as a standard. The analysis is based on the reaction of bromocresol green (BCG) with BSA at acidic conditions (pH 4.5) making the BCG color change from green to blue (Fig. S11). The plot of relative hue degree as the color change from green to blue *versus* BSA concentration showed an increasing signal when the BSA concentration increased (Fig. 6B). A linear calibration curve for the albumin analysis was obtained in the albumin concentration range 3–20 mg/mL of albumin (y = 19.5x + 5.2, R^2 = 0.999). The

Table 1
Determination of glucose and albumin in control samples.

Analytes	HumaTrol N		HumaTrol P	
	Certified value	μCAD	Certified value	μCAD
Glucose (mM)	5.72 ± 0.92	5.72 ± 0.76[a]	12.3 ± 2.0	11.9 ± 1.0[a]
Albumin (mg/mL)	33.8 ± 7.8	35.4 ± 2.4[a]	41.7 ± 9.6	41.2 ± 7.5[a]

[a] Standard deviation of three measurements.

reproducibility of the analysis of albumin in the linear concentration range was < 10% (n = 5) with the LOD of 2.77 mg/mL. This LOD is sufficient to quantify albumin in human serum which has normal levels of 34–54 mg/mL [37].

To further demonstrate the real-world application of the devices, the μCADs were used to measure the glucose and albumin concentrations in clinical control serum samples, including HumaTrol N (normal range) and HumaTrol P (abnormal range) (Human GmbH, Germany). These control samples are commonly used for determining the accuracy of diagnostic assays in a biologically relevant matrix without the problem of blood borne pathogens. The results are shown in Table 1. Using a paired *t*-test, the concentrations of glucose and albumin obtained from the μCAD were not significant difference at the 95% confidence level from the certified values obtained in clinical control samples, both in normal and abnormal ranges (two tailed $P = 0.765$). The high degree of equivalence between these values indicates that the μCAD developed is appropriate for analyzing glucose and albumin in serum samples. Although the analytical performances of the developed μCAD are similar to those previously reported by μPAD for glucose and albumin detections [38–41], by taking the advantages of μCAD for its robustness and the ability to be close contact to human skin, one of the potential applications would be the development of μCAD toward the glucose and albumin sensors in sweat samples. Various reports have demonstrated the development cloth-based sensors for biomarker detection in real human sweat to offer real time monitoring of chemical level by embedding them into daily textile products. μCAD for lactate sensor in real human sweat samples was developed to indicate the performance of the athletes [42]. A wearable sensor employing active electrodes and supercapacitor coated on cloth was developed for real time monitoring of electrolyte in real human sweat [43]. A cloth-based potentiometric pH sensor was developed for wearable application by printing sensitive (thick film graphite composite) and reference electrodes (Ag/AgCl) on cellulose-polyester blend cloth [44]. Therefore, the simple, low-cost and one-step fabrication method developed in this work could potentially contribute to the development of cloth-based sensor for wearable application in the future.

4. Conclusions

A polymer screen-printing method has been demonstrated for its capability as a simple, low-cost and rapid method for the fabrication of μCADs requiring only polystyrene and a patterned screen. Under optimal fabrication conditions, the method produced well-defined hydrophilic channels and hydrophobic barriers with high device-to-device repeatability without the requirement of any sophisticated processes or expensive instruments. Various patterns can be created on the μCADs by adjusting the screen patterns. Cloth treatment by hot water, Na_2CO_3 and NaOH did have an effect on the wicking rate of the fabricated μCADs. Application of the fabricated μCADs for real world analyses was successfully demonstrated for glucose and albumin in control human serum. The results showed no significant differences between the glucose and albumin concentrations obtained from the μCAD and the certified levels of the control samples at the 95% confidence intervals. These results demonstrated that the polymer screen-printing method is a novel alternative method for fabrication of μCADs, which is suitable for developing countries. It is capable of further modifications for other biological assays, environmental monitoring, cell-based studies as well as complex chemical analyses.

CRediT authorship contribution statement

Benjarat Tasaengtong: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-original draft. **Yupaporn Sameenoi:** Supervision, Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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