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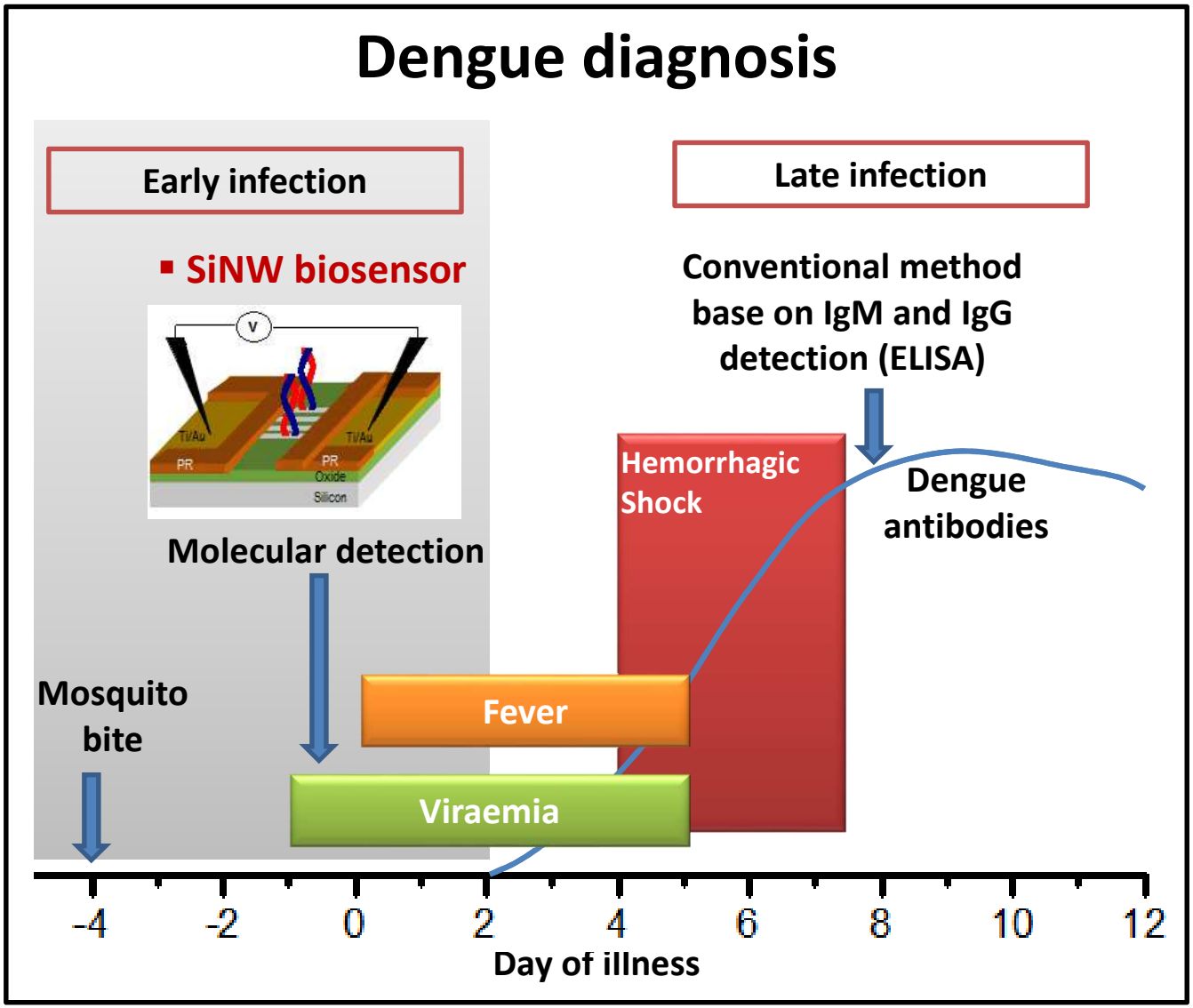
Dengue diagnosis
Early infection
Late infection
SiNW biosensor
Conventional method base on IgM and IgG detection (ELISA)
V
Ti/Au
PR
Oxide
Silicon
Molecular detection
Hemorrhagic Shock
Dengue antibodies
Mosquito bite
Fever
Viraemia
-4
-2
0
2
4
6
8
10
12
Day of illness

ACCEPTED MANUSCRIPT

Enhanced Sensing of Dengue Virus DNA Detection using O_2 Plasma Treated-Silicon Nanowire based Electrical Biosensor

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Abstract
Dengue Virus (DENV) has become one of the most serious arthropod-borne viral diseases, causing death globally. The existing methods for DENV detection suffer from the late stage treatment due to antibodies-based detection which is feasible only after five days following the onset of the illness. Here, we demonstrated the highly effective molecular electronic based detection utilizing silicon nanowire (SiNW) integrated with standard complementary metal-oxide-semiconductor (CMOS) process as a sensing device for detecting deoxyribonucleic acid (DNA) related to DENV in an early stage diagnosis. To transform the fabricated devices as a functional sensing element, three-step procedure consist of SiNW surface modification, DNA immobilization and DNA hybridization were employed. The detection principle works by detecting the changes in current of SiNW which bridge the source and drain terminal to sense the immobilization of probe DNA and their hybridization with target DNA. The oxygen (O_2) plasma was proposed as an effective strategy for increasing the binding amounts of target DNA by modified the SiNW surface. It was found that the detection limit of the optimized O_2 plasma treated-SiNW device could be reduced to 1.985×10^{-14} M with a linear detection range of the sequence-specific DNA from 1.0×10^{-9} M to 1.0×10^{-13} M. In addition, the developed biosensor device was able to discriminate between complementary, single mismatch and non-complementary DNA sequences. This highly sensitive assay was then applied to the detection of reverse transcription-polymerase chain reaction (RT-PCR) product of DENV-DNA, making it as a potential method for disease diagnosis through electrical biosensor.

Keywords: silicon nanowire; Dengue diagnosis; DNA hybridization detection; plasma surface treatment; electrical detection

1.0 Introduction

The dengue virus (DENV) continues to be an unmet major public health concern with an estimated 50 million infections leading to at least 22000 deaths globally each year [1]. Dengue illness is caused by the viruses of the *Flaviviridae* family and transmitted to human bodies by the *Aedes aegypti* mosquito. The early symptoms of dengue infections are usually non-specific and are similar to those of influenza, measles, malaria, typhus and other virus infections [2]. However, the late detection of DENV may progress from dengue fever (DF) into more severe and potentially fatal illnesses known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [3]. To date, there is no specific vaccine nor has an effective treatment been developed yet to combat the disease. Public education and mosquito eradication strategies have been widely adopted by many countries where the virus is endemic but these attempts are

reported to have met with limited success in controlling the spread of the disease [3]. Thus, there is an unmet and increasingly urgent need for reliable diagnostic methods for both epidemiological surveillance and clinical diagnosis to identify the disease rapidly and accurately, and subsequently to treat the DENV infection in an early stage [4].

Conventional methods used for DENV infection diagnosis are virus isolation, detection of antibodies against dengue and detection of envelope protein in the blood of susceptible patients [5]. The former of these, virus isolation, although a reliable confirmation method, takes time as well as requiring specialized equipment and a high level of technical expertise [6]. A range of serological tests based on detection of virus-specific antibodies are commercially available and have been extensively evaluated [7]. The most common of these methods is enzyme-linked immunosorbent assay (ELISA), detection based on immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to DENV [8]. The IgM antibody is the first immunoglobulin isotype to appear and according to the Pan American Health Organization (PAHO) guidelines, by day five of the illness, 80% of cases exhibit detectable IgM antibodies. Anti-dengue IgG can be detected in a low titer at the end of the first week of the infection and thereafter increases slowly [4][5]. Even though this method has resulted in good approaching for routine dengue diagnosis, it could not provide an early detection since this antibodies-based detection demands an appropriate time frame (after five days of onset of infections) in order to mount sufficient immune response to produce detectable antibodies in patients for diagnosis. Moreover, the tests are also susceptible to being compromised by cross-reactivity with other flaviviruses [3].

More recently attention has been focused on molecular assays based on nucleic acid amplification for DENV detection. The molecular-based diagnostic assays is preferred as it is more sensitive and can provide reliable results in shorter assay time compared to serological techniques [9][10]. The hybridization technique for detection of viral nucleic acid has attracted the enormous efforts in the development of DNA biosensor [11][12]. DNA biosensors consist of an immobilized DNA strand to detect the complementary sequence by hybridization process. The binding of the surface-confined probe and its complementary target strand is translated into a useful signal can be determined by several transduction methods that have been reported in the literature [13].

From the point of view of the electrical properties, a conformational change in a biological or chemical event often causes the change in the electrical properties of the substances [14][15]. Therefore, DNA biosensors based on electrical detection could be more simple, rapid and portable detection platforms. The advancement of nanotechnology has opened up the opportunities of using electrical system for biomolecule. The SiNW has become a great candidate for use in miniaturized biosensors devices [16][17] and have been proven as a powerful platforms for highly sensitive label-free detection of biological species, such as proteins [18], viruses [19] and DNA [20]. These advantages are significant for the development of molecular electronics based on SiNW device for early detection of DENV through DNA hybridization detection.

Central to the entire discipline of the formation of a DNA duplex on the SiNW surface is the concept of DNA hybridization. It is known that, the sensitivity of SiNW device is affected by

binding amounts of target DNA (formation of DNA duplex) on the nanowire surface [21]. The binding amount of target DNA could be improved by increasing the amounts of immobilized probe DNA and lead to enhanced sensitivity of biosensors based on the electrical detection. In this regard, much effort has been made on demonstrating nanostructures integrated on top of SiNW surface such as nanoforest [22], nanoparticles [23][24][25] and nanoribbons [26] in order to increase the surface area of the device and thus increase the amount of analyte binding.

The oxygen (O_2) plasma treatment typically used to remove the organic contaminants on the silicon-based substrate [27][28]. Moreover, surface hydroxylation is expected to be achieved as the oxygen radicals preferentially break Si-O bonds at the SiO_2 surface, which introduced the generation of chemically active dangling bonds and hydroxyl groups at the surface [29]. Accordingly, this process activated the surface with high density of silanol groups (Si-OH) that are uses as anchoring sites for the next silanization reaction [30]. According to the literature, the surface hydroxylation process is of paramount importance in surface chemistry [31]. Sharma and group demonstrated the use of Fenton and nitric acid (HNO_3) boiling to prepare highly activated Si-OH groups on the surface [32]. There also a few reported on using piranha solution that is a strong oxidizing agent for sample cleaning as well as surface hydroxylation [31][33]. Even though these solution based method demonstrated an effective way to activated the Si-OH groups, however, it is worth pointing out that the O_2 plasma treatment shows the advantages of simplicity technique, increased effectiveness, environmental safety and little effect on other devices during the process compared to the chemical based treatment using strong acids. More generally, it should be possible to extend this approach as an effective method to improve the

amount of chemical-link available on the sensing area and consequently enhance the number of receptors immobilized on the wire surface.

To the best of our knowledge, the use of O_2 plasma surface treatment as enhancing platforms for increasing the binding amounts of target DNA has not been widely studied. As the sensitivity of the SiNW biosensor derived from the high surface-to-volume ratio of the SiNW, for the first time, we report herein the potential of using O_2 plasma treatment in enhancing the device sensitivity for the detection of DENV DNA target species with extremely low concentrations. Without using a complex wet chemistry process to enhance the surface area of SiNW, the sensitivity of the sensor device is tunable simply by this method and the role of O_2 plasma treatment is elucidated.

2.0 Material and methods

2.1 Fabrication of SiNW biosensor device

Commercially available 4 inch (100 mm in diameter) p-type silicon on insulator (SOI) wafer which initially had a 50 nm silicon layer on a 200 nm buried-oxide (BOX) insulating layer was used as the starting material in this work. Standard cleaning procedure using Radio Corporation of America (RCA 1) composed of hydrogen peroxide (H_2O_2), ammonium hydroxide (NH_4OH) and di-ionized water (DIW) in the ratio of 1: 1: 5 was introduced to remove organic residues and particles on the wafer surface. Subsequently, the buffered oxide etches (BOE) with composition of hydrofluoric acid (HF) and DIW in the ratio of 1:50 was used to remove the native oxide. Electron beam lithography (EBL) coupled with optical lithography was implemented in this work to fabricate Si-based nanoscale sensor device. The SiNW with

designed 60 nm in width and length of 400 µm was defined using EBL and dry etching system. Subsequently, a thin layer of chromium (Cr) and gold (Au) with thickness of 300 Å and 500 Å, respectively were deposited onto the fabricated SiNW sample using thermal evaporator method in order to fabricate the source and drain contact pad. After photolithography process, the unwanted layers of Au/Cr were removed by freshly mixed of nitric acid (HNO_3) and hydrochloric acid (HCl) in a volumetric ratio of 1:3, which denoted as "aqua regia" in room temperature. Finally, the samples were coated with PR1-2000A resist as passivation layer with thickness approximately 2.5 µm and patterned via optical lithography to electrically isolate the metal contact regions from the electrolyte samples for DNA measurement as schematically illustrated in Fig. 1(a).

Fig. 1

2.2 Chemically modified SiNW surface

Prior to modification process, the devices were preserved in a clean room for 5 days to ensure the thickness saturation of the native oxide. It is suspected that the surfaces of SiNW tend to oxidize and thus introduce the formation of thin layer oxide. The SiNW surface covered with an oxide layer was then functionalized with 3-aminopropyltriethoxysilane (APTES, 99%) obtained from Sigma-Aldrich. The solution was prepared by mixing of 2% APTES in a mixture of ethanol and DIW for 2 hours at room temperature. The samples were washed with DIW and dried on a hot plate at 120 °C for 30 min. The surface reaction occurred between the silanol groups (-OH) occurred on naturally oxidized SiNW surface with triethoxysilane APTES ($(NH_2$-$(CH_2)3$-$Si(OC_2H_5)3$). This silanization process terminated the surface with $–NH_2$ functional

groups. Next, the APTES functionalized SiNW samples were immersed with 2.5 % glutaraldehyde solution for 1 hour at room temperature [34]. The solution was formed by diluting 25 % glutaraldehyde (Sigma-Aldrich) with phosphate-buffered saline (PBS, pH 7.4). Subsequently, the samples were rinsed with PBS solution to remove excess of glutaraldehyde (GA) and dried with blowing air. GA which constitutes of two aldehyde groups (CHO-(CH_2)3-CHO) is used as a linker. One end will have interactions with amine-terminated APTES and the other one is free for further reaction with amine-terminated probe DNA. At this stage, the SiNW surface was covered by –COOH functional group and can be further attach with the amine-terminated probe of single-stranded DNA (ssDNA) as depicted in Fig. 1 (b).

2.3 DNA immobilization and hybridization

The synthetic amine-terminated probe of DENV DNA at 5’ position was used to bind with the available aldehyde groups on the NW surface. The 27-mer probe DNA with sequence of 5’-NH_2-C_6-AAC AGC ATA TTG ACG CTG GGA GAG ACC-3’ was diluted using phosphate buffer solution (PBS, ph 7.4) to provide 1.0 x 10^{-6} M solution of probe DNA. For immobilization process, a drop of 10 µL of this probe DNA was placed onto SiNW surface and incubated overnight at room temperature. The samples were rinsed with PBS buffer to remove the un-reacted ssDNA (probe) and subsequently dried with blowing air. Then, 10 µL of 27-mer complementary target DNA were applied onto the immobilized sample to form double stranded DNA (dsDNA) as depicted in Fig. 1 (c). The samples were incubating at room temperature for 2 hour to allow hybridization process. The unbound target DNA was washed away with PBS solution and then dried with blowing air. The complementary target DNA that used in this work has a sequence of 5’-TGG CTC CCT GAC CGT CAA TAT GCT GTT-3'. In addition, the non-

complementary target DNA with sequence of 5'-CTT TGT GTT AGT ATC TGG CCG ATG TCC-3' and one-base mismatched target DNA with sequence of 5'-GGT CTT TCC CAG CGT CAA TAT GCT GTT-3' were used as a control to ensure the selectivity of the device as a sensor. All the sequences employed were synthesized by Integrated DNA Technologies (IDT), Inc.

2.4 Detection procedure of RT-PCR product of DENV DNA

2.4.1 Preparation of RT-PCR product

QIAampMinElute® Spin Kit was used for purification of viral RNA from cell-culture media by following the manufacturer's protocol. Aedesalbopictus mosquito clone cells line propagated in C6/36 (ATCC® CRL-1660™) cell-culture was kindly provided by Department of Medical Microbiology & Parasitology, Universiti Sains Malaysia. The oligonucleotide primers used in this work were 5'- AAG GAC TAG AGG TTA KAG GAG ACC C-3' for forward and 5'- GGC GYT CTG TGC CTG GAW TGA TG-3' for reverse transcription. Then, MyTaq™ One-Step RT-PCR kit was used for RT-PCR process. The reaction mixture contained 12.5 µl of 2 x MyTaq One-Step Mix, 2.0 µl of 10 µM primer mix, 0.25 µl of Reverse Transcriptase, 0.5 µl of RiboSafe RNase Inhibitor, 4.75 µl of DEPC-H_2O and 5.0 µl of Dengue RNA extraction (template). The reverse transcription was employed at 48 °C for 30 min (1 cycle) and followed by polymerase activation at 95 °C for 5 min. Then, the 35 amplification cycles each at 95 °C for 15 sec (denaturation step), 60 °C for 30 sec (annealing step) and 70 °C for 1 min (extension step). Then the extension step was remained for 10 min at 70 °C and incubating at 4 °C.

2.4.2 Hybridization of RT-PCR product to the ssDNA-functionalized SiNW

The RT-PCR product of DENV DNA was diluted with PBS buffer (pH 7.4) to concentration of 1.2 x 10^{-7} M. The sample was then denatured into single-stranded DNA (ssDNA) by immersing in heated DIW at 90 °C for 5 min, and subsequently, placed in ice bath for 5 min. For hybridization process, 10 µl of prepared target ssDNA solution was employed onto the ssDNA-functionalized SiNW and incubated for 2 hour at room temperature. Afterwards, the sample was washed with PBS solution to remove excess target DNA and dried with blowing air. The electrical measurement was performed to evaluate successful bound of RT-PCR product to the 27-mer probe ssDNA-functionalized SiNW.

2.5 Oxygen plasma treated-SiNW surface

In an attempt to enhance the device sensitivity using surface plasma treatment in comparison to the direct functionalized of SiNW device, the fabricated SiNW samples were plasma-treated with oxygen (O_2) in a chamber of plasma-preen system (Plasma-Preen H-862, Plasmatic Systems, Inc.). It is believed that, the O_2 plasma treatment cleaned the sample surface and maximizes the –OH groups available to bond the silane groups [30]. During plasma treatment, the power was maintained at 100 W with oxygen pressure of 10 torr, while the process time was varied for 30 sec, 60 sec and 90 sec respectively. The effect of Si surface corresponds to the duration of plasma treatment was evaluated using atomic force microscope (AFM) and water contact angle measurement, consequently. Then, the similar procedure for SiNW surface modification was carried out for DNA hybridization detection. The efficiency of the DNA hybridization on the plasma-treated surface was observed based on the electrical response.

2.6 Characterization

Field emission scanning electron microscope (FESEM) in combination with energy-dispersive x-ray (FESEM/EDX-Hitachi H7100) was carried out to inspect the fabricated structures and identification of elements. Contact angle measurement (Water Surface Analysis System VCA 3000, AST Products) was done to study the hydrophilicity of modified silicon surface. For confirmation of chemical bonding during surface modification, fourier transform infrared spectroscopy (FTIR, Perkin Elmer Spectrum 400 spectrometer) has been done in order to observe the stretching vibration of the elements that exist on the sample surface. Atomic force microscopy (AFM, SPA400-SPI3800. Seiko Instruments Inc.,Japan) was used to investigate the surface profile of the plasma treated-Si surface. Due to the difficulties of analysis on the SiNW surface, a planar silicon samples (2 cm x 2 cm) with various surface modification was carried out for observation using contact angle measurement, FTIR and AFM respectively. Finally, electrical characterizations of developed SiNW devices were performed using Keithley 6487 Picoammeter Probe Station. During the measurement set-up, two SMU probes are individually aligned on to the two terminal NW devices by supplying sweep voltages of 2V to the source region and obtain the output current at the drain region (Fig. 1 (d)). The electrical performance of the sensor was observed when operating in dry conditions.

3.0 Result and discussion

3.1 Characterization of fabricated SiNW device

The top-down fabricated SiNW device was characterized using FESEM image as shown in Fig. 2 (a). The image was taken for the developed SiNW structure with a line-width of 60 nm. The result showed that the nanowire pattern was successfully transferred onto the Si layer that

covered with buried oxide layer (BOX). The SiNW was etched anisotropically producing smooth sidewalls with a bottom dimension that has about the same equal in width to the top dimension, which originally drawn by the electron beam on the ma-N2403 resist mask. The SiNW structure was formed as an island structure on the buried oxide layer (BOX) after the introduction of the 15 sec RIE process. The height measurement is approximately 50 nm of the fabricated SiNW and it was obtained corresponding to the top Si layer thickness of the SOI substrate as a starting material. The top active Si region is electrically isolated from the Si handle with 200 nm of the buried oxide layer.

Fig. 2

Fig. 2 (b) shows the optical images of the final fabricated patterns consists of electrode pads and fluidic channel. The design allowed three independent NWs segments (three devices) per measurement. The devices were coated with passivation layer and patterned via photolithography to open windows exposing the contact pads and the nanowire channels in order to isolate the metal contact pads during the aqueous sensing part and prevent short circuits as well. The close-up view of the SiNW underlying the passivation layer is depicted in Fig. 2 (c), while the insert image of Fig. 2 (c) shows the well aligned of a single SiNW with contact pad illustrated in dark field image of optical view. In such a sensing device, the two metal electrodes which are designated as source (S) and drain (D) of a device is bridged by SiNW.

Next, the elemental analysis of the developed SiNW sensor was determined by the energy dispersive x-ray (EDX) equipped with FESEM. The EDX spectra show strong peaks of silicon

(Si) with the weak peaks of oxygen (O) as illustrated in Fig. 2 (d).The specific peaks of Si and O elements with the mean percentage of 77.82 % and 22.18 % respectively that appeared on the EDX profile confirmed that the Si wire was covered with a relatively thin layer of oxide on top of the wire surface. The results are in conformity with the earlier reports of silicon surfaces that have been exposed in an ambient environment such as air or solutions will oxidize spontaneously [35]. This thin oxide layer can be used for surface functionalization of the analyte detection using chemical modification protocols in order to provide the SiNW structure as a sensing element of the device.

3.2 Optimization of O_2 plasma treated-SiNW device

For biosensor applications, the oxide surface needs to be functionalized with specific catcher molecules that bind reliably to the probe molecules. The quality of the functionalization process such as silanization also depends on the initial treatment of the SiO_2 surface. Amino silane (APTES) was used to create a covalently bound onto SiO_2 surface for bio-functionalized surface. Generally, the surfaces are treated with O_2 plasma for cleaning of organic contaminations and activation of Si-OH groups for a good coverage of the silane molecules on the surface [36]. In some cases, this can also be used for immobilization strategy of ssDNA functionalized SiNW in order to enhance the amount of target molecules attached as well as boost the device sensitivity.

Fig. 3

Fig. 3 (a) shows the water contact angles measured on the treated Si surface prepared after 30 sec, 60 sec and 90 sec of O_2 plasma treatment. This quantitative analysis has been done on planar Si surface in order to optimize the procedure that used in this work by analyzing the hydrophilicity of the surface. Contact angle data indicates that the wettability of Si surface depends on the amount of silanol (Si-OH) groups existed on the surface after each plasma treatment. As expected, the surface becomes more hydrophilic as the treatment time increases. Compared to untreated Si surface, the contact angle decrease from 24° to 13° after employed 30 sec of O_2 plasma. Then, the measurement decrease to 11° and 9° after further plasma treatment for 60 sec and 90 sec, respectively.

The characterization of the treated Si surface using O_2 plasma requires an investigation of surface topography. Therefore, the AFM analysis was carried out on the samples after different times of plasma treatments as shown in Fig. 3 (b). Morphological inspection gives a qualitative picture of the effect of plasma treatment, whereas mean roughness values, Rms, provide a quantitative parameter of surface structure. Based on the three dimensional AFM images, it can be seen that the surface roughness increases when increasing the treatment time. The Rms value for plasma treated Si surface using 30 sec, 60 sec and 90 sec are 0.717nm, 0.912nm and 1.195nm, respectively. It shows that, increasing the time of O_2 plasma treatment will increase the surface roughness of samples.

Fig. 4

To verify the improvement of the O_2 plasma treatment onto SiNW surface, the electrical performance of the SiNW device was carried out in response to the GA/APTES surface modification as shown in Fig. 4 (a). It was found that the surface chemistry has altered the electrical properties of device. Significant in current changes was observed for the GA/APTES modified SiNW as compared to the bare SiNW. This suggested that the modification process generated a layer of negative surface charge (COO^-) on the surface and thus creating an accumulation of hole carrier in the p-type SiNW channel (increase the current signal).

Moreover, enhance in current measurement was observed for the O_2 plasma treated-SiNW device as compared to the untreated-SiNW device. However, the electrical characteristics of the samples after 90 sec of O_2 plasma treatment exhibit non-conductive signal, implying that the oxide layer on the sensor surface might have been damaged during plasma process [37][21]. This founding was supported by previously research [38], believed that the nanowire become more fragile after certain time of plasma treatment due to the hot-carrier endurance and thermal stability degraded after exposure. Although samples with 90 sec of O_2 plasma treatment exhibit the highest hydrophilic surface that indicates more silanol groups available, however the electrical performance of the device degrades after 90 sec of treatment. Thus, 60 sec of O_2 plasma treatment is sufficient for improving efficiency of SiNW device when considering both surface analysis and electrical results.

To further confirm our observation, a FTIR analysis was conducted on the plasma treated sample and untreated sample in response to the GA/APTES surface modification, respectively as shown in Fig. 4 (b). The result clearly demonstrated the signal enhancement at peak 1743cm^{-1}

which attribute to C=O stretching vibration for GA-modified Si surface in correspondence with the increased binding amount of the amine-terminated group of APTES-modified Si surface for the plasma treated-SiNW sample. This is in agreement with the reported work [39], suggesting that the initial silanization step is the most crucial since it subsequently determines the properties of the final target DNA detection. Moreover, the obtained results signify successful attachment of GA to amine terminated surface that provides aldehyde groups as linkers between the SiNW surfaces (sensing element) and the biomolecules (DNA).

3.3 Analytical performance of bio-specific interaction on developed SiNW device

The electrical characterizations of the developed devices in response to the DNA detection were investigated as shown in Fig. 5 (a). For this purpose, 1.0 x 10^{-6} M of ssDNA-probe was immobilized on the samples and subsequently complementary targets DNA with concentration of 1.0 x 10^{-6} M was employed. Based on the gradient of the I-V curves at 2 V, the current measurements of the device before and after hybridization were approximately 7.0 nA and 14.0 nA respectively, corresponding to a 50% change in resistance. The decrease in resistance value coincides well with previous reports suggesting that the hybridization process could be monitored by observation of current changing with a SiNW sensor [40]. The build-up of an electric field after the DNA hybridization due to phosphates groups (PO_4^-) in DNA backbone at SiNW surface caused the accumulation of charge carrier (holes) in the p-type SiNW channel and resulting in the decrease of electrical resistance as schematically depicted in Fig. 5 (a).

Fig. 5

Previous study showed that, it is still possible that the DNA detection could be achieved not only by specific hybridization between complementary target DNA and probe DNA, but also by physical adsorption of DNA molecules to the Si surface and by nonspecific binding between non-complementary target DNA and probe DNA molecules [41][42]. To further verify that the current change due to hybridization of the complementary target DNA to the immobilized probe DNA, two additional tests were employed. First, 1.0×10^{-6} M of complementary target DNA was tested with non-functionalized probe DNA (GA/APTES SiNW surface modification) in order to study the effect of physical adsorption of DNA molecules to the SiNW surface. Second, a detection of 1.0×10^{-6} M non-complementary target DNA was used to hybridize onto the ssDNA-functionalized SiNW surface for confirming the effect of non-complementary target DNA-ssDNA probe binding. After incubation and washing process, both of these tests showed no significant current change as compared to the initial signal, suggesting the absence of nonspecific binding of target DNA to the SiNW surface. This proves that the DNA detection occurred by hybridization of complementary target DNA with ssDNA-functionalized SiNW.

Furthermore, in the presence of one-base mismatched target DNA, approximately 52% resistance change was visible in comparison with hybridization complementary target DNA as presented in Fig. 5 (b). This implies that only specific binding of mismatched target DNA with ssDNA-functionalized SiNW bind to each other to form a duplex structure and induce the changes in current. Overall, we can conclude that the current signals of the SiNW biosensor increased in the following order with the presence of targets; without target DNA < non-complementary < one-base mismatch and complementary. This observation is consistent with

those previously studied in monitoring DNA hybridization detection using p-type SiNW devices [43][12].

The regeneration of the developed sensor devices were investigated by denaturation of the fully complementary DNA pair and resulting in only single-stranded probe DNA present on the SiNW surface. This process was carried out by washing the sample with hot DI water (85 °C) to separate the hybridized target DNA from the probe surface. As reconfirmed by measuring the I-V relations, the curve returned to almost the same level as the immobilization curve, indicating that the denaturation was completed. Subsequently, re-hybridization of the same concentration of complementary target DNA (1.0×10^{-6} M) was performed on the sample. The proposed sensor is capable of generating the current response repeatedly between 7.2 nA and 6.6 nA across three-generation attempts as illustrated in Fig. 5 (c). Remarkably, it was found that the covalent binding is sufficiently stable for repeatable and reproducible biosensing owing to the stable amide bonding. This indicates the developed biosensor have a promising potential in monitoring DNA hybridization detection with stable and good repeatability performance.

3.4 Sensitive detection of plasma treated-SiNW biosensor

Previous work on DNA detection has shown that the coated native oxide on the SiNW surface without introduced plasma treatment could be used directly as sensing element for bio-molecule detection [44][45]. The effect of O_2 plasma treatment on the devices sensitivity were evaluated by performing measurements at different concentrations of complementary targets DNA in the range of 1.0×10^{-9} M to 1.0×10^{-13} M in response to the immobilized ssDNA-probe SiNW samples. To verify that the high performance of developed devices was caused by the

plasma treatment method, the relative resistance changes among various concentrations of complementary target DNA and a non-complementary target DNA with concentration of 1.0 x 10^{-9} M (control) has been characterized as depicted in Fig. 6 (a).

Fig. 6

The obvious resistance change approximately 23% was observed as the 1.0 x 10^{-9} M complementary target DNA was introduced for the untreated-SiNW sensor device. A descending trend of device response approximately 14% and 9% could be observed for decreasing concentration of complementary target DNA to 1.0 x 10^{-10} M and 1.0 x 10^{-11} M, respectively. Meanwhile, merely 5% resistance change was recorded when 1.0 x 10^{-12} M concentration was employed, which is distinguishable from the control signal (non-complementary target DNA). Barely any resistance change was measured for further dilution of DNA concentration (1.0 x 10^{-13} M) as compared to the control signal, pointing the capability of the untreated-SiNW devices to detect the target concentration down to 1.0 x 10^{-12} M.

Interestingly, it was observed that the relative change of resistance was enhanced approximately 12%, 11%, 9% 6% and 5% for plasma treated-SiNW devices as compared to untreated-SiNW devices in response to the 1.0 x 10^{-9} M to 1.0 x 10^{-13} of complementary target DNA. There were two possible explanations considered from the results. First, the O_2 plasma treatment roughens the SiNW sensor surface as verified by using AFM. The rough surface results in an increase in surface area and enlarges the possible number of surface-bonded silanol groups, which were assumed to be the essential surface species of hydrophilic Si surface to form

strong siloxan-bond [21]. The second reason was attributed to the presence of silanol-groups of the plasma-treated Si surface resultant to the increased binding sites for chemical linkers (as proved in Fig. 4 (b)) and more DNA probes were immobilized [30]. Thus, the binding amount of target DNA could be improved and lead to enhanced sensitivity of the devices.

The correlation between the device responses and the target DNA concentrations as obtained in Fig. 6 (b) indicates that the detection responses of the SiNW devices were linear with the value of the complementary target DNA in the concentrations ranged of 1.0 x 10^{-9} M to 1.0 x 10^{-13} M with the linear regression of R^2= 0.989. In biosensing, the limit of detection (LoD) was defined as the lowest detectable molar concentration of target DNA and can be calculated as $Y_{lod} = Y_{blank} + 3SD_{blank}$; where Y_{blank} is mean of the blank measurement and SD_{blank} is the standard deviation of the blank measurement [46]. Thus, the associated concentration for LoD of the plasma treated-SiNW was approximately 1.985 x 10^{-14} M, indicates that extremely low concentrations of target DNA can effectively be detectable with the devices used in this work. Remarkably, the detection limit demonstrated was clearly increased compared to the initial SiNW with detection limit of 4.131 x 10^{-13} M, proving that the sensor performance could be improved by modified the SiNW surface using O_2 plasma at the optimal conditions. It is envisioned that the method described here potentially be used as an effective strategy to enhance the device performance due to its simplicity, rapid processing and endowed with the lowest detection limit compared to the previously reported in the literatures as shown in **Table 1**.

Table 1: Strategies for enhancing device sensitivity in application of DNA biosensor.

Device specification	Strategies / Method	Advantages	Remarks	Reference
p-type SiNW (40 nm width) LoD=2.0 x 10^{-7} M	Reduce wire size using etching under angle technique	Increase surface-to-volume ratio, improved the sensitivity	Need to highly control of etching process	[47]
n-type SiNW (20 nm width) LoD=1.0 x 10^{-8} M	Remove oxide layer by etching process for directly passivated SiNW	Increase the signal response, improved the sensitivity	Possibility to etch over buried oxide (BOX) layer of SOI sample	[48]
p-type SiNW (20 nm width) LoD=1.0 x 10^{-10} M	Reduce wire size using plasma trimming and oxide etching technique	Increase surface-to-volume ratio, improved the sensitivity	Required additional fabrication steps	[44]
p-type SiNW (80 nm width) LoD=1.0 x 10^{-13} M	Gold nanoparticles (GNPs) embedded SiNW using RF sputter and post-annealing process	Increase the binding site, increase the signal response	Size nanoparticles affect probe loading capacity	[25]
p-type SiNW (60 nm width) LoD=2.0 x 10^{-14} M	Modified SiNW surface using optimized oxygen plasma surface treatment	Increase the binding site, improved the sensitivity	Straightforward modification on sensing surface	This work

*LoD = limit of detection

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3.5 Detection of RT-PCR product of DENV DNA

The developed sensor device was then applied in the real sample detection of DENV. The agarose gel electrophoresis and UV-visible spectrophotometer was employed to characterize the concentration and purification of the resulting RT-PCR product. A single band of the PCR amplification product of DENV, with molecular size of approximately 140bp was clearly observed in lane 2 as shown in Fig. 7 (a). The DNA concentration was estimated by measuring absorbance at 260 nm (Fig. 7 (b)), since all nucleotides (ssDNA and dsDNA) absorb at 260 nm which contribute to the total absorbance of the sample. The concentration of the RT-PCR product obtained is approximately 570µg/ml, which equals to 12 x 10^{-3} M. Whereas, the good-quality of RT-PCR product has been obtained with the purity of 1.8 which was determined by measuring the ratio value of absorbance 260 nm and 280 nm(Abs_{260nm}/ Abs_{280nm}) [49].

Fig. 7

The RT-PCR product may contain a mixture of the primers, the samples, the target amplicons and protein as well [2]. Thus, in order to confirm that the current changes is caused by the hybridization of RT-PCR product that contains sequences complementary to the probe region, rather than other sequences in the RT-PCR mixture, a different probe DNA sequence which are non-complementary to the target was run as a control. For this purpose, the 22-mer amine-terminated non-complementary probe DNA with sequence of 5'-NH_2-C_6-GGATGACAAATATCTGCGCTGC-5' which is designed probe for *Escherichia coli* detection [50] was used as a control to determine the specificity of detection between RT-PCR product DNA target with the DNA probe. This non-complementary probe was synthesis by AIT Biotech.

Fig. 7 (c) illustrated the current change of the device after introduced PCR-target DNA (real samples). For reference, the response of the device in the case of 27-mer fully complementary target DNE (synthetic) with the same concentration was tested. An obvious change in resistance approximately 30% was observed for the devices that functionalized with the complementary probe DNA in response to the RT-PCR product of target DNA. Due to the DNA hybridized to the probe immobilized SiNW surface, more negative charge is introduced and resulting in increasing of current. However, it is noted that the signal response for the real samples product (140bp) is much smaller than that of the 27-mer complementary DNA. This might be due to the limitation of Debye length could be found on using long DNA sequence of RT-PCR product (140bp) which affected the device sensitivity [2]. The issues related to the effect of dielectric function on the analyte sensing ability of sensor have been extensively investigated previously [51][52][53]. The works suggest that the sensitivity of electrical biosensing assays rely on the buffer ionic strength and surface charge density. The other factor that affect the hybridization efficiency is the coiling up of molecule resultant from the longer sequences of DNA (more than 30 bases) upon binding on the sensing element [54]. Therefore, the expected hybridization signal may be reduce depending on the oligonucleotide length. The result obtained here is in agreement with the previously reported work [55][56], proving the versatility of this sensor for detection of longer sequences target DNA in real sample application.

In contrast, no significant change was observed when the same target was introduced to the non-complementary DNA probe-functionalized SiNW. The data suggest that nonspecific sensor could not response to the obtained PCR product, supporting that the current changes were indeed caused by the specific sequences of the target to the complementary probe region. From

this viewpoint, we have verified that our developed ssDNA-functionalized SiNW biosensor is highly specific towards DENV through DNA hybridization detection.

4.0 Conclusion

We have demonstrated the molecular electronic based detection of DENV DNA utilizing modified-SiNW surface as a highly selective and ultrasensitive DNA biosensor. The modification of SiNW surface by O_2 plasma treatment represents an effective strategy in improving the SiNW sensing ability. By using this enhancer technique, the results show that the limit of detection for the 60 sec plasma treated-SiNW device could be reduced to 1.985×10^{-14} M as compared to 4.131×10^{-13} M for the untreated-SiNW device. We believe that, these findings could greatly promote the use of plasma treatment as enhancing platforms to enable high-throughput DNA detection. Moreover, the modified SiNW biosensor could distinguish different sequences of 27-mers base targets DENV DNA oligomer and is stable towards the denaturation and re-hybridization circle. The developed biosensor was also able to detect RT-PCR product of DENV in real samples, indicating its potential applications as point-of-care disease diagnostics tool.

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FIGURE LEGENDS

Fig. 1. A schematic diagram of procedure used to develop SiNW biosensor for DNA hybridization detection. (a) A 3D model illustrated the device fabrication based on top-down process using EBL coupled with conventional photolithography. (b) Chemically modified SiNW surface using APTES and GA. (c) DNA immobilization and hybridization steps. (d) Electrical measurement detection using Keithley 6487 Picoammeter probe station based on I-V relations.

Fig. 2. (a) A cross-section view of the etched Si layer formed as nanowire. (b) The optical view of final fabricated sample with three independent NWs segments per measurement. (c) FESEM image of a well aligned SiNW underlying of passivation layer.(d) EDX profile shows a thin oxide layer covers on the SiNW surface.

Fig. 3. (a) Results of contact angle measurement for (i) untreated Si sample (θ=24°), (ii) 30 sec O2 plasma treated Si surface (θ=13°), (iii) 60 sec O2 plasma treated Si surface (θ= 11°) and (iv) 90 sec O2 plasma treated Si surface (θ=9°). (b) AFM images for the (i) 30 sec O_2 plasma treated Si surface (b) 60 sec O_2 plasma treated Si surface and (c) 90 sec O2 plasma treated Si surface.

Fig. 4. (a) The I-V characteristics and (b) FTIR spectra of the plasma treated-SiNW sensors in response to GA/APTES modified.

Fig. 5. Electrical measurement of SiNW biosensor. (a) The current-voltage relations in response to the different target DNA with concentration of 1.0 x 10^{-6} M. (b) Histogram of the relative resistance changes demonstrated the hybridization specificity of complementary, one-base mismatched and non-complementary target DNA at voltage of 2.0 V.

Fig. 6. The plasma treated-SiNW device compared with untreated-SiNW device. (a) The device response towards different concentration of complementary target DNA. (b) Calibration curve of the relative change in resistance with estimated LOD.

Fig. 7. (a) The gel electrophoresis of RT-PCR product of DENV. Lane 1 shows 50 bp DNA ladder (molecular weight in base pair, bp) and Lane 2 shows the approximately 140 bp RT-PCR product. (b) Absorbance spectra of RT-PCR product. (c) Specificity of the RT-PCR product as the complementary target to the different DNA-probe functionalized SiNW biosensor.

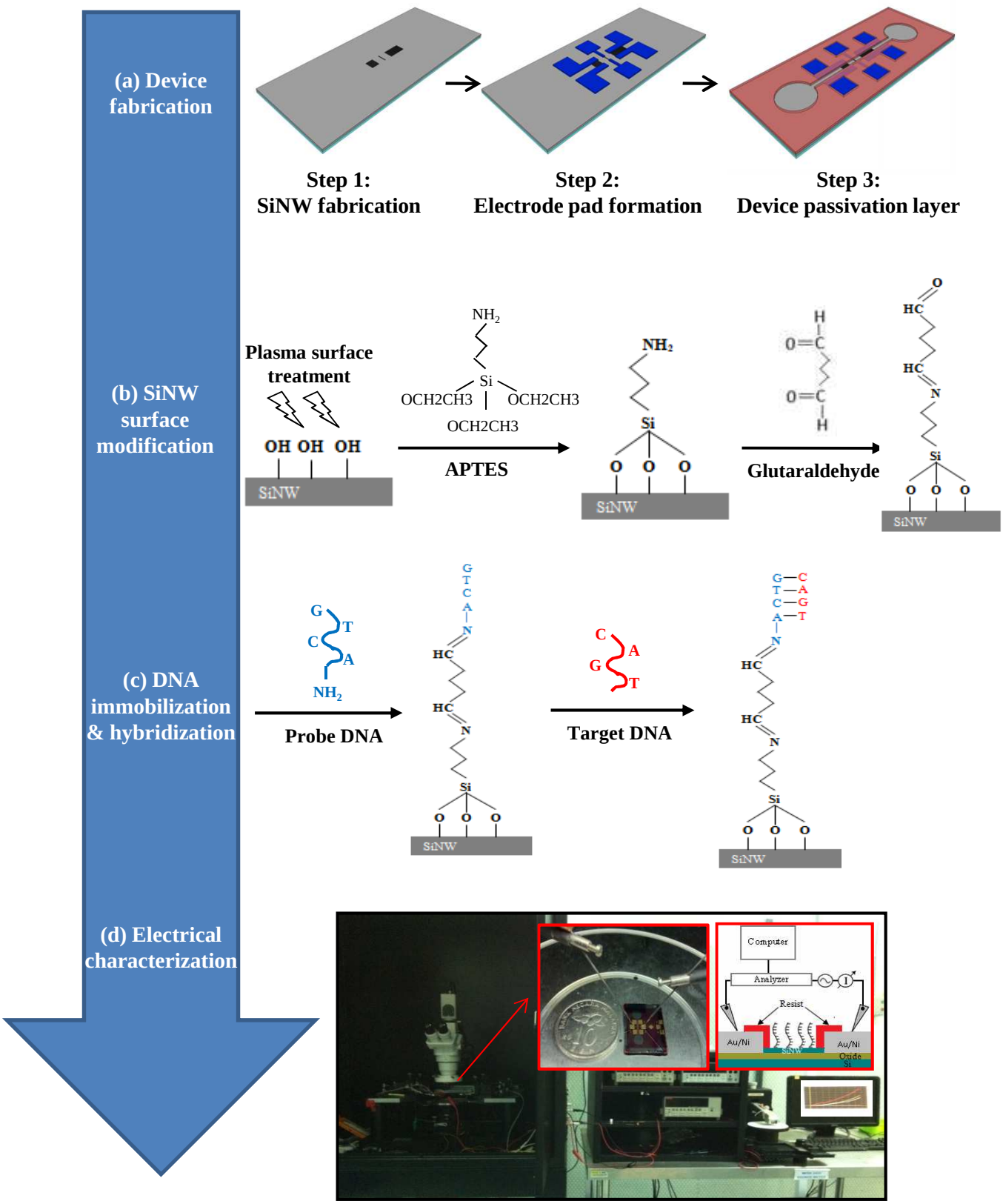
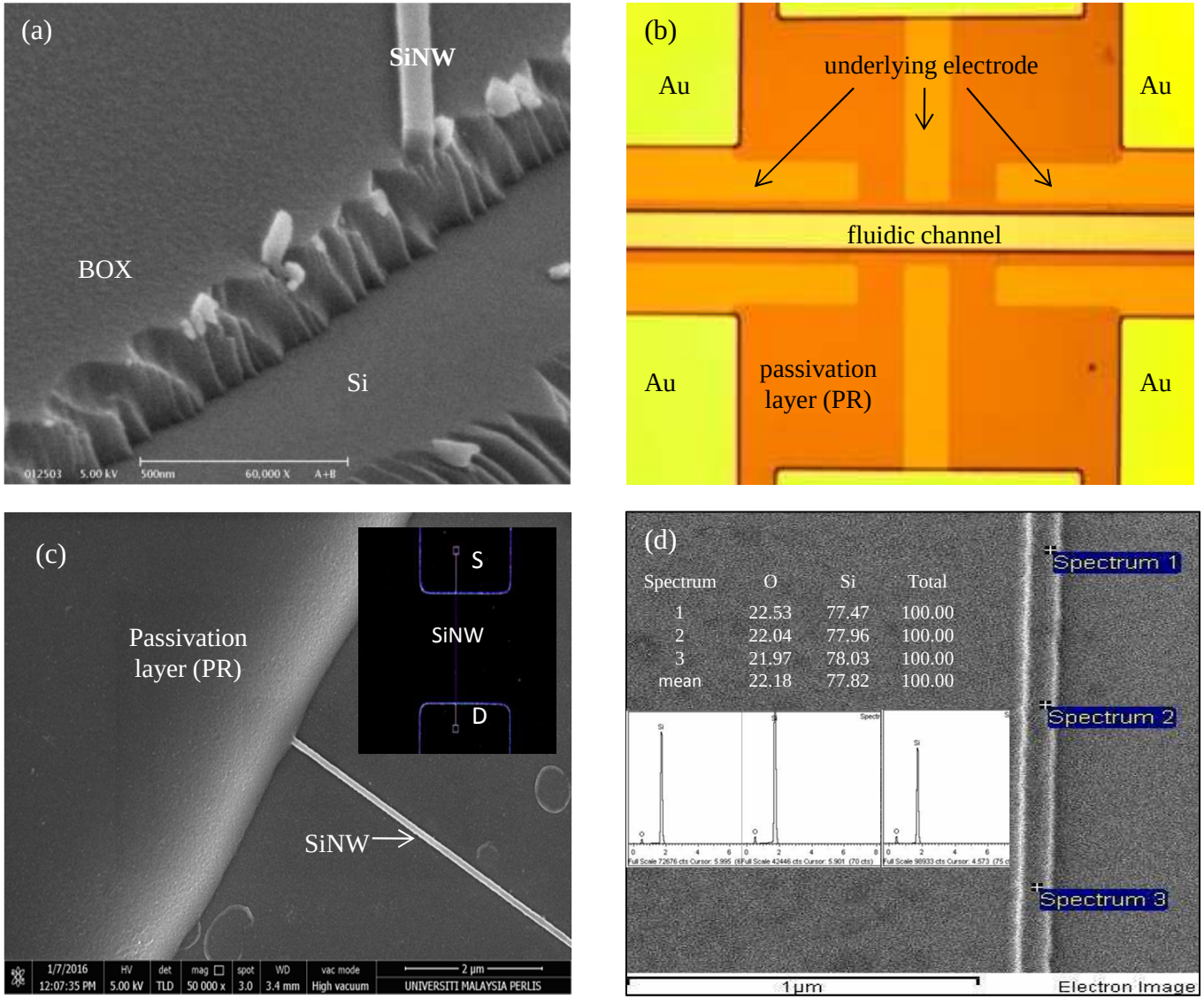
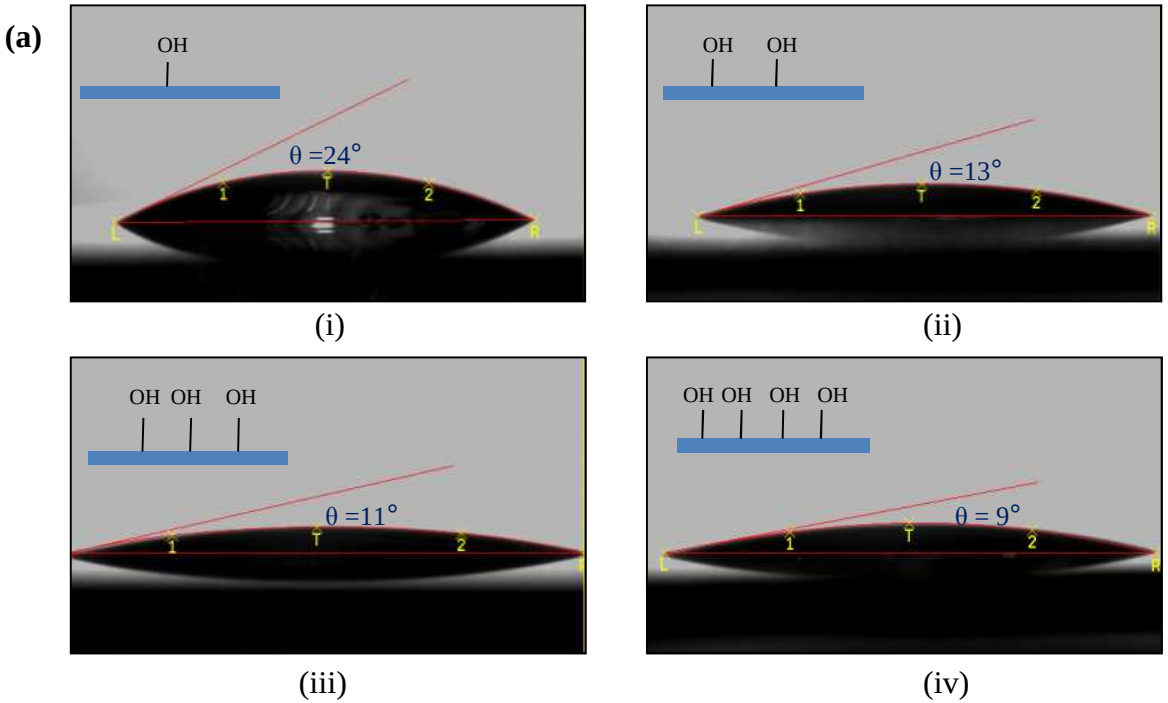

(a) Device fabrication
Step 1: SiNW fabrication
Step 2: Electrode pad formation
Step 3: Device passivation layer
(b) SiNW surface modification
Plasma surface treatment
OH OH OH
SiNW
NH2
Si
OCH2CH3
OCH2CH3
OCH2CH3
APTES
Glutaraldehyde
(c) DNA immobilization & hybridization
Probe DNA
Target DNA
(d) Electrical characterization
Computer
Analyzer
Resist
Au/Ni
Au/Ni
Oxide
Si

Fig. 1



Spectrum	O	Si	Total
1	22.53	77.47	100.00
2	22.04	77.96	100.00
3	21.97	78.03	100.00
mean	22.18	77.82	100.00

Fig. 2



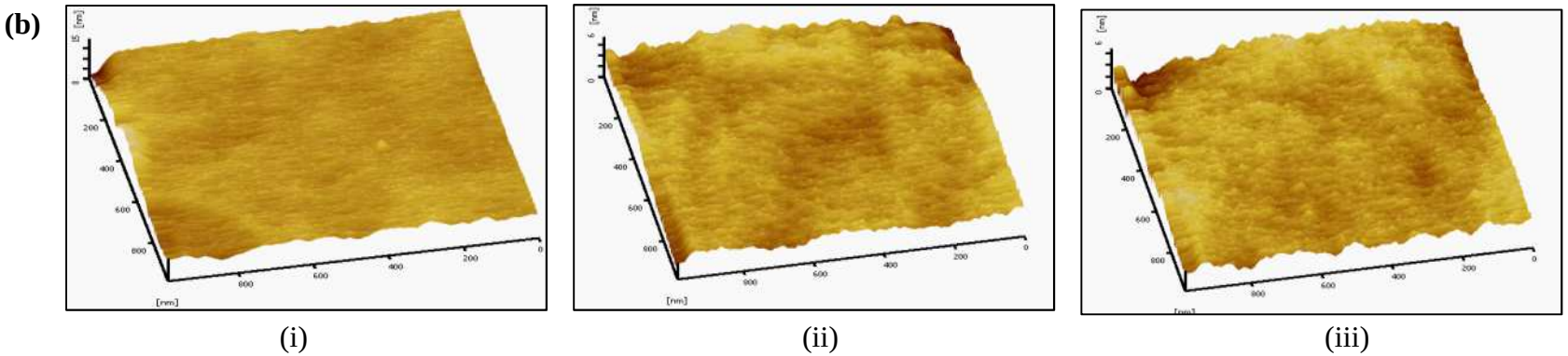


Fig. 3

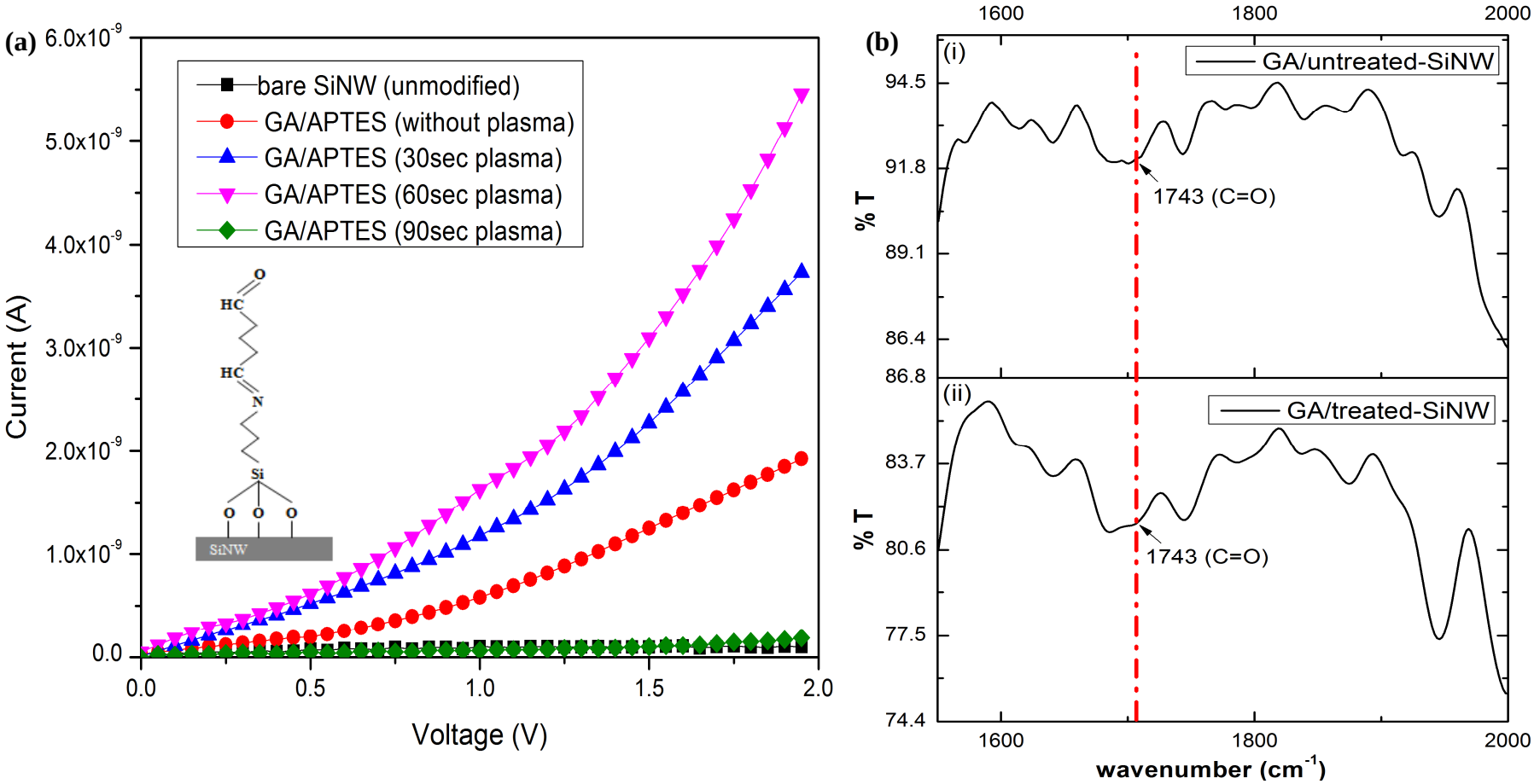

(a)
bare SiNW (unmodified)
GA/APTES (without plasma)
GA/APTES (30sec plasma)
GA/APTES (60sec plasma)
GA/APTES (90sec plasma)
Current (A)
Voltage (V)
SiNW
(b)
(i)
GA/untreated-SiNW
1743 (C=O)
(ii)
GA/treated-SiNW
1743 (C=O)
% T
wavenumber (cm-1)

Fig. 4

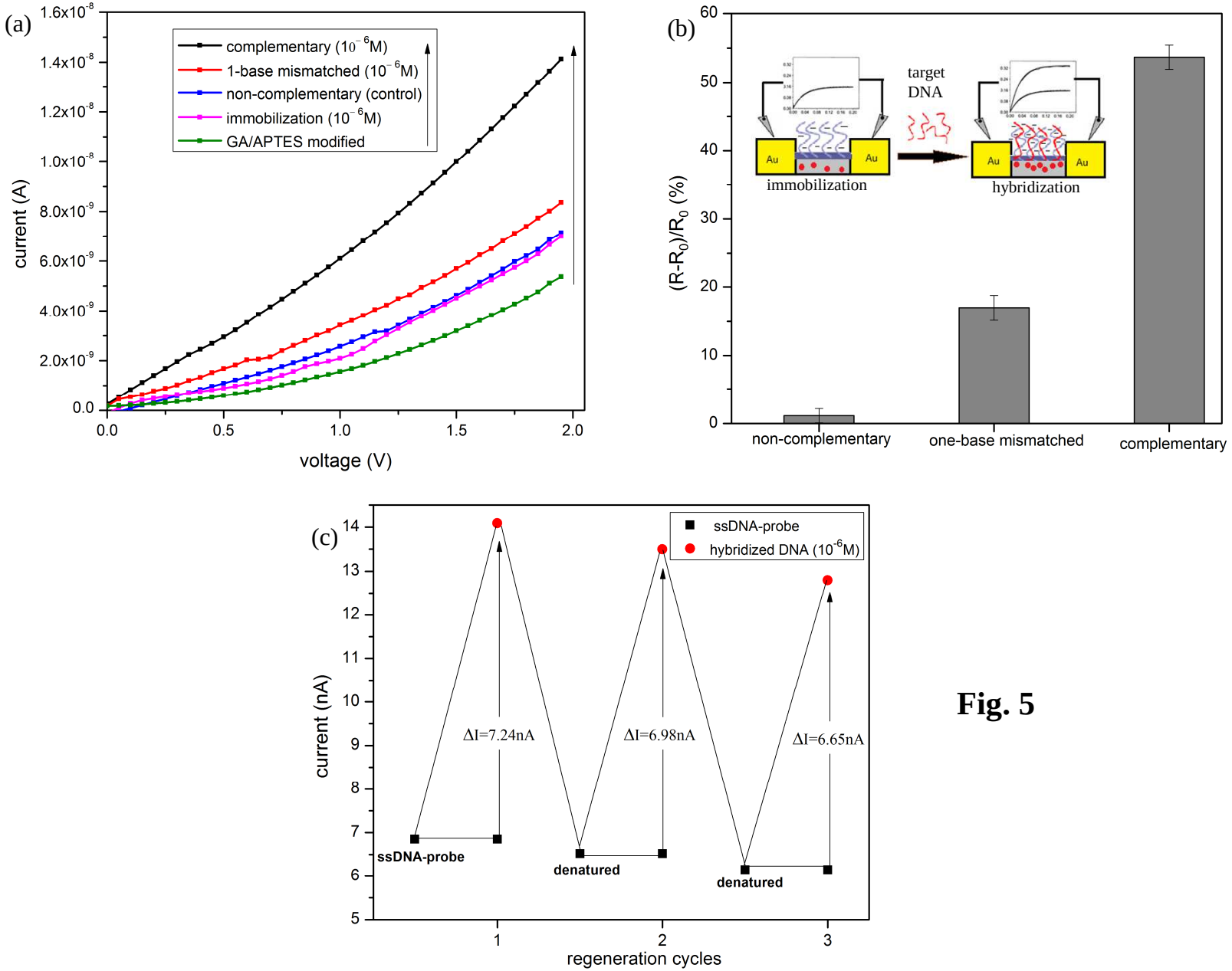

(a)
complementary (10^{-6}M)
1-base mismatched (10^{-6}M)
non-complementary (control)
immobilization (10^{-6}M)
GA/APTES modified
current (A)
voltage (V)
(b)
target DNA
Au
immobilization
hybridization
$(R-R_0)/R_0$ (%)
non-complementary
one-base mismatched
complementary
(c)
ssDNA-probe
hybridized DNA (10^{-6}M)
ΔI=7.24nA
ΔI=6.98nA
ΔI=6.65nA
ssDNA-probe
denatured
denatured
current (nA)
regeneration cycles

Fig. 5

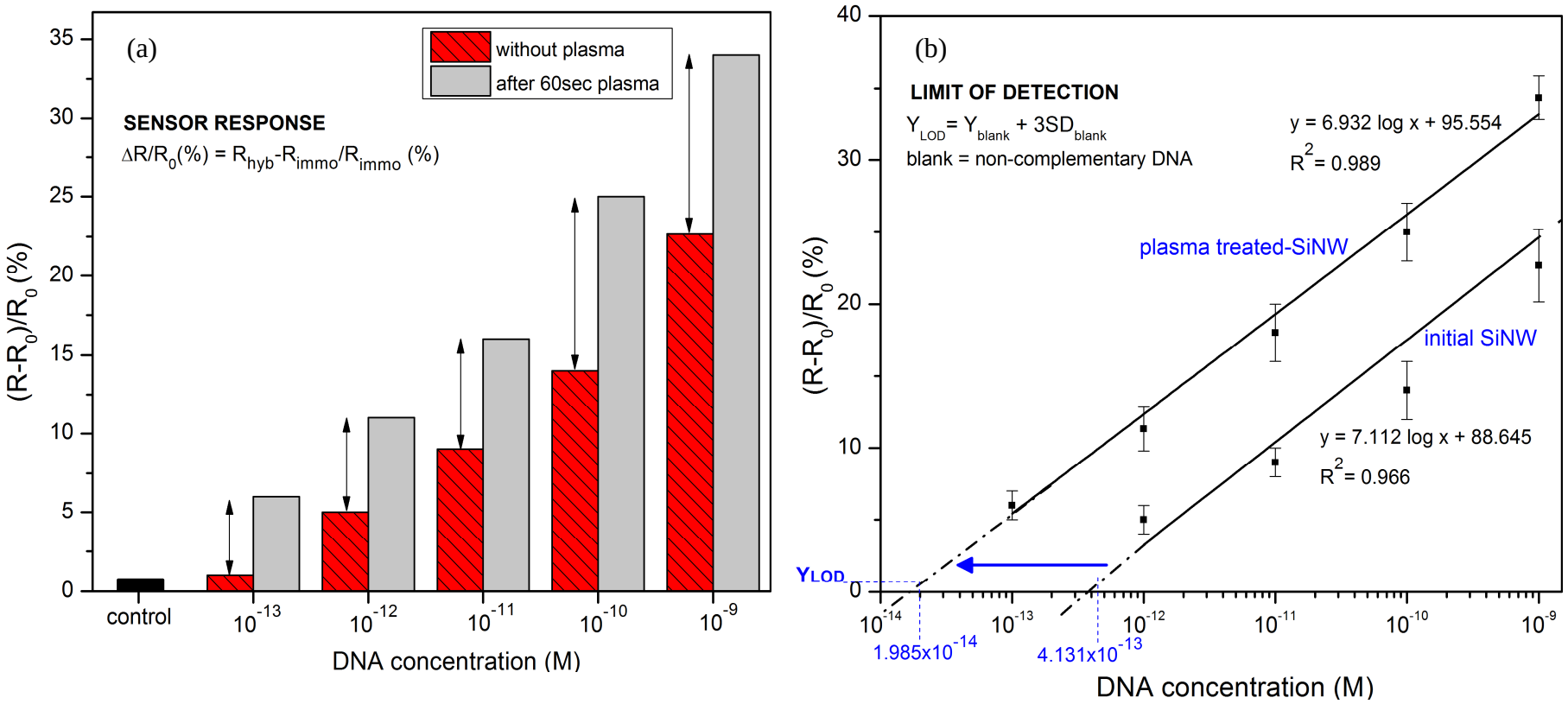

(a)
without plasma
after 60sec plasma
SENSOR RESPONSE
ΔR/R0(%) = Rhyb-Rimmo/Rimmo (%)
(R-R0)/R0 (%)
control
10-13
10-12
10-11
10-10
10-9
DNA concentration (M)
(b)
LIMIT OF DETECTION
YLOD = Yblank + 3SDblank
blank = non-complementary DNA
y = 6.932 log x + 95.554
R2 = 0.989
plasma treated-SiNW
initial SiNW
y = 7.112 log x + 88.645
R2 = 0.966
YLOD
1.985x10-14
4.131x10-13
DNA concentration (M)

Fig. 6

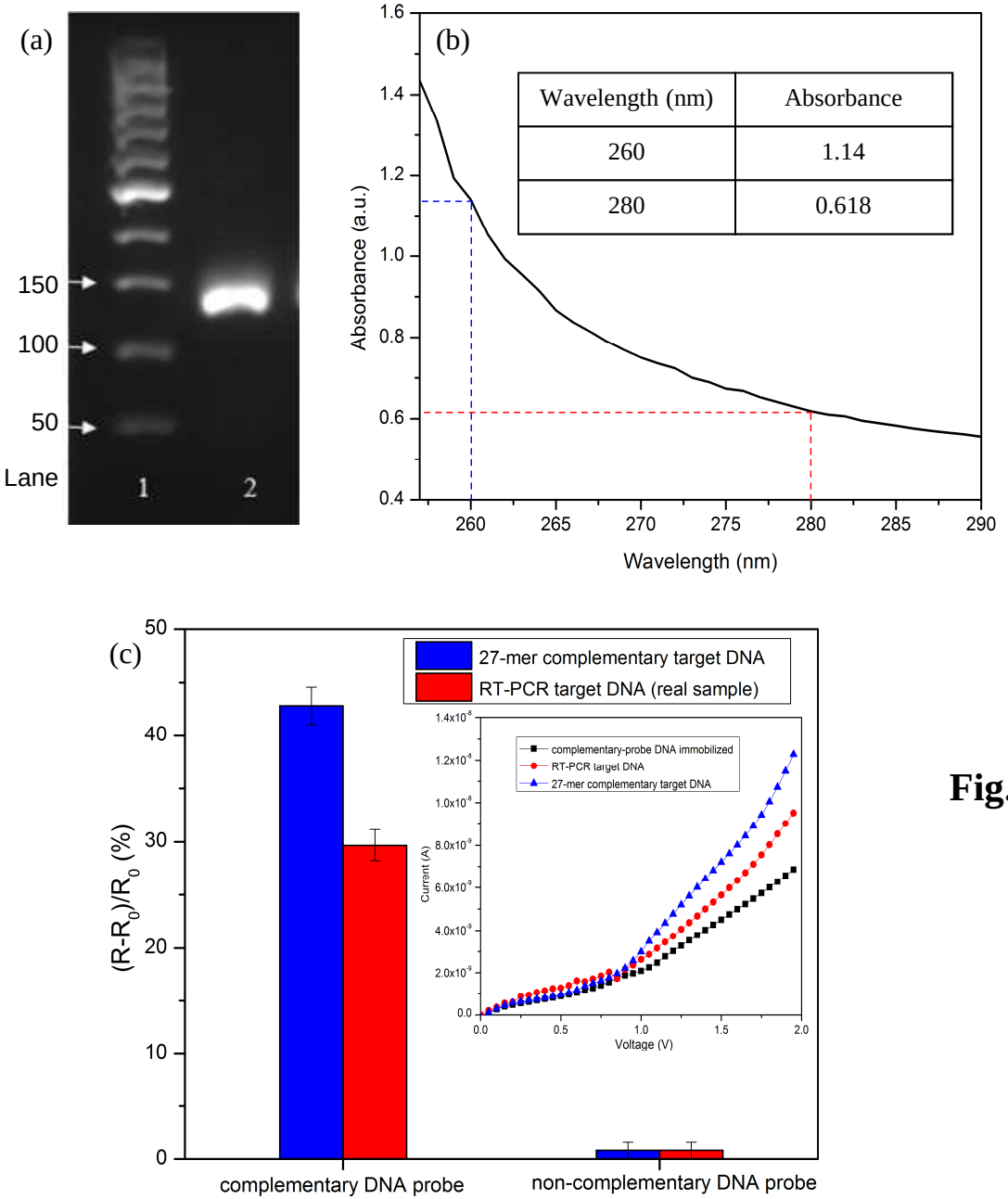

(a)
150
100
50
Lane
1
2
(b)
Wavelength (nm) | Absorbance
260 | 1.14
280 | 0.618
Absorbance (a.u.)
Wavelength (nm)
(c)
27-mer complementary target DNA
RT-PCR target DNA (real sample)
complementary-probe DNA immobilized
RT-PCR target DNA
27-mer complementary target DNA
Current (A)
Voltage (V)
(R-R0)/R0 (%)
complementary DNA probe
non-complementary DNA probe

Fig. 7

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Highlights

- Molecular electronic detection of Dengue Virus (DENV) DNA using SiNW biosensor is presented.
- Oxygen plasma surface treatment as an enhancer technique for device sensitivity is highlighted.
- The limit of detection (LoD) as low as 1.985 x 10^{-14} M for an optimized plasma treated-SiNW device is reported as compared to 4.131 x 10^{-13} M for the untreated-SiNW device.
- The developed sensor is feasible for application of real sample DENV DNA detection.

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