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A simple and label-free genosensor for BRCA1 related sequence based on electrospinned ribbon conductive nanofibers

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ABSTRACT

The Breast cancer is the second most common cancer in women. So early detection of cancer is very important. We prepared a novel electrochemical genosensor for label-free, sensitive and selective detection of BRCA1 gene sequence. The developed sensor capture probe (DNA-p) hybridize to target BRCA1 gene sequence (DNA-t) on electrospinned ribbon conductive nanofibers (RCNFs) nanofiber electrospinned ribbon conductive nanofibers of polyethersulfone and nanotubes (RCNFs - MWCNTs) modified carbon paste electrode. This platform has a conductive and high mechanical strength and the surface to electrode volume ratio has increased 2 times. The construction of the platform for the sensor is very simple and cost-effective and the nanofiber can be spun in one step on the electrode surface. The BRCA1 gene sequence could be specifically recognized by capture aptamer pre-immobilized on modified carbon paste surface. Under optimum conditions, the electrochemical impedance spectroscopy (EIS) signals were proportional to the BRCA1 gene sequence concentration from 5 to 14,000 pM with a detection limit of 2.4 pM. The genosensor also exhibited high selectivity, stability and reproducibility with recoveries between 101.5 and 105.2%, indicating the developed biosensor would be a potential alternative tool for BRCA1 gene sequence detection in real biological samples.

1. Introduction

Breast cancer 1 (BRCA1) gene, which is located on the long arm of chromosome 17q determined by genetic linkage in 1990 [1]. It is a human caretaker gene that is expressed in the cells of breast and other tissues and is responsible for the majority of families containing multiple cases of breast and ovarian cancer. It has been observed that mutations in a number of genes cause increasing of the susceptibility to breast cancer. Among the genes with high-risk families, BRCA1 is one of the most important genes affecting especially human breast cancer [2, 3].

A number of researches have been published that show mutations in BRCA1 gene are responsible for approximately 40% of inherited breast cancers [4]. Similarly, > 80% of inherited breast and ovarian cancers has been observed in women who carry mutations in BRCA1 gene [5, 6].

In the past decade, aptamers have been widely employed as bio-recognition elements for detection of biological macromolecules especially protein and DNA [7]. The unique properties that make aptamer come in many uses are due to the advantages of high stability of aptamers, specific recognition as the target, easy and straightforward synthesis, simple labeling, and good stability [8–13].

Recently, for BRCA1 detection, several genosensors have been introduced based on electrochemical method. Such studies have attracted great attention because of the favorable attributes of electrochemical techniques such as low cost, simplicity, rapidity, high sensitivity, and low power requirement [14–16].

Carbon nanotubes have also widely been used in electrochemical-based biosensors, and continue to receive remarkable attention [17]. The reason for this special attention is their substantial electronic conductivities (depending on the atomic structure, carbon nanotubes can behave electrically as a conductor or as a semiconductor), large specific surface areas, and good biocompatibility [18, 19]. However, despite the great progresses which have taken place in using carbon nanotubes in electrochemical sensors, achieving effective aptasensors based on carbon nano materials with impressive sensitivities and selectivities is still challenging and hard. Moreover, carbon nanotubes can facilitate the electron transfer rate between electroactive species and electrode surfaces and this suggest that carbon nanotubes have the ability to use as modifier [20]. Therefore, carbon nanotubes have been

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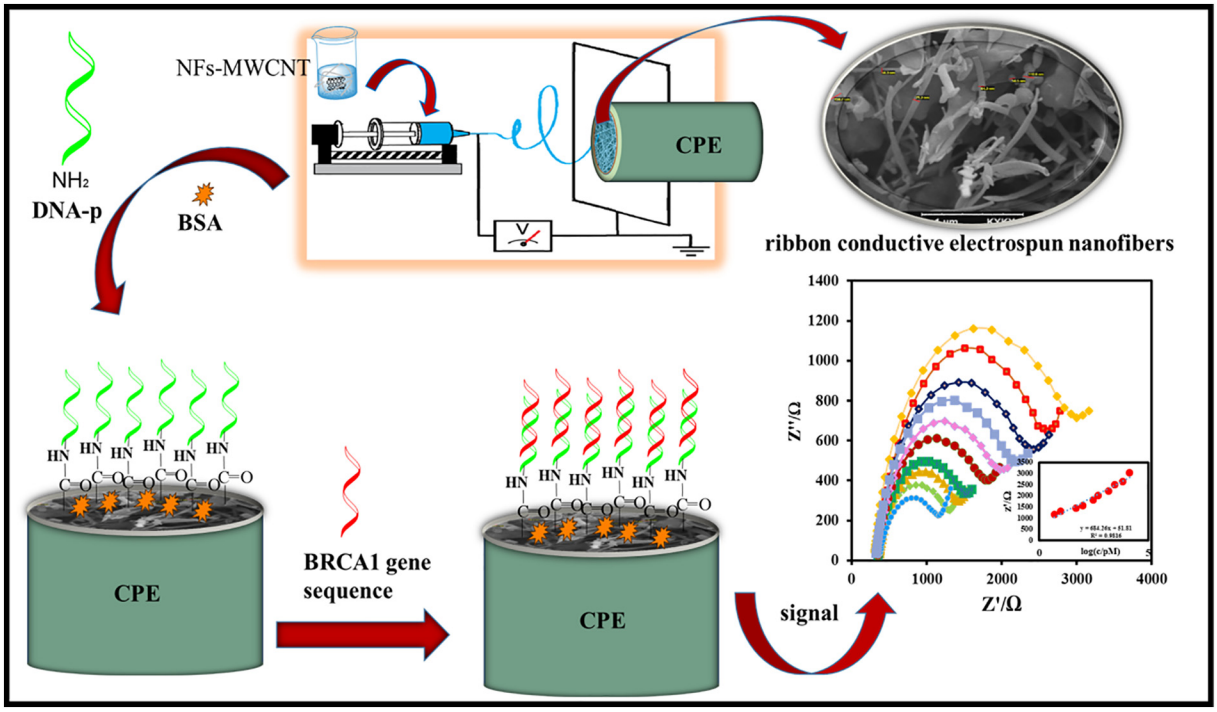

Scheme 1. The schematic illustration for fabrication of genosensor.

used as a robust and advanced carbon electrode material for the design of electrochemical sensors [21]. MWCNT has had a significant impact on the development of new techniques and instrumentation for chemical analysis especially for electrochemical sensors [22, 23]. One of the most advanced materials and one of the most interesting material member of carbon families, is electrospun polymer nanofiber. Electrospun polymer nanofiber can be a promising electrode modifier material due to its high electronic properties, mechanical strength and electrochemical performances. Nanofibers have interesting features such as light weight, high level, and a porous structure [24]. There are a wide range of synthetic polymers, natural polymers or a blend of both that used in electrospinning such as polyvinylpyrrolidone [25], poly (vinyl alcohol) [26], chitosan [26], polystyrene (PS) [26] and poly(ethylene-co-vinyl alcohol) (PEVA) [27]. They are able to form fibers within the nano meter range.

In this study, a sensitive electrochemical aptamer platform is introduced based on electrospinned ribbon conductive nanofibers of polyethersulfone and nanotubes (RCNFs-MWCNTs) modified carbon paste electrode. Then, the genosensor was applied for measurement of BRCA1 gene sequences using impedimetric technique. The stability, reproducibility, and repeatability of the nanocomposite genosensor were considered and it was used for the monitoring of BRCA1 gene sequences in serum as real samples.

2. Experimental

2.1. Reagents and chemicals

N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride $C_8H_{17}N_3$·HCl (EDC·HCl) and N-hydroxysuccinimide (NHS, 98%) were prepared from Sigma-Aldrich Corporation, St. Louis, MO USA. Potassium ferrocyanide ($K_4Fe(CN)_6$) and potassium ferricyanide ($K_3Fe(CN)_6$) were purchased from Merck (Darmstadt, Germany). Phosphate buffer saline (PBS, pH 7.4) containing NaCl (100 mM), KCl (2.0 mM), Na_2HPO_4 (6.4 mM), and KH_2PO_4 (1.0 mM) was employed as an incubation buffer. The functional multiwall carbon nanotubes (MWCNTs) were obtained from Nanoport. Co. Ltd., Shenzhen, China. Bovine serum albumin (BSA) was also purchased from Sigma-Aldrich Corporation, St. Louis, MO USA.

The synthetic short oligonucleotide sequences which are used in this study purchased from Fazabiotech Co. (Tehran, Iran). The base sequences of used oligonucleotides are as follows:

Capture probe (DNA-c): 5′-(NH_2)-GAT TTT CTT CCT TTT GTT C-3′

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

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

Double-base mismatched probe: 5′5′ CAA CAA AAG CAA GAA AAT C 3′

Three-base mismatch probe: 5′ CAA CAA AAG CAA CAA AAT C 3′

It should be noted that the target DNA sequence is the gene sequence associated with breast cancer.

2.2. Apparatus and measurements

All the cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) experiments were conducted using an Autolab PGSTAT204 potentiostat/galvanostat, controlled via a Nova 1.11 software. A three-electrode setup was used for all experiments. In this setup a modified carbon paste electrode was used as a working electrode, an Ag/AgCl/KCl (3.0 M) was applied as a reference electrode, and a Pt wire employed as a counter (auxiliary) electrode. EIS experiments were performed in a solution containing $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1, 5 mM) as a redox couple, with an AC voltage amplitude of 5 mV and a frequency range of 10 kHz to 0.01 Hz at an open circuit potential. All voltammograms were recorded at room temperature.

A laboratory scale electrospinning setup was used equipped with a syringe pump with the accuracy of 0.1 mL/h, and a 23 gauge flat-end needle. This setup was used a high DC voltage power supply (0–30 kV).

2.3. Procedure

Graphite powder (0.7 g) and appropriate amount of mineral oil (paraffin 0.3 g) was used for preparation of carbon paste electrode thorough hand mixing in a mortar and pestle. Then, a portion of prepared composite mixture was packed into the end of a syringe (3.0 mm