Chapter 20

Label-Free Direct Detection of MiRNAs with Poly-Silicon Nanowire Biosensors

Jing He, Jianjun Zhu, Bin Jiang*, and Yulan Zhao*

Abstract

The diagnostic and prognostic value of microRNAs (miRNAs) in diseases becomes promising. Owing to fast response and high sensitivity, silicon nanowire (SiNW) biosensor has been considered a potential tool for miRNAs detection. Here, we describe a booming method to detect miRNAs with poly-silicon nanowire biosensors. Standard and real miRNA samples are applied in this study. The results show a limitation of 1 fM in the detection of standard miRNA sample with our poly-nanowire devices. Meanwhile, one-base mismatched sequence could be distinguished. Furthermore, these poly-SiNW arrays can detect snRNA U6 in total RNA samples extracted from HepG2 cells with a detection limitation of 0.2 μg/mL.

Key words miRNA, SiNW

1 Introduction

MicroRNAs (miRNAs) are a class of highly conserved noncoding RNAs within 18–25 nucleotides. MiRNAs play a vital role in post-transcriptional level of gene expression through binding to 3′ UTR or coding region of mRNA [1, 2]. They are involved in various physiological and pathological processes such as cell differentiation, proliferation, and apoptosis [3, 4]. Dysregulation of miRNAs is related to cancers [5], atherosclerosis [6], and other diseases. The level of miRNAs in body fluid including serum has been revealed to alter in diseases, indicating their diagnostic and prognostic value in clinic [7, 8]. However, due to their characteristics like small size, low level, and sequence similarity among members, the clinical application of miRNAs is limited so far.

Current methods for miRNAs detection are mainly divided into two groups: methods based on amplification or hybridization [9]. In general, methods such as quantitative real-time PCR and

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microarray do not match perfectly with the requirements of clinical detection, such as easy utility, fast response, low cost, high sensitivity, and specificity. Silicon nanowire (SiNW) biosensors have advantages such as label-free detection, high sensitivity, rapid response, and good selectivity. Zhang et al. first reported SiNW biosensors could detect 1 fM miRNA directly with peptide nucleic acid (PNA) probe [10]. Dorvel et al. were able to achieve 100 fM detection level of miR-10b with using SiNW and hafnium oxide dielectrics, and claimed a theoretical limit of 1 fM [11]. Importantly, they took single-stranded DNA (ssDNA) as probe for cost-saving. Notably, miRNAs detected in these studies are standard samples.

Here, we introduce our poly-SiNW biosensors to detect both standard and real miRNA sample with ssDNA as probes. Since polysilicon is the major materials used in commercial manufacture of SiNW, our study could provide experimental evidence for poly-SiNW application for miRNA detection.

2 Materials

1. Milli-Q water.
2. ssDNA and RNA oligonucleotides (Table 1).
3. Poly-silicon nanowire biosensors surrounded with a SiO_2 layer were (provided by Shanghai Integrated Circuit Research & Development Center, China) (*see* Fig. 1).
4. 2% APTES: Add 1 mL APTES to 49 mL 95% ethanol.
5. 1.25% glutaraldehyde: Add 1.25 mL 50% glutaraldehyde to 48.75 mL H_2O.
6. 1× SSC solution: 150 mM NaCl, 15 mM sodium citrate.

Table 1
Sequences of standard sample and probe

Standard sample and probe	Sequence
Let-7b	5′-UGAGGUAGUAGGUUGUGUGGUU-3′
Let-7c	5′-UGAGGUAGUAGGUUGUAUGGUU-3′
Mismatch (MM)	5′-AUGCAUGCAUGCAUGCAUGCAA-3′
Let-7b probe	5′-AACCACACAACCTACTACCTCA-3′
snRNA U6 probe	5′-TGCTAATCTTCTCTGT-3′

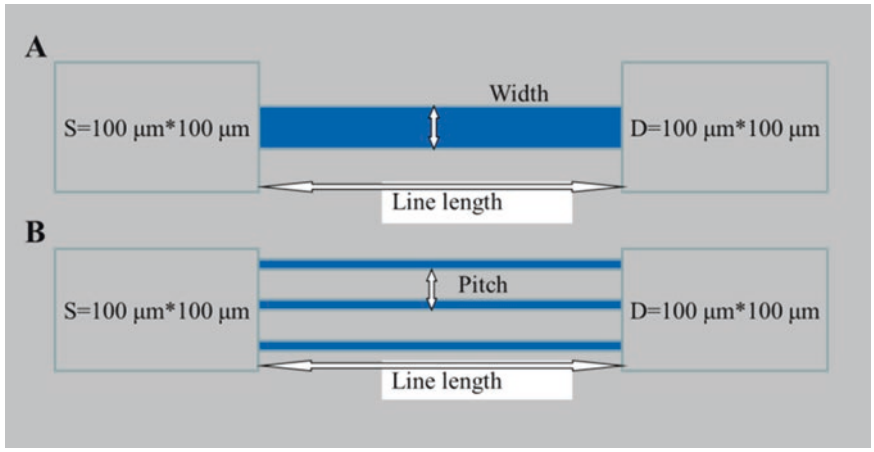

Fig. 1 Structures of SiNW biosensor. S: Source, D: Drain. SiNW length is 100 μm. Pitch is width sum of silicon nanowires and lateral blank area. (**a**) Schematic diagram of RR17, RR19, and RR20; (**b**) Schematic diagram of FF47, FF49, FF50, FF57, FF59, and FF60

3 Methods

3.1 Modification of Poly-SiNW Biosensors

1. Clean the SiNW biosensors with isopropyl alcohol and ethanol (*see* **Note 1**).
2. Immerse SiNW biosensors into 2% APTES solution for 2 h under 253.7 nm UV light (*see* **Note 2**).
3. Wash the SiNW device for three times with absolute ethanol and air-dry for 10 min.
4. Place the SiNW device in 1.25% glutaraldehyde for 1 h (*see* **Note 3**).
5. Wash the device for three times with Milli-Q water and air-dry for 10 min.

3.2 Probe Preparation and Hybridization

1. Incubate SiNW device with 100 nM ssDNA probe in 1× SSC at room temperature overnight (*see* **Note 4**).
2. Wash the device with 1× SSC for three times (*see* **Note 5**).
3. Before hybridization, measure the voltage-current curve of SiNW device and calculate the resistance (R_0) (*see* **Note 6**).
4. Add enough target sample in 0.01× SSC to the device surface and incubate for 1 h (*see* **Note 7**).
5. Wash the device with 0.01× SSC for three times and air-dry for 10 min.
6. Measure the voltage-current curve to calculate the resistance (R).
7. Compare R_0 and R and calculate R/R_0 (*see* **Note 8**).

4 Notes

1. Clean the SiNW biosensors with isopropyl alcohol and ethanol help remove contaminants.
2. Silanation is carried out by reaction with 2% APTES in 95% ethanol solution for 2 h to convert surface silanol groups to amines.
3. Treatment of 1.25% glutaraldehyde for 1 h helps create the aldehyde-modified SiNW surface so that the ssDNA probe can bind to the device (*see* Fig. 2).
4. Instead of PNA, we use ssDNA as probe which is of lower cost.
5. Wash the device with 1× SSC for three times to remove unreacted probe and air-dry for 10 min.
6. Voltage-current curves of each sample are measured in Cascade probe station (Cascade Microtech) within a certain range (0–5 V).
7. A series of standard samples of let-7b, let-7c, and mismatch miRNA sequences are prepared by adding miRNA powders in 0.01× SSC solution. Total RNA samples were extracted from cultured human liver cancer cell line HepG2 using Trizol by standard protocol.
8. Changes in resistances reflect hybridization efficiency. Data are analyzed through comparing changes in resistances. After preliminary screening, we found the current-voltage curves (I–V

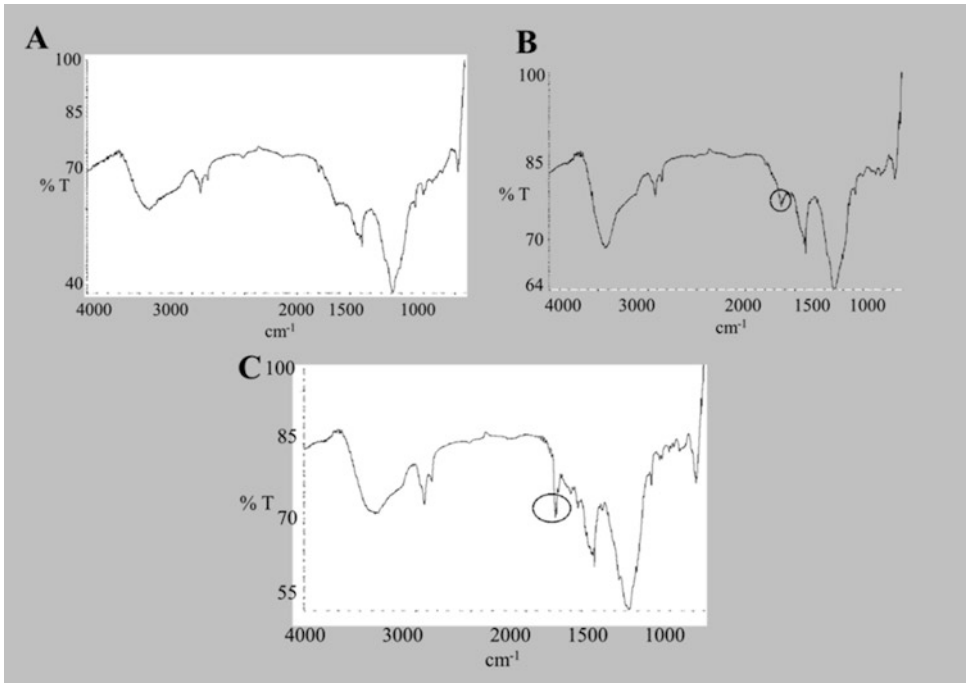

Fig. 2 Chemical modification of SiNW. (**a**) SiNW before chemical modification. (**b**) After modification of 2% APTES, bending vibration of N-H is enhanced in 1650 cm^{-1}. (**c**) After modification of 1.25% glutaraldehyde, stretching vibration of C-O is enhanced in 1730 cm^{-1}

curves) were commonly not straight lines, suggesting resistant of each sample was not a constant. Hence, we analyzed the resistance changed by the following process: (1) to obtain the current-voltage function (cubic function is suitable according to our experience) according to the raw curve, which is defined as $f(I) = aU^3 + bU^2 + cU + d$; (2) to obtain the conductance-voltage function by derivation $f(G) = dI/dU = 3aU^3 + 2bU^2 + c$; (3) to calculate the area under curve (AUC) of the conductance-voltage curve in a certain range (0–5 V in this study) $\int_0^5 f(G)$ to indicate the conductance of each sample; (4) to calculate the resistance change (R/R_0) by the following equation

$$R / R_0 = \frac{\int_0^5 f(G_0)}{\int_0^5 f(G)}.$$

Here, U is the voltage; I is the current; G_0 is the conductance of a biosensor before sample loading; G is the conductance of this biosensor after miRNA sample hybridization; and a, b, c, and d are constants (*see* Fig. 3).

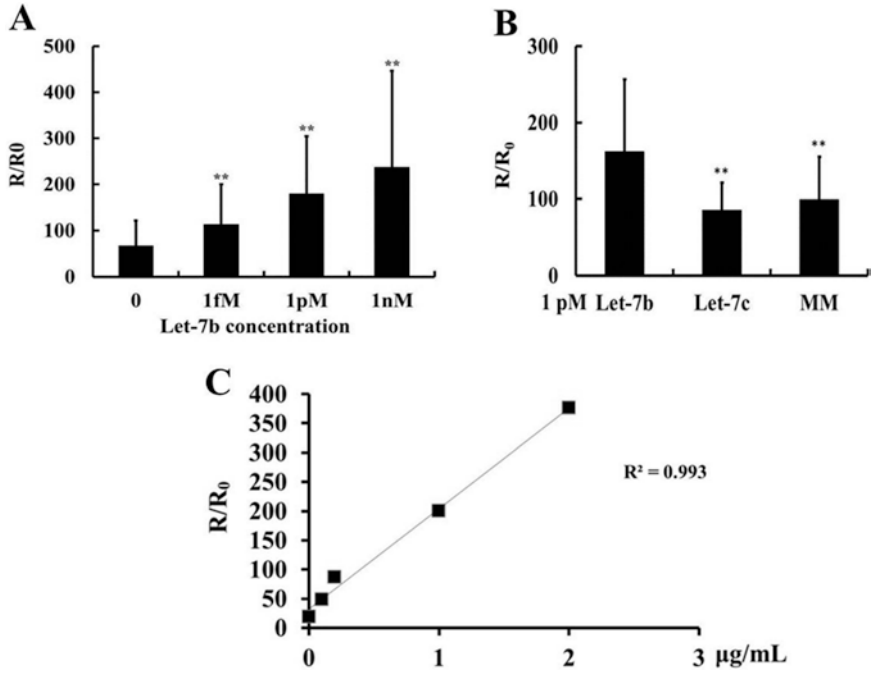

Fig. 3 (**a**) Detection of microRNA standard sample. Let-7b standard sample: 1 nM, 1 pM, 1 fM, and 0. Probe: 100 nM Let-7b probe. The detection limit for Let-7b standard sample is 1 fM. (**b**) Detection of one-base mismatched microRNA standard sample: 1 pM Let-7b, 1 pM Let-7c, and 1 pM mismatch (MM). Probe: 100 nM Let-7b probe. Single base difference between Let-7b and Let-7c is significantly identified at 1 pM concentration level. (**c**) Detection of snRNA U6 in total RNA from HepG2 in five concentrations. There is a well linear relationship among a series of concentrations

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