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The utilization of SiNWs/AuNPs-modified indium tin oxide (ITO) in fabrication of electrochemical DNA sensor

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ABSTRACT

This work describes the incorporation of SiNWs/AuNPs composite as a sensing material for DNA detection on indium tin-oxide (ITO) coated glass slide. The morphology of SiNWs/AuNPs composite as the modifier layer on ITO was studied by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). The morphological studies clearly showed that SiNWs were successfully decorated with 20 nm-AuNPs using self-assembly monolayer (SAM) technique. The effective surface area for SiNWs/AuNPs-modified ITO enhanced about 10 times compared with bare ITO electrode. SiNWs/AuNPs nanocomposite was further explored as a matrix for DNA probe immobilization in detection of dengue virus as a bio-sensing model to evaluate its performance in electrochemical sensors. The hybridization of complementary DNA was monitored by differential pulse voltammetry (DPV) using methylene blue (MB) as the redox indicator. The fabricated biosensor was able to discriminate significantly complementary, non-complementary and single-base mismatch oligonucleotides. The electrochemical biosensor was sensitive to target DNA related to dengue virus in the range of 9.0–178.0 ng/ml with detection limit of 3.5 ng/ml. In addition, SiNWs/AuNPs-modified ITO, regenerated up to 8 times and its stability was up to 10 weeks at 4 °C in silica gel.

1. Introduction

Nucleic acid detection based on DNA hybridization in the field of biosensor has gained attention by some researchers for various applications as clinical diagnostic [1,2], gene analysis [3,4], environmental monitoring [5,6], forensic analysis [7] and food monitoring [8]. DNA biosensors can offer an accurate and rapid detection, fast responses, easy operation, and low detection in real samples [9,10]. There are many kinds of DNA sensors based on signal transducers [11,12]. Electrochemical sensors describe as high sensitive, selective, portable, simple, cost effective, good stability, low-cost instrumentation, small dimensions, on-site monitoring and ease to miniaturization [13]. In electrochemical detection of DNA, single stranded DNA (ssDNA) acts as recognition molecules and immobilizes on the surface of working electrode that captures specific DNA target. The hybridization event converts into measurable voltammetric signal. Therefore, the signal difference generated upon hybridization process is the main analytical response in electrochemical biosensors.

Since the electrochemical DNA sensor consists of an active sensing material incorporated within a working electrode and a signal transducer, the selection of an active sensing material should be emphasized. With the tremendously growing of nanotechnology, many kinds of sensing nanomaterials with unique properties have been fabricated and explored for new electrochemical sensor development. For instance, the utilization of one dimensional structure (1D) of nanowires for electrode modification can enhance the performance of DNA electrochemical sensors in terms of sensitivity and conductivity. A few studies demonstrated the application of nanowires in electrochemical DNA sensors such as polyaniline nanowires [14], diamond nanowires [15], graphene/polyaniline nanowires [16], GaN nanowires [17], ZnO nanowires [18], Au nanowires [19] and CoPtP/Au nanowires [20].

In recent years, silicon nanowires (SiNWs) have been employed as sensing nanomaterials for the construction of ultrasensitive sensors due to owning high surface area [21]. According to Rashid et al. [22] SiNWs exhibited excellent electrical and optical properties, fast electron transfer, and favorable biocompatibility making great promises in the future for sensing applications. Most DNA sensors employed SiNWs based on field effect transistor (FET) detection [23] and Surface-Enhanced Raman Spectroscopy (SERS) techniques [24]. Up to now, there isn't any report in literatures on the functionalization of SiNWs for DNA hybridization detection using electrochemical biosensors. This is probably due to the challenging steps on immobilization of

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ssDNA probes on the surface of SiNWs based electrodes with suitable orientation and stability to capture DNA targets. In this project, the surface of SiNWs was decorated with gold nanoparticles (AuNPs) in order to enhance the amount of immobilized ssDNA probe due to the binding of AuNPs with thiolated-DNA [25]. Yan et al. [26] have developed a novel non-enzymatic hydrogen peroxide (H_2O_2) sensor utilized nickel/SiNWs as composite working electrode. The incorporation of SiNWs/nickel showed high catalytic electro-oxidation towards H_2O_2 and improved sensitivity. Similarly, the utilization of SiNWs in electrochemical sensors enhanced the conductivity of electrode for electron transfer in detection of dopamine and ascorbic acid [27], glucose [28], glutamate [29] and ethanol [30].

To our knowledge, there are no reports on the development of DNA electrochemical sensor utilized SiNWs decorated AuNPs on indium tin oxide (ITO) surface as sensing material. In this work, self-assembly monolayer technique based on thiol–AuNPs interaction was used for binding AuNPs to SiNWs. Then, thiolated probes with different sequences bonded on gold surfaces for loading more immobilized DNA probes on ITO substrate. The complementary target DNA was introduced onto the probe DNA–SiNWs/ITO for hybridization process using methylene blue as the redox indicator.

2. Experimental section

2.1. Materials and reagents

Indium tin oxide coated glass slide was fabricated in Laboratory and Scientific Enterprise (Malaysia) with sheet resistance and thickness of <7 Ω/sq and 1.1 mm, respectively. Silicon nanowires in isopropyl alcohol suspension (D × L; 150 nm × 20 μm), gold chloroauric acid salt ($HAuCl_4 \cdot 4H_2O$), potassium ferricyanide (III), sodium citrate, (3-aminopropyl) triethoxysilane (APTES), 3-3′-dithiopropionic acid (DTPA), hydrogen peroxide (H_2O_2) (30% w/w in H_2O) and ammonium hydroxide (NH_4OH) (30% w/w), were purchased from Sigma-Aldrich (USA). Methylene blue (MB) was purchased from R&M Chemicals (Essex, UK). Synthetic DNA sequences were purchased from First BASE Laboratories Sdn Bhd, (Selangor, Malaysia) as probe ssDNA (5′-SH-$(CH_2)_6$-AAC AGC ATA TTG ACG CTG GGA GAG ACC-3′); target complementary DNA (5′-GGT CTC TCC CAG CGT CAA TAT GCT GTT-3′); one-base mismatched DNA (5′-GGT CTT TCC CAG CGT CAA TAT GCT GTT-3′) and non-complementary (5′-TTC TGT GTT AGT ATC TGG GCC ATG TCC-3′). Synthetic DNA solutions (100 μM) were prepared in a TE buffer solution (10 mM Tris-(hydroxymethyl) aminomethane–HCl (Tris–HCl) + 1 mM EDTA (pH 8.0)) (Sigma, USA) and kept frozen. All other chemicals were of analytical reagent grades. All aqueous reagents were prepared in sterilized ultra pure water (Mili Q ultrapure water system, Millipore Billerica, MA, USA).

2.2. Apparatus

Electrochemical potentiostat (μAUTOLAB, Metrohm, Netherlands) controlled by general purpose electrochemical system (GPES) software version 4.9 (Eco Chemie) was used for electrochemical measurements. The electrochemical experiments were carried out in 50 ml cell consisting of conventional three electrodes system with ITO coated glass slide as working electrode, platinum (Pt) as an auxiliary electrode and a Ag/AgCl (3 M KCl) reference electrode. The characterization of SiNWs/AuNPs/ITO surface was performed using scanning electron microscopy–energy dispersive X-ray (SEM–EDX) (Philips XC30-ESTM, USA).

2.3. Synthesis of citrate capped gold nanoparticles (AuNPs)

The citrate capped gold nanoparticles (AuNPs) with the average diameter of 20 nm were prepared according to the modified method by Nasir and Hadi [31]. Briefly, 0.04 g of gold chloroauric salt ($HAuCl_4 \cdot 4H_2O$) was dissolved in 100 ml of distilled water and stirred followed by heating to boiling temperature. Then, 10 ml sodium citrate (38.8 mM) solution was added to the boiling $HAuCl_4 \cdot 4H_2O$ solution until the color of solution changed from yellow to dark blue and finally turned to deep red color due to the successful reduction of gold (III) ions to gold nanoparticles. The citrate capped AuNPs solution was stirred at boiling temperature for 5 min and cooled down to room temperature before keeping it in a refrigerator (4 °C).

2.4. The modification of ITO substrate with SiNWs and AuNPs

ITO coated glass slides were cut in 0.5 cm × 2.0 cm using a diamond glass cutter. Each ITO slide was sonicated sequentially in acetone for 20 min, 2-propanol (5 min) and deionized water (5 min) following dried under nitrogen stream. The pretreated ITO was soaked in a mixture solution containing H_2O, NH_4OH (30%) and H_2O_2 (30%) in the ratio of 5:1:1 for 10 min before rinsing with deionized water and dried under N_2 gas. The stock suspension of SiNWs was diluted in APTES solution to make SiNWs suspension in 0.5% APTES. 10 μl of SiNWs in 0.5% APTES was drop casted onto an exposed area of ITO surface (0.5 cm × 0.5 cm) and incubated for 24 h at room temperature. Then, it was washed thoroughly with ethanol and calcinated at 100 °C for 30 min. The working ITO–SiNWs electrode was then modified with self-assembly monolayer (SAM) of DTPA by immersion of the exposed area of ITO–SiNWs in 5 mM DTPA solution for 24 h in dark room at room temperature. Subsequently, the working ITO–SiNWs electrode was decorated by AuNPs by incubation in AuNPs suspension for 2 h then rinsed with deionized water and dried under nitrogen stream. The electrochemical characterization of SiNWs/AuNPs-modified ITO was carried out using cyclic voltammetry (CV) in 1.0 mM $[Fe(CN)_6]^{3-/4-}$ containing Tris–HCl buffer (pH 7.8) in the potential range of −0.6 to 0.6 V with a scan rate of 0.1 V/s. The SiNWs/AuNPs-modified ITO surface was then characterized and verified by SEM and EDX techniques.

2.5. Immobilization and hybridization of DNA onto SiNWs/AuNPs-modified ITO

The immobilization of ssDNA probe on the surface of SiNWs/AuNPs-modified ITO surface was carried out by drop casting 20 μl of thiolated ssDNA probe (3 μM) in TE buffer solution (pH 8.0) for 24 h at room temperature. It was then washed with TE buffer to remove any unbounded thiolated ssDNA probe and then was denoted as ITO/SiNWs/AuNPs/ssDNA. For the hybridization events, 20 μl of target complementary DNA in TE buffer (pH 8.0) was incubated on the surface of ITO/SiNWs/AuNPs/ssDNA for 2 h at 40 °C [32]. It was then followed by washing the excess of target DNA complementary with TE buffer solution, distilled water and dried with N_2 gas.

2.6. Electrochemical DNA detection using methylene blue as the redox hybridization indicator

At first, the constructed biosensor ITO/SiNWs/AuNPs/DNA was immersed into 50 μM MB containing Tris–HCl solution (pH 7.8) for 20 min in an open circuit condition. Secondly, the hybridized DNA modified ITO was rinsed with 50 mM Tris–HCl buffer (pH 7.8) to remove the excess of non-bonded MB and dried with N_2 gas. Thirdly, the modified ITO electrode quickly soaked in the blank supporting electrolyte and its electrochemical signal was recorded by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). DPV measurement was carried out in the potential range of −0.5 V to 0.0 V, step potential of 0.005 V, and modulation amplitude of 0.5 V with the interval time of 0.64 s at room temperature.

3. Results and discussions

3.1. The fabrication of electrochemical DNA biosensor based on SiNWs/AuNPs modified ITO

Fig. 1 shows the fabrication process of SiNWs/AuNPs modified ITO. The cleaning bare ITO was soaked in a mixture solution of H_2O, NH_4OH and H_2O_2 to activate hydroxyl groups (–OH) on ITO surface, then modified with APTES-SiNWs suspension via a silanization process. The modified ITO was denoted as SiNWs-ITO. Self-assembly monolayer technique based on thiol–AuNPs interaction was used in this study due to an ultra thin and well-ordered layer of thiol group on SiNWs surface for gold nanoparticles decoration. The modification process was carried out by introducing thiol groups and dithiopropionic acid (DTPA) solution which has two functional groups of thiol at the terminated end of SiNWs surface. The thiolated probe ssDNA was immobilized on the surface of AuNPs/SiNWs-modified ITO by bonding AuNPs to thiol groups. The DNA immobilization strategy based on Au–S bonding has been previously reported as a simple technique, and can provide a strong covalent bonding between thiolated probe DNA and gold surface for loading more immobilized DNA probes on AuNPs surface. The complementary target DNA was introduced into the probe DNA–SiNWs/ITO for hybridization process. The hybridization process was transfered into electrochemical signal using the redox indicator, methylene blue which is able to incorporate at DNA surface. The peak current of MB decreased significantly after hybridization process as a result of different properties of MB binding on DNA surface.

3.2. SEM image and EDX analysis of SiNWs/AuNPs modified ITO

SiNWs/AuNPs composite material was used to modify bare ITO template in order to enhance DNA sensing performances. Fig. 2(b) shows the morphology surface of SiNWs/AuNPs-modified ITO. It was observed that the distribution of SiNWs/AuNPs composite is not homogenous, which results to some aggregated areas on ITO surface. This is probably due to the fact that amine groups on SiNWs tend to aggregate to each other when functionalized on ITO surface in the silanization process. Also, it maybe due to the weak dispersion of SiNWs in 0.5% APTES solution during sonication. It was supported by Rashid et al. [22] who found out that it is quite a challenge to control the nanowires suspension distribution (align) uniformly onto the electrode surface. Meanwhile, Fig. 2(b) inset demonstrated the decoration of AuNPs on SiNWs-modified ITO surface, for the purpose of binding site for ssDNA probe and thus promoting the hybridization event. The presence of SiNWs/AuNPs composite was additionally confirmed using energy dispersive X-Ray spectroscopy (EDX). The amount of Si and Au elements was achieved around 35.81% and 6.46% from the total percentage weight, respectively. The highest of EDX peak for AuNPs decorated SiNWs was shown (Fig. 2(b)) at 1.8 KeV, indicating that SiNWs/AuNPs-modified ITO was successfully fabricated.

3.3. Electrochemical properties of SiNW/AuNP-modified ITO in $[Fe(CN)_6]^{3-/4-}$ solution

Potassium ferricyanide solution was used to evaluate the electrocatalytic performance of SiNWs/AuNPs composite coated ITO electrode using CV technique. Ferrocyanide ion has been widely known as a good redox indicator for the characterization of redox properties of modified electrodes [33] due to its reversibility and fast electrochemical responses.

3.3.1. Effect of supporting electrolyte

The role of supporting electrolyte in electrochemistry is threefold: to increase conductivity, eliminate the transport of electroactive species by

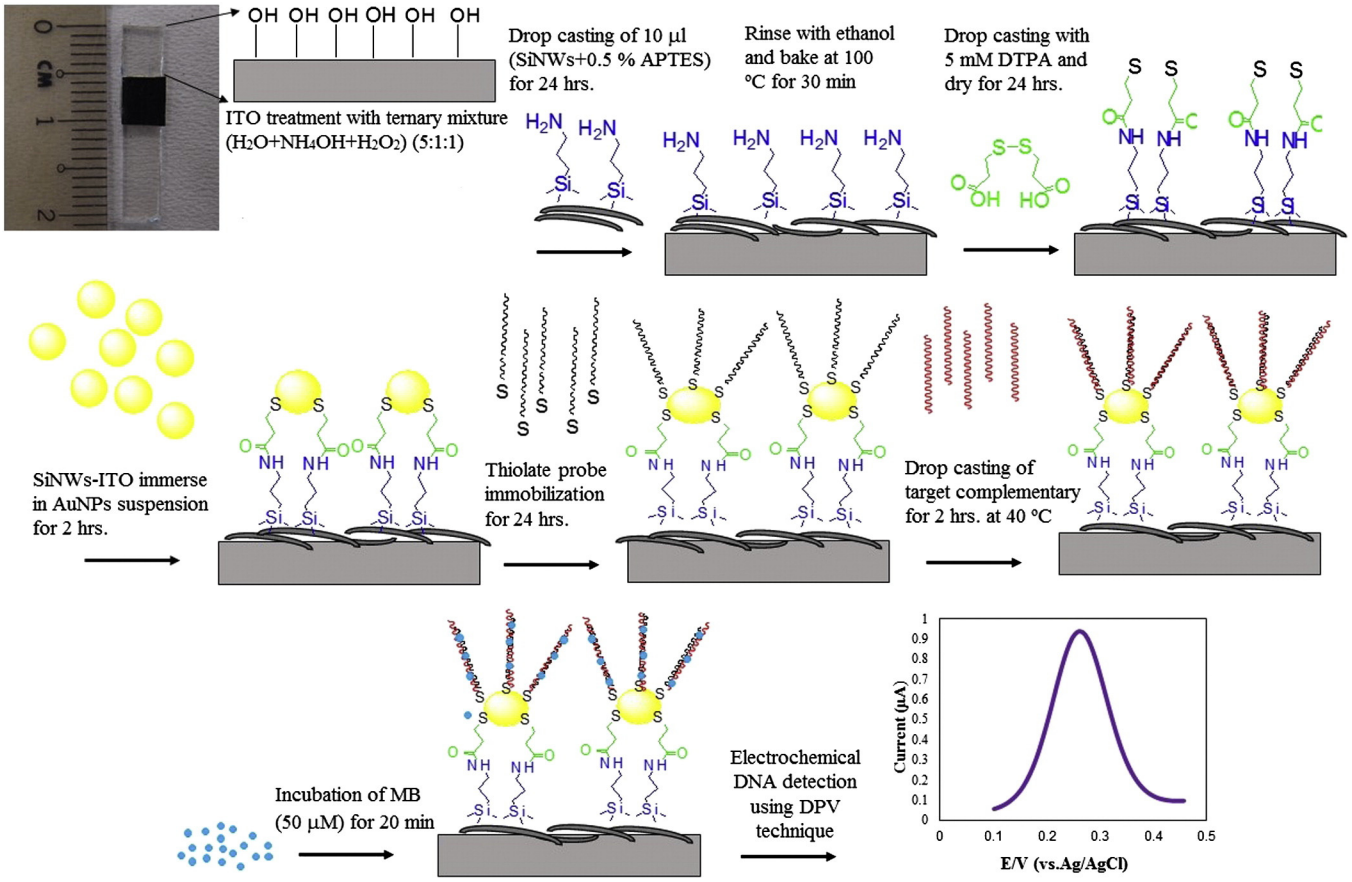

Fig. 1. The fabrication process of SiNWs/AuNPs modified ITO electrode for electrochemical biosensor.

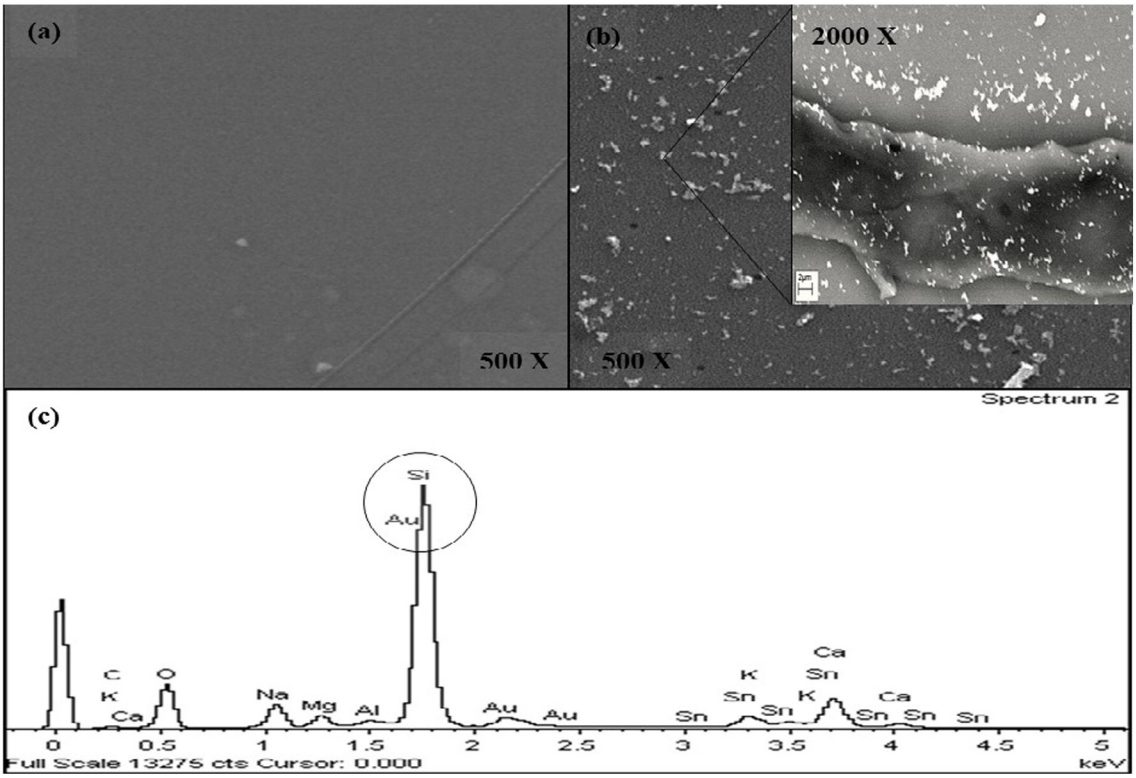

Fig. 2. a) SEM image of bare ITO; b) SEM image of SiNWs/AuNPs modified ITO morphology at magnification of 500×. Inset is SiNWs/AuNPs modified ITO at the magnification of 2000×; c) EDX spectrum from the surface of SiNWs/AuNPs modified ITO.

ion migration in the electric field and maintain pH and ionic strength. The reduction and oxidation peaks of $[Fe(CN)6]^{3-/4-}$ ion at SiNWs/AuNPs-modified ITO surface are significantly affected by using different kinds of supporting electrolytes, herein Tris–HCl buffer solution produced peak currents of 110.39 ± 1.32 μA and 114.4 ± 0.84 μA for reduction and oxidation reactions respectively (Table 1). Therefore, Tris–HCl buffer solution was chosen as the optimized supporting electrolyte to enhance higher sensitivity. In general, the degree of redox currents was shown in the following order for different supporting electrolytes:

Tris–HCl > KCl > PBS > Tris–EDTA > NaCl.

3.3.2. Effect of pH

The effect of pH on the redox peak currents of potassium ferrocyanide on the surface of SiNWs/AuNPs nanocomposite coated ITO electrode was carried out in 50 mM Tris–HCl solution in the pH range of 2–12. Generally, the reduction peak current of $[Fe(CN)_6]^{3-/4-}$ was significantly increased from pH 2.0 to 8.0 and then decreased at pH > 8.0 (Fig. 3). Therefore, pH of 8.0 was selected as the optimized pH value for DNA sensor application.

Table 1
Oxidation and reduction peaks for 1.0 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 50 mM of different supporting electrolytes.

Supporting electrolyte	SiNWs/AuNPs-ITO	
	Reduction peak current (I_{pc})	Oxidation peak current (I_{pa})
KCl	98.22 ± 1.99	112.76 ± 5.36
Tris–HCl	110.39 ± 0.19	114.40 ± 0.84
PBS	87.52 ± 2.73	98.65 ± 4.77
Tris–EDTA	77.54 ± 3.97	89.48 ± 3.54
NaCl	72.31 ± 0.43	76.38 ± 1.17

3.3.3. Stability of fabricated biosensor

The stability of SiNWs/AuNPs modified ITO was performed in 30 successive cyclic voltammetries in the potential range of −0.4 V to +0.8 V with the scan rate of 100 mV/s in 1 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 50 mM Tris–HCl. The results showed that the oxidation and reduction peaks for $[Fe(CN)_6]^{3-/4-}$ on SiNW/AuNP modified ITO were stable for more than 30 cycles due to the covalently binding of SiNWs/AuNPs on ITO electrode surface.

3.4. The electrochemical characteristic of modified ITO electrode

The electrochemical behavior of SiNWs/AuNPs modified ITO electrode have been studied by cyclic voltammetry in $[Fe(CN)_6]^{3-/4-}$ (1.0 mM) containing 50.0 mM Tris–HCl at pH 8.0. Fig. 4 shows that

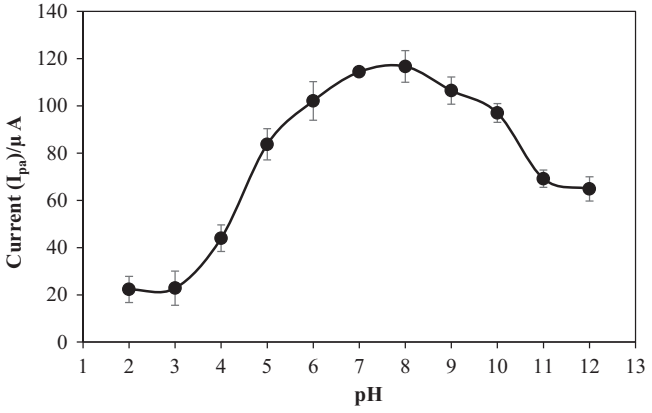

Fig. 3. Effect of pH on the reduction peak current of $[Fe(CN)_6]^{3-/4-}$.

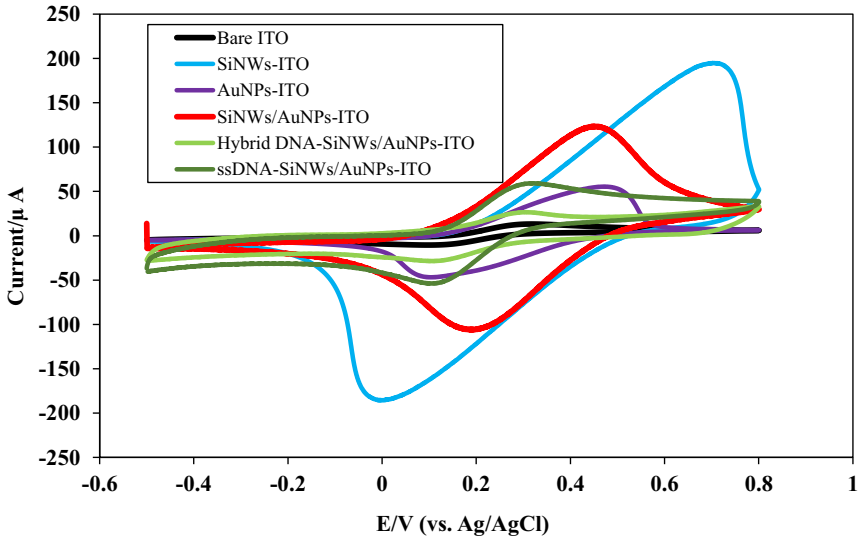

Fig. 4. Cyclic voltammetry responses of different kinds of modified ITO electrodes in 1.0 mM potassium ferrocyanide containing 50 mM Tris–HCl solution at scan rate of 100 mV/s.

bare ITO electrode produced a pair of well-defined redox peaks due to the oxidation and reduction of $[Fe(CN)_6]^{3-/4-}$. Both cathodic and anodic peak currents were greatly enhanced after modification with SiNWs due to the excellent electrical properties of SiNWs with peak separation of 0.72 V. The strategy of using SAM of dithiopropionic acid (DTPA) for AuNPs decorated on SiNWs surface resulted on a decrease in redox peak current of $[Fe(CN)_6]^{3-/4-}$ due to the formation of thiol monolayer on electrode surface that can hinder the electron transfer between redox indicator species and the modified electrode [34]. However, a smaller peak separation of 0.27 V after decoration of SiNWs with AuNPs indicated the catalytic effect of AuNPs in electron transfer with SiNWs.

The role of SiNWs on enhancement of electrode conductivity was confirmed by a control experiment without using SiNWs (AuNPs-modified ITO) where the redox peak currents of potassium ferrocyanide on AuNPs-modified ITO were lower than SiNWs/AuNPs-modified ITO (Fig. 4). The decrease in redox peak currents happened after the immobilization of thiolated probe DNA on the surface of SiNWs/AuNPs-modified ITO. This is attributed to the introduction of negative charge phosphate backbone of probe DNA contributed to the repulsion of $[Fe(CN)_6]^{3-/4-}$ ion on the surface of SiNWs/AuNPs-modified ITO. It was clearly showed that the thiolated probe DNA was successfully immobilized on modified electrode surface. As shown in Fig. 4, upon the hybridization of target DNA and thiolated probe DNA, a further decrease in peak current for $[Fe(CN)_6]^{3-/4-}$ was obtained due to more electrostatic repulsion force between ferrocyanide and SiNWs/AuNPs-modified ITO electrode. Besides that, the formation of a monolayer of DNA complementary would probably limit the diffusion of $[Fe(CN)_6]^{3-/4-}$ ion on modified electrode which results to the suppression of redox peak currents.

The effective surface area of bare ITO and SiNWs/AuNPs-modified ITO was calculated based on the Randles–Sevcik equation [35] (Fig. 5). The anodic peak current (I_{pa}) of $[Fe(CN)_6]^{3-/4-}$ increased linearly with square root of scan rate in 1 mM $[Fe(CN)_6]^{3-/4-}$ solution. The effective surface area values of bare ITO and SiNWs/AuNPs-modified ITO were calculated as 0.0503 cm^2 and 0.542 cm^2. It was clearly showed that the utilization of SiNWs/AuNPs composite as electrochemical sensing material can enhance the effective surface area for about 10 times. Fig. 5 shows that the reduction peak current of $[Fe(CN)_6]^{3-/4-}$ on SiNWs/AuNPs modified ITO was proportional to the square root of scan rate ($\nu^{1/2}$) which indicated that the electrochemical reaction of SiNWs/AuNPs modified ITO is under a diffusion control process [35].

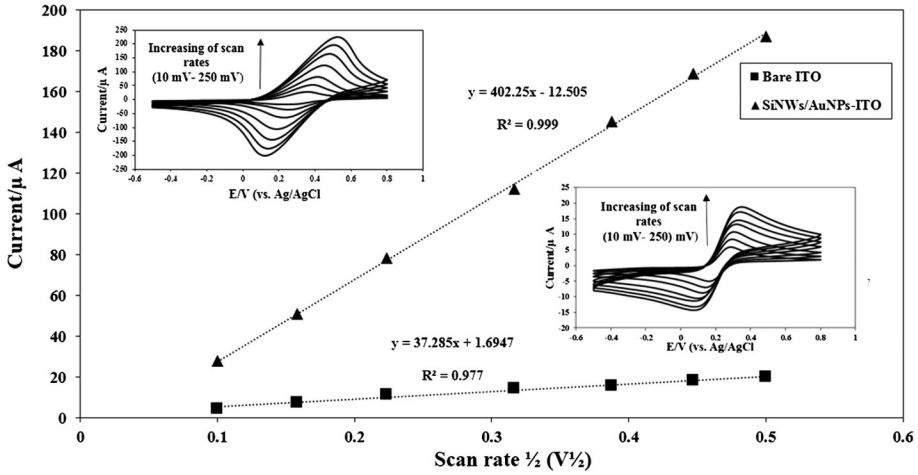

Fig. 5. The plot of reduction peak current of $[Fe(CN)_6]^{3-/4-}$ against square root of scan rate. Inset is CV of $[Fe(CN)_6]^{3-/4-}$ at different scan rates on SiNWs/AuNPs-modified ITO.

3.5. The application of SiNWs/AuNPs-modified ITO based-electrochemical biosensor in DNA dengue detection using methylene blue

Differential pulse voltammetry (DPV) technique was used in this study to monitor the current changes of DNA labeled with MB on modified electrode to provide higher selectivity and sensitivity for fabricated electrochemical biosensor. Methylene blue has been reported by the work of Erdem et al. [36], who found out that the utilization of MB as redox indicator in electrochemical sensor was cost effective, require simple operation and provide rapid detection. As shown in Fig. 6, the highest DPV peak current of MB was obtained after immobilization of thiolated single stranded DNA onto SiNWs/AuNPs-modified ITO surface. However, upon the hybridization with dengue target DNA at the concentration of 0.88 ng/ml, the peak current of MB obviously reduced from 39.24 ± 3.28 μA to 12.9 ± 3.19 μA (Fig. 6). This finding assumed that MB was more accumulated on the surface of ssDNA–SiNWs/AuNPs-modified ITO (before hybridization) compared with the dsDNA–SiNWs/AuNPs-modified ITO. It was supported by the previous work, MB showed strong affinity to the exposed of free guanine bases at ssDNA surface and also adsorbed by the electrostatic binding to negative charge of ssDNA [35,36]. Therefore, the suppression of MB peak current after hybridization (inset Fig. 6) was probably due to the less accumulation of MB on dsDNA–SiNWs/AuNPs-modified ITO since the interaction between guanine bases and MB was inaccessible. In contrast, there are other previous studies with different outcomes where the current of MB was higher after DNA hybridization process on modified electrode [36]. Most of the previous studies reported that MB has different modes of binding towards DNA where the intercalative and groove binding take places among dsDNA and MB, leading to high accumulation of MB on dsDNA surface. Some experimental conditions (ionic strength, pH, type of buffer, DNA concentration) affect the mode of interaction between MB and DNA [37].

In the presence of non-complementary dengue target DNA, the DPV peak current of MB didn't show any obvious changes in comparison with dengue target DNA (Fig. 6), indicating that there was no hybridization process. The peak current of MB decreased in the following order in the presence of targets, "without dengue target DNA > non-complementary dengue target DNA > one-base mismatch > dengue target DNA". This result indicated that the utilization of MB as the redox indicator in the developed sensor enable us to detect dengue target DNA.

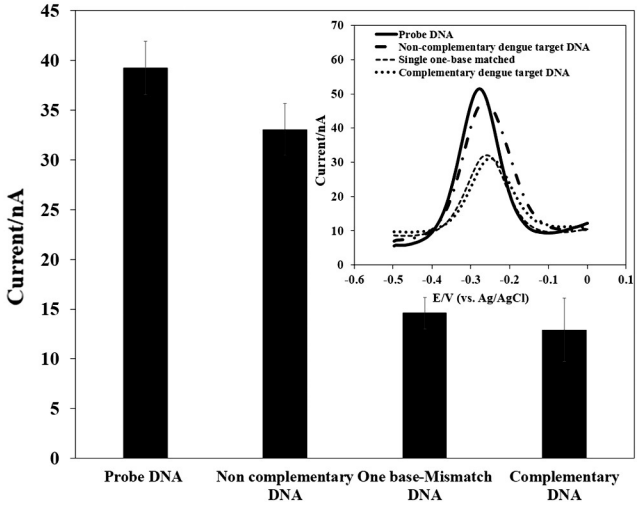

Fig. 6. Histogram of the MB reduction peak current using DPV at different types of DNA in 50 mM Tris–HCl and 50 μM of MB at scan rate of 100 mV/s. Inset is DPV voltammograms in the same conditions.

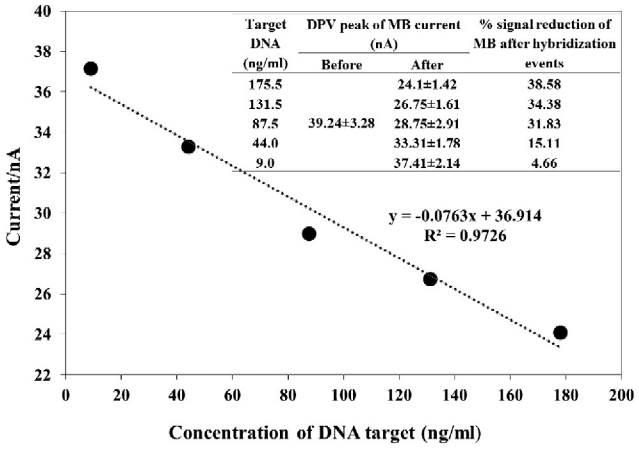

Fig. 7. The calibration curve using SiNWs/AuNPs-modified ITO at different DNA concentrations. Inset is the reduction peak current of MB (%) after hybridization with complementary target at different concentrations.

3.6. Calibration curve

The sensitivity of DPV using the developed sensor towards different concentrations of dengue target DNA was studied in the range of 9.0 ng/ml to 178 ng/ml. In general, the DPV current of MB decreased linearly with increasing target DNA (Fig. 7). The regression equation was $Y = -0.0763X + 36.914$ ($R = 0.9726$), where Y is DPV peak current of MB (nA), and X is the amount of target DNA (ng/μl). Using the $3\sigma/m$ measurements(σ, standard deviation of blank solution; m; slope) ($n = 9$) [38–40], the detection limit for the fabricated sensor was calculated as 3.5 ng/ml.

3.7. Regeneration and stability of SiNWs/AuNPs-modified ITO based electrochemical sensor

The regeneration of the developed sensor was performed by denaturation the complementary DNA-modified ITO by conversion to ssDNA-modified ITO. The regeneration process was carried out by soaking complementary DNA-modified ITO in hot water at 80 °C for 10 min followed by rapid cooling in ice bath. It was found that the regenerated ssDNA-modified electrode could maintain the hybridization activity for about 92.3% of its original response after regenerating for 8 times (RSD 5.78%, $n = 5$). In addition, after keeping ssDNA-modified ITO electrode in a petri dish containing silica gel at 4 °C for 10 weeks, the constructed biosensor produced 86.34% (RSD; 6.34%; $n = 5$) of the original signal showing good stability and life time.

4. Conclusion

In this study, we fabricated a biosensor based on SiNWs/AuNPs composite incorporated on an ITO electrode substrate for detection of dengue DNA target. It has been proven that the utilization of SiNWs/AuNPs composite improved the sensitivity of bare ITO. We found that the hybrid nanomaterial composite showed a good biocompatibility for immobilization of 27 base pairs of thiolated DNA probe on SiNWs/AuNPs modified ITO. The fabricated biosensor can be store at 4.0 °C for 10 days. It was found that the regenerated biosensor could maintain the hybridization activity for about 92.3% of its original response after 8 times with relative standard deviation of 5.78% ($n = 5$).

Conflict of interest

The authors declare that there is no conflict of interest.

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