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A lateral flow biosensor for detection of nucleic acids with high sensitivity and selectivity†

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A lateral flow biosensor based on isothermal strand-displacement polymerase reaction and gold nanoparticles has been developed for the visual detection of nucleic acids with a detection limit of 0.01 fM.

The detection of nucleic acids is of central importance for the diagnosis and treatment of genetic diseases, detection of infectious agents,[1] biowarfare agents,[2] and drug discovery.[3]

Various strategies and technologies have been developed for the detection of nucleic acids such as Southern blot, agarose gel electrophoresis, and polyacrylamide gel electrophoresis. However, such conventional methods are time-consuming and labor-intensive, which have been hampering their implementation for wider and more versatile applications. The advent of new methods and technologies including real-time polymerase chain reaction (RT-PCR),[4] DNA microarrays,[5] surface plasmon resonance (SPR)[6,7] and isothermal strand-displacement polymerase reaction (ISDPR) based fluorescence method[8] or chemical method[9] offer fast and sensitive tools to detect nucleic acids. However, complicated instrumentation and highly trained personal are needed, which prevents their use in many laboratories. So, continuing efforts have been made to seek ideal tools for fast, sensitive, low-cost, and easy-to-use detection of nucleic acids.

Recently, the development of point-of-care (POC) nucleic acid biosensors has attracted a great deal of research interest and the emerging lateral flow nucleic acid (LFNA) test offers a promising approach to realize POC nucleic acid detection. In the past two years, we have developed lateral flow nucleic acid biosensors (LFNABs) for the detection of protein,[10,11] cancer cells,[12] and copper.[13] In this communication, for the first time, we presented a LFNAB for detecting nuclei acids with ISDPR and Au-nanoparticles (AuNPs). The sensor has the following features: (1) high sensitivity with a detection limit of 0.01 fM nuclei acid, (2) high selectivity, and (3) no complex and expensive instrumentation is needed.

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Based on the ISDPR method reported by Guo Q. *et al.*,[8] we designed a new hairpin probe and sequence tagged-primer for detection of nucleic acids with AuNPs. As shown in Fig. 1, the biosensor was composed of three parts: a sample pad, a strip of nitrocellulose membrane, and an absorbent pad. A 5′-thiol-modified, 3′-biotin-modified 41-mer DNA hairpin probe (probe 1, Thiol-5′-TCTTGGACACAACTAACGCCATGGCTAGACTGTGTCCAAGA-3′-Biotin) containing the target DNA binding site (underlined) was coated with AuNPs. Another 23-mer DNA probe (probe 2, CTTTCTATCTTTCTATCTTGGAC) was used as a strand displacement primer with a sequence tag at the 5′ end (underlined). A third 28-mer target DNA probe (probe 3, GTCTAGCCATGGCGTTAGTTGTGTCTTT) was used for recognition and hybridization with probe 1, triggering an ISDPR to generate large numbers of sequence tagged duplex DNA complexes. The ISDPR solution consisted of probe 1, probe 2, probe 3, Klenow polymerase exo-, dNTPs and buffer solution. When

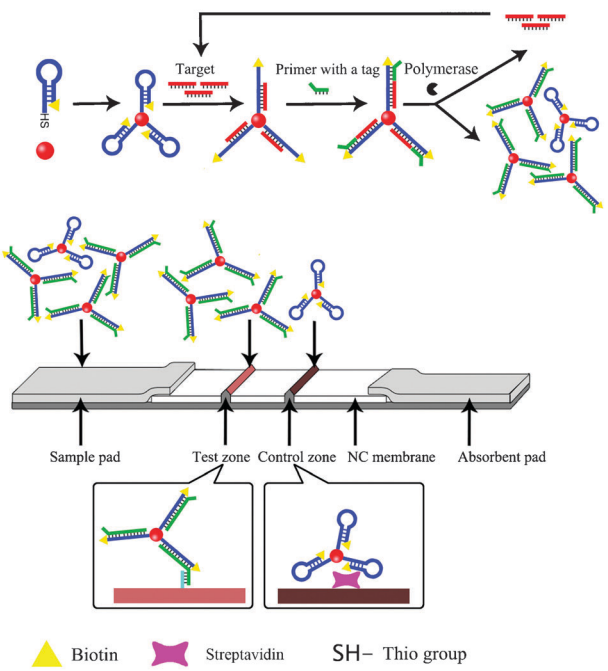

Fig. 1 Schematic illustration of the production of sequence tagged-duplex DNA based on ISDPR and the structure of a lateral flow biosensor.

probe 3 was added, it recognized and hybridized with probe 1, causing probe 1 to undergo a conformational change and leading to stem separation. Following this step, probe 2 annealed with the open stem and triggered an extension reaction in the presence of Klenow polymerase exo-. During primer extension, the target is displaced by the polymerase extension reaction, leading to the formation of more sequence tagged-duplex DNA complexes and triggering additional rounds of polymerase reaction. A large number of sequence tagged-duplex DNA complexes were produced throughout this repeating process. The biotin on the 3′ end of probe 1 was used to bind to streptavidin fixed on the control zone of the nitrocellulose membrane. The resulting sequence tagged-duplex DNA complexes were then captured by an anti-tag DNA sequence (probe 4 GAAAGATAGAAAGAT) fixed on the test zone of the nitrocellulose membrane. The red band at the control zone indicates that the biosensor works properly, and the red band at the test zone indicates the presence of target nucleic acid. Qualitative analysis is performed by observing the color change caused by AuNPs of the test zone, and quantitative detection can be realized by recording the optical density of the test line with a portable "strip reader".

We have examined the sensitivity of the LFNAB using synthetic target DNA at different concentrations. First, the synthetic target DNA was dissolved in ultra-pure water at seven concentrations as follows: 1 μM, 10 nM, 100 pM, 1 pM, 10 fM, 0.1 fM, 0.01 fM and 0 fM (negative control). For each test, 5 μL of synthetic target at different concentrations was added to 20 μL ISDPR reaction solution. After being incubated at 42 °C for 30 min, the ISDPR products were loaded to the LFNAB. Fig. 2A shows typical images along with the corresponding optical responses of the biosensors loaded with different amounts of synthetic target DNA. There is no red band observed on the test zone of the LFB in the absence of target DNA (negative control). The red bands on the test zone were observed with as low as 0.01 fM of target DNA, which can be considered the limit of detection (LOD) and this LOD is 1000 times better than previously published LFB methods,[14] and compared to other reported methods based on ISDPR, the sensitivity of the AuNP based LFNAB developed here is comparable to or higher than that of fluorescence and colorimetric approaches. Moreover, the usage of AuNPs, instead of fluorescent dyes or radio isotopes, enables visual detection of the results. Semi-quantitative analysis was performed by recording the intensities of the test line with a portable strip reader. Well defined peaks were observed and the peak areas increased along with the increase of target DNA concentration (Fig. 2B). The resulting calibration plot of the peak areas *vs.* target DNA concentration was linear between 1 fM to 1 pM (Fig. 2B).

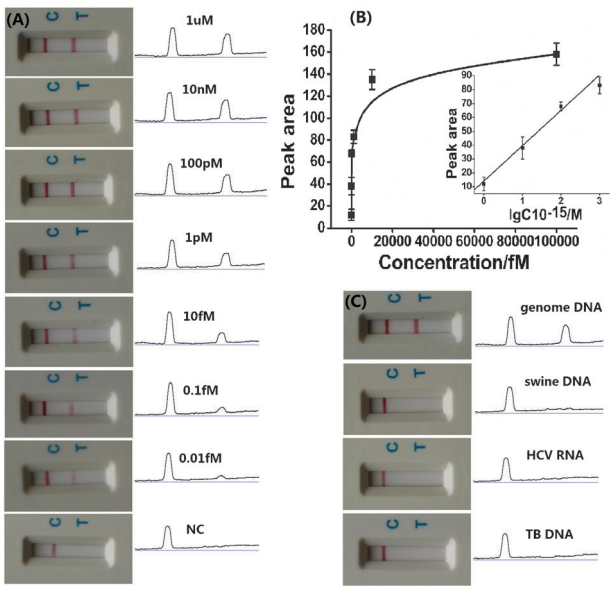

Fig. 2 (A) Typical photo images (left) and corresponding intensities (right) of a LFNAB in the presence of different concentrations of synthetic target DNA and negative control. (B) Calibration curve of a LFNAB with different concentrations of target DNA. Error bars represent standard deviation, $n = 3$. (C) Typical photo images (left) and corresponding intensities (right) of LFB for detection of human genome DNA; swine DNA, HCV RNA, and TB DNA were used as negative controls.

To test the performance of the LFNAB, human genomic DNA was tested, hepatitis C virus (HCV) RNA, tuberculosis (TB) DNA and swine DNA were used as negative controls. Forty human clinical blood samples were kindly supplied by Guangzhou blood center and human genome DNA was extracted using a commercial DNA extraction kit. HCV RNA and TB DNA samples were supplied by Guangzhou Chest Hospital and swine blood samples were supplied by Guangzhou Tianhe Meat Process Factory and swine DNA was extracted using a commercial DNA extraction kit. Another hairpin DNA probe (probe 5,Thiol-5′-TCTTGGACACAATTAGACAACAGAGG-AGACATGTGTCCAAGA-3′-Biotin) was used to prepare the AuNP–DNA conjugates for the detection of human genomic DNA. Before the test, the extracted human genomic DNA was denatured by treating with boiling water for about 5 min, and then promptly chilled in ice for another 15 min. The denatured human genomic DNA was mixed with 20 μL ISDPR reaction solution, incubated at 42 °C for 30 min and then applied to the LFNAB for detection along with 20 μL 4X SSC. Fig. 2C shows that a visible red line was observed when 1 μL human genome DNA was used, but no test line was observed in the absence of human genome DNA, or when swine genome DNA, HCV RNA, or TB genome DNA were used. All 40 clinical blood samples were tested and gave positive results. The results were confirmed by PCR assays. As shown in Fig. S2 (ESI†), except for the negative control, all 40 clinical samples had bands after gel electrophoresis, correlating with the results observed on the LFNAB. The sensitivity of the LFB for human genome DNA was examined using a serial dilution method. The concentration of the extracted human genome DNA was calculated with an optical density (OD) value measured using a UV spectrometer, and the DNA sample was diluted in ultra-pure water at six concentrations as follows: 1 μg mL^{-1}, 500 ng mL^{-1}, 100 ng mL^{-1}, 50 ng mL^{-1}, 25 ng mL^{-1}, 0 ng mL^{-1} (negative control). All 6 diluted samples except for the negative control were tested to be positive (Fig. S3, ESI†). The detection limit of human genome DNA was then estimated to be 25 ng mL^{-1}. The concentration of DNA extracted from blood samples is usually on the order of micrograms per millilitre, the sensitivity of 25 ng mL^{-1} is sufficient for nucleic acid detection in blood samples.

This test provides an extremely rapid method for detecting nucleic acids. It can be performed within 30 min, which is

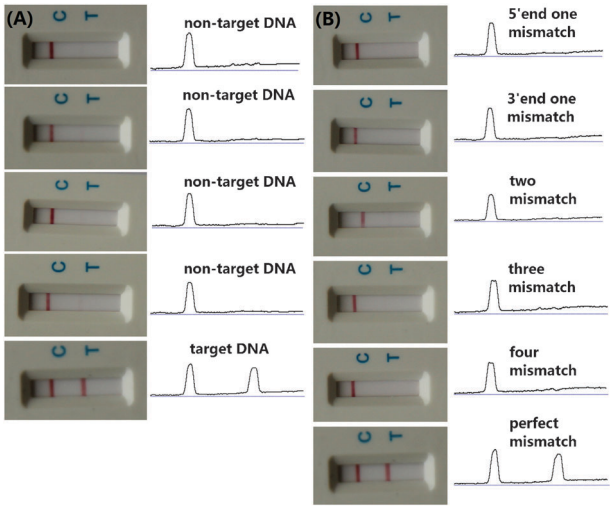

Fig. 3 (A) Photo images (left) and corresponding intensities (right) of the LFNAB for specificity analysis with target DNA and non-target DNA (DNA with random sequences). (B) Photo images (left) and corresponding intensities (right) of the LFNAB tested with target DNA and target DNA with one or more mismatched bases.

significantly less time than that required for PCR, realtime-PCR, and other Southern blot based methods. Although the biosensor is less sensitive than PCR and realtime-PCR, it is less time-consuming, requires no complicated machine, and is easy to use. Furthermore, the usage of AuNPs, instead of fluorescent dyes or radio isotopes, enables visual detection of the results.

For specificity assay, 5 μL of 1 μM synthetic target DNA or synthetic DNA with random sequences were tested. Fig. 3A shows that a visible red line was observed on the test zone of the biosensor when target DNA was tested, but no test line was observed when tested with three DNA samples of different random sequences. The specificity was further tested by using a number of synthetic target sequences with one, two, three, and four mutations. The original sequence and mutated sequences are as follows: 5′-GTCTACCCATGGCGTTAGTTGTGTCTTT-3′, 5′-GTCTAGCCATGGCGTTTGTTGTGTCTTT-3′, 5′-GTCTACCCTTCCCGTTAGTTGTGTCTTT-3′, 5′-GTCTACCCTTCCCCTTAGTTGTGTCTTT-3′, 5′-GTCTACCCTTCCCCTTTGTTGTGTCTTT-3′. The mutated bases are underlined. As shown in Fig. 3B, no test line was observed with target DNA having one or more mismatched bases. Only perfectly matched target DNA can recognize and hybridize with the AuNP–DNA and produce a clear red band on test zone, demonstrating high specificity using the LFB. These results demonstrate that the biosensor can differentiate one base mutation and, therefore, it can provide an alternative SNP detection method for genetic diseases, personalized medicine, cancer related mutations, and drug resistant mutations of infectious agents.

In conclusion, we have developed a rapid, ultrasensitive, and specific biosensor for detection of nucleic acids based on ISDPR and AuNPs. Compared to other reported methods based on ISDPR, the sensitivity of the AuNP based LFNAB developed here is comparable or higher than that of fluorescence and colorimetric approaches. Moreover, this biosensor is easy to use, no trained personnel or complex and expensive instruments are needed. By using this assay, target DNA as low as 0.01 fM could be detected within 30 min by naked eyes. The response of the optimized LFB is highly linear over a range of 1 fM–1 pM target DNA. This new nucleic acid detection method provides a rapid, low cost, and semi-quantitative tool for the detection of nucleic acid samples. Also, it can provide an alternative SNP detection method for genetic diseases, personalized medicine, cancer related mutations, and drug resistant mutations of infectious agents. It shows great promise for in-field and point-of-care diagnosis of genetic diseases as well as detection of infectious agents or warning against biowarfare agents.

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