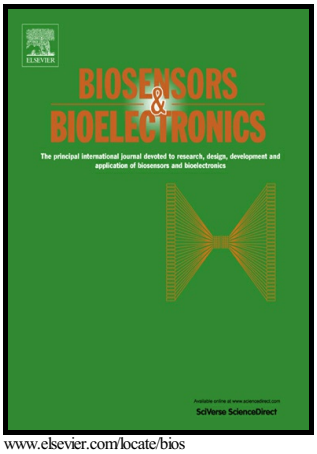

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Superhydrophilic cotton thread with temperature-dependent pattern for sensitive nucleic acid detection

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Abstract

Cotton thread is promising in fabricating biosensors for diagnostic application due to its excellent characteristics. However, the enrichment of the capture molecules on a narrow zone of the cotton thread based biosensor is a big challenge because of its superhydrophilicity. Here, we report a simple, low-cost and accurate cotton thread based nucleic acid biosensor with temperature-dependent pattern. Liquid wax is used to fabricate temperature-dependent pattern to restrict the test zone in a narrow area. This biosensor enables visual and quantitative detection of target DNA by accumulation of gold nanoparticles (GNPs) on the test zone with a detection limits of 0.75 nM. In addition, the cotton thread based biosensor needs less sample than previous reported lateral flow strip and the sample solution wicks faster at the cotton thread which can lead to a shorter detection time. This simple, low-cost and fast detection method holds great potential to improve healthcare services in the developing regions.

Keywords: cotton thread; temperature-dependent pattern; enrichment; nucleic acid; visual detection;

1. Introduction

Recently, point-of-care (POC) diagnostic technology has attracted much interest and is of great importance in biochemical analysis and clinical diagnostic (Dixit et al. 2016; Kaushik et al. 2016; Kumar et al. 2015; Shi et al. 2014). The requirement of

advanced diagnostic technologies and devices in developing countries promote us to study low-cost materials in the development and application of POC diagnostic devices (Engel et al. 2015; Hu et al. 2014; Niemz et al. 2011; Sharma et al. 2015). These devices show excellent merits such as portable, user-friendly, long-term stable and time-saving. In the last decade, one-dimensional biosensors such as lateral flow strips (Anfossi et al. 2013; Gao et al. 2016; Kuang et al. 2013), cotton thread (Nilghaz et al. 2013) have attracted much attention. Cotton thread has been reported to be an attractive material for fabrication of low-cost, low-volume, easy-to-use microfluidic device, which has potential applications in human health diagnostics, food safety analysis and environmental monitoring (Li et al. 2010; Nilghaz et al. 2013; Reches et al. 2010; Safavieh et al. 2011). Recently, cotton threads have been used as a capillary substrate for rapid blood grouping (Ballerini et al. 2011a, b; Nilghaz et al. 2014) and the detection of protein (Mao et al. 2015; Mao et al. 2014; Zhou et al. 2012), nucleic acid (Du et al. 2015; Mao et al. 2014). To meet the requirement of POC detection, the performance of cotton thread based biosensors including sensitivity, reproducibility, selectivity should be improved dramatically.

In the fabrication of biosensors, the immobilization of capture molecules is one of the most important procedures and the wettability of biosensing interface may have great effect on it. The wettablilty can be defined by contact angles, superhydrophilic surfaces showing a water contact angle less than 10^{o} and hydrophobic surfaces has a water contact angle exceeding 90^{o}. The surface wettablility was dependent on its chemical composition and nanostructure. The high surface energy of cotton thread endows it with hydrophilicity. And there are many nanogaps between cotton fibers. Water will fully permeate inside structural gaps, resulting superhydrophilicity of cotton threads. The superhydrophilicity of cotton thread is beneficial for sample flow in the detection process. But as for the immobilization of capture molecules, the superhydrophilicity of cotton thread makes the droplets containing capture molecules diffuse easily. The wide distribution of capture molecules is not favored the enrichment of the detecting signal and limits the detection sensitivity. Several groups have reported the enrichment of a few molecules from highly diluted solution, which

was induced by wettability difference and provided an efficient strategy to improve the detection sensitivity (De Angelis et al. 2011; Hou et al. 2014; McLane et al. 2015). Our group has reported a novel superwettable biochip based on two-dimensional superhydrophilic/superhydrophobic pattern for ultratrace nucleic acid detection (Xu et al. 2015). The capture molecules can be enriched in superhydrophilic microwells and thus the performance of DNA biosensor can be improved dramatically. The superhydrophilic/superhydrophobic pattern are also expected to be used to the one-dimensional thread based biosensors, which can help to immobilize the capture molecules in a confined area. However, these superhydrophilic/superhydrophobic pattern is difficult for sample flow during the detection. To resolve this conflict, tunable change of surface wettability of cotton thread is desirable to ensure the enrichment of the capture molecules on the thread and keep the thread hydrophilic at the process of detection simultaneously.

In this paper, we present a cotton thread based nucleic acid biosensor with temperature-dependent pattern (pCTNAB) for visually sensitive nucleic acid detection. Temperature-dependent liquid wax was used to realize the pattern on the thread. Before the immobilization of capture molecules, liquid wax was dropcasted onto cotton thread to form superhydrophilic/hydrophobic pattern. The liquid wax was solid at the temperature below 19 ^{o}C and these wax modified thread was turned to be hydrophobic, which is beneficial for the concentration of capture molecules in the test zone after evaporation. The detection process was carried out at room temperature, in this situation, the wax changed to be liquid again and wicked towards the absorption pad with the running buffer, which doesn't interfere with the detection result. Benefitting from the tunable state of the liquid wax, the conflict between the concentration of the capture molecules at the test zone and the sample flow at the detection process can be resolved. Due to the enrichment of capture molecules, the signal of pCTNAB was improved to achieve more sensitive detection.

2. Experimental

2.1 Apparatus and Reagents

The thread was purchased from a thread store (Beijing). The thread is produced by Linzhi Co., Ltd. (Yiwu, China) and the diameter is 430±17 μm. Glass fibers (GF-06), cellulose fiber pads (JN8025) were purchased from Jiening Biological Technology Co., Ltd. (Shanghai, China). Nitrocellulose membrane (HFB18002) was purchased from Millipore (Billerica, MA). The DT1032 portable strip reader was purchased from Shanghai Goldbio Tech. Co., Ltd. (Shanghai, China). Vacuum plasma reactor (PDG-MG) was purchased from Chengdu Mingheng Sci. & Tech. Co., Ltd. (Chengdu, China). Water contact angles were measured on an OCA20 system (DataPhysics, Germany).

The liquid wax was purchased from Beijing Innochem Tech. Co., Ltd. (Beijing, China) and the melting point of the liquid was 19 ^{o}C. Streptavidin, $HAuCl_4$, trisodium citrate, $Na_3PO_4{\cdot}12H_2O$, NaCl, $MgCl_2$, sucrose, Tween-20, sodium dodecyl sulfate (SDS), bovine albumin (BSA), PBS (PH=7.4), Tris-HCl (PH=7.5), sodium chloride-sodium citrate (SSC) buffer (PH=7.0) were purchased from Sigma-Aldrich and used without any purification. The oligonucleotides were obtained by Sangon Biotech (Shanghai, China). The oligonucleotide sequences used in this study were as follows:

Target DNA: 5'-AGA CCA TCC TGG CTA GTC TGT TGT CTC TAC TAA AAA TA-3'

Detection probe (probe1): 5'-Thio / $(CH_2)_6$ / GGG GTT TCA CCG TGT TAG CCA GGA TGG TCT-3'

Capture probe (probe 2): 5'-biotin/CGC CCG GCT AAT TTT TTG TAT TTT TAG TAG AGA C-3'

Single-base mismatched strand: 5'-AGA CCA TCC TCG CTA GTC TGT TGT CTC TAC TAA AAA TA-3'

Three-bases mismatched strand: 5'-AGA GCA TCC AGG CTA GTC TGT TGT CTC TAG TAA AAA TA-3'

Complementary mismatched strand: 5'-ACA CGT CTA ACG CCA ATA TCC TCA GCG AGA CCG ATG ACG TGT-3'

2.2 Fabrication of temperature-dependent pattern on the thread.

The main component of the thread are cellulose which has many hydrophilic hydroxyl groups. First of all, cotton threads were treated with air plasma for 5 min at 450 mTorr at a power of 150 mW. To fabricate temperature-dependent pattern on the thread, two aliquots of 0.3 μL liquid wax was added to two sites of the cotton thread, respectively and the gap between the two sites was 10 mm, then the cotton thread was transferred to the refrigerator to make the wax become solid, thus the superhydrophilic /hydrophobic pattern on the thread was formed. Subsequently, the center of gap was coated with certain amounts of streptavidin-biotinylated capture probe, and dried in the refrigerator to make capture probe concentrated into this area after evaporation. For applying streptavidin-biotinylated probe to the cotton thread without pattern, streptavidin-biotinylated capture probe was applied as several aliquots of 0.2 μL to decrease the effect of diffusion, with 5 min drying at room temperature after each step.

2.3 Synthesis of gold nanoparticles (GNPs) and GNPs/DNA conjugate.

GNPs with an average diameter of 15 ± 3 nm were synthesized according to the reported methods with slight modifications (Grabar et al. 1995; Mao et al. 2009). Briefly, 4 mL 1% trisodium citrate solution was added to the boiling $HAuCl_4$ solution (100 mL, 0.01%, w / v) with vigorous stirring. The color of the solution firstly turned deep blue and then changed to wine-red. The obtained solution was heated for another 15 min and then the heating source was removed to make the solution cool down to the room temperature. Finally, the obtained GNPs solution was stored at 4^oC for further use. The transmission electron microscopy (TEM) image of GNPs was showed in Fig. S1.

The GNPs/DNA conjugate was prepared according to the reported methods with slight modifications (Gao et al. 2016; Li et al. 2008; Mao et al. 2009). Certain amounts of 100 μM activated thiolated DNA was added into 200 μL tenfold-concentrated GNPs and then incubated at room temperature for 16 hours. Subsequently, 20 μL of 1 M NaCl / 10 mM Na_3PO_4 buffer solution (PH=7.4) was

dropped into the solution in an hour (2 μL every 6 min). 4 μL 1% SDS was added to the solution and the mixture was incubated for another 4 hours. 20 μL of 10% BSA solution was added to passivate GNPs for 30 min. The mixture was centrifuged (10000 rpm, 10 min) and the supernatant was removed. Then the GNPs/DNA pellet was resuspended in 200 μL PBS, this procedure was repeated for three times to remove the unbound DNA. Finally, the as-obtained red pellets were dispersed in 200 μL stock solution containing 20 mM $Na_3PO_4{\cdot}12H_2O$, 5% BSA, 0.25% Tween-20, and 10% sucrose and stored at 4 ^oC for further use.

2.4 Preparation of Streptavidin-Biotinylated DNA conjugate.

The streptavidin-biotinylated DNA conjugate was prepared according to the reported methods (Gao et al. 2014). 5 nmol biotinylated DNA (probe 2) was mixed with 20 μL 2.5 mg/mL streptavidin and incubated on shaker for 1 hour at room temperature. After adding 50 μL PBS, the solution was transferred to the dialysis tube and centrifuged for 20 min at 6000 rpm under 4^oC. The procedure was repeated for 3 times and the remaining solution in filter was about 100 μL.

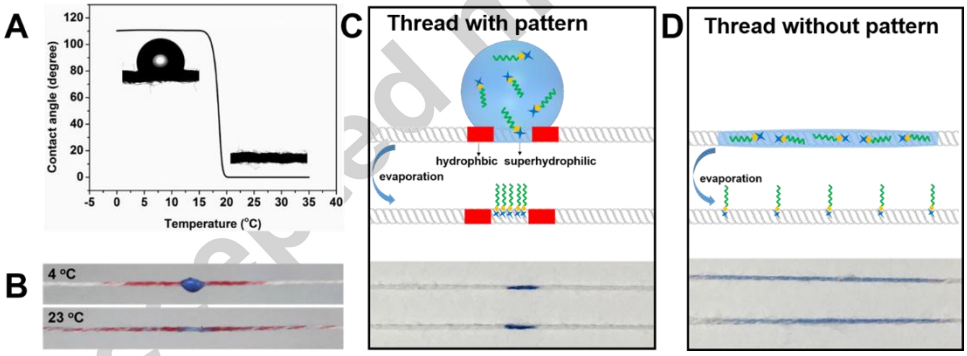


Fig. 1. (A) Contact angle of the liquid wax blocked thread at different temperature. The insets were photographs of a droplet (3 μL) on liquid wax blocked cotton thread at 4 ^oC and 23 ^oC, respectively. (B) Photographs of temperature-dependent pattern at 4 ^oC and 23 ^oC. The water droplet was colored by blue dye, and liquid wax was colored by oil red. (C) Illustration for the enrichment of probe molecules at superhydrophilic/hydrophobic pattern and a photograph of water droplet (colored with blue food dye, 2 μL) on the cotton thread with temperature-dependent pattern. (D) Illustration for application of the probe molecules to the cotton thread without pattern and a photograph of water droplet (colored with blue food dye, 2 μL) applied to the cotton thread without pattern.

2.5 Fabrication of pCTNAB.

Double faced adhesive tapes were stuck to a clean plastic pad with a space wide about 4 cm parallelly. The space between the double faced adhesive tapes was made to be lower than two sides to make the test zone hang in the air. Capture probe coated cotton threads were attached to the plastic pad and ensure the test zone was in the middle. A glass fiber (8mm×8mm) was placed at head end as the conjugate pad and sample pad, an absorption pad (8mm×8mm) was placed at the downstream end of the thread to sever as a capillary pump to draw the liquid.

2.6 pCTNAB assay procedure.

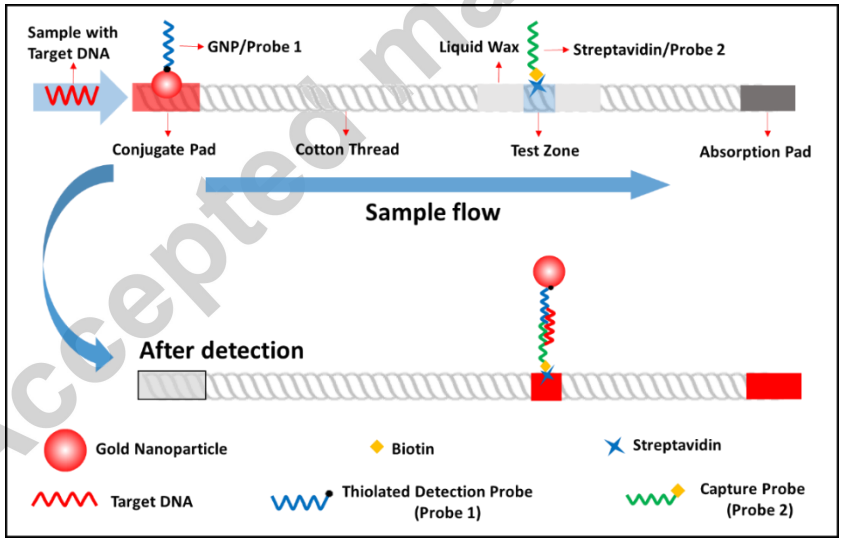
To perform an assay, certain amount of GNPs/DNA conjugate was firstly loaded on the conjugate pad, then 20 μL sample solution with desired amount of target DNA was added, then the mixed solution migrated toward the absorption pad. After 15 min, a characteristic red band could be visible by naked eyes. For quantitative measurements, the optical intensity of the red band was read using the portable "strip reader" instrument combined with "GoldBio strip reader" software

3. Results and Discussion

3.1 Cotton thread with temperature-dependent pattern.

The wettability of cotton thread was changed based on the state of liquid wax at different temperature. As shown in Fig. 1A, at the temperature below 19 ^{o}C, the state of the wax was solid, and the cotton thread modified by wax was hydrophobic with a contact angle of about $110^{o} \pm 5^{o}$ due to the low surface energy of wax. When the temperature rose to 19 ^{o}C, the wax turned to be liquid and easily spread, and the air/water/cotton thread contact line prevailed, thus the contact angle of the thread decreased to about 0^{o}. The insets in the Fig. 1A demonstrated a droplet (3 μL) on liquid wax modified cotton thread at temperature below and up 19 ^{o}C, respectively. The tunable state of the liquid wax was exploited to fabricate temperature-dependent pattern on the thread. The superhydrophilic/hydrophobic liquid pattern was fabricated at 4 ^{o}C. As shown in Fig. 1B, the water droplet (colored by blue dye) was confined at the gap between the liquid waxes (colored by oil red) due to the difference in

wettability. The increasing of the temperature to 23 ^{o}C resulted in melting of the wax, the cotton thread was superhydrophilic again and the liquid wax started to wick to both ends along with water solution. Fig. 1C illustrated the enrichment of probe molecules at superhydrophilic/hydrophobic pattern. At 4 ^{o}C, the droplet of probe molecules covered the entire superhydrophilic gap and part of the hydrophobic wax covered thread. During the evaporation, the droplet was pinned to the superhydrophilic gap because of the wettability contrast. After evaporation, the probe molecules was enriched at the gap between the waxes. The water droplet (colored with blue food dye, 2μL) was employed to study the enrichment effect of cotton thread with superhydrophilic/hydrophobic pattern and the blue dye molecules were concentrated at the gap. In comparison, for the cotton thread without pattern, the dye molecules diffused along the whole thread, thus the color intensity of food dye is lower due to the lack of enrichment. (Fig. 1D). The wax treated cotton thread with superhydrophilic/hydrophobic pattern exhibits great enrichment effect and may have great potential in fabricating sensitive biosensor.



Scheme 1. Schematic illustration of the configuration and measurement principle of the pCTNAB and the test result at the presence of target DNA.

3.2 Principle of pCTNAB.

The principle of the pCTNAB is based on sandwich type of "DNA-Target DNA-DNA /GNPs" hybridization reactions, and the protocol is illustrated in Scheme

1. In this study, GNPs are used as a tracer to monitor DNA hybridization event. The thiolated detection probe (probe 1) was immobilized on the GNPs surface by self-assembling via the Au-S bond to form the GNPs/DNA conjugates and is firstly applied to the conjugate pad before the assay. The biotinylated DNA probe (probe 2) is pre-immobilized on the thread through biotin-streptavidin interaction to form test zone, liquid wax is added to the thread at both sides of the test zone to make the probe 2 concentrated in the test zone without diffusion, thus the test zone can be confined in a narrow area. To perform assay, sample solution with target DNA is applied to the conjugate pad to rehydrate GNPs/DNA conjugate and the target DNA hybridizes with the thiolated detection probe of the GNPs/DNA conjugates. Then the hybrids wick through the thread by capillary and are captured at the test zone by the reaction between the target DNA and the capture probe (probe 2). The accumulation of GNPs at the test zone produced a characteristic red band at the test zone, enabling visual detection of target DNA. However, no red band can be observed at the test zone without the target DNA. For quantitative measurements, the portable "strip reader" instrument combined with "GoldBio strip reader" software is used to read the optical intensities of the red bands.

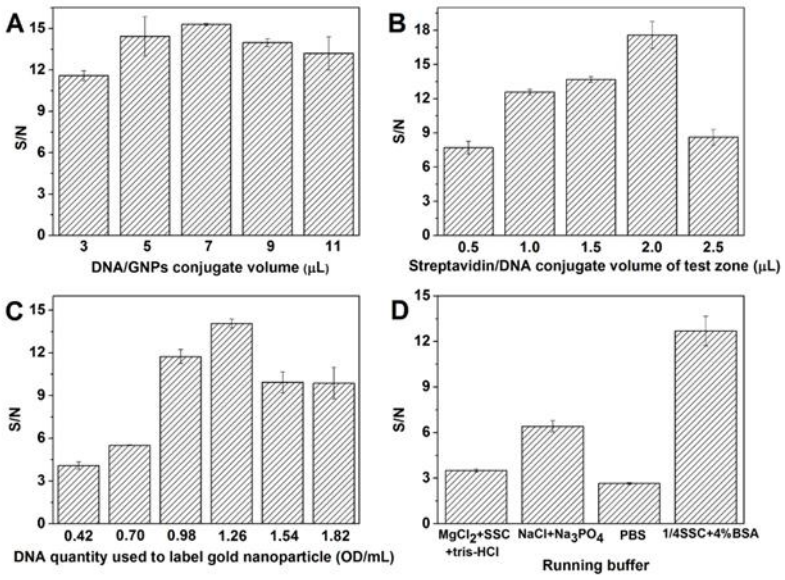


Fig. 2. (A) Effect of the volume of DNA/GNPs conjugate on the S/N ratio of the pCTNAB; (B) Effect of the volume of streptavidin/DNA conjugate on the S/N ratio of the pCTNAB; (C) Effect of the quantity of detection probe used to label gold nanoparticle; (D) Effect of the

running buffer on the S/N ratio of the pCTNAB.

3.3 Optimization of pCTNAB parameters.

Various factors could affect the accumulation of GNPs on the test zone and then affect the sensitivity and reproducibility of the pCTNAB. Four factors including the volume of GNPs/DNA conjugate loaded on the conjugate pad, the volume of the streptavidin-biotinylated capture probe applied to the test zone, the amount of detection probe (probe 1) on the surface of GNPs and the running buffer were investigated by comparing the analytical performance of 50 nM target DNA.

As depicted in Fig. 2A, the volume of the GNPs/DNA conjugate loaded on the conjugate pad has a great effect on the intensities of the test zone. With the increase of the GNPs/DNA conjugate volume, the S/N ratio increased, and then decreased after the volume reached 7 μL due to the nonspecific adsorption on the test zone. Therefore, the best volume of the GNPs/DNA conjugate was 7 μL, and was used in the following study.

The amount of the streptavidin-biotinylated probe on the test zone also plays an important role in the performance of the target DNA detection. Fig. 2B demonstrated that 2 μL of the streptavidin-biotinylated capture probe was the optimal amount, further increasing of the streptavidin-biotinylated probe would decrease the S/N ratio because of the increased background. Thus 2 μL of the streptavidin-biotinylated capture probe was adopted in the following experiments.

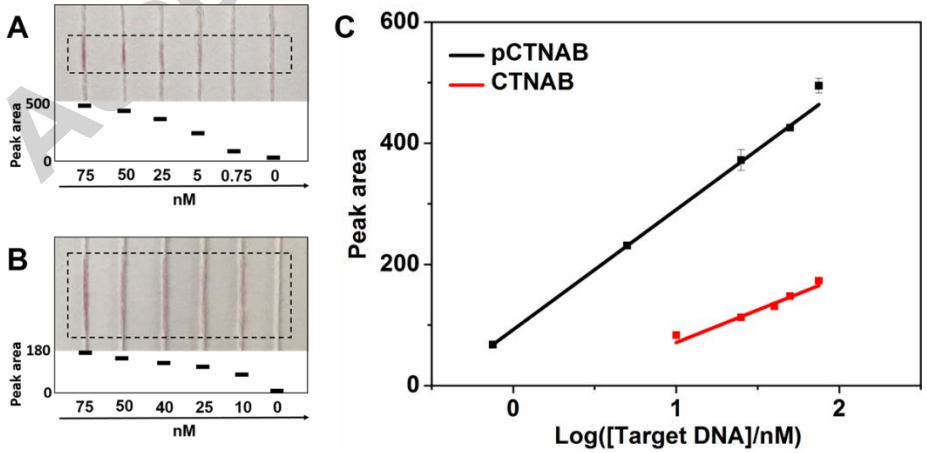


Fig. 3. (A) Typical photo images of the pCTNAB after applying different concentration of target DNA; (B) Typical photo images of the CTNAB after applying different concentration of target DNA; (C) The resulting calibration curve of the intensities of the test zone (peak area)

versus target DNA concentration for both pCTNAB and CTNAB. All of the photo images of the pCTNABs and CTNAB were recorded with a digital camera while the optical responses of test zone on the pCTNABs and CTNABs were recorded with a strip reader. Running buffer: 1/4 SSC +4% BSA; the volume of the streptavidin-biotinylated capture probe of the test zone: 2 μL; GNPs/DNA conjugate volume loaded on the conjugate pad: 7 μL; DNA quantity used to label gold nanoparticle: 2.0 OD/mL; total assay time: 20 min.

Different amount of detection probe on the surface of GNPs will influence the hybridization of detection probe and target DNA, and then influence the intensities of the test zone. As shown in Fig. 2C, the S/N ratio of the pCTNAB increased with the increase of the detection probe on the surface of the GNPs. When the concentration of the detection probe was 1.26 OD/mL, the S/N ratio was maximum. Then the S/N ratio decreased with more detection probe on the surface of GNPs due to the nonspecific adsorption. So the optimal concentration of the detection probe was 1.26 OD/mL and was used in the following study.

The response of the pCTNAB is also relevant to the running buffer. An appropriate buffer can help improve the sensitivity, reproducibility and selectivity, as well as decrease the nonspecific adsorption of GNPs/DNA conjugate. In this study, four kinds of running buffer including $MgCl_2$+Tris-HCl, NaCl+Na_3PO_4, PBS, 1/4 SSC+4% BSA were selected to compare the analytical performance of the biosensor on detecting 50 nM Target DNA. The best S/N ratio was obtained with 1/4 SSC+4% BSA buffer. (Fig. 2D).

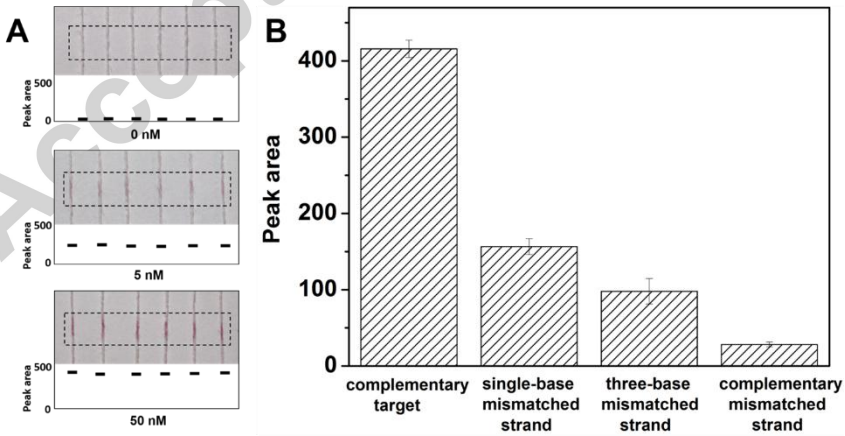


Fig. 4. (A) Typical photo images of the pCTNABs with different concentrations of target DNA. (B)The optical intensities (peak area) of the pCTNAB test zones analyzed in the presence of different 50 nM strands (complementary target, single-base mismatched strand, three-base mismatched strand, complementary mismatched strand). Experimental conditions

were the same as in Fig. 3.

3.4 Analytical characters.

The analytical performance of the pCTNAB was assessed in detecting different concentration of target DNA ranging from 0.75 nM to 75 nM under the optimized conditions. Fig. 3A presents the typical photo images of the pCTNAB in the presence of different concentration of target DNA ranging from 0.75 nM to 75 nM. For quantitative measurement, the intensity of the test zone was recorded by the portable strip reader. The calibration curve of the intensities of the test zone (peak area) versus DNA concentration was linear in the range from 0.75 nM to 75 nM and was appropriate for quantitative detection. The detection limit of 0.75 nM (based on S/N = 3) was estimated (Fig. 3C black one).

Reproducibility is another important criteria to evaluate the performance of the pCTNAB. Sample solutions containing 0 nM, 5 nM and 50 nM of target DNA were used to examine the performance of the pCTNAB. Each concentration was tested 6 times, respectively. The result was shown in Fig. 4A, similar responses were obtained against the same concentration level, and the RSD values for the concentration of 0 nM, 5 nM and 50 nM were 7.8%, 4.3% and 2.6%, respectively, indicating an excellent analytical reproducibility.

To assay the selectivity of the pCTNAB, three other kinds of DNA sequences including single-base mismatched strand, three-base mismatched strand and complementary mismatched strand at 50 nM had been tested at the pCTNABs. As shown in Fig. 4B, no distinct responses were obtained with mismatched strands, however, perfectly matched target DNA could accurately bind to the two DNA probes (capture probe and detection probe) and produced a clear red band on the test zone, demonstrating high selectivity of the pCTNAB.

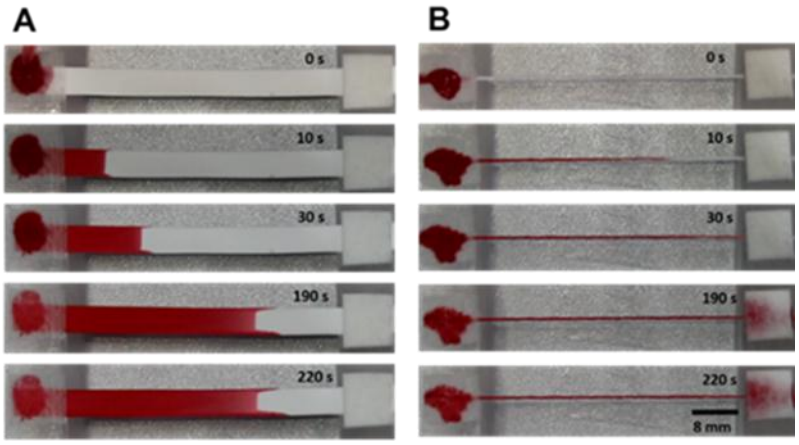


Fig. 5. Images extracted from a video illustrating the wicking properties of red ink on (A) nitrocellulose membrane and (B) cotton thread.

We also compared the sensitivity of the pCTNAB with the cotton thread based nucleic acid biosensors (CTNAB) without pattern (Fig.3B). To reduce the influence of the diffusion when fabricated the CTNAB, we applied the streptavidin-biotinylated probe as several aliquots of 0.2 μL, with 5 min drying at room temperature after each step. Even so, the test zone of the CTNABs was still longer than that of the pCTNAB. The calibration curve of the intensities of the test zone (peak area) versus DNA concentration was fabricated for the CTNAB. The linear range is from 10 nM to 75 nM, which is much narrower than that of pCTNAB. The detection limit of CTNAB is 7.2 nM (based on S/N = 3), and about ten times higher than that of pCTNAB (Fig.3C). Based on the comparison, the performance of pCTNAB is obvious superior to CTNAB, which can be ascribe to the confined diffusion of capture probe in the superhydrophilic/hydrophobic pattern. As been discussed, the wax treated cotton thread with superhydrophilic/hydrophobic pattern exhibits great enrichment effect. The capture probe was confined at the superhydrophilic gap between the liquid waxes, which resulted in smaller distribution area. After the adding of target DNA, GNPs were captured and accumulated at the test zone. For the given amount of target DNA, the number of GNPs captured at test zone should be the same theoretically. Due to the narrower distribution area of capture probe, the accumulation area of GNPs is more concentrated in pCTNAB, which resulted in higher signal intensity. In comparison, for the CTNAB, the capture probe diffused along the thread, which result in the wide distribution of GNPs after the detection. Therefore, the CTNAB has low signal

intensity (Fig.3B), and it's hard to discern the signal from the background. The confined distribution of capture probe in superhydrophilic/hydrophobic pattern plays a critical role in improving the sensor sensitivity of pCTNAB.

Since conventional lateral flow strips based on nitrocellulose membrane are widely used in various fields, the detection sensitivity of the pCTNAB was compared with the lateral flow strips (Hu et al. 2013; Mao et al. 2009; Rastogi et al. 2012). The sensitivity of the two kinds of biosensors was comparable. Then the wicking properties of the cotton thread was compared with that of the nitrocellulose membrane which is the main part of the lateral flow strip, and the most widely used width of the nitrocellulose membrane at the lateral flow strip is 4 mm. As demonstrated in Fig. 5, 20 μL red ink was added to the left end of the cotton thread and 4 mm-wide nitrocellulose membrane, respectively. It can be easily found that the red ink wicked more quickly at the cotton thread than that at the nitrocellulose membrane and much less sample is needed for the cotton thread. Considering these advantages, the cotton thread is very promising in the fabrication of nucleic acid biosensor.

4. Conclusion

In summary, we developed a cotton thread based nucleic acid biosensor with temperature-dependent superhydrophilic/hydrophobic pattern for simple visual-sensing and quantification of target DNA. Liquid wax was used to fabricate temperature-dependent pattern on the thread, thus the capture probe could be enriched in a narrow test zone. Due to the successful application of the superhydrophilic/hydrophobic pattern to the one-dimensional thread based biosensors, the sensitivity of the pCTNAB was improved compared to the CTNAB. Under optimal conditions, a visual and quantitative detection limit of 0.75 nM in aqueous solutions was obtained. In addition, the requirement of sample solution for the pCTNAB is much less than previous reported lateral flow strip due to the small size of the thread, and the sample solution wicks faster at the pCTNAB, which had shorted the detection time. Such attractive pCTNAB provides an alternative tool for DNA detection in developing countries due to its simple preparation, low-cost, user-friendly

characters, have potential applications in POC diagnostic devices.

Notes

The authors declare no competing financial interest.

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Highlights

- A simple, low-cost and accurate cotton thread based nucleic acid biosensor (CTNAB) with temperature-dependent pattern was developed.
- Liquid wax with tunable state was used to fabricate the temperature-dependent pattern to enrich the capture molecular on a narrow test zone without influencing the sample flow during the detection process.
- The sample wicked faster at the CTNAB and less sample was needed compared to the previous reported lateral flow strip.
- The proposed sensor showed fine reproducibility and excellent anti-interference ability.