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Reduced graphene oxide-yttria nanocomposite modified electrode for enhancing the sensitivity of electrochemical genosensor

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ABSTRACT

Reduced graphene oxide-yttria nanocomposite (rGO:Y) is applied as electrochemical genosensor platform for ultrahigh sensitive detection of breast cancer 1 (BRCA1) gene for the first time. The sensor is based on the sandwich assay in which gold nanoparticle cluster labeled reporter DNA hybridize to the target DNA. Glassy carbon electrode modified with rGO-yttria serves as the immobilization platform for capture probe DNA. The sensor exhibited a fine capability of sensing BRCA1 gene with linear range of 10 attomolar (aM) to 1 nanomolar (nM) and a detection limit of 5.95 attomolar. The minimum distinguishable response concentration is down to the attomolar level with a high sensitivity and selectivity. We demonstrated that the use of rGO:Y modified electrode along with gold nanoparticle cluster (AuNPC) label leads to the highly sensitive electrochemical detection of BRCA1 gene.

1. Introduction

DNA sensors are widely used in diagnostic tests for early cancer and mutation detection, analysis of gene sequences, forensic investigation, and assessment of medical treatment. Electrochemical genosensors are particularly attractive owing to their simplicity, low cost and high sensitivity (Cai et al., 2002; Wang, 2006; He et al., 2005; Lucarelli et al., 2008). These genosensors generally uses a conducting and biocompatible platform for efficient immobilization of single stranded DNA (ssDNA). Graphene/reduced graphene oxide (rGO) is widely used in sensors due to its high surface area, robust mechanical properties and tailored electrical properties (Du et al., 2013). Non-covalent interactions such as π-π stacking or hydrogen bonding between rGO and biomolecules allow efficient immobilization of various biomolecules (Feng et al., 2011; Jayakumar et al., 2012; Shao et al., 2010; Lin et al., 2011; Huang et al., 2011; Fu and Li, 2010). It is established that ssDNA immobilizes on the graphene/reduced graphene oxide through π electron interactions. Recently graphene oxide/rGO- metal oxide nanocomposites have received considerable attention in electrochemical sensing (Zhang et al., 2012; Wang et al., 2010; Ma et al., 2012).

Yttria nanoparticles have good chemical stability and narrow band gap which may greatly facilitate the electron transfer, and thus it can provide excellent electrochemical activity for the sensing applications (Yang et al., 2016). In rGO:Y nanocomposites, the reduced graphene oxide serves as a high-performance support and metal oxides are dispersed on graphene oxide/rGO. The charge transfer at the interface of these hybrid materials can exhibit a synergistic effect to induce properties that are different from those of individual components (Yu et al., 2013; Zhang et al., 2014). Thus, reduced graphene oxide- yttria nanocomposites may enhance the electron transfer rate and allow efficient biomolecule immobilization due to the high surface area leading to sensitive detection of immobilized biomolecules (Yang et al.,2016).

In general, DNA sensors use the reporter probe DNA conjugated to electroactive mediator to improve the sensitivity (Kong et al., 2014, Xia et al., 2010; Shi et al., 2014). A variety of nanomaterials especially gold nanoparticles (AuNPs) have been widely explored as signal amplification agents in various electrochemical sensors (Wu et al., 2014; Wang, 2012; Oh and Lee, 2011; Cao et al., 2011). Recently, we have reported reduced graphene oxide based electrochemical sensor for femtomolar detection of BRCA1 gene using AuNP as an electrochemical label for reporter probe DNA (Rasheed and Sandhyarani, 2014). We also noted that on using gold nanoparticle cluster (AuNPC) as electrochemical label the sensitivity could be increased (Rasheed and Sandhyarani, 2015).

Herein for the first time, a sensitive and simple genosensor is fabricated effectively using rGO:Y modified glassy carbon electrode. The reported genosensor is based on a “sandwich” detection strategy, which involves the immobilized DNA-c on rGO:Y composite. On this, the target BRCA1 gene and the AuNPC labeled

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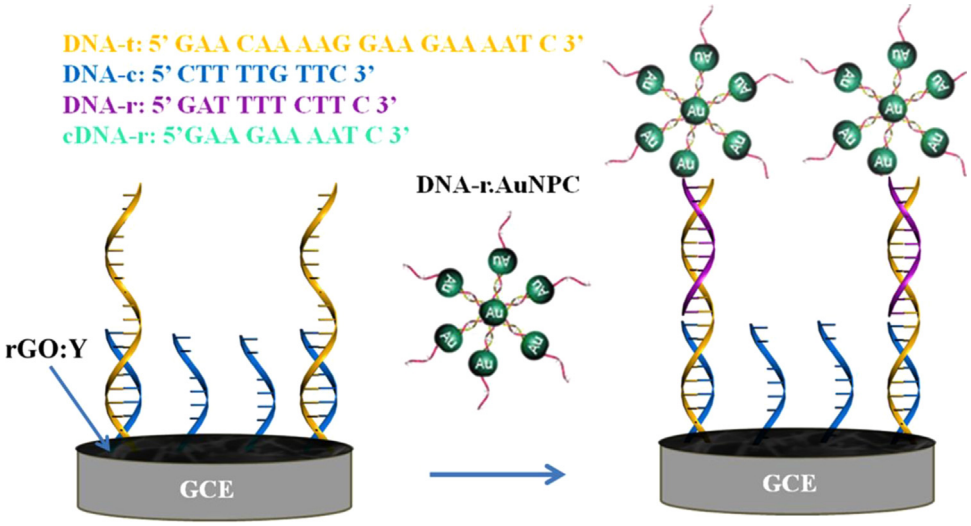

Scheme 1. Schematic representation of the sensor.

reporter probe DNA get hybridized as shown in Scheme 1. These AuNPCs on the surface exhibit strong oxidation signal of gold in perchloric acid ($HClO_4$) which in turn indicates the concentration of target gene. We have evaluated the effect of rGO:Y in the sensitivity of the sensor by comparing with our previous study which uses rGO as the substrate (Rasheed and Sandhyarani, 2015). Presence of yttria on the rGO enhances the sensitivity due to the enhanced electrochemical activity of the nanocomposite. Dahal et al. reported the n-type doping of graphene by growing yttria on the surface, thereby tailoring the electrical properties of graphene (Dahal et al., 2013). The enhancement in the sensitivity is believed to arise due to the charge doping of reduced graphene oxide with yttria.

2. Experimental

2.1. Materials

The oligonucleotides were purchased from Integrated DNA Technologies, USA. The base sequences are as follows.

Capture probe (DNA-c): 5′ CTT TTG TTC 3′

Target probe (DNA-t): 5′ GAA CAA AAG GAA GAA AAT C 3′

Reporter Probe (DNA-r): 5′ GAT TTT CTT C 3′

Complementary reporter probe (cDNA-r): 5′GAA GAA AAT C 3′

Non complementary probe (NC): 5′CCT TGT TGG ACT CCC TTC T 3′

Three base mismatch complementary probe (3MM): 5′ GAA TAA AAG CAA TAA AAT C 3′

Single base mismatch complementary probe (1MM): 5′ GAA CAA AAG GAA GCA AAT C 3′

Graphite powder($>20\,\mu m$), O-(3-Carboxypropyl)-O′-[2-(3-mercaptopropionylamino)ethyl]-polyethylene glycol (CPEG), N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride(EDC), 3 sulfo-N-hydroxysuccinimide (NHS), $Y(NO_3)_3 \cdot 6H_2O$ (Aldrich, 99.9% purity) were purchased from Sigma Aldrich India. Chloroauric acid was purchased from SRL chemicals, India. Other chemicals used were of analytical reagent grade and they were supplied from Fischer Scientific and Merck. Ultrapure and deionized water was used in all experiments. Conjugation buffer and hybridization buffer used were 0.1 M NaCl PBS buffer and 0.3 M NaCl PBS buffer respectively (Storhoff et al., 2002; Park et al., 2002). 0.1 M NaCl PBS buffer consists of 0.1 M NaCl in 10 mM phosphate buffer (pH 7) and 0.3 M NaCl PBS buffer consist of 0.3 M NaCl in10 mM phosphate buffer (pH 7). The glassy carbon electrode was used as the sensor surface for electrochemical measurements.

2.2. Instruments

Cyclic voltammetric (CV), differential pulse voltammetry (DPV) and chronoamperometry measurements were performed with a CHI 660E electrochemical workstation, USA. Electrochemical Impedance Spectroscopy (EIS) and chronocoulometric (CC) measurements were carried out with a CHI 760E electrochemical workstation, USA. A three-electrode system was employed with Pt wire as auxiliary electrode, calomel electrode as reference electrode, and glassy carbon electrode (GCE) or the modified glassy carbon electrode as the working electrode. The cyclic voltammetry analysis was performed in 0.2 M perchloric acid at room temperature with a scan rate of 0.1 V/s and chronoamperometry analysis was performed in 0.2 M perchloric acid at an applied potential of 1.1 V. The DPV analysis of the sensor was monitored in 0.2 M perchloric acid from 0.7 to 1.2 V at an amplitude of 0.05 V, a pulse width of 0.06 s and pulse period of 0.5 s. EIS was recorded in 10 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl. The frequency range used is from 1 Hz to 1 MHz at amplitude of 0.1 V. Chronocoulometric curves were recorded for the reduction of 0.5 mM $K_3[Fe(CN)_6]$ with 0.1 M KCl. Scanning electron microscope (SEM) images and energy dispersive x-ray spectroscopy (EDS) spectra were obtained using SU6600 field emission scanning electron microscope (Hitachi, Japan). Elemental analysis of the composite was performed using Axis Ultra x-ray photoelectron spectroscopy (Shimadzu, Japan). Raman spectra were recorded using Thermo-Nicolet 6700 Raman spectrometer.

2.3. Synthesis

Reduced graphene oxide (rGO) was synthesized by a reported procedure (Thomas et al., 2014). Low temperature exfoliation was used for the conversion of graphene oxide (GO) to reduced graphene oxide. The GO powder was taken in a quartz crucible and kept at 180 °C for 3 h in a hot air oven. The synthesized reduced graphene oxide was found to be few layered. The rGO:Y nanocomposite was synthesized by a hydrothermal method. 20 mg of reduced graphene oxide was dissolved in 40 ml distilled water with ultrasonic treatment 10 min to form a well dispersed suspension. Subsequently, 86 mg of $Y(NO_3)_3 \cdot 6H_2O$ and 12 g of NaOH was added into the suspension and stirred for 30 min. Then the mixture was transferred into the teflon lined stainless steel