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ARTICLE TYPE

A Siphonage Flow and Thread-based Low-cost Platform Enables Quantitative and Sensitive Assay†

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In concept of pump-free, material abundant, portable, and easy-to-operate low-cost microfluidics, a siphonage flow microfluidic thread-based analytical device (S-μTAD) platform enabling quantitative and sensitive assay was designed. Renewable and continuous siphonage flow allowed the replicate sampling and detection on one channel/device, obviating some possible inconsistencies among channels or devices. The Y-shaped channels were fabricated with polyester cotton blend thread for its best chemiluminescent sensitivity in comparison with that of cotton and polyester threads. S-μTAD sensors for glucose and uric acid were fabricated by using oxidase-immobilized cotton thread as the sample arm of channels. The acceptable reproducibility and high sensitivity, demonstrated by the relative standard deviations of less than 5% in all cases and the detection limits of 4×10^{-8} mol/L hydrogen peroxide, 1×10^{-7} mol/L glucose, and 3×10^{-6} mol/L uric acid, showed the feasibility of S-μTAD for quantitative assays. Good agreements between S-μTAD/sensor results and hospital results for blood glucose and uric acid assays indicated the capability of S-μTAD/sensors for analysis of real samples. These findings proved the utility of siphonage for low-cost microfluidics and the availability of our S-μTAD design for quantitative assays.

Introduction

Lateral-flow format and dipstick diagnostic assays have taken the concept of low-cost, potable, disposable, and user friendly technology in past three decades. However, the perceived performance limitations in reproducibly and sensitivity have challenged their demand and expanding.[1,2] Paper-based microfluidic platform (microfluidic paper-based analytical devices, μPADs), pioneered by Whitesides et al., [3] has been emerging as an attractive technology to achieve the quantitative low-cost assay with controlled manipulation fitting for the challenges which defy the conventional laboratory analysis such as in situ point-of-care (POC) medical diagnostics, food safety assay as well as pollutant monitoring. [4-6] Pump-free capillary flow offers μPADs the capabilities of small volume sample and reagent delivery and distribution, defined mixing, simultaneous array detections. In addition, μPADs possess two desirable traits in their construction and manipulation, (1) fabricated with the low-cost, abundant and disposable materials; (2) simple and portable instrumentation and viable non-professional operation. Various paper channel fabrication techniques, such as photolithography, [3] printing, [7-10] oxygen plasma and laser treatment [11,12] are extensively acknowledged. Three-dimensional μPADs (3D-μPADs) are also investigated with the purpose to introduce vertical flow into paper channel and realize sufficient mixing.[13,14] Colorimetric, [3,15] electrochemical, [16] electrochemiluminescent, [17] chemiluminescent, [18] and mass spectrometric [19] techniques are employed for μPADs signal acquisition. Following the analogous concept of μPADs, multifilament threads and textiles have been investigated as alternative inexpensive materials to fabricate microfluidic thread-based analytical devices (μTADs). [20-28] Taking advantages of greater tensile strength and better flexibility, threads allow easy channel fabrication since the unnecessary to pattern the hydrophobic barriers, in particular for the 3D structures. [21,22,29] These advantages also facilitate μTADs to realize some interest improvements, including the better control on mixing and splitting of fluids by knotting or twisting threads, the timing of reactions by on/off fashion switch, and the accurate localization of detection zone. [21,22,24,29] The analytical capabilities of μPADs and μTADs in multi-disciplinary fields have been demonstrated, [29-31] sensible and imaginative scientists have flocked to develop various innovative low-cost microfluidic techniques. [32]

Pump-free low-cost platforms are the state-of-art designs in terms of minimal/no power consumption, viable fabrication, and better acceptance in both academics and industry. Despite the proved quantitative assays of such platforms, some undesirable limitations affecting the reproducibility have been perceived. According to commentators, [2,31] these limitations on reproducibility mainly attribute to the inconsistencies in flow rate, sampling, and mixing. As known, the effective concentration of the analyte/reagent presented in the channel is inversely proportional to the square of the change in flow rate. [33] For porous materials of thread and paper, both their innate non-uniformity and acquired non-uniformity from the stretching, pressing and folding in processes of fabrication, dispatch, storage and end use, cause the change in capillary flow rate. When such change in flow rate is uneven among channels in a device, the

limited reproducibility is caused. The same is true when a quantitative assay, including the standard calibration and analyte detection, has to be conducted by several devices. Inconsistent sampling efficiency caused by solvent evaporation and sample retention in channel is another factor that affects the reproducibility. [24,28,31] Moreover, the insufficient passive mixing of sample and reagent by capillary flow is also regarded as a factor leading to the limited reproducibility. [2] As such, to ensure the quantitative detection, careful managements from the device fabrication to the end use are required; the inherent advantage in convenience of low-cost microfluidics is thus weakened.

Accomplishing replicate detections on one channel/device can be an alternative to obviate most of aforementioned inconsistencies. This strategy will become feasible if a refreshable channel/device is available. To refresh the channel/device, continuous and renewable fluid driving force besides capillary action is of necessity. Bearing in mind pump-free fluid movement of low-cost microfluidics, siphonage seems the best available candidate to induce continuous and renewable fluid flow. In addition, by using continuous siphonage flow the inconsistencies aroused from solvent evaporation and sample retention can be significantly relieved. To withstand the replicate sampling, reaction and refreshment, threads are supposed the proper material to fabricate the channels for their high wet-strength. By obliging two thread streams to merge into one, vertical flow can be introduced; sufficient and repeatable mixing is expected. Furthermore, cellulose thread permits easy surface chemical modification, e.g. aldehyde functionalization, to prepare a universal matrix for immobilization of molecule recognition elements such as enzyme, protein, nucleic acid. So, sensor format microfluidic devices can be designed by pre-immobilization of molecule recognition elements on the cotton threads.

Following aforementioned strategy, a microfluidic siphonage flow and thread-based analytical device (S-μTAD) platform aiming at improving detection reproducibility for trace analyte was developed. Chemiluminescent (CL) detection was employed in light of its high sensitivity and proved suitability for opaque matrix-based microfluidic platforms. [18,34] Siphonage flow was found to provide the controlled and renewable delivery of sample and reagent. Localized fluids mixing and detection zone were realized by thread knotting and cutting. Hydrogen peroxide, glucose, and uric acid were selected as the model analytes to testify the feasibility of our design. The S-μTAD detection of trace hydrogen peroxide and glucose suggests the acceptable reproducibility for quantitative assays. With the success of S-μTAD quantifying human blood glucose, S-μTAD sensors were fabricated by immobilizing appropriate oxidases on the thread; glucose and uric acid contents in blood samples were determined. The presented S-μTAD prototypes are low-cost, portable, and easy-to-operate. Our S-μTAD design offers an effective alternative technique for pump-free microfluidics, particularly low-cost and disposable devices for trace healthcare substance assays and POC diagnostics.

Experimental

Materials and chemicals

Cotton thread, polyester-cotton blend thread, and polyester thread were purchased from the central textile market of Xi'an city, China. Prior to thread channels fabrication, natural wax and dirt on the thread were removed by ultrasonic cleaning in 0.1 mol/L NaOH solution for 1 hr to promise the thread wettability. [35] After removing NaOH with water, the thread was dried at ambient air and then kept in seal bags for further use. Polymethyl methacrylate (PMMA) was purchased from Acryl China Co., Ltd. All chemicals were of analytical grade except the oxidases; the solutions were prepared with doubly distilled water (SZ-93 automatic de-ionized, distilled water system, Shanghai Yarong Biochemistry Instrument Factory, China). Luminol was purchased from Shanghai Chemical Reagent Co., Ltd, China. Hydrogen peroxide (30%), glucose, potassium ferricyanide, thymol blue, Coomassie Brilliant Blue G-250, hydroxylamine hydrochloride, sodium borate, sodium carbonate (Na_2CO_3), and acetic acid (HAc) were purchased from Sinopharm Chemical Reagent Co., Ltd, China. Uric acid, glucose oxidase (GOD, 147900 U/g), and uric acid oxidase (UOD, 4700 U/g) were purchased from Sigma-Aldrich, USA. Sodium hydroxide (NaOH) and sodium acetate (NaAc) were obtained from Tianjin Hengxing Chemical Preparation Co. Ltd, China. Sodium bicarbonate ($NaHCO_3$) was purchased from Shanghai Hongguang Chemical Reagent Co. Ltd, China.

Preparation of oxidase-functionzalized cotton thread

Cotton cellulose containing abundant active groups was extensively used as a carrier to immobilize biomolecule, such as enzymes. The cotton thread was firstly treated with periodate to convert 2,3-vicinal diol of the cellulose glucose units into 2,3-dialdehyde cellulose by soaking cotton thread (1.0 g) in 20.0 mL of 0.2 mol/L sodium periodate (pH 6.0) with oscillating at 40 °C for 8 h. [36] Excessive oxidant was removed by immersing cotton thread in 20.0 mL of 0.1 mol/L glycerol at 40 °C for 30 min. Finally, the oxidized cotton thread was washed with doubly deionized water and dried at ambient temperature. The aldehyde content of oxidized cotton thread was determined to be 90 mmol/g by a modified hydroxylamine hydrochloride method. [37] Oxidase immobilization was conducted via covalent cross-linking reaction between amino group of enzyme and aldehyde group of the oxidized cotton thread by immersing aldehyde functionalizd thread into GOD phosphate buffer (pH 5.0) or UOD borate buffer (pH 8.5) for overnight at 4 °C. [38] The free GOD or UOD were removed by washing threads with phosphate buffer (pH 7.4) or borate buffer (pH 7.4), respectively. The obtained oxidase functionalized cotton threads were stored in a -18 °C refrigerator.

Bradford method, a preferred method for quantifying protein, was used to testify if oxidase was successfully immobilized on the cotton thread. The assay is based on the fact that the absorbance maximum of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein. [39] The oxidase functionalized cotton threads were incubated in 0.1 g/L Coomassie Brilliant Blue G-250 solution for 5 min. After incubating with Coomassie Brilliant Blue G-250, the oxidase functionalized cotton threads displayed significantly deeper blue than that of control (Fig. S1, inset). This result suggests that the oxidase was covalently immobilized on the cotton thread.

To examine the enzyme activity of immobilized oxidase, the static CL method was conducted. The oxidase functionalized cotton threads were incubated with 50.0 μL of 5.0×10^{-5}mol/L

substrates (glucose and uric acid) at ambient temperature for 30 min to convert them into hydrogen peroxide. And then 400 μL of the mixed solution of luminol and potassium ferricyanide was injected into the resultant solution to initiate the CL reaction. As shown in Fig. S1, the CL signal taken from the oxidase functionalized cotton threads was much higher than that of the control. This experiment indicated that the immobilized oxidases are capable of converting the glucose and uric acid into hydrogen peroxide.

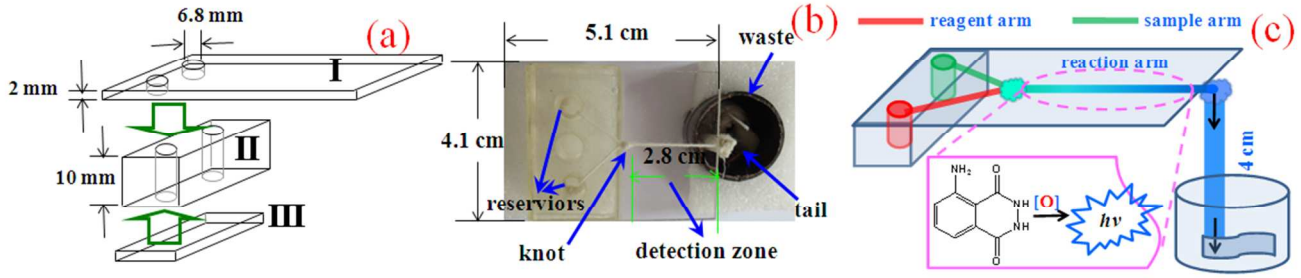

Fig. 1 The structural principle of a Y-shaped channels S-μTAD. (a) Scheme of PMMA support backing; the assembly of support backing was completed by gluing blocks of I, II, and III together as indicated by green arrows. (b) A vertical view photo of a Y-shaped channels S-μTAD; the PMMA support backing was mounted on a 3 cm high polystyrene foam pedestal with acrylic self-adhesive; detection zone was arranged just above the window of photomultiplier tube (PMT). (c) A lateral view scheme illustrating the siphonage flow generation by a superfine fiber tail and the CL generation in the detection zone. The actual appearance of the superfine fiber tail is illustrated in Video S2.

Support backing fabrication

As schemed in Fig. 1a, the support backing for S-μTAD was made up of three blocks of PMMA. Their sizes were of 5.1 cm length × 4.1 cm width × 2.0 mm height (block I) and 2.5 cm length × 4.1 cm width ×10 mm/1.0 mm height (block II/III), respectively. As showed in Fig. 1a, the block II was glued on one end of block I using epoxy resin at 50 °C for 12 h. On this end, two or three holes with the diameter of 6.8 mm and the deepness of 1.2 cm was punched and used as sample and reagent reservoirs. Finally, block III was glued on the bottom of the holes with epoxy resin at 50 °C for 12 h. The volume of each reservoir was estimated to be 435 μL.

S-μTAD and S-μTAD sensors fabrication

As schemed in Fig. 1b-c and in Video S1, two polyester cotton blend threads were knotted into Y-shaped channels and used as sample arm, reagent arm, and reaction arm (including the detection zone and tail connection zone), respectively. The lengths for sample arm and reagent arm are 2.0 cm, and for reaction arm is 2.8 cm. The ends of sample and reagent arms were fastened on polyethylene rings (o.d. 6.8 mm) to promise their inlets being submerged in the bottom of the reservoirs. The end of reaction arm was connected with a superfine polyester fiber tail which was prepared by putting a bunch of polyester superfine fiber into a 3 mm i.d.× 4 cm length plastic tube. The procedures for superfine fiber tail connection with reaction arm and the assembly of thread channels with support backing were illustrated in Video S2. To refresh the superfine fiber tail, fibers were taken out from the plastic tube, cleaned with 0.1 mol/L NaOH solution and followed washing with water, and dried for use. S-μTAD sensors were fabricated with the same procedure as aforementioned except that the sample arm was replaced with an oxidase functionalized cotton thread.

Procedure for flow rate measurement

Fig. S2 illustrated the setup for measuring siphonage flow rate along polyester cotton blend thread. A laboratory jack was used to hoist the S-μTAD to the appropriate height (10, 20, and 40 cm). The capillary channels in thread were initially filled with water by the capillary action till the end of thread tail prior to measurement with the aim to induce continuous siphonage flow. The two reservoirs were respectively pipetted with 400 μL of water without replenishing during the measurements. The outflow of water was recorded using a BSA124S electronic analytical balance (Sartorius). Data acquisition began when first drop of water was outflowed and continued for 20 min. The mass and the time that took for each drop of outflow water were recorded. The volume of outflow water was obtained by dividing the mass of the outflow water recorded with the density of water (1 g/cm^3). The flow rate was calculated by dividing the volume of the outflow water with the time that taken for.

S-μTAD and S-μTAD sensor detection procedure

After water wetting thread channels, 400.0 μL of CL reagent and 400.0 μL of hydrogen peroxide were pipetted into sample and reagent reservoirs, respectively (Video S-2). The CL emission produced was recorded by a CR105 PMT (Beijing Hamamatsu Photo Techniques Inc.) with biasing high voltage of the PMT at -600 V. CL data acquisition and treatment were performed using MPI-A data processing system (Xi'an Remex Electronic Science-Tech Co. Ltd, China). After the inflexion of the second CL peak appeared, the program was paused. Sample and reagent residues were removed; the S-μTAD channels were cleaned with 400.0 μL of doubly distilled water added into reservoirs until the CL signal declined to the baseline. The success of refreshment was proved by the immediate blank examination. For each standard/analyte concentration, triplicate measurements were performed unless specified otherwise. The relative CL intensity (ΔI), net CL peak height against that of blank, was used for standard calibration and analyte quantification.

Prior to human blood assays, serum proteins were removed by adding 1.0 mL of 95% ethanol into 1.0 mL of serum and centrifuging at 11000 r/min for 20 min. Off-thread conversion of glucose into hydrogen peroxide was performed by incubating 10.0 μL of supernatant/standard with 5.0 mL of 15 U/mL GOD in 0.05 mol/L HAc-NaAC buffer solution (pH 4.5) at 35°C for 30 min. According to the same procedure described for hydrogen peroxide, blood glucose was determined.

For S-μTAD sensor blood glucose assay, the supernatant was diluted 50-fold with water before sampling. 50.0 μL of sample/ standard in the sample reservoir was driven forward along the GOD arm by siphonage to convert glucose into hydrogen peroxide for 10 min at ambient temperature. After the conversion, 400.0 μL of the doubly deionized water was added into sample reservoir to elute the produced hydrogen peroxide to the threat knot and reacted with the CL reagent in the detection zone. The CL emission produced was detected by the PMT biased at -800 V. The relative CL intensity was used for glucose calibration and quantification. The procedure for blood uric acid assay by uric acid sensor was similar to that for glucose by replacing glucose standard with uric acid standard.

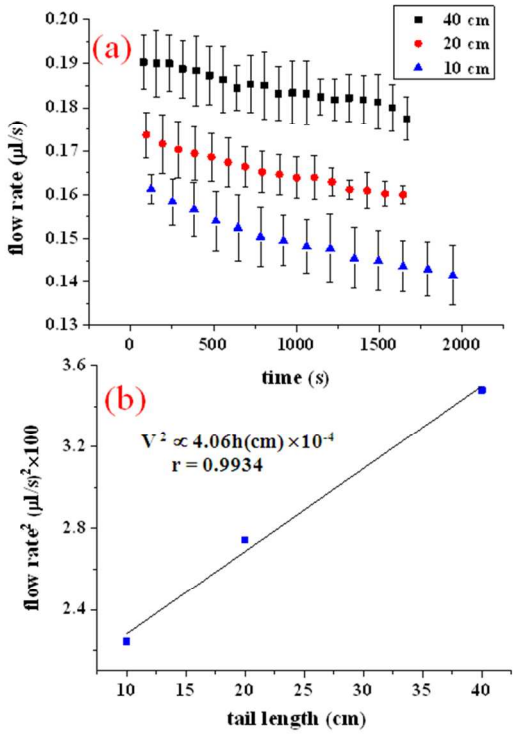

Fig. 2 (a) Water flow rate as a function of time corresponding to the tail length of 10 cm, 20 cm and 40 cm polyester cotton blend tail; Error bars represent the ± SD of triplicate measurements. (b) Depiction of the relationship between the square of flow rate and the length of tail. *V* is the average of flow rates with respect to each tail length; h is the thread tail length as well as the siphonage height difference; r is the linear correlation coefficient.

Results and discussion

Flow behavior and S-μTAD construction

To construct the S-μTAD, the first step was to investigate the basic behavior of fluid flow along the thread. A naturally drooped polyester cotton blend thread tail was used to induce siphonage flow; the length of thread tail was also the height difference between the surface of the upper reservoir and the drain point (Fig.S2). The flow rate of water as a function of time was measured by weighing the mass of effluent water with respect to the tail length of 10 cm, 20 cm, and 40 cm with 400 μL water in two reservoirs for all measurements, respectively. Initially, the thread capillary channels were filled with water by the capillary action till the end of tail, after which continuous siphonage flow was induced. As showed in Fig. 2a, the longer the length of the thread tail was used, the higher the flow rate was obtained. Almost constant flows were observed for all tail lengths. With regard to the average flow rates, a linear relationship was found between the square of flow rate and the length of tail (Fig. 2b), which almost followed the Bernoulli's equation ($V \propto h^{1/2}$, V, flow rate; h, height difference as well as the tail length, Fig. 2b).

$$H = k_1 t^{1/2} \quad (1)$$

then $\frac{dH}{dt} = k_2 t^{-1/2}$

flow rate $V = \frac{dH}{dt}$

so $V \propto t^{-1/2} \quad (2)$

Ballerini et al. demonstrate a linear relationship between water capillary rise height (H) and the square root of time as summarized by equation 1. [20] If consider H also as the weight/mass of water, it can be inferred that the capillary flow rate is inversely proportional to the square root of time (equation 2). In comparison, the water flow behavior in this work obviously obeys Bernoulli's equation. Above results suggest that the continuous water flow is dominated by the siphonage. The slight decrease of flow rate is attributed to the variation of thread's porosity during wetting and the decrease of water level. So that, thread channels were pre-wetted in following experiments to achieve repeated flow rate.

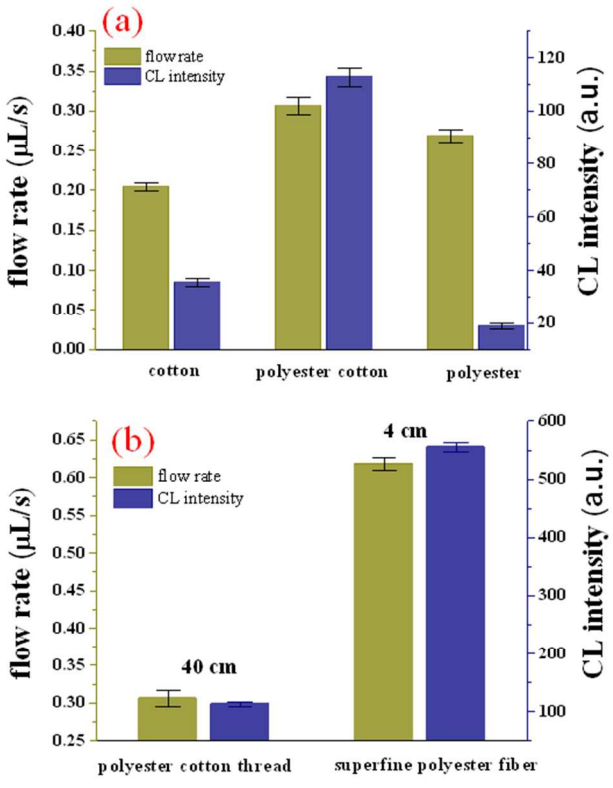

Fig. 3 Effects of thread tail materials on siphonage flow rate and CL response. (a) Flow rate and CL response comparison among three thread tail materials of cotton, polyester cotton blend and polyester; All the tails

are single thread in 40 cm length; error bars represent the ± SD for triplicate measurements. (b) The enhanced flow rate and CL response by superfine polyester fiber tail in comparison with the single polyester cotton blend tail. Here, the flow rate is the average flow rate obtained by dividing the total water volume in two reservoirs with the time consumed for water draining off with respect to each material. CL experiments were performed with a luminol-NaClO reaction system, concentrations of luminol, NaClO, NaOH were 1.0×10^{-4} mol/L, 1.0×10^{-4} mol/L, 0.01 mol/L, respectively.

Repeatable and sufficient mixing of reagent and sample directly determines the reproducibility and sensitivity for reaction dynamic relevant detection. In this work, the merge of reagent and sample was accomplished by knotting two threads (Video S-1). By so doing, the voids between fibers of thread are distorted, leading to the vertical flow as well as the improved mixing of sample and reagent. This mixing is further strengthened by cutting one of the two knotted threads; two streams are obliged to merge into one. In addition, the thread knot also defines the starting point of CL detection zone.

Attribute of thread material has been known as a key factor determining the flow rate. [25] Siphonage flow behaviors of three threads, including cotton, polyester cotton blend, and polyester, were examined. The measured flow rates with respect to 40 cm tail length of each material were showed in Fig. 3a. It is found that the polyester cotton blend contributes the highest flow rate. To investigate the material suitability for CL detection, CL response with respect to three threads were measured as also showed in Fig. 3a. Clearly, polyester cotton blend exhibits significantly higher CL response than that of cotton and polyester.

The concept and importance of flow control for μTADs have been detailed by Ballerini et al. [20] Besides the acknowledged significance in timing the fluid flow and reaction, localizing the mixing and detection zone, and promising a reasonable reporting time, the flow control greatly affects the sensitivity of CL detection. Our experiments confirmed that the higher the flow rate the higher the CL signal and the better sensitivity. Such results are reasonable since the high flow rate results in the sufficient mixing of reagent and sample. Obviously, 40 cm or longer thread tail benefits sensitive CL detection. The implementation of single thread tail induced siphonage flow has a fundamental challenge that even a 20 cm length (height) tail would greatly daunt S-μTAD being a potable setting. Fortunately, a bunch of superfine polyester fiber with 4 cm height difference (Fig. 1c) was found to generate 0.62 μL/s water flow, which was much higher than that of 0.31 μL/s generated by a 40 cm polyester cotton blend tail, as well as the much stronger CL response (Fig. 3b). This superfine fiber tail can be readily and repeatedly connected with the reaction arm of S-μTAD as illustrated in Video S2. As a result, the portable S-μTAD was constructed with the polyester cotton blend channels, a superfine fiber tail, and a PMMA support backing as what described in Fig. 1.

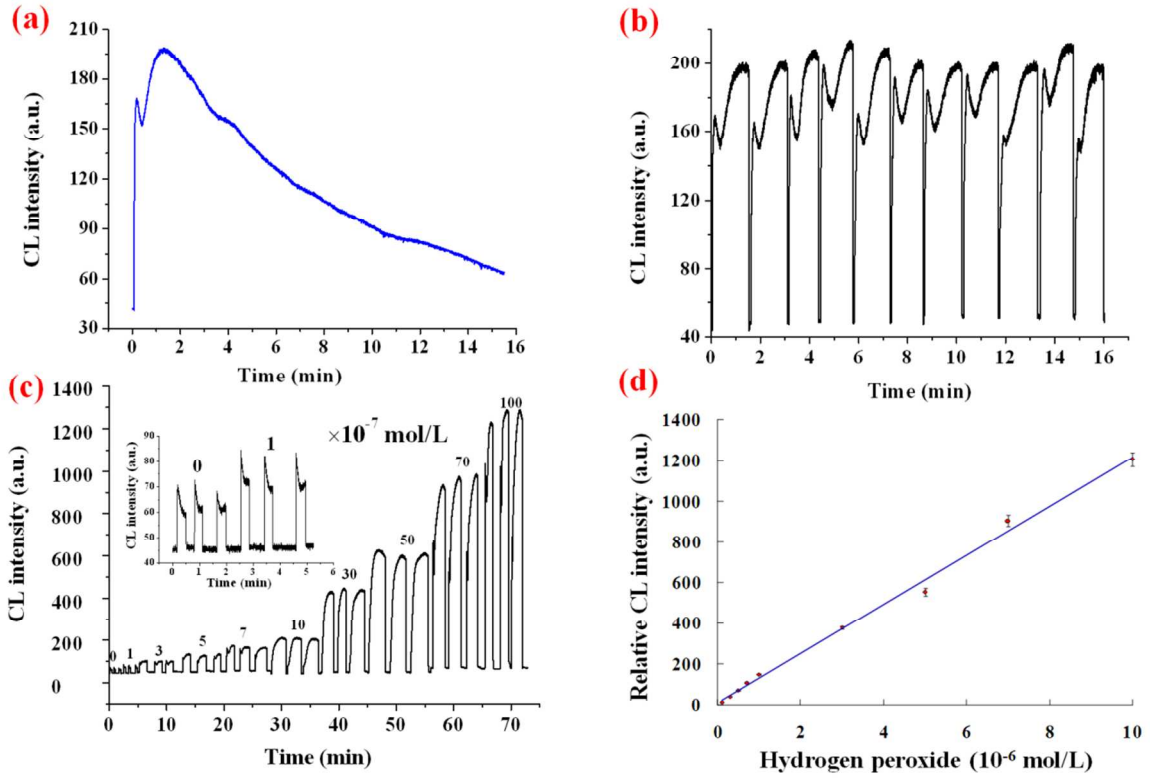

Fig. 4 Results of investigations on S-μTAD reproducibility, sensitivity and analytical characteristics with hydrogen peroxide as an analyte. (a) A representation of full CL profile obtained from 1.0×10^{-6} mol/L hydrogen peroxide standard. (b) CL responses of 11 times measurement to 1.0×10^{-6} mol/L hydrogen peroxide standard for reproducibility investigation. (c) Records of CL signals for standard calibration in the concentration range of 1.0×10^{-7} to 1.0×10^{-5} mol/L (n=9); inset is an enlarged view for blank and 1.0×10^{-7} mol/L hydrogen peroxide. (d) The linearity relationship between CL signal and hydrogen peroxide presence. All experiments were conducted with the reagent mixture of 5.0×10^{-4} mol/L luminol and 1.0×10^{-2} mol/L potassium

ferricyanide at pH 10.5 by 0.1 mol/L Na_2CO_3-$NaHCO_3$ buffer; the biased potential applied to PMT was -600V; error bars represent the ±SD for triplicate measurements.

Given the consideration of on-thread conversion of glucose or uric acid into hydrogen peroxide to construct S-μTAD sensors, cotton thread is chosen for its proved compatibility and easy functionalization. So, the Y-shaped channels of a S-μTAD sensor were fabricated with polyester cotton blend thread except the sample arm with the enzyme-immobilized cotton thread as described in experimental section. It is supposed that our S-μTAD/sensor can be readily fabricated and assembled by a non-professional person with the aid of described schemes and Video S1-2.

S-μTAD reproducibility and sensitivity

Hydrogen peroxide was chosen to verify S-μTAD efficacy in reproducibility and sensitivity. Here, a polyester cotton blend fabricated S-μTAD incorporated with the potassium ferricyanide-luminol CL system was used for hydrogen peroxide detection. [40] A representative CL signal profile for hydrogen peroxide is showed in Fig. 4a; two CL peaks can be observed. The first CL peak appeared within 12 s once the sample and reagent were pipetted. Its peak height exhibited poor reproducibility and was strongly dependent on the interval time between channel refreshment and the next fluid loading. In hydrophobic channels of superfine polyester fiber tail, water drainage took place in above interval, leading to a capillary flow that diminishing with the water refill of these channels. So, the emergence of the first CL peak is thought the compromise of above temporary capillary flow and the concentration increase of reactants at the early stage of once detection. Despite its CL peak height responds to the amount of hydrogen peroxide, the need of accurate interval time management to achieve the acceptable reproducibility greatly daunt the practical operation. On the other hand, the second CL peak reached its maximum at about 1.5 min and its peak height exhibited the accepted reproducibility as showed in Fig. 4b. Therefore, the second peak height was used to calculate the net CL signal by subtracting blank. The subsequent slow decay of CL is attributed to the drop out of the CL reagent and sample.

To examine the detection reproducibility with one S-μTAD against multiple devices, CL detections for a 5.0×10^{-5} mol/L hydrogen peroxide standard with three S-μTADs were carried out. Observing the listed results in Table 1, it is found that the same device detection exhibits better performance in reproducibility. This find implies the easily available quantitative detection by using one S-μTAD.

Table 1 A comparison in reproducibility between one device measurements and that of three devices

Signal	device 1	device 2	device 3	RSD%
1	2090	1941	1993	3.8
2	1971	1866	2068	5.1
3	1936	1827	2133	7.9
RSD%	4.0	3.1	3.4	

Optimizations of experimental variables for hydrogen peroxide detection were conducted with one S-μTAD and detailed in Fig. S3a-c. It is estimated that a S-μTAD can be refreshed for about 50 times for the water removable analytes. Thanks to the high sensitivity to hydrogen peroxide, as low as concentration of 1.0×10^{-6} mol/L standard was continuously measured for eleven times to investigate the detection reproducibility (Fig. 4b). The 3.2% relative standard deviation is acceptable for quantitative assay of trace hydrogen peroxide. As showed in Fig. 4c and 4d, hydrogen peroxide in the concentration range from 1.0×10^{-7} to 1.0×10^{-5} mol/L was found being linear to relative CL intensity following the equation of $\Delta I=120.52c\ (10^{-6}\ mol/L)+11.51\ (10^{-6}\ mol/L)+11.51$ (n=9, the square of linear correlation coefficient $r^2=0.995$). The obtained 4×10^{-8} mol/L detection limit (3σ) for hydrogen peroxide, which matched with the sensitivity level from bulk and flow methods, [40] indicates the sufficient mixing of sample and reagent. This high sensitivity is of significance for the less sample requirement and the potential for healthcare analytes presented at the ppm or lower concentration levels, for example, the blood uric acid.

Table 2 Applications of S-μTAD/sensor to human blood glucose and uric acid in contrast to hospital results.

Analytes	Sample No.	This work (mol/L±SD)[a]	Hospital (mol/L)	Difference (%)
Glucose[b]	1	$(5.13\pm0.20)\times10^{-3}$	5.39×10^{-3}	-4.8
	2	$(5.31\pm0.17)\times10^{-3}$	5.36×10^{-3}	-0.9
Glucose[c]	1	$(5.55\pm0.26)\times10^{-3}$	5.39×10^{-3}	+3.0
	2	$(5.29\pm0.10)\times10^{-3}$	5.36×10^{-3}	-1.3
Uric acid[c]	3	$(2.03\pm0.07)\times10^{-4}$	1.98×10^{-4}	+2.5
	4	$(1.98\pm0.06)\times10^{-4}$	2.07×10^{-4}	-4.3

[a]triplicate measurements

[b]off-thread glucose conversion

[c]S-μTAD sensor results

Blood glucose determination

The capability of sensitive quantifying hydrogen peroxide is of significant potential in clinical and biological applications, because a variety of physiologically important substances (e.g. glucose, uric acid, etc.) can be stoichiometricaly converted into hydrogen peroxide by corresponding oxidase. Here, blood glucose, the most frequently diagnosed indicator was chosen as an example to test the quantitative capability of S-μTAD. The experimental conditions and operations were the same as those for hydrogen peroxide along with the optimized GOD concentration (Fig. S3d). Blood glucose was converted into hydrogen peroxide as described in detection procedure section. The relative CL intensity was found to be linearly related to glucose concentration in the range of 1.0×10^{-6}-1.0×10^{-4} mol/L following the equation of $\Delta I=25.95\ c\ (10^{-6}\ mol/L)-4.10$ (n=9, the square of linear correlation coefficient $r^2=0.998$). The detection limit (3σ) was 1×10^{-7} mol/L glucose and the relative standard deviation was 3.8% (5.5×10^{-6} mol/L glucose, n=7). Above analytical characteristics show the competence of S-μTAD in both reproducibility and sensitivity for blood glucose determination. At last, two human blood samples obtained from a local central hospital were determined, the S-μTAD results matched well with those offered by the hospital (Table 2). In contrast with the glucose standard, when measuring a blood sample exceeds nine times, decreased CL with the relative error more than 5% against the average of initial seven measurements were found. The CL decrease is attributed to the block of thread bundle by absorbing protein and suspended particles on cellulose.

Thus, for the analysis of cellulose strong absorbing samples or the infectious samples, for example the blood sample, a S-μTAD is preferred to be disposable for one sample.

Glucose and uric acid S-μTAD sensors

In case of low-cost microfluidics being fabricated in central labs and dispatched to remote communities, a S-μTAD sensor enabling the on-device analyte conversion into detectable species would provide the best convenience to the end users. Here, glucose and uric acid were selected as the model analytes to test the feasibility and universality of the design on S-μTAD sensor. GOD was immobilized on cotton thread via aldehyde functionalization and Schiff base reaction [38]. By replacing the sample arm of Y-shaped channels with GOD immobilized cotton thread, a glucose S-μTAD sensor was fabricated. At 28°C ambient temperature, glucose standard or diluted sample was allowed to drain away along the GOD arm. The produced hydrogen peroxide was eluted by water and merged with the CL reagent (luminol and potassium ferricyanide mixture) at the knot. With the same experimental conditions and operations as described for hydrogen peroxide detection, this S-μTAD sensor displayed a linear CL response to glucose in the concentration range of 2.0×10^{-6}-6.0×10^{-5} mol/L following equation of ΔI=4.99 c (10^{-6} mol/L)+10.85 (n=8, the square of linear correlation coefficient r^2=0.998). The detection limit (3σ) was determined to be 5×10^{-7} mol/L with a relative standard deviation of 4.2% (5.5×10^{-6} mol/L glucose, n=7). Such analytical characteristics show the capability of this glucose sensor to quantify the highly diluted blood sample. Similarly, the effectiveness of glucose S-μTAD sensor was verified by determining blood glucose levels in two human samples against the hospital determinations, agreements between their results had been found (Table 2).

Uric acid S-μTAD sensor was fabricated with the same procedure except for the use of UOD instead of GOD as glucose sensor. The optimizations of experimental conditions for uric acid sensor were performed and the results were showed in Fig. S4. A relative standard deviation was 3.2% when parallel detecting a 5.0×10^{-5} mol/L uric acid standard for seven times. The net CL signals were proportional to uric acid concentration extending from 1.0×10^{-5} mol/L to 1.0×10^{-4} mol/L with the linear equation of ΔI=39.94c (10^{-5} mol/L)-8.45 (n=5, the square of linear correlation coefficient r^2=0.991). The detection limit (3σ) was determined to be 3×10^{-6} mol/L uric acid. Above results in reproducibility, sensitivity and calibration range show the potential of this sensor for quantifying blood uric acid. As a result, uric acid levels in two human blood samples from a local central hospital were determined. The agreements of S-μTAD sensor results with hospital results showed the effectiveness of this sensor (Table 2).

Conclusions

We have demonstrated a new pump-free S-μTAD platform by the siphonage fluid movement. Renewable siphonage flow was found to render the S-μTAD refreshment, which allows replicate sampling and detection on one device, and thus evading the inconsistencies among channels or devices. Polyester cotton blend thread showed its best suitability to fabricate microfluidic channels for CL detection. The designed S-μTAD can be portable and facilely fabricated by little trained personnels. Our S-μTAD proved the acceptable reproducibility for quantitative assays of hydrogen peroxide and glucose. S-μTAD sensors were co-fabricated with polyester cotton blend and enzymes pre-immobilized cotton thread. S-μTAD sensors of glucose and uric acid exhibited their convenience and capability in diagnostic and POC use. The presented S-μTADs and sensors can be disposable to complete an analysis including the standard calibration and detection for one sample. The general approach of this work can be transformed into new designs for quantitative assays of substances interested in diagnostics, environment, and food safety. The proved utility of siphonage fluid movement is also expected to find a wider range of applications to lab-on-a-chip, particular in 3D low-cost microfluidic platforms.

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† Electronic Supplementary Information (ESI) available: Four figures of the verification of success oxidase immobilization, the siphonage flow rate measuring setup, optimum experimental variables for hydrogen peroxide and off-device glucose measurements, and for sensor uric acid measurement. Two movies of thread channels fabrication, device assembly and operation demonstration. See DOI: 10.1039/b000000x/

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