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A novel adenosine-based molecular beacon probe for room temperature nucleic acid rapid detection in cotton thread device

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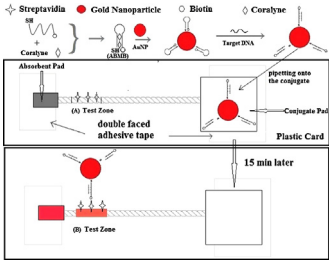
HIGHLIGHTS

- Natural cotton thread-based point-of-care diagnosis devices.
- A room temperature nucleic acid detection method.
- A novel molecular beacon technique based on poly adenosine and coralyne interaction.
- The test device is capable of discrimination single base mismatched sequences.
- Simple preparation, low cost, rapid detection, easy to handle.

GRAPHICAL ABSTRACT



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ABSTRACT

We used cotton thread as substrate to develop a novel room temperature DNA detection device for low-cost, sensitive and rapid detection of a human genetic disease, hereditary tyrosinemia type I related DNA sequences. A novel adenosine based molecular beacon (ABMB) probe modified on gold nanoparticle was used as reporter probe. In the presence of coralyne, a small molecule which can react with adenosines, the ABMB would form a hairpin structure just like traditional molecular beacon used extensively. In the presence of target DNA sequences, the hairpin structure of ABMB modified on gold nanoparticles will be opened and the biotin group modified at one end of the DNA probes will be released and react with the streptavidin immobilized on the test zone of the cotton thread. The response of the thread based DNA test device is linear over the range of 2.5–100 nM complementary DNA. The ability of our developed device for discriminating the single base mismatched DNA related to a human genetic disease, hereditary tyrosinemia type I, was improved comparing with previous report. It is worth mentioning that the whole assay procedure for DNA test is performed under room temperature which simplified the assay procedures greatly.

1. Introduction

Tyagi and Kramer [1] first reported the fluorescent molecular beacon (MB) in 1996 as nucleic acid probe, being able to initiate conformational change spontaneously due to the hybridization with complementary nucleic acid target. In recent years, nanomaterials including gold nanoparticle and carbon nanotube were employed as

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substrates to immobilize molecular beacons. Bockisch et al. developed a novel DNA stem-loop structured probe for enzymatic detection of nucleic acid target based on the molecular beacon immobilized on the surface of microplate and electrode [2,3].

The study of interaction between adenosine and coralyne was first reported by Ren and Chaires in 1999 [4]. In 2012, Lin and Tseng utilized coralyne, a special molecule able to interact with adenosine, to design a novel molecular beacon. In the report, the fluorescence method was used to realize quantitative detection of target DNA molecules successfully [5]. However, their obtained results indicated that the discrimination of single base mismatched target DNA sequences were not satisfied. We have improved the performance by using microplate as substrate for fluorescence test based on the similar strategy [6].

Whitesides and Shen have proposed cotton thread as a support for making microfluidic circuits and rapid diagnostic tests [7,8]. Natural cotton thread has also been reported by David as a support for transporting and mixing liquids in lateral flow immunochromatographic assays for quantitative high-sensitivity immunoassays [9,10]. Lateral flow strip was used extensively in biomarker and disease biosensors due to its assay formats [11–19]. These cotton thread based devices own some advantages of lateral flow strip, but even possess some superior advantages due to its internal characters mentioned above. All these make it an ideal candidate for point-of-care and on-site diagnosis applications.

Here we explored a cotton thread-based device for rapid, sensitive and quantitative detection of human genetic disease related DNA. The ability of our developed cotton thread-based DNA detection device for discriminating the single base mismatched DNA related to a human genetic disease, hereditary tyrosinemia type I [20] was realized by using a novel adenosine based molecular beacon probes (ABMB) which was labeled on gold nanoparticles. In the presence of target DNA sequences, the hairpin structure of ABMB modified on gold nanoparticles will be opened and the biotin group modified at one end of the DNA probes will be released and react with the streptavidin immobilized on the test zone of the thread. The thread was pasted on two parallel placed double faced adhesive tapes, and an absorbent pad was applied at the downstream end. The glass fiber loading gold nanoparticle conjugate was attached to the other end of the thread. The immunoassay format of the device is more like a lateral flow strip biosensor: sample solution and running buffer were dropped directly on the glass fiber to rehydrate the conjugates. After waiting for fifteen minutes, a red band would appear in the presence of specific analyte, which can be used as a qualitative mean. Quantitative detection can be realized by recording the color intensity of the test zone with a scanner and "ImageJ" software. The response of the thread based DNA test device is linear over the range of 2.5–100 nM complementary DNA. The ability of our developed device for discriminating the single base mismatched DNA related to a human genetic disease, hereditary tyrosinemia type I, was improved comparing with previous report. It is worth mentioning that the whole assay was conducted at room temperature in less than 0.5 h, which greatly simplify the assay procedures.

2. Experimental

2.1. Materials and reagents

Cotton thread (100% mercerized) was purchased from a thread store (Xi'an). Albumin bovine serum (BSA) and human serum were supplied by Dingguo Biological Products (Beijing, China). Streptavidin from Streptomyces Avidin, and coralyne were obtained from Sigma–Aldrich (Shanghai, China). 3-mercapto propionic acid (MPA) was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). DNA sequences (Table 1)were supplied by Sangon Biotech (Shanghai, China). 0.1 M HEPES (pH 7.0, 0.2 M NaCl) was used as incubating buffer. All other chemicals were of analytical reagent grade. All buffer solutions were prepared using ultrapure water.

Table 1
DNA sequences of ABMB and tested DNA.

Name	Sequence (5′–3′)
DNA_1	SH-AAAAAAAATGGACCAGATACTCACCGAAAAAAAAA-Biotin
DNA_2	CCGGTGAGTATCTGG
DNA_3	CCGGTGAATATCTGG
DNA_4	CCGGTGATTATCTGG
DNA_5	CCGGTGACTATCTGG
DNA_6	TCAGTGGGGTTGGACGGGATGGTGCCTGAA

2.2. Instrumentation

Basic pH meter was purchased from Sartorius (Beijing, China). Sterilization kettle was supplied by Shanghai Boxun Industry & Commerce Co. Ltd. (Shanghai, China). High pure water distiller was obtained from Changzhou Guohua electric appliance Co. Ltd. (Jiangsu, China). CanoScan 9000F was supplied by Canon Co. Ltd. (Thailand). Drying oven was supplied by Yiheng technology Co., Ltd. (Shanghai, China).

2.3. Synthesis of gold nanoparticles (Au NPs)

Au NPs with the diameter of 40 ± 5 nm was prepared using the citrate reduction method, as described briefly as follows. 1.5 mL 1% trisodium citrate solution for synthesis of 40 ± 5 nm Au NPs was rapidly added into a stirred boiling $HAuCl_4$ (100 mL, 0.01%, w/v) solution. After several minutes, the color of the solution changed from violet to deep red. The obtained solution was heated for another 15 min to ensure a stable color followed by slow cooling to room temperature under stirring. The obtained Au NPs solutions were stored at 4 °C for future use.

2.4. Pre-treatment of adenosine-based molecular beacon and its immobilization

A well designed DNA probe modified a thiol group and a biotin group on each end was titled as DNA_1 (Table 1). DNA_1 was immobilized on the surface of Au NPs (40 ± 5 nm) through self assembling. It is worth mentioning that 14 μL of 5 μM DNA probe used should be treated subsequently at 95 °C and ice-cold water bath for 10 and 30 min, respectively, followed by adding 0.7 μL of 2 mM coralyne and 55.3 μL of 0.1 M HEPES (pH 7.0, 0.2 M NaCl) and reacting at room temperature for 30 min to form a hairpin structure just like traditional molecular beacon probe. The formed hairpin structure DNA probe was titled as adenosine based molecular beacon probe (ABMB). The above ABMB was added to 1 mL Au NP solution (pH 6.5) to stand for 24 h at 4 °C, the conjugate was slowly aged with addition of BSA until reach a final concentration of 1%, then 1.12 μL 375 μM MPA was added, followed by adding NaCl to reach 0.05 M final concentration. The solution was allowed to stand for another 24 h at 4 °C, followed by 10 min centrifugation at 6500 rpm to concentrate the conjugates by two times. After the supernatant was discarded, the precipitated Au NP conjugate was resuspended in 1 mL of dispersion buffer (0 .1 M HEPES, pH 7.0, 0.2 M NaCl, 1% BSA), and then stored at 4 °C before use. The formed gold nanoparticle conjugate was titled as Au NP-ABMB.