Chapter 14

Lateral Flow Biosensors for the Detection of Nucleic Acid

Lingwen Zeng, Puchang Lie, Zhiyuan Fang, and Zhuo Xiao

Abstract

The detection of nucleic acid is of central importance for the diagnosis of genetic diseases, infectious agents, and biowarfare agents. Traditional strategies and technologies for nucleic acid detection are time-consuming and labor-intensive. Recently, isothermal strand-displacement reaction-based lateral flow biosensors have attracted a great deal of research interest because they are sensitive, simple, fast, and easy to use. Here, we describe a lateral flow biosensor based on isothermal strand-displacement polymerase reaction and gold nanoparticles for the visual detection of nucleic acid.

Key words Lateral flow biosensor, Nucleic acid, Gold nanoparticle

1 Introduction

The detection of nucleic acid is of central importance for the diagnosis of genetic diseases, infectious agents [1], and biowarfare agents [2].

Various strategies and technologies have been developed for the detection of nucleic acid 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) [3], DNA microarrays [4], surface plasmon resonance (SPR) [5, 6], and isothermal strand-displacement polymerase reaction (ISDPR)-based fluorescence method [7, 8] or chemical method [9] offers fast and sensitive tools to detect nucleic acid. However, complicated instrumentation and highly trained personal are needed, which prevent 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 acid.

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Lateral flow biosensor is a strip-type biosensor. It is commonly composed of three parts: sample pad, nitrocellulose membrane, and absorbent pad. Through the aggregation of indicator such as functionalized gold nanoparticle, the analytical result can be read from observing the color change of test zone on the strip.

Variety of lateral flow biosensors have been developed for the detection of different analytes, such as proteins [10, 11], cancer cells [12], and copper [13]. This analytical assay has four advantages: (1) It allows visual detection of analytes, requiring no expensive or complicated instruments; (2) It is easy to use. It does not involve multiple incubation and washing steps commonly performed in most other assays; (3) This assay can be performed in a short period of time; (4) Without use of expensive instruments, it is cost effective. Thus this assay is suitable for in field and point-of-care diagnosis, having great potential for the detection of genetic diseases, cancer-related mutations, and infectious agents.

Here, we describe a lateral flow biosensor based on ISDPR and gold nanoparticles for the visual detection of nucleic acid.

2 Materials

Ultrapure water (prepared by purifying deionized water to attain a resistivity of 18MΩ.cm at 25 °C) and analytical grade reagents are used to prepare all solutions. All reagents are prepared and stored at room temperature (unless indicated otherwise). All waste disposal regulations are diligently followed when disposing waste materials.

2.1 Colloidal Gold

1. 1 % $HAuCl_4$ solution. Weigh 1 g of $HAuCl_4$ to a beaker. Add water to a volume of 90 mL. Mix gently and adjust volume to 100 mL. The solution can be stored at 4 °C for 3 months.
2. 1 % Trisodium citrate solution. Weigh 1 g of Trisodium citrate to a beaker. Add water to a volume of 90 mL. Mix gently and adjust volume to 100 mL. Prepare this solution every time before use (*see* **Note 1**).
3. Bovine serum albumin (BSA).

2.2 AuNP–DNA Conjugates Preparation

1. Streptavidin (SA): Weigh 1 mg of SA in a 1.5-mL Eppendorf tube. Add ultrapure water to a volume of 1 mL. Store at 4 °C.
2. Biotinylated thiol-modified hairpin probe (probe 1, Biotin-5′-TCTTGGACACAACTAACGCCATGGCTAGACTGTGTCCAAGA-3′-thiol).
3. Target DNA. GTCTAGCCATGGCGTTAGTTGTGTCTTT.
4. Non-target DNA with random sequences (random sequence 1, GGTAGAAGGGAGGGCTAGTTGTGTCTTT; random sequence 2, GTCAGTTCCCCTTGGTTCTGTGTCTTT;

and random sequence 3, AGTGTGGATTCGCACTCCTT GTGTCTTT).

5. Rinsing buffer: 20 mM Na_3PO_4, 5 % BSA, 0.25 % Tween-20, 10 % sucrose, 0.1 % NaN_3.
6. Suspension solution: 100 g/L BSA, 0.5 g/L NaN_3, 38 mM $Na_2B_4O_7$. Weigh 10 g of BSA, 0.05 g of NaN_3, and 0.762 g of $Na_2B_4O_7$ to the beaker. Add water to a volume of 90 mL. Mix gently and adjust volume to 100 mL. Store at 4 °C after filtration by 0.22 μm Millipore membrane.
7. AuNPs solution.
8. Aging solution: 100 mM phosphate buffer, pH 7.0, 1 % sodium dodecyl sulfate, 1.5 M NaCl.

2.3 Lateral Flow Biosensor Construction

1. Nitrocellulose membrane, 25 mm × 30 cm, capillary rate: 140 s, thickness: 145 μm.
2. Sample pad buffer: 1 % Triton, 1 % BSA, 2 % glucose, 50 mM boric acid, pH 8.0.
3. Fiberglass, 16 mm in width. Fiberglass was treated by soaking in a sample pad buffer, dried, and stored in low-humidity at room temperature.
4. Absorbent paper, 17 mm in width.
5. Lateral flow dispenser (Shanghai Kinbio, Shanghai, China).
6. Paper cutter (Programmed high speed cutter, Shanghai Kinbio, Shanghai, China).

2.4 Isothermal Strand-Displacement Reaction (ISDR)

1. Polymerase Klenow fragment exo-, 5 U/μL.
2. Deoxynucleoside triphosphates (dNTPs), 2.5 mM each.
3. Reaction solution: 50 mM Tris–HCl, pH 8.0, 50 nM tag-primer (probe 2), 3 U polymerase Klenow fragment exo-, 50 μM dNTPs, 6 % DMSO, 0.1 % BSA, 1 mM DTT, 5 mM $MgCl_2$, and 1× enzyme reaction buffer.

2.5 Detection of Nucleic Acid by Lateral Flow Biosensor

1. 4× SSC: 0.6 M NaCl, 50 mM trisodium citrate, pH 7.0. Adjust to pH 7.0 with HCl.
2. Hand-held strip reader (Shanghai Kinbio, Shanghai, China).

3 Methods

Carry out all procedures at room temperature unless otherwise specified.

3.1 Preparation of AuNPs and AuNP–DNA Conjugates

1. Mix 4 mL of 1 % $HAuCl_4$ solution to 400 mL of ultrapure water in a 500 mL round bottom flask (*see* **Note 2**). Heat and stir to boil for 5 min (*see* **Note 3**).

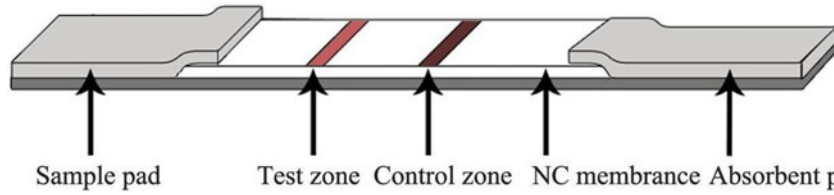

Fig. 1 Construction of lateral flow biosensor. The biosensor composed of three parts: sample pad, nitrocellulose membrane, and absorbent pad, two lines are dispensed onto the nitrocellulose membrane to form a test zone and a control zone

2. Add 6.8 mL of 1 % trisodium citrate solution rapidly to that boiling $HAuCl_4$ solution (0.01 %). After the solution turns red, boil it for 10 additional min, then cool to room temperature with gentle stirring.
3. Store the resulting AuNPs solution at 4 °C (*see* **Note 4**) and use it for preparation of AuNP–DNA conjugates.
4. To prepare AuNP–DNA conjugate, add 100 μL of 100 μM 5′-biotinated 3′-thiolated DNA hairpin probe to 500 μL of fourfold concentrated AuNPs solution, and shake the mixture gently at 4 °C for 24 h.
5. Subject 500 μL of the DNA-coated AuNPs to "aging" by adding 55 μL of aging solution. Keep the solution at 4 °C for 12 h.
6. Centrifuge the aged DNA-coated AuNPs at 13400 × g for 20 min and rinse them three times with rinsing buffer to remove any unbound DNA. Resuspend the red pellet in 500 μL of rinsing buffer and store the suspension in a refrigerator at 4 °C until use.

3.2 Construction of Lateral Flow Biosensor

1. Dispense 30 μL of 100 μM 15-mer DNA probe (probe 4 GAAAGATAGAAAGAT) and 30 μL of 1 mg/mL streptavidin (SA) onto the nitrocellulose membrane simultaneously to form a test zone and a control zone, respectively, with a lateral flow dispenser (Fig. 1).
2. Dry the membrane at room temperature for 12 h.
3. Attach a strip of sample pad, nitrocellulose membrane, and absorbent pad along the long axis of an adhesive plate with an overlap of 2–3 mm, according to the layout shown in Fig. 1. Then cut the plate into 0.4-cm wide strips using a paper cutter (*see* **Note 5**).

3.3 Detection of Nucleic Acid by Lateral Flow Biosensor

1. To detect nucleic acid using the lateral flow biosensor, mix 2 μL of hairpin probe (probe 1)-conjugated AuNPs with different amounts of synthetic target DNA, non-target DNA with random sequences, extracted human genome DNA, hepatitis C

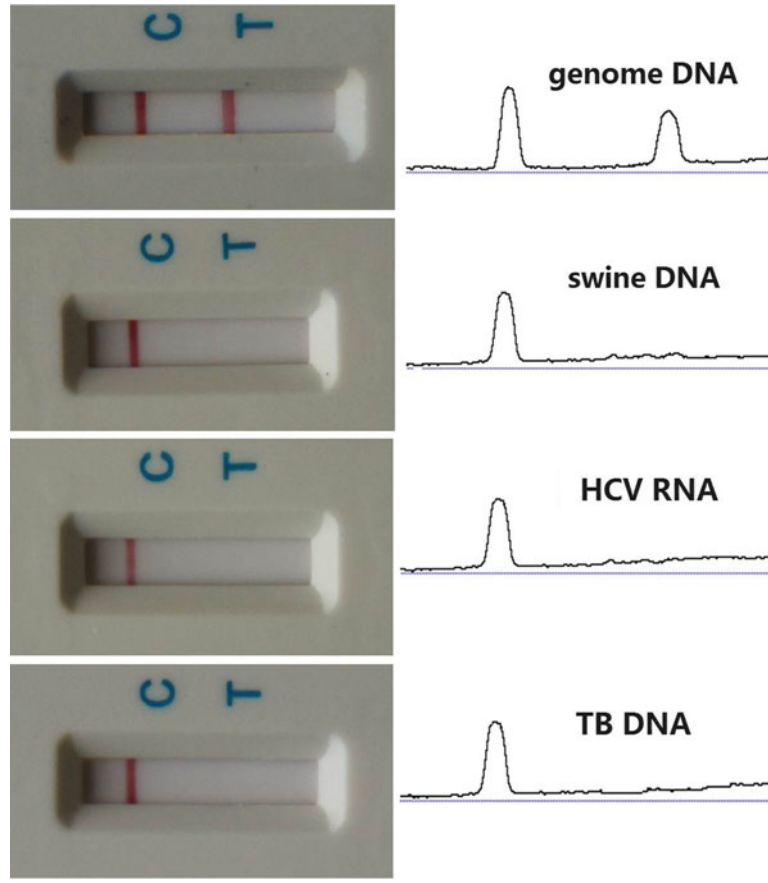

Fig. 2 Typical photo images (*left*) and corresponding intensities (*right*) of lateral flow biosensor for detection of human genome DNA, swine DNA, HCV RNA, and TB DNA were used as negative control

virus (HCV) RNA, swine DNA, or tuberculosis (TB) DNA in 20 μL reaction solution (*see* **Note 6**) (Figs. 2 and 3).

2. Incubate the mixture at 42 °C for 30 min (*see* **Note 7**).
3. Load 20 μL of ISDPR product onto the sample pad along with 20 μL of 4× SSC. Scan the biosensor using a hand-held strip reader 10 min later.

4 Notes

1. We find that it is best to prepare this fresh each time.
2. The round bottom flask must be very clean to guarantee the pureness, avoid any floating and sedimentation of the sythesized colloidal gold solution.

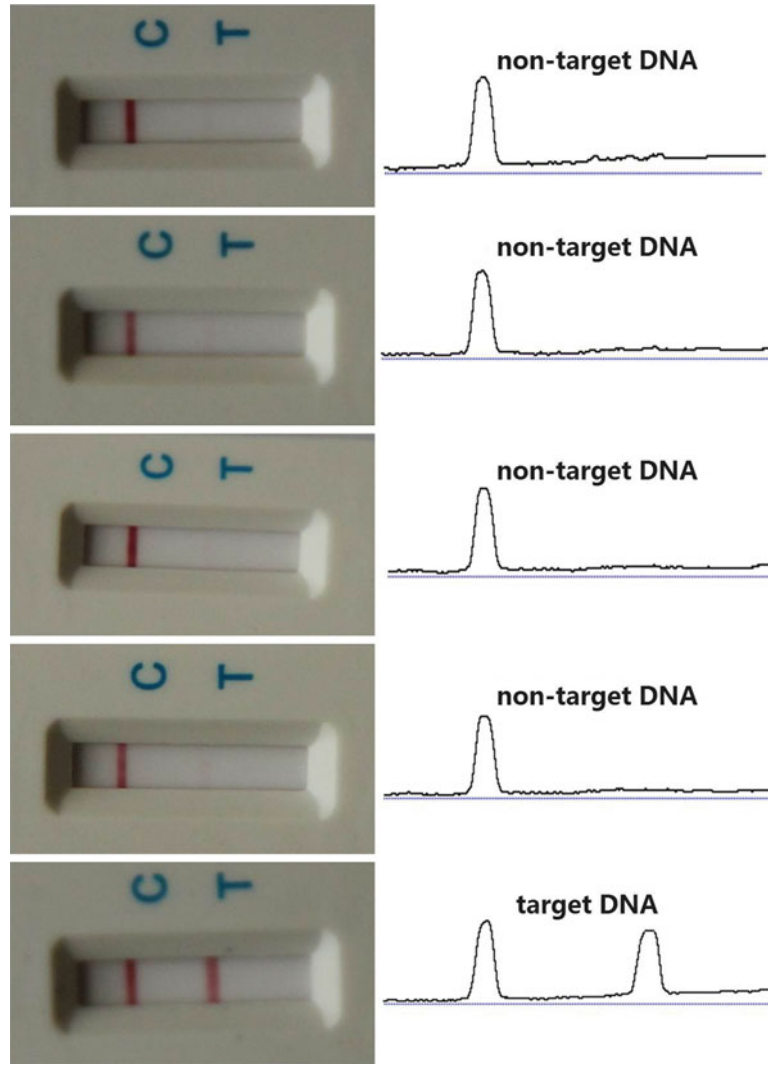

Fig. 3 Photo images (*left*) and corresponding intensities (*right*) of the lateral flow biosensor for specificity analysis with target DNA and non-target DNA (DNA with random sequences)

3. In order to make the size of sythesized colloidal gold uniform, the agitating speed is set to be 600 rpm. So the intensity field of the magnetic stirrer is.
4. We find that sythesized colloidal gold can be stored at 4 °C for at least 1 year.
5. The environment for construction of lateral flow biosensor must be strictly controlled, temperature 25–27 °C, humidity is lower than 30 %.

6. To reduce the operation error for multiple sample detection, we: prepare a mix of reaction buffer first and then subdivide into each sample.
7. We find that 2 μL of AuNP–DNA conjugate is the optimum concentration and 30 min is the optimum reaction time.

Acknowledgments

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