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Silicon nanowire biosensor for highly sensitive and rapid detection of Dengue virus

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ABSTRACT

The paper presents an innovative silicon nanowire (SiNW)-based sensor for highly sensitive and rapid detection of reverse-transcription-polymerase chain reaction (RT-PCR) product of Dengue serotype 2 (DEN-2). A specific peptide nucleic acid (PNA) was covalently attached onto the SiNW surface. A complementary fragment of DEN-2 (69 bp) was obtained through one-step RT-PCR amplification, and applied to the PNA-functionalized SiNW. The hybridization event was verified by measuring the resistance change of the SiNW before and after binding of the RT-PCR product of DEN-2 to the PNA sequence. It is found that the SiNW sensor can detect below 10 fM concentration of the amplicons within 30 min. The approach shows potential of eliminating the demand for laborious methods by integrating the SiNW sensor with RT-PCR based on silicon technology platform. Consequently, this system-scale integration for a point-of-care medical device will facilitate the diagnostic applications.

1. Introduction

Dengue, a commonly prevalent arthropod-borne viral infection throughout the world, is part of the *Flavivirus* genus of *Flaviviridae* family. It is generally transmitted to human through the infection of *Aedes agypti* or *Aedes albopictus* mosquitoes. The four antigenically related, but distinct serotypes are capable of inducing a wide spectrum of non-specific symptoms that led to Dengue fever (DF) or its more severe case Dengue hemorrhagic fever/Dengue shock syndrome (DHF/DSS) [1,2]. Infection with any of the four serotypes of Dengue virus may cause a DF. Dengue virus is recognized as one of the most important arthropod-spread virus affecting human survival. It is estimated that globally 2.5 billion people are at risk, with 50 million infections each year, 500,000 cases of DHF and at least 22,000 deaths.

Infection with the Dengue virus may be subclinical (no apparent symptoms), and its symptoms are similar to those of influenza, malaria, measles, typhus, and other virus infections. No effective vaccine or specific therapeutic treatment, to date, is available for preventing or curing the disease caused by Dengue virus. Hence diagnostic methods are required to identify the disease reliably and rapidly, and subsequently treat Dengue virus infection in an early stage. Virological detection of specific viral molecules and serological detection of specific anti-Dengue antibodies are the two commonly used techniques for diagnosis of Dengue virus infections. Serological tests allow for identification of IgM and IgG antibodies to the Dengue virus [3,4]. However, sufficient amount of antibodies need to be produced at least 5 days after the onset of illness. Further more, the tests suffer from cross-talking with other flaviviruses. Currently virological techniques, in particular reverse-transcription-polymerase chain reaction (RT-PCR) and *TaqMan* RT-PCR, are preferred as they are more sensitive, with reduced risk of contamination and shorter assay time compared to serological techniques [5–9]. The detection is eventually visualized utilizing stained agarose gel electrophoresis, whose process is, however, time-consuming and hazardous. With the advancement of today's technology, the rise of biosensor is replacing such conventional approaches. Biosensors based on nucleic acid hybridization and liposome signal amplification have recently been developed for inexpensive and rapid detection of RNA molecules [10–12]. The target RNA molecules were amplified by the isothermal nucleic acid sequence-based amplification (NASBA) technique. However, this assay needs a sandwich format, in which the amount of RNA present in the sample was identified by the number of liposome caught on the surface indirectly.

Miniaturized disease diagnostic devices which are inexpensive, portable and field-ready with high sensitivity and specificity, play a significant role because small and low-cost medical devices are

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urgently needed to diagnose large populations suffering from infectious diseases. Silicon nanowires (SiNWs), which act as a field-effect transistor (FET) sensor, are employed as a solution to these limitations. This electrical based biosensor integrates biomolecules with complementary metal-oxide semiconductor (CMOS) compatible technologies to allow the detection of change in charges when target analyte binds onto the immobilized probe. Due to one dimensional structure and high surface-to-volume ratio, SiNWs prove to be a highly sensitive, direct and specific detection for a wide spectrum of species, which includes metal ions [13,14], proteins [15–17], nucleic acids [18–24], and viruses [25] coupled with the versatility of single or multiplex format. Its fast response time and low detection limit (up to femtomolar range), in addition to the elimination of labelling make this mode of detection intriguing. Until now, only oligomer detection has been reported by SiNW biosensor, implying that the oligomer was synthesized by designing. As we demonstrated the microRNA detection previously [24], the sequence of microRNA is 22-bp long. To make real application possible, the target DNA sequence would be longer like PCR products or genome sequences. Because of limitations on Debye length, etc., little has been known on detection of long sequence of DNA by SiNW biosensor.

Dengue virus contains a single-stranded RNA (ssRNA), which allows the amplification and hence detection of Dengue viral genome through RT-PCR. RT-PCR allows the identification of different serotypes through the usage of serotype-specific primers. As a proof-of-concept, we describe in this paper the development of a novel SiNW biosensor platform based on peptide nucleic acid (PNA)–DNA hybridization for highly sensitive and rapid detection of Dengue virus. Studies in Southeast (SE) Asia suggest that secondary infection with Dengue serotype 2 (DEN-2) virus is more likely to cause severe disease than do other serotypes [26]. A specific fragment (69 bp) of DEN-2 genome sequences was chosen and amplified by RT-PCR. Functionalization of a carefully selected PNA probe sequence on the nanowire surface enables the hybridization between PNA probe and the RT-PCR product sequence, and thus the detection of DEN-2. Instead of a negatively charge phosphate backbone that DNA possesses, PNA is a nucleic acid with a neutral peptide backbone. Therefore, hybridization with a complementary sequence of DNA would bring about an accumulation of negative charges on the surface of the nanowire solely contributed by the sequence. This change in charge upon hybridization further induces an increase in resistance which constitutes the basis of this detector's sensing mechanism. This work, for the first time, investigates the feasibility of Dengue virus detection on a n-type FET nanowire sensor, offering a rapid, specific and simple diagnosis of Dengue viruses that are difficult to cultivate.

2. Materials and methods

2.1. Materials

Viral RNA mini kit (Qiagen Cat. 52904), Qiagen one-step RT-PCR kit (Cat. No. 210212), and QIAquick PCR purification kit (Cat. No. 28104) were purchased from Qiagen. Chemicals used for surface modification, namely aminopropyltriethoxysilane (APTES) and glutaraldehyde, were purchased from Sigma–Aldrich. The synthetic PNA probe utilized was purchased from BIOSYNTHESIS (Lewisville, Texas). The primers were synthesized by Sigma-Proligo. Product of RT-PCR, both purified and un-purified, were used for hybridization. The sequences of the PNA, the primers and the RT-PCR product are shown in Table 1. As a control, negative RT-PCR product was used.

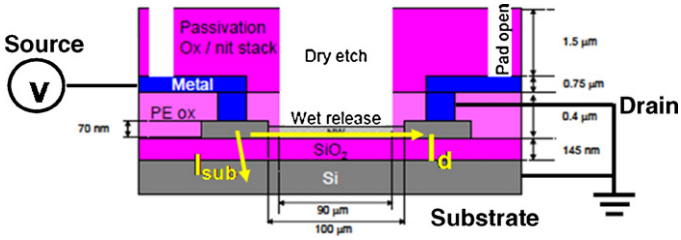

Fig. 1. Schematic diagram of a cross-sectional view of the SiNW sensor with electrical connection illustrated.

2.2. Silicon nanowire fabrication

The silicon nanowire sensor was fabricated on a silicon-on-insulator (SOI) wafer with a 145 nm buried oxide layer. The wafers were n-doped with an implant dose of 5×10^{13} cm^{-2} at 30 keV and the dopants activated via rapid thermal annealing. Using deep ultraviolet lithography, a 50–80 nm silicon layer was patterned and etched to define the fins from which the nanowire arrays were generated. Individual nanowires were further formed via the thermal oxidation of the fin structure at 900 °C in O_2 for ~4 h, followed by n+ doping of the nanowire ends for contact metal definition and subsequent passivation with Si_3N_4/SiO_2. Finally, the nanowires were released by dry etching of the Si_3N_4/SiO_2 passivation layers followed by wet etching of the remaining SiO_2. A cross-sectional view of the SiNW sensor is schematically illustrated in Fig. 1.

2.3. Surface modification of SiNWs

The SiNW surface was first functionalized using APTES, which functions as a facilitator to immobilize biomolecules on the Si–OH surface, as described previously [24]. SiNWs were immersed with 2% APTES in 5% water and 95% ethanol for 2 h, and rinsed with ethanol. Since APTES is amine-terminated, a bifunctional linker was required to bind the amine-terminated PNA probe onto the surface. The bifunctional linker employed in this study is glutaraldehyde, which constitutes of two aldehyde terminals. One end would bind to the anime-terminated APTES and the other end free to immobilize the amine-terminated PNA. Hence, the SiNWs were treated with 2.5% glutaraldehyde in H_2O for 1 h, and rinsed with pure H_2O. Acrylic well was then pasted onto the chip to enclose the area where SiNWs reside. This provided the convenience for localizing application of analytes. Ten micromolars of PNA in citrate buffer (15 mM sodium citrate, 150 mM NaCl, pH 7.4) was then applied into the well and incubated overnight in a moist environment at room temperature. After incubation, excess PNA were rinsed free from the surface by washing the chip thrice (5 min each time) with the same buffer.

Table 1
PNA sequence for immobilization on the SiNW surface, the primer sequences for RT-PCR of the Dengue virus serotype 2, and the whole sequence of the RT-PCR product.

Name	Sequences
PNA	N-CATGGCCCTKGTGGCG-C
PNA (control)	N-AACCACACAACCTACTACCTCA-C
DEN-2-forward	5′-CATGGCCCTKGTGGCG-3′
DEN-2-reverse	5′-CCCCATCTYTTCAGTATCCCT G-3′
RT-PCR product of DEN 2 (69 bp)	5′-CCCCATCTCTTCAATATCCCTGCTGTTGGTGGGATTGTTAGGAAACGAAGGAACGCCACCAGGGCCATG-3′

K stands for G or T.