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Visual detection of microRNA with lateral flow nucleic acid biosensor

Xuefei Gao [a,b], Hui Xu [b], Meenu Baloda [b], Anant S. Gurung [b], Li-Ping Xu [a], Tao Wang [b], Xueji Zhang [a,*], Guodong Liu [b,c,**]

[a] Research Center for Bioengineering and Sensing Technology, University of Science & Technology Beijing, Beijing 100083, PR China
[b] Department of Chemistry and Biochemistry, North Dakota State University, Fargo, ND 58105, USA
[c] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 35002, PR China

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ABSTRACT

We report a DNA-gold nanoparticle (DNA-GNP) based lateral flow nucleic acid biosensor for visual detection of microRNA (miRNA)-215 in aqueous solutions and biological samples with low-cost and short analysis time. Sandwich-type hybridization reactions among GNP-labeled DNA probe, miRNA-215 and biotin-modified DNA probes were performed on the lateral flow device. The accumulation of GNPs on the test zone of the biosensor enables the visual detection of miRNA-215. After systematic optimization, the biosensor was able to detect a minimum concentration of 60 pM miRNA-215. The biosensor was applied to detect miRNA-215 from A549 cell lysate directly without complex sample treatment, and the detection limit of 0.148 million cells was obtained. This study provides a simple, rapid, specific and low-cost approach for miRNA detection in aqueous solutions and biological samples, showing great promise for clinical application and biomedical diagnosis in some malignant diseases.

1. Introduction

MicroRNAs (miRNAs) are a class of small (~18–25 nucleotides) noncoding RNAs that serve as post-transcriptional regulators of gene expression in a broad range of plants, viruses and mammals (Heidenreich et al., 2010; Wightman et al., 1993). Mature miRNAs are considered to regulate gene expression by catalyzing the cleavage of messenger RNA and incorporation into RNA-induced silencing complex (RISC) where they interact with complementary sites on messenger RNA as well as downstream regulation of the expression of target messenger RNA molecules (Engels and Hutvagner, 2006; Meister and Tuschl, 2004). Especially, some miRNA expression patterns are associated with some malignant diseases such as liver-related diseases, cardiovascular diseases and cancers. Therefore, miRNAs are considered as potential biomarkers specific to corresponding tissues or diseases (Bartel, 2004). The detection of microRNA is a challenge due to its instability, short sequence, trace-amounts, and the complex interferences from biological samples miRNA (Engels and Hutvagner, 2006; Meister and Tuschl, 2004). Conventional methods, such as real-time polymerase chain reaction (RT-PCR), northern blotting and microarray analysis, offer high accuracy and sensitivity for miRNAs measurement (Heidenreich et al., 2010; Peng and Gao, 2011). However, most of these methods require time-consuming sample pretreatment, tedious and complicated procedures and harsh experimental conditions as well as high experimental cost (Jia et al., 2010; Li et al., 2011; Linsen et al., 2009; Válóczi et al., 2004; Wark et al., 2008). These issues dramatically restrict their further practical applications. As a result, it is highly desirable to develop a facile, rapid, and economical approach for accurate detection of miRNA.

Nowadays, with the achievements of nanotechnology, nanoparticles-based biosensors have aroused great interests, because nanoparticles could favor the signal amplification to achieve high sensitivity and selectivity for target analysis (Dong et al., 2013; Su et al., 2013; Zhang et al., 2013). A kind of lateral flow strip biosensor that combines nanoparticles with conventional immunoassay has attracted significant attention in biological analysis and clinical diagnosis in recent years (Singer and Plotz, 1956; Zhang et al., 2006). The lateral flow strip biosensors are considered as one of the most promising technologies owing to their simplicity, rapid analysis, low costs, high sensitivity and specificity (Zhang et al., 2006). In addition, they show less interference and long-term stability over a wide range of climates (Cho et al., 2005). To date, several lateral flow strip biosensors have been developed to detect many objects such as DNA, mRNA, proteins (Mao et al., 2009; Xu et al., 2008), biological agents (Fisher et al., 2009; Liu et al., 2009; Nakasone et al., 2007; O'Keeffe et al., 2003;

* Corresponding author. Tel.: +86 10 8237 6993.
** Corresponding author at: Department of Chemistry and Biochemistry, North Dakota State University, Fargo, ND 58105, USA. Tel.: +1 701 231 8697; fax: +1 701 231 8831.
E-mail addresses: zhangxueji@ustb.edu.cn (X. Zhang), guodong.liu@ndsu.edu (G. Liu).

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Xia et al., 2009) and chemical contaminants (He et al., 2011; Li et al., 2007). Nevertheless, there are very few reports on miRNA analysis using the lateral flow strip biosensors.

Herein, we report a lateral flow nucleic acid biosensor (LFNAB) for specific qualitative (visual)/quantitative analysis of miRNA in aqueous solutions and biological samples. MiRNA-215 was chosen as the target analyte, which plays crucial regulatory roles in A549 cell cycle progression through coordinately regulating the expression of key cell cycle transcripts (Georges et al., 2008). Experimental conditions were optimized on LFNABs by testing miRNA-215 in aqueous solution. Under the optimal conditions, target miRNA-215 was successfully detected by LFNABs in A549 cell lysate without complex sample treatment. The promising properties of the approach are reported in the following sections.

2. Experimental

2.1. Production of cell lysates

CCRF-CEM (CCL-119, Peripheral Blood, Human/Homo sapiens) and A549 (CCL-185, Lung Carcinoma, Human/Homo sapiens) were purchased from the American Type Culture Collection (ATCC) and cultivated followed the cell culture methods of ATCC.

Cell layer was firstly washed by phosphate buffered saline (PBS, pH 7.0), and then rinsed with 3 mL of Trypsin-EDTA solution (0.25% (w/v) Trypsin-0.53 mM EDTA) at 37 °C until cell layer was dispersed (usually within 5–15 min). After the incubation, the dispersed cells were centrifuged at a rate of 1800 rpm for 5 min at 10 °C, and then washed with 10 mL of PBS twice. After discarding the supernatant, the resulted cells were re-suspended in cell lysis buffer, and the solution was harvested by QIA shredder (Mini spin column). The resulting cell lysate was obtained through QIA shredder and collected in 1.5 mL EP tube. The cell lysate should be stored at −80 °C for further use.

2.2. Apparatus and reagents

The Airjet AJQ 3000 dispenser, Biojet BJQ 3000 dispenser, Clamshell Laminator, and the Guillotine cutting module CM 4000 were purchased from Biodot LTD (Irvine, CA). The DT1030 portable strip reader was purchased from Shanghai Goldbio Tech. Co., LTD (Shanghai, China). Labconco class II biosafety cabinet (LFBSC class II type A2) was provided by Labconco (USA). Cellometer Auto T4 plus cell counter was provided by Nexcelom Bioscience (USA).

Streptavidin was purchased from Streptomyces avidinii. $HAuCl_4$, sucrose, hydroxylamine, $Na_3PO_4 \cdot 12H_2O$, Tween 20, Triton X-100, trisodium citrate, deoxyadenosine triphosphate (dATP), bovine serum albumin (BSA), sodium chloride-sodium citrate (SSC) buffer (pH=7.0), PBS (pH=7.4) trypsin, EDTA and trypan blue solution (0.4%) were purchased from Sigma-Aldrich and used without further purification. Glass fibers (GFCP000800), cellulose fiber sample pads (CFSP001700), laminated cards (HF000MC100), and nitrocellulose membranes (HFB18004 and HFB 24004) were purchased from Millipore (Billerica, MA). Target miRNAs and oligonucleotide probes used in this study were obtained from Integrated DNA Technologies, Inc. (Coralville, IA) and have the following sequences:

Target miRNA-215 (*has-miR-215)*: 5'–AUG ACC UAU GAA UUA ACA GAC-3' (Georges et al., 2008; Pichiorri et al., 2010)

Target miRNA-224 *(has-miR-224)*:5'–AAA AUG GUG CCC UAG UGA CUA CA-3' (Feitelson and Lee, 2007; Wang et al., 2008)

Detection probe (probe 1): 5'-/ThioMC6-D/GTC TGT CAA-3'.
Capture probe (probe 2): 5'–ATA GGT CAT/Biotin/-3'.
Control probe (probe 3): 5'-/Biotin/TGG ACA GAC-3'.

2.3. Preparation of DNA/GNPs conjugate

The DNA/GNPs conjugate was prepared according to the reported methods with slight modifications (He et al., 2011; Zhao et al., 2007). dATP was added into 1 mL of concentrated GNPs solution (final concentration of dATP is 7.05 μM, GNPs with the average diameter of 15 ± 3.5 nm were synthesized following the procedures reported previously (Xu et al., 2008)). The mixture was incubated at room temperature for 20 min. 15 μL of 1% of SDS was slowly added into the mixture, and incubated on shaker for 10 min. 50 μL of 2 M of NaCl was dropped into the mixture at a rate of 2 μL/2 min. Then 0.25 OD of thiolated DNA (probe 1) was added and the mixture was incubated for 3 hours in water bath at 60 °C. The use of dATP was to protect GNPs from salt-induced aggregation by the mononucleotide (A) adsorption in the preparation of thiol-DNA/GNPs conjugate (Zhao et al., 2007). After the incubation, the mixture was centrifuged at 12,000 rpm for 15 min, and the supernatant was discarded, then washed with 1 mL of PBS for 3 times, the as-obtained ruby sediments were re-suspended in 1 mL of Eluent Buffer (20 nM of $Na_3PO_4 \cdot 12H_2O$ containing 5% BSA, 0.25% Tween 20 and 10% sucrose).

2.4. Preparation of streptavidin-biotinylated DNA conjugate

Two hundred microlitre of 2.5 mg/ml of streptavidin was mixed with 50 nmol biotinylated DNA probe (probe 2/probe 3). The mixture was incubated on shaker for 1 h. After adding 500 μL PBS into the mixture, the solution was centrifuged in dialysis tube for 20 min at 6000 rpm under 4 °C. The above step was repeated for 3 times. The remaining solution in filter was diluted to 600 μL with PBS.

2.5. Preparation of LFNAB

The LFNAB consisted of four components: sample application pad, conjugate pad, nitrocellulose membrane, and absorbent pad. All components were laminated into a sheet of plastic orderly using the Clamshell Laminator (Biodot, Irvin, CA). The sample application pad (17 mm × 30 cm) was made from glass fiber (CFSP001700, Millipore) and saturated with a Tris–HCl buffer (pH 8.0) containing 0.23% of Triton X-100, 0.05 M of Tris–HCl and 0.15 M of NaCl. Then, the pad was dried at 37 °C for 2 h and stored in desiccators at room temperature (RT) (Liu et al., 2009; Mao et al., 2009). The test zone and control zone on the nitrocellulose (NC) membrane were prepared by dispensing streptavidin-biotinylated capture probe and streptavidin-biotinylated control probe solutions, respectively. The distance between the test and control zones was 3 mm. The membrane was then dried at 37 °C for 1 h and stored at 4 °C in a dry state. Finally, the sample pad, conjugate pad, nitrocellulose membrane, and absorption pad were assembled on a plastic adhesive backing (60 mm × 30 cm) using the clamshell laminator (He et al., 2011). Each part overlapped 2 mm to ensure that the solution could migrate through the strip during the assay. Strips with a 3-mm width were cut using the Guillotin CM 4000 cutting module. The DNA/GNPs conjugate was dropped on the conjugate pad.

2.6. Assay procedure

In a typical miRNA test on LFNAB, 100 μL of running buffer (25% SSC buffer containing 4% BSA) with the desired amount of target miRNA-215 was applied to the sample pad. 5 μL of DNA/GNPs conjugate was loaded on the conjugate pad. During the assay process, the solution migrated up by capillary force. The test zone and control zone were evaluated visually within 20 min. For quantitative measurements, the optical intensity of the red band