Sensors and Actuators B 264 (2018) 320–326

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Lateral flow test for visual detection of multiple MicroRNAs

Wanli Zheng[a], Li Yao[a], Jun Teng[a], Chao Yan[a], Panzhu Qin[a], Guodong Liu[b,*], Wei Chen[a,*,1]

[a] School of Food Science & Engineering, Hefei University of Technology, 230009, Anhui, PR China
[b] Department of Chemistry & Biochemistry, North Dakota State University, Fargo, ND 58105, USA

ARTICLE INFO

Article history:
Received 10 August 2017
Received in revised form 18 February 2018
Accepted 22 February 2018

Keyword:
Lateral flow biosensor
microRNA
Simultaneous detection
Multiplex detection
Serum sample

ABSTRACT

The authors describe a rapid and low-cost approach for multiplex microRNA(miRNA) assay on lateral flow nucleic acid biosensor (LFNAB). The principle of assay is based on sandwich-type nucleic acid hybridization reactions to produce gold nanoparticle (GNP)-attached complexes (ssDNA-microRNA-ssDNA/GNPs), which are captured and visualized on the test zone of LFNAB. By designing three different test zones on LFNAB, simultaneous detection of microRNA-21, microRNA-155 and microRNA-210 was achieved with an adding-measuring model by using GNP as visual tag. The method was challenged by testing the microRNAs in spiked serum samples with satisfied results. In our perception, the test is a particularly valuable tool for clinical application and biomedical diagnosis, particularly in limited resource settings.

1. Introduction

MicroRNAs (miRNA) are class of short (usually ~18–25 nucleotides), single-stranded and endogenous noncoding RNA molecules, which are derived from long primary transcripts by the catalysis of Dicer enzyme [1,2]. It is of crucial importance in regulating post-transcriptional gene expression in a broad range of animals, plant, and viruses [3–5]. More importantly, there is accumulating evidence that the expression levels of microRNAs are associated with a variety of pathological conditions including cancers, such as lung cancer, hepatocellular carcinoma, breast cancer and gastric carcinoma [2]. MicroRNAs have become a vital class of potential biomarker candidates for clinical and early diagnosis [6,7]. MicroRNA detection is challenging due to its low abundances, small size, and high degree of sequence similarity presenting obstacles in the use of conventional analytical approaches [8,9]. Meanwhile, some studies have also demonstrated that the variation of microRNA in cancer includes both downregulated and overexpressed microRNAs with putative tumor suppressive and oncogenic functions. For qualitative analysis, the amount of microRNA in suspicious samples is compared to that of normal cells or tissues. For quantitative measurement, the detection limit is the bottleneck for detection of microRNA downregulation [9]. Taking these issues into account, considerable effort has been devoted to the development of methods with both high sensitivity and specificity for microRNA detection [10,11]. Sequencing-based methods have been used for microRNA detection, such as Northern blotting [12], reverse transcriptase-polymerase chain reaction (RT-PCR) [13], rolling circle amplification (RCA) [14] and microarrays [6,15,16]. Northern blotting protocol is highly quantitative and has been considered as the "gold standard" method in microRNA profiling, but it requires large amounts of sample (~10–30 μg) and is highly cumbersome and time-consuming, often taking days for completion [10,17,18]. RT-PCR has high dynamic range and is sensitive, but has low throughput and is less specific compared to standard PCR [19]. Microarray-based methods were limited in terms of sensitivity, selectivity, and specificity although they have the property of high-throughput [20]. In general, although these methods performed well on microRNA analysis with high sensitivity, high selectivity, or high throughput, they suffered from time-consumption, expensive equipment and complex operations [21,22] which dramatically limited their further applications on point-of-care diagnostic testing at low-resource settings. Therefore, facile, rapid, straight-forward and economical experimental approaches are urgently needed for microRNA analysis [23]. Recently, with the extraordinary achievements of nanotechnology, nanomaterial-based biosensors have sprung out and been greatly developed due to the unique electronic, optical and catalytic prop-

* Corresponding authors.
E-mail addresses: guodong.liu@ndsu.edu (G. Liu), chenweishnu@163.com (W. Chen).
[1] Researcher ID: F-4557-2010, http://www.researcherid.com/rid/F-4557-2010.

https://doi.org/10.1016/j.snb.2018.02.159
0925-4005/

Table 1
Sequences of all the oligonucleotide probes used in this research.

Name of probes	Sequences Information
miRNA-21 (miR-21)	5′-UAG CUU AUC AGA CUG AUG UUGA-3′
miRNA-155 (miR-155)	5′-UUA AUG CUA AUC GUG AUA GGGU-3′
miRNA-210 (miR-210)	5′-CUG UGC GUG UGA CAG CGG CUGA-3′
C line probe (probe 1)	5′-Biotin-ACA CGG TGT CTA GGG GG-3′
T-21 probe (probe 2)	5′-CTG ATA AG C TAC CCCC-Biotin-3′
T-155 probe (probe 3)	5′-GAT TAG CAT TAA CCC CC-Biotin-3′
T-210 probe (probe 4)	5′-ACA CGC ACA GCC CCC-Biotin-3′
Detection probe to miRNA-21 (probe 5)	5′-thiol-CCC CCT AGA CAC CGT GTT CAA CATC AGT-3′
Detection probe to miRNA-155 (probe 6)	5′-thiol −CCC CCT AGA CAC CGT GTA CCC CTA TCAC-3′
Detection probe to miRNA-210 (probe 7)	5′-thiol- CCC CCT AGA CAC CGT GTT CAG CCG CTGT-3′

erties of the nanomaterials, which offer the signal amplification to achieve high sensitivity [3,23].

Lateral flow biosensors have been extensively used as an effective tool for detecting bacteria [24], parasite antigens [25,26], and hormones [27,28] owing to their obvious advantages of user-friendly format, short assay time, and low testing cost [29,30]. Gold nanoparticles (GNPs) are the most widely used tag on lateral flow assays for colorimetric analysis [31]. Furthermore, lateral flow nucleic acid biosensors (LFNABs) which inspired by the traditional immunochromatography test were reported for rapid visual detection of DNA or RNA segments [32,23]. The principle of LFNAB is based on sandwich-type DNA hybridization reactions, which were implemented on the traditional lateral flow device. Recently, Liu et al. applied LFNABs to detect microRNA 215 (miRNA-215) with a detection limit of 60 pM [23]. Disease diagnostics usually requires accurate measurement of a panel of biomarkers, while only single-microRNA detection is usually inadequate for accurate diagnosis of cancer [33–36]. To the best of our knowledge, simultaneous detection of multiple microRNAs on lateral flow device with satisfied sensitivity has not been reported.

Herein, we present a lateral flow nucleic acid biosensor for multiplex microRNA assay in aqueous solution and spiked serum samples. MicroRNA-21 (miR-21), microRNA-155 (miR-155) and microRNA-210 (miR-210), which are related with cancers and considered as potential biomarkers of diseases, were chosen as model targets to evaluate the multiplex feasibility of LFNAB. The promising properties of the approach are reported in the following sections.

2. Materials and methods

2.1. Reagents

Streptavidin (SAV), bovine serum albumin (BSA), trisodium citrate, tween-20, deoxyadenosine triphosphate (dATP) and tris (2-carboxyethl)phosphine (TCEP) were purchased from Sangon Biotech (Shanghai) Co., Ltd. and used directly without further purification. $HAuCl_4$ was purchased from J&K Chemical Scientific Ltd. (Shanghai, China). Cellulose fiber sample pads, conjugate pads, and laminated cards were purchased from Millipore (USA). Nitrocellulose membranes including CN 95 and Vivid 170 were purchased from Sartorisu Stedim Biotech GmbH (Goettingen, German) and PALL Corporation (New York, USA), respectively. All the solutions used in this research were prepared with double distilled water (>18 MΩ). Target microRNAs used in this study were synthesized by Takara Biotechnoly (Dalian) Co., Ltd. All other deoxy-oligonucleotide probes used in this study were ordered from Sangon Biotech (Shanghai) Co., Ltd. The sequences of DNA probes and microRNAs are presented in Table 1.

2.2. Preparation of concentrated (10-fold) gold nanoparticles (GNPs)

Gold nanoparticles (GNPs) with average diameter of 25 nm were prepared by the classic trisodium citrate reduction method with slight modifications [30]. Briefly, 50 mL of 0.01% $HAuCl_4$ were brought into a 400-mL conical flask and heated to boiling with vigorous stirring, and then 950 μL of 1% trisodium citrate was added rapidly. The color of the solution changed from dark red to purple, and finally to wine red. The solution was kept heating and stirring for another 10 min and then cooled to room temperature.

One milliliter above prepared GNP solution was centrifuged at a relative centrifugal force (RCF) of 9200*g* for 7 min, and 900 μL of supernatant was decanted. The remaining pellet in solution was eddied to obtain the 10-fold GNP solution. The 10-fold GNPs were stored at 4 °C for subsequent use.

2.3. Preparation of GNP-X ssDNA conjugates (X means miRNA-21, miRNA-155 and miRNA-210)

Firstly, a mixture solution containing three thiol-modified ssDNA probes (Table 1) was prepared. The final concentration of each ssDNA probe in the mixture solution was 2 μM. Then 100 μL of the mixture solution was activated for 1 h in dark by adding 2 μL of 1 mM TCEP at room temperature (RT), which was prepared in 0.5 M acetate buffer. Following, 100 μL of 10-fold concentrated GNPs were added and the solution was kept stirring for 1 h. Afterward, 10 μL of 10 mM dATP was added to inhibit the nonspecific absorption on the GNP surface, and the solution was kept stirring for another 45 min. The GNP-X ssDNA conjugates were aged by adding 20 μL of 0.1 M NaCl and stayed at RT for 1 h. The solution could stand at 4 °C for additional 6 h. The conjugates were purified by centrifugation at 8600*g* for 7 min and the pellet was re-suspended in 100 μL of re-suspension solution containing 1 mM Tris-HCl (pH 8.0), 5% BSA, 0.25% Tween-20, and 10% sucrose.

2.4. Preparation of lateral flow nucleic acid biosensor (LFNAB)

The diagram of LFNAB is shown in Scheme 1. It is composed of four parts including an absorbent pad, a nitrocellulose membrane, a glass fiber membrane (conjugated pad) and a sample pad. Before assembling, the sample pad and the conjugated pad were treated with different buffer solutions. The buffer solution containing 50 mM Tris-HCl (pH 8.0), 0.15 mM NaCl and 0.25% Tritonx-100 was used to treat the sample pad. While the buffer solution to treat the conjugated pad was 10 mM PBS (pH 7.4) containing 5% sucrose, 1% Trehalose, 0.25% PEG, and 0.3% Tween 20. After the pretreatments, the sample pad and conjugated pad were dried at 37 °C for 6 h and stored in desiccator at RT. The biotin-modified ssDNA probe (T-21 probe, T-155 probe, T-210 probe and control line probe) was mixed with streptavidin in PBS (pH 7.4) solution. After purifi-