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Dry-reagent nucleic acid biosensor based on blue dye doped latex beads and lateral flow strip

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ABSTRACT

In the manuscript, a quantitative lateral flow nucleic acid biosensor (Lateral flow nucleic acid biosensor, LFNB) based on blue dye doped latex beads was proposed and its feasibility for detecting deoxyribonucleic acid (DNA) in plasma was investigated. A 60-mer DNA sequence (T1) was selected as model to demonstrate the protocol. Blue dyes doped latex bead bearing DNA probe would be captured on the corresponding test line in the presence of target DNA, to form an evident blue band. Although qualitative tests are realized by observing the color change of the test zone, quantitative data are obtained by recording the optical responses of the test zone with a portable "Strip Reader" instrument conveniently. The strip has been applied for the detection of synthesized DNA sample in human plasma sample with a detection limit of 3.75 fmol. Interference was not evident even the target DNA was spiked with 50 μL plasma which indicated the well shielding of the latex bead reporters and quantified chromatographic separations of unwanted materials of the strip comparing with traditional gold nanoparticle based LFNB platforms.

1. Introduction

The nucleic acid test is of central importance to the diagnosis and treatment of genetic diseases, to the detection of infectious agents, drug discovery, or warning against bio-warfare agents [1–4]. Developing sensitive and specific DNA detection platforms for use in fundamental research and clinical applications cause extensive attentions in recent years. Several strategies, including real-time PCR, DNA microarrays and surface plasmon resonance [5–7], are widely used for DNA test. While powerful, these approaches are frequently time- and labor-intensive and require expensive instrumentation, which limits their application in laboratory and point-of-care settings.

Lateral flow test strip (LFTS) were first developed for antibody-based pregnancy test in 1990, since then, it was widely applied in detecting of various biomolecules such as protein, enzyme and cells [8]. Comparing with traditional immunoassay technologies, LFTS based immunoassay offer a simple, rapid and cost-effective tool for in-field test. Considering these unique advantages of LFTS, recently, research has concentrated on the developing of POC nucleic acid biosensors based on LFTS technologies.

As we know, the kinetics of nucleic acid hybridization in lateral flow are quite different and more complex as compared with the formation of the antigen–antibody complex used in common immunochromatography assay, most of reports to detect nucleic acid sequences with LFNA test strips are based on the formation of hapten–antibody or hapten–protein (e.g., biotin-avidin) complexes when the PCR products are amplified using a hapten labeled primmer. The other alternative way is based on hybridization reactions of PCR products and specific sequences. These methods have enough sensitivity for amplified DNA detection. For example, Fong et al. [9], Piepenburg et al. [10] and Corstjens et al. [11] use a lateral flow immunoassay for the detection of DNA amplification product. Baeumer et al. reported a dipstick-type LFNA biosensors with nanomolar detection limits based on dye-encapsulating liposome labels [12,13]. The biosensors have been applied to detect Dengue virus in blood samples [14] and viable *Escherichia coli* in drinking water [15]. Ioannou and Christopoulos's group reported a dry-reagent strip biosensor based on oligonucleotide functionalized gold nanoparticles for visual detection of DNA [16]. The biosensors have been used for visual detection of genetically modified organisms, [17] leukemia-related chromosomal translocations, [18] molecular diagnosis of bacterial infection [19] and genotyping of single-nucleotide polymorphisms [20]. In these reported LFNA test strips, the actual hybridization reaction is generally performed before the flow. The pre-hybridization reaction time usually takes 10–30 min. Some tests [11,21] require special and expensive hardware for quantitative detections. We developed some LFTS technologies for DNA tests by using gold nanoparticle as reporter probes for visual test of special DNA

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sequences recently [22,23]. Due to the unique optical characters of gold nanoparticle and enzyme based second amplification protocol, the sensitivity of the LFTS based DNA test tools were greatly improved. Moreover, pre-amplification procedures based on PCR is not necessary and the whole assay time is less than 20 min with detection limits of pM levels.

Considering the relative higher price of gold nanoparticles, some researchers used common dye as reporter probe for proteins detection on LFTS platforms, but the detection limit is pretty high [24,25]. In this study, a quantitative lateral flow nucleic acid biosensor based on blue dyes doped latex bead and lateral flow strip technology (Lateral flow nucleic acid biosensor, LFNB) is proposed and its feasibility for detecting deoxyribonucleic acid (DNA) in plasma is investigated. A pair of DNA probes was designed as capture and reporter probes which were immobilized on nitrocellulose membrane as test line and latex bead, respectively. All reagents used in the test were pre-immobilized and dried on LFNB, so for the test, we just need drop the sample solution on LFNB, waiting for ten more minutes, visual discrimination of the blue band on test line give a "yes" or "no" results. The sensitivity could be further increased by quantitative determinate DNA within several seconds with a portable "Strip Reader" instrument, which is comparable with the performance of our previous reported gold nanoparticle based strip biosensors. The strip biosensor also shows great assay performance for detection of synthesized DNA sample spiked in human plasma sample.

2. Experimental

2.1. Apparatus

Airjet AJQ 3000 dispenser, Biojet BJQ 3000 dispenser, Clamshell Laminator and the Guillotine cutting module CM 4000 were from Biodot LTD (Irvine, CA). Portable strip reader DT1030 was purchased from Shanghai Goldbio Tech. Co., LTD (Shanghai, China).

2.2. Reagents

Streptavidin from *Streptomyces avidin*, sucrose, Tween 20, dithiothreitol (DTT), Triton X-100, bovine serum albumin (BSA) and sodium chloride-sodium citrate (SSC) Buffer 20× concentrate (pH 7.0), phosphate buffer saline (PBS, PH 7.4, 0.01 M) were purchased from Sigma-Aldrich. Glass fibers (GFCP000800), cellulose fiber sample pads (CFSP001700), laminated cards (HF000MC100) and nitrocellulose membranes (HFB18004 and HFB 24004) were purchased from Millipore (Billerica, MA). Human plasma samples were purchased from Golden West Biologicals (Temecula, CA). DNA oligonucleotides were obtained from Integrated DNA Technologies, Inc. (Coralville, IA) and had the following sequence:

Target DNA: 5′-TTCCCTAGCCCCCCCAGTGTGCAAGGGCAGTGA-AGACTT
GATTGTACAAAATACGTTTTG-3′
DNA probe 1: 5′-amino-C6-D/CAA AAC GTA TTT TGT ACA AT-3′
DNA probe 2: 5′-ACA CTG GGT GGG CTA GGG AA/Biotin/-3′
DNA probe 3: 5′-Biotin/ATT GTA CAA AAT ACG TTT TG-3′
Noncomplementary DNA: 5′-ATG GCA TCG CTT AGC TGC CAG TAC ACT GAT TGA AGA CAT CAT AGT GCA GAC AAG CAT ATC-3′

All chemicals used in this study were analytical reagent grade. All other solutions were prepared with ultrapure (> 18 MΩ) water from a Millipore Milli-Q water purification system (Billerica, MA).

2.3. Preparation of latex bead-DNA conjugates

Blue dyes doped latex beads (modified with carboxyl group) with average diameter 390 nm were purchased from Signanoprobe company. An oligonucleotide modified with amino group (DNA probe 2) was used for conjugation with latex beads. Before conjugation reaction, the latex beads was activated by the following procedure: 25 μL of 5 mg/mL latex beads was washed with 25 mM MES (pH 6.0) for two times, and dispensed in 500 μL MES buffer containing 25 mg EDC and NHS to react for 30 min. The activated beads were washed three times with 500 μL MES buffer, dispensed in 500 μL MES buffer containing 80 nmol DNA probe 1 to react for 1.5 h. After centrifuge at 8000 rpm for 6 min and discard the supernatant, the DNA modified latex beads were dispensed in 500 μL 50 mM Tris–HCl buffer to react for 15 min to block the active sites of latex beads. Additional three washes with 10 mM pH 7.40 PBS buffer were used to remove the excess DNA probes. Finally, the obtained conjugates were dispensed in a buffer containing 20 mM Na_3PO_4, 5% BSA, 0.25% Tween and 10% sucrose.

2.4. Preparation of lateral flow nucleic acid strip

A schematic diagram of the LFNA test strip is shown in Fig. 1. The LFNA test strip consists of four components: sample application pad, Latex bead-DNA conjugate pad, nitrocellulose membrane and absorbent pad. All of the components were mounted on a common backing layer (typically an inert plastic, e.g., polyester) using the Clamshell Laminator (Biodot, CA, USA). The sample application pad (17 mm × 30 mm) was made from glass fiber (CFSP001700, Millipore) and saturated with a buffer (pH 8.0) containing 0.25% Triton X-100, 0.05 M Tris–HCl and 0.15 M NaCl. Then it was dried and stored in a desiccator at RT. The conjugate pad (8 mm × 30 mm) was prepared by dispensing a desired volume of latex bead-DNA conjugate solution onto the glass fiber pad with the dispenser Airjet AJQ 3000, and then drying it at RT. The pad was stored in a desiccator at 4 °C. Nitrocellulose membrane (25 mm × 30 mm) was used to immobilize the capture DNA probes (DNA probe 1) and control DNA probes (DNA probe 3) at different zones to form test line and control line, respectively. To facilitate its immobilization on the nitrocellulose membrane, streptavidin was used to react with the biotinylated DNA probes to form the streptavidin-biotin DNA conjugates. Briefly, 60 μL of 1 mM biotinylated DNA probes and 140 μL of PBS were added to 300 μL of 1.67 mg/mL streptavidin solution, and the mixture was incubated 1 h at RT. The excess DNA probes were removed by

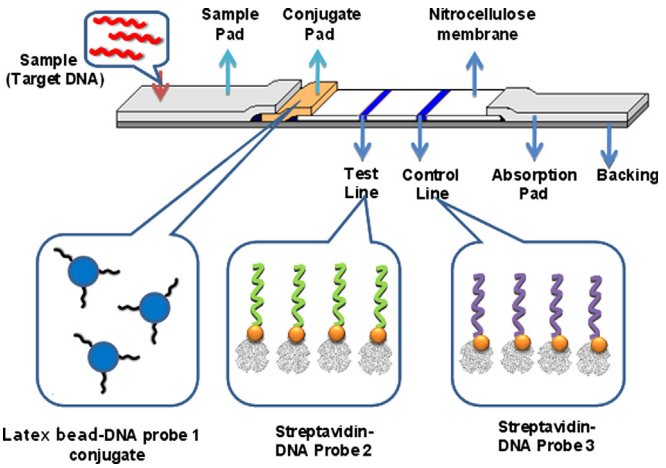

Fig. 1. Components of blue latex beads based lateral flow nucleic acid test strip. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)