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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 GAC 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.