www.elsevier.com/locate/talanta

Comparison of impedimetric detection of DNA hybridization on the various biosensors based on modified glassy carbon electrodes with PANHS and nanomaterials of RGO and MWCNTs

Ali Benvidi, Marzieh Dehghan Tezerjani, Shahriar Jahanbani, Mohammad Mazloum Ardakani, Seyed Mohammad Moshtaghioun

PII: S0039-9140(15)30409-4
DOI: http://dx.doi.org/10.1016/j.talanta.2015.10.043
Reference: TAL16051

To appear in: *Talanta*

Received date: 31 May 2015
Revised date: 13 October 2015
Accepted date: 15 October 2015

Cite this article as: Ali Benvidi, Marzieh Dehghan Tezerjani, Shahriar Jahanbani Mohammad Mazloum Ardakani and Seyed Mohammad Moshtaghioun Comparison of impedimetric detection of DNA hybridization on the variou biosensors based on modified glassy carbon electrodes with PANHS an nanomaterials of RGO and MWCNTs, *Talanta* http://dx.doi.org/10.1016/j.talanta.2015.10.043

Comparison of impedimetric detection of DNA hybridization on the various biosensors based on modified glassy carbon electrodes with PANHS and nanomaterials of RGO and MWCNTs

Ali Benvidi[a1], Marzieh Dehghan Tezerjani[a], Shahriar Jahanbani[a], Mohammad Mazloum Ardakani[a], Seyed Mohammad Moshtaghioun[b]

[a]Department of Chemistry, Faculty of Science, Yazd University, Yazd, I. R. Iran

[b]Department of Biology, Faculty of Science, Yazd University, Yazd, I. R. Iran

Abstract

In this research, we have developed lable free DNA biosensors based on modified glassy carbon electrodes (GCE) with reduced graphene oxide (RGO) and carbon nano tubes (MWCNTs) for detection of DNA sequences. This paper compares the detection of *BRCA1* 5382insC mutation using independent glassy carbon electrodes (GCE) modified with RGO and MWCNTs. A probe (*BRCA1* 5382insC mutation detection (ssDNA)) was then immobilized on the modified electrodes for a specific time. The immobilization of the probe and its hybridization with the target DNA (Complementary DNA) were performed under optimum conditions using different electrochemical techniques such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The proposed biosensors were used for determination of complementary DNA sequences. The non- modified DNA biosensor (1-pyrenebutyric acid-N-hydroxysuccinimide ester (PANHS)/GCE), revealed a linear relationship between ΔR_{ct} and logarithm of the complementary target DNA concentration ranging from 1.0×10^{-16} mol L^{-1} to

[1] Corresponding author: E-mail addresses: abenvidi@yazd.ac.ir, benvidi89@gmail.com ;
Tel.: +98 353 812 2645; Fax: +98-353-821064

1.0×10^{-10} mol L^{-1} with a correlation coefficient of 0.992, for DNA biosensors modified with multi wall carbon nano tubes (MWCNTs) and reduced graphene oxide (RGO) wider linear range and lower detection limit were obtained. For ssDNA/PANHS/MWCNTs/GCE a linear range 1.0×10^{-17} mol L^{-1} - 1.0×10^{-10} mol L^{-1} with a correlation coefficient of 0.993 and for ssDNA/PANHS/RGO/GCE a linear range from 1.0×10^{-18} mol L^{-1} to 1.0×10^{-10} mol L^{-1} with a correlation coefficient of 0.985 were obtained. In addition, the mentioned biosensors were satisfactorily applied for discriminating of complementary sequences from noncomplementary sequences, so the mentioned biosensors can be used for the detection of *BRCA1*-associated breast cancer.

Keywords: 1- pyrenebutyric acid-N- hydroxysuccinimide ester (PANHS); DNA-biosensor; Immobilization; Breast cancer; Reduced graphene oxide

1. Introduction

For genetic detection of cancers in initial steps and analysis of gene sequences, DNA biosensors in diagnostic tests are important. Breast cancer is a kind of malignant tumor, which starts in the cells of the breast. A malignant tumor contains a group of cancer cells that can grow into circumambient tissues or spread to the other areas of the body. This disease occurs in women more than men, but men can get it, too. The BRCA1 5382insC mutation increases the risk of getting breast cancer 10 times and ovarian cancer 20 times, compared with the general population. Detection of breast cancer like other cancers in the initial stages is so important, consequently different DNA biosensors based on different detection strategies have been developed [1–4]. This disease can be detected by different methods [5], among them, electrochemical biosensors have been improved due to having some advantages such as being highly sensitive, selective and rapid [6–9].

As known, by development of nanotechnology and nano science, different nanomaterials have been introduced to improve sensing performance of electrochemical biosensors. For example, Wang groups [10, 11] have fabricated several electrochemical DNA biosensors based on nanomaterials. Obviously, nanotechnology has exhibited a main role in electrochemical DNA diagnostics, and among nanomaterials, graphene is a single atom with two-dimensional sheet of sp^2-bonded carbon. Due to its structure, it has specific attributes such as high mobility of charge carriers, good biocompatibility, high electrocatalytic property and large specific surface area. Thus, it can be used in the fabrication of electrochemical biosensors [12-13]. Todays, electrochemical reduction of graphene oxide (Go) on the electrode surface has been proposed. Electrochemical reduction of graphene oxide can be achieved with different techniques such as cyclic voltammetry or potentioststic method [14].

In addition, nanomaterials which have been widely applied as an ideal material to fabricate electrochemical biosensors, are multi wall Carbon nanotubes (MWCNTs). Recently, because of owing the properties of each synergistic effect, composite materials based on MWCNTs and polymers have gained more interest [15, 16]. The first example of electrochemically assisted covalent modification of carbon was reported by Barbier [17] and after that many works have been reported about this general approach. These kinds of biosensors have been improved effectively due to having suitable sensitivity and selectivity.

PANHS (1- pyrenebutyric acid-N- hydroxysuccinimide ester) is one of the linkers which can be used to fabricate some electrochemical biosensors. PANHS is commonly found in organic chemistry or biochemistry where it is used as an activating reagent for carboxylic acids. This material has been used as a scaffold molecule comprising an anchor group and a terminal group. Due to hydrophobic π system group, PANHS has strong affinity with the base plane of the

carbon sheets and carbon tubes through π-stacking [18], and this material with succinimid ester group reacts strongly to nucleophilic substitution by amine groups on the proteins [19].

Electrochemical impedance spectroscopy (EIS) is a powerful technique for investigation of electrode surface to follow detection of immobilized DNA probes and hybridization of complementary DNA target at the electrode surface. This technique enables researchers to investigate different properties of interfaces of electrode and solution, so it can be used for detection of events occurred on the electrode surface. One of the benefits of this technique is being label-free detection method, which is helpful due to no need to additionally biomolecule markers, such as fluorescent dyes, enzymes, or other redox labels [20].

Several electrochemical biosensors have been designed for genetic diagnoses in literature [21, 22]. In our recently works [23-25], some electrochemical DNA biosensors were designed. In reference [23], fabrication of a modified carbon paste electrode with gold nanoparticles was investigated for sex determination. In papers [24, 25], a label free biosensor based on a glassy carbon electrode modified with graphene and gold nanoparticles for detection of breast cancer and a simple and label-free detection of DNA hybridization on a modified graphene nanosheets electrode were fabricated, respectively.

In the present paper, we reported new electrochemical biosensors based on modified glassy carbon electrodes which named ssDNA/PANHS/MWCNTs/GCE and ssDNA/PANHS/RGO/GCE. Glassy carbon electrode was modified with nano particles of RGO and MWCNTs which owing some benefits such as using synthesized nano materials of RGO and MWCNTs and using PANHS provide a suitable base for attaching the DNA strands and consequently a better hybridization process can occur at the electrode surface. The results showed that EIS responses versus the concentration of complementary oligonucleotides for

ssDNA/PANHS/RGO/GCE was obtained from 1.0×10^{-18} to 1.0×10^{-10} mol L^{-1} with a detection limit of 3.5×10^{-19} mol L^{-1}, linear range and the limit of detection for ssDNA/PANHS/GCE and ssDNA/PANHS/MWCNTs/GCE electrodes were obtained, too. Also, we examined complementary sequences from noncomplementary sequences under the optimum conditions.

To the best of our knowledge, there are few papers which used PANHS to graft DNA strands to the surface of electrode [26]. One of the advantages of using PANHS is decreasing the electrode preparation processes (not necessary to use NHS/EDC (1-ethyl-3-(3-dimethyl amino propyl) carbo-diimide hydrochloride (EDC), N-hydroxysuccinimide (NHS)) for activation of carboxylic acid groups). Other advantages of this work are making electrochemical biosensor without label, using nanomatrials (reduced graphen oxide and carbon nano tubes) to improve linear range and detection limit. Also, the designed biosensors revealed high stability and reproducibility for detection of sequences of complementary and noncomplementary of *BRCA1* strands. The proposed approach for immobilization of probe DNA and hybridization with target oligonucleotides is illustrated in Scheme 1.

HERE Scheme. 1

1. Experimental

1.1. Reagents and Apparatus

Specific primers and probes were designed based on GenBank breast cancer database. Primers were dissolved in water to form 1 mg ml^{-1} stock solution and were kept frozen at -20 °C. Tween 20 (Polyoxy ethylenesorbitan monolaurate), potassium ferrocyanide, potassium ferricyanide, graphite, 1- pyrenebutyric acid-N- hydroxysuccinimide ester (PANHS), MWCNTs, 1-ethyl-3-(3-dimethyl amino propyl) carbo-diimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and other chemical reagents were purchased from commercial

sources (Merck or Sigma) and were applied in analytical grade. Double distilled water and all buffers were sterilized by using an autoclave. All solutions were prepared with deionized water (DI: 18 MΩ cm^{-1} resistivity). Graphene oxide (GO) nanosheets were prepared according to the papers [27, 28]. The sequences of probe and complementary were as follows:

Probe sequence (ssDNA=P1):

5'-AAGCGAGCAAGAGAATTCCAG-3'

Complementary sequence (dsDNA=P1C):

5'-GTGAAAGTATCTAGCACTGCTGGAATTCTCTTGCTCGCTT-3'

Noncomplementary sequence (noncom dsDNA=P1nC):

5'-TGTGAAAGTATCTAGCACTGTGGGAATTCTCTTGCTCGCT-3'

EIS and electrochemical measurements were performed with Autolab potentiostat/galvanostat model PGSTAT 302 N (Eco Chemic, Utrecht, Netherlands) and NOVA 1.7 software at laboratory temperature (25±1°C). The applied three-electrode system was composed of a glassy carbon as a working electrode (surface area of 7.07 mm^2), an Ag/AgCl (1.0 mol L^{-1} KCl) reference electrode, and a platinum wire as an auxiliary electrode. The pH measurements were performed using a Metrohm model 691 pH/mV meter.

2.2 Preparation of nonmodified electrode (dsDNA/PANHS/GCE)

Before fabrication, the surface of a GCE was polished by 0.05 µm alumina slurry to mirror like, and then the mirror surface of GCE was washed successively with anhydrous alcohol and water. Then 20 µL of PANHS solution (1 µmol L^{-1}) was dropped on the surface of electrode overnight in a saturated chamber with DMF vapor. For immobilization of DNA probe to the electrode surface, 20 µL of DNA probe (ssDNA) solution (1 µmol L^{-1}) was pipetted on the electrode surface for 30 min in a wet chamber with water to fabricate ssDNA/PANHS/GCE. The

prepared electrode was kept in a 0.1% Tween solution at room temperature for 30 min to remove nonspecific adsorption of DNA probes and also to fill the bare areas of the electrode surface that have not been covered by DNA probe molecules. The electrode was washed with buffer after each step and each step was followed using CV and EIS techniques. For hybridization, 100 µL of complementary DNA (1.0×10^{-15} mol L^{-1}) was dropped on the fabricated electrode for 50 min at 30 ℃. This electrode was named dsDNA/PANHS/GCE.

2.3. Preparation of modified electrodes

2.3.1 Preparation of dsDNA /PANHS/MWCNTs/GCE

Before fabrication, like the mentioned electrode, bare GCE was polished. A 20 µL of homogeneously dispersed solution of multi wall carbon nano tubes (MWCNTs) (0.050 g mL^{-1}) was then pipetted onto the surface of GCE and dried under ambient condition. After that, 20 µL of PANHS solution (1 µmol L^{-1}) was dropped on the surface of electrode for 3 h in a saturated chamber with DMF vapor. For immobilization of DNA probe on the electrode surface, 10 µL of DNA probe (ssDNA) solution (1 µmol L^{-1}) was dropped on the electrode surface for 30 min in a wet chamber with water to fabricate ssDNA/PANHS/MWCNTs/GCE. The electrode was then kept in a 0.1% Tween solution at room temperature for 30 min to remove nonspecific adsorption of DNA and fill the non-covered areas of ssDNA/PANHS/MWCNTs/GCE by DNA molecules. For hybridization of ssDNA/PANHS/MWCNTs/GCE, 100 µL of complementary DNA (1.0×10^{-15} mol L^{-1}) was dropped on the fabricated electrode for 50 min at 30 °C. This electrode was named dsDNA /PANHS/MWCNTs/GCE.

2.3.2. Preparation of dsDNA/PANHS/RGO/GCE

First, bare GCE was polished as described for non-modified electrode, then it was modified with reduced graphene oxide by electrochemical method. In this method, graphene oxide (GO) on a glassy carbon electrode was reduced at a constant potential of −0.9 V for 40 s [24] in a PBS (0.1 mol L^{-1}, pH=7.4) solution by constant potential colometry (CPC) technique. The RGO/GCE was washed with double-distilled water, 20 µL of a PANHS solution (1 µmol L^{-1}) was then dropped on the surface of electrode for 3 h in a saturated chamber with DMF vapor. For immobilization of DNA probe to the electrode surface, 10 µL of DNA probe (ssDNA) solution (1 µmol L^{-1}) was dropped on the electrode surface for 30 min in a wet chamber with water to fabricate ssDNA/PANHS/RGO/GCE. To remove nonspecific adsorption of DNA molecules, the electrode was kept in a 0.1% Tween solution at 30 ℃ for 30 min. For hybridization of PANHS/RGO/GCE, 100 µL of complementary DNA (10×10^{-16} mol L^{-1}) was dropped on the fabricated electrode for 50 min at 30 ℃. This electrode was named dsDNA/PANHS/RGO/GCE, and modification steps were followed by using CV and EIS techniques, too.

2.4. BRCA1 mutation investigation

To investigate BRCA1 5382insC mutation detection efficiency, the non-modified electrode (dsDNA /PANHS/GCE) and modified electrodes of dsDNA/PANHS/MWCNTs/GCE and dsDNA/PANHS/RGO/GCE were fabricated and the hybridization of complementary and noncomplementary sequences (P1C and P1nC) were followed for every electrode.

3. Result and Discussion

3.1. Characterization of fabricated modified electrode

3.1.1. Characterization by SEM, cyclic voltammetry and EIS techniques

In this experiment, the morphology of bare GCE, MWCNTs and RGO was studied by SEM (Fig. 1). As shown in Fig. 1A, in SEM image of bare GCE, no considerable amorphous carbon particles were observed and the surface of bare electrode is smooth but in Fig.1B and Fig. 1C the electrode surfaces are rougher than non-modified glassy carbon electrode, it can mean that RGO and MWCNTs act as a nanowire between sample solution and the surface of glassy carbon electrode. So, the modification of GCE with RGO and MWCNTs increases the loading amount of DNA probes that can cause the improvement of immobilization and hybridization processes.

EIS and CV methods were also used to check the fabrication process of the modified biosensor (ssDNA/PANHS/RGO/GCE). All experiments were carried out in a solution containing 0.5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ (1:1) in a 0.1 mol L^{-1} PBS as a redox probe**.** Fig. 2 shows the cyclic voltammograms of different electrodes in 0.5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ at a scan rate of 100 mV/s. As shown in this figure, an obvious increase in the peak current (i_p) of RGO/GCE electrode is observed (curves a and b) compared with the CV response of bare GCE. This enhancement of peak current can be due to the huge surface, high electric transfer kinetics and excellent conductivity of RGO [29]. By immobilizing probe on the surface of electrode, peak current decreased and peak potential difference (ΔE_p) enhanced significantly due to repulsion of $[Fe(CN)_6^{3-/4-}]$ by the negative charged phosphate backbone of probe DNA and preventing $[Fe(CN)_6^{3-/4-}]$ ions from reaching to the electrode surface (curve d). By adding complementary DNA, the negative charge of redox species increases more, which leads to decrease of peak current. On the other hand, it has been revealed that DNA immobilization and hybridization could be affected on the current of fabricated electrode surface (Fig.2, curve f) [30].

The similar results were obtained for the other modified electrode (ssDNA/PANHS/MWCNT/GCE), too. EIS signals for different modification steps were in good agreement with the obtained results by CV experiments. As shown in Fig. 3, by modification of bare electrode with RGO, the surface of electrode is improved and due to high conductivity of RGO and its high surface area, R_{ct} decreases compared with bare GCE (Fig. 3 curves a and b). The amount of R_{ct}, increases by immobilization of DNA probe on the PANHS/RGO/GCE surface (Fig. 3, curve d). In the last step, hybridization of DNA probe on the RGO modified electrode (ssDNA/PANHS/RGO/GCE) with complementary DNA enlarged the diameter of the semicircle of impedance spectrum due to more repulsion between negatively charge of hybridized DNA and $[Fe(CN)_6]^{3-/4-}$ anions (Fig. 3, curve f). In addition, hybridization of non-complementary DNA (P1nC) probe on ssDNA/PANHS/RGO/GCE was examined (curve g). As shown in this curve, there is no significant change in ΔR_{ct} which indicating no hybridization was occurred. These experiments were done for the other modified electrode with MWCNTs (ssDNA/PANHS/MWCNTs/GCE), while the obtained results had a good agreement with ssDNA/PANHS/RGO/GCE.

Here Figures1-2-3

3.2. Optimization of experimental conditions

3.2.1. Investigation of immobilization time and Tween incubation time

To investigate the detection ability of DNA biosensor, the immobilization amount of probe is important. The changes in the charge transfer resistance ($\Delta R_{ct} = (R_{ct})_{final} - (R_{ct})_{initial}$) was adopted as the measurement signal, where $(R_{ct})_{initial}$, is the R_{ct} before immobilization step and $(R_{ct})_{final,}$ is R_{ct} after each step in all optimization steps. We examined different times of immobilization time on the electrode surface from 1 h to 24 h. It was observed that by increasing

time, ΔR_{ct} increases significantly. Anyway, when time is more than about 16 h, ΔR_{ct} starts to level off after 16 h (Fig. 4A), this can be due to the full coverage of the surface of electrode by the DNA probe after 16 h. Therefore, an immobilization time of 16 h was selected for experiments, considering the largest ΔR_{ct}. ssDNA/PANHS/RGO/GCE electrode was incubated in a solution of 0.1% Tween in different times to remove nonspecifically adsorbed probe molecules from the surface of electrode. It is possible for Tween to attach to the nonspecific sites on ssDN/PANHS/RGO/GCE and remove nonspecifically adsorbed DNA molecules from the fabricated electrode surface. It was observed that ΔR_{ct} increased with increasing time between 5 min to 30 min, and then response signal has been stable (Fig. 4B). Therefore, the optimum time of 30 min was chosen for the incubation of Tween.

3.2.2. Effect of time and temperature of hybridization

The effect of time and temperature on the hybridization process were tested. As shown in Fig. 4C, hybridization time was changed from 5 min to 1 h while keeping hybridization temperature and complementary target DNA concentration constant at 25 ^{o}C and 10×10^{-16} mol L^{-1}, respectively. The impedance value of target was as a function of hybridization time. As shown in Fig. 4C, 50 min was obtained as an optimum hybridization time.

The other parameter for hybridization reaction is temperature of hybridization. The obtained results indicated that the ΔR_{ct} value increased by increasing temperature up to 35 ^{o}C and rapidly decreased with the increase of temperature. Reduction of ΔR_{ct} occurs due to removing some molecules from the electrode surface in higher temperature, so the temperature of 35°C was selected as the optimum hybridization temperature (Fig. 4D).

Here figure 4

3.3. Calibration investigation on modified electrode

Fig. 5 shows EIS responses of the fabricated biosensor (ssDNA/PANHS/RGO/GCE) through the addition of different concentrations of complementary target DNA. According to this figure, with increasing the concentrations of complementary target DNA, R_{ct} increases. Inset A of Fig. 5 reveals the used Randles equivalent circuit for EIS measurements. As known, in this equivalent circuit, R_s is the electrolyte resistance, W_{01}, is Warburg impedance resulting from the ions diffusion, CPE is a constant phase element and R_{ct} is the electron transfer resistance. We used the recorded R_{ct} value for each experimental steps, because electron transfer process between the redox indicator solution of $[Fe(CN)_6]^{3-/4-}$ and electrode surface has been influenced by electrode modification and hybridization processes.

The obtained results in Fig. 6A show that for non-modified electrode PANHS/GCE) ΔR_{ct} versus concentration of complementary DNA strands is constituted from 1.0×10^{-16} mol L^{-1} to 1.0×10^{-10} mol L^{-1} with a regression equation of ΔR_{ct} =1.278 Log C (mol L^{-1}) +23.035 (R^2 =0.992). The detection limit based on $3s_{bl}$ (s_{bl} was the relative standard deviation of 10 replicate measurements of blank solution) was obtained to be 3.7×10^{-17} mol L^{-1}. For PANHS/MWCNTs/GCE modified electrode, ΔR_{ct} for target DNA concentration is evaluated from 1.0×10^{-17} mol L^{-1} to 1.0×10^{-10} mol L^{-1} with a regression equation of ΔR_{ct} =1.268 Log C (mol L^{-1}) +23.902 (R^2 =0.993) and the limit of detection (based on $3s_{bl}$) was obtained to be 3.1×10^{-18} mol L^{-1} (curve B of Fig.6).

Also, for PANHS/RGO/GCE modified electrode, ΔR_{ct} via target concentration is made from 1.0×10^{-18} mol L^{-1} to 1.0×10^{-10} mol L^{-1} with a regression equation of ΔR_{ct} =1.350 Log C (mol L^{-1}) +25.90 (R^2 =0.985) and the lower detection limit of 3.5×10^{-19} mol L^{-1} was obtained (curve C of Fig. 6). These results show that using RGO and CNTs as modifiers due to their

unique properties exhibit high sensitivity. Some characteristics of the proposed electrode (ssDNA/PANHS/RGO/GCE) with the wider linear range and lower detection limit were compared with similar reported electrodes [21, 25, 26, 30-32] in the Table 1. According to this table, obtained detection limit and linear range in this work for PANHS/RGO/GCE modified electrode, are comparable and in some cases better than other works.

Here Fig. 5

Here Fig. 6

Here Table 1

3.4. The selectivity and analytical performance of the modified DNA sensor

One of the significant aims of designing new DNA biosensors is recognition of differences between the targets DNA from others. Achieving this purpose, selectivity of the designed electrochemical biosensor (ssDNA/PANHS/RGO/GCE with the higher sensitivity than other fabricated biosensors ssDNA/PANHS/MWCNT/GCE and ssDNA/PANHS/GCE) was tested by using prepared modified electrode to hybridize with different kinds of DNA sequences (target P1C and non-complementary P1nC). The results revealed that after hybridization with complementary and non-complementary DNA sequences, the proposed electrode has different EIS responses. With P1C sequence high signal was obtained and for P1nC sequence, a small change in EIS response was observed. According to Table 2, EIS results show that ΔR_{ct} values of complementary P1C increased more compared with those of non-complementary DNA sequence P1nC. So, these results can reveal that selectivity of the designed electrodes with two different modifiers (RGO, MWCNTs) is excellent and it can be offered that the fabricated biosensor applies in the next works to detect *BRCA1* mutation in real samples in the next works.

Here Table 2

3.5 Repeatability, reproducibility and stability of the DNA sensors

For repeatability investigations (the electrode capability for the generation of a repeatable response) of the fabricated DNA sensors, hybridization of ssDNA/PANHS/RGO/GCE were performed with 1.0×10^{-16} mol L^{-1} target DNA, five times and a relative standard deviation of 3.2% was obtained. Also, repeatability investigations for ssDNA/PANHS/GCE and ssDNA/PANHS/MWCNT/GCE electrodes were done and the relative standard deviations of 3.0% and 4.2% were obtained. For reproducibility investigations, three independently ssDNA/PANHS/RGO/GCE were prepared and the fabricated ssDNA/PANHS/RGO/GCE electrodes were hybridized with 1.0×10^{-16} mol L^{-1} target DNA. The results showed the ΔR_{ct} values of 6.15, 6.32 and 6.80 KΩ with a relative standard deviation of 5.2% (n=3) indicating a satisfactory reproducibility of this electrochemical biosensor. Also, the mentioned steps were tested for ssDNA/PANHS/GCE and ssDNA/PANHS/MWCNT/GCE electrodes, and relative standard deviations of 4.8% and 5.0% were obtained, respectively. In addition, the stability of the proposed modified electrodes were investigated, too. When the modified electrodes of ssDNA/PANHS/RGO/GCE, and ssDNA/MWCNT/PANHS/GCE, and non-modified electrode of ssDNA/PANHS/GCE were stored in a 0.1 mol L^{-1} PBS buffer solutions (pH 7.4) at 4 °C for 5 days, the responses of electrodes were retained about 93%, 91%, 90% of their primary responses respectively, so the stability of the proposed DNA biosensors are suitable.

4. Conclusion

In this study, we described the fabrication of DNA label-free electrochemical biosensors for BRCA1 mutation detection, based on PANHS, glassy carbon electrode with two modifiers (RGO, CNTs) for DNA immobilization and hybridization processes. Under optimum conditions,

for non-modified electrochemical biosensor (ssDNA/PANHS/GCE) a detection limit of 3.7×10^{-17} mol L^{-1} and a linear range of 1.0×10^{-16} - 1.0×10^{-10} mol L^{-1} were obtained, respectively. For the modified biosensor of ssDNA/PANHS/MWCNTs/GCE a linear range of 1.0×10^{-17} - 1.0×10^{-10} mol L^{-1} and a detection limit of 3.1×10^{-18} mol L^{-1} were estimated. For the electrochemical biosensor modified with RGO (ssDNA/PANHS/RGO/GCE) a wider linear range (1.0×10^{-18} - 1.0×10^{-10} mol L^{-1}) and a lower detection limit of 3.5×10^{-19} mol L^{-1} were obtained by EIS technique. So, the last electrochemical biosensor, (ssDNA/PANHS/RGO/GCE), has a wider linear range and lower detection limit compared to non-modified and modified biosensor with MWCNTs. Also, ssDNA/PANHS/RGO/GCE biosensor with higher selectivity and sensitivity than the other proposed biosensors, was successful in detection of *BRCA1* mutation and employed in determination of complementary and non-complementary sequences.

Acknowledgments

The authors wish to thank Yazd University Research Council for financial support of this research.

References

[1] S. J. Park, T. A. Taton, C. A. Mirkin, Array-based electrical detection of DNA with nanoparticle probes, Science 295 (2002) 1503–1506.

[2] F. Li, Y. Huang, Q. Yang, Z. T. Zhong, D. Li, L. H. Wang, S.P. Song, C.H. Fan, A graphene-enhanced molecular beacon for homogeneous DNA detection, Nanoscale 2 (2010) 1021–1026.

[3] Z. F. Ma, L. Tian, T.T. Wang, C.G. Wang, Optical DNA detection based on gold nanorods aggregation, Anal. Chim. Acta 673 (2010) 179–184.

[4] C.J. Yu, Y.J. Wan, H. Yowanto, J. Li, C.L. Tao, M.D. James, C.L. Tan, G.F. Black-burn, T.J. Meade, Electronic detection of single-base mismatches in DNA with ferrocene-modified probes, J. Am. Chem. Soc. 123 (2001) 11155–11161.

[5] F. Lisdat, D. Schafer, The use of electrochemical impedance spectroscopy for biosensing, Anal. Bioanal. Chem. 391 (2008) 1555–1567.

[6] J. Wang, Nanoparticle-based electrochemical DNA detection, Anal. Chim. Acta 500 (2003) 247–257.

[7] A. Erdem, Nanomaterial-based electrochemical DNA sensing strategies, Talanta 74 (2007) 318–325.

[8] M. T. Casta˜neda, S. Alegret, A. Merko, Electrochemical sensing of DNA using gold nanoparticles, Electroanalysis 19 (2007) 743–753.

[9] Y. Y. Shao, J. Wang, H. Wu, J. Liu, I. A. Aksay, Y. H. Lin, Graphene based electrochemical sensors and biosensors: a review, Electroanalysis 22 (2010) 1027–1036.

[10] J. Wang, A. N. Kawde, M. Musameh, Carbon-nanotube-modified glassy carbon electrodes for amplified label-free electrochemical detection of DNA hybridization, Analyst 128 (2003) 912–916.

[11] J. Wang, A. N. Kawde, A. Erdem, M. Salazar, Magnetic bead-based label-free electrochemical detection of DNA hybridization, Analyst 126 (2001)2020–2024.

[12] D. Chen, L. Tang, J. Li, Graphene-based materials in electrochemistry, Chem. Soc. Rev. 39 (2010) 3157–3180.

[13] J. Zhao, G. F. Chen, L. Zhu, G. X. Li, Graphene quantum dots-based platform for the fabrication of electrochemical biosensors, Electrochem. Commun. 13 (2011) 31–33.

[14] M. Zhou, Y. L. Wang, Y. M. Zhai, J. F. Zhai, W. Ren, F. Wang, S.J. Dong, Controlled Synthesis of large-area and patterned electrochemically reduced grapheme oxide films, Chem. Eur. J. 15 (2009) 6116–6120.

[15] T. Yang, W. Zhang, M. Du, K. Jiao, DNA Hybridization sensors based on electrochemical impedance spectroscopy as a detection tool, Talanta 75 (2008) 987–994.

[16] C. Jiang, T. Yang, K. Jiao, H. W. Gao, A DNA electrochemical sensor with poly-l- lysine/single-walled carbon nanotubes films and its application for the highly sensitive EIS detection of PAT gene fragment and PCR amplification of NOS gene, Electrochim. Acta 53 (2008) 2917–2924.

[17] B. Barbier, J. Pinson, G. Desarmot, M. Sanchez, Electrochemical bonding of Amines to carbon fiber surfaces toward improved carbon-Epoxy composites, J. Electrochem.Soc. 137 (1990) 1757.

[18] Y. X. Xu, H. Bai, G. W. Lu, C. Li, G. Q. Shi, Flexible graphen films via filtration of water- souble noncovalent functionalized graphen sheets, J. Am. Chem. Soc. 130 (2008) 5856 - 5857.

[19] R. J. Chen, Y.G. Zhang, D.W. Wang, H. J. Dai, Noncovalent sidewall functionalization of single-walled carbon nanotubes for protein immobilization. J. Am. Chem.Soc. 123 (2001) 3838- 3839.

[20] A. Benvidi, N. Rajabzadeh, M. Mazloum-Ardakania, M. M. Heidari, A. Mulchandani, Simple and label-free electrochemical impedance Amelogenin gene hybridization biosensing based on reduced graphene oxide, Biosens. Bioelectron. 58 (2014) 145-152.

[21] X. M. Li, J. P. Xia, S. S. Zhang, Label-free detection of DNA hybridization based on poly(indole-5-carboxylic acid) conducting polymer, Anal. Chim. Acta 622(2008) 104–110.

[22] X. X. Xiuhua Weng, A. Liu, Q. Lin, C. Wang, W. Chen, X. Lin, Electrochemical genosensor for detection of human mammaglobin in polymerase chain reaction amplification products of breast cancer patients, Anal. Bioanal. Chem. 405 (2013) 3097–3103.

[23] M. Mazloum-Ardakani, N. Rajabzadeh, A. Benvidi, M. M. Heidari, Sex determination based on amelogenin DNA by modified electrode with gold nanoparticle, Anal. Biochem. 443 (2013) 132–138.

[24] A. Benvidi, A. Dehghani Firouzabadi, M. Dehghan Tezerjani, S.M. Moshtaghioun, M. Mazloum Ardakani, Ultra-sensitive DNA sensor based on AuNPs/RGO/GCE, Anal. Biochem. In Press.

[25] A. Benvidi, N. Rajabzadeh, H. Molaye-Zahedi, M. Mazloum-Ardakani, M. M. Heidari, L. Hosseinzadeh, Simple and label-free detection of DNA hybridization on a modified graphene nanosheets electrode, Talanta 137 (2015) 80–86.

[26] J. Tian, P. X. Yuan, D. Shan, S. N. Ding, G. Y. Zhang, X. J. Zhang, Biosensing platform based on graphen oxide via self- assembly induced by synergic interactions, Anal. Biochem. 460 (2014) 16-21.

[27] A. Benvidi, N. Rajabzadeh, M. Mazloum-Ardakani, M. M. Heidari, Ashok Mulchandani, imple and label-free electrochemical impedance Amelogenin gene hybridization biosensing based on reduced graphene oxide, Biosens. Bioelectron. 58 (2014) 145-152.

[28] D. C. Marcano, D.V. Kosynkin, J. M. Berlin, A. Sinitskii, Z. Sun, A. Slesarev, L.B. Alemany, W. Lu, M. Tour, Improved synthesis of graphene oxide, ACS Nano 4 (2010) 4806–4814.

[29] A. E. Radi, J. L. Acero Sachez, E. Baldrich, C.K. O'Sullivan. Reagentless, Reusable, Ultrasensitive electrochemical molecular beacon aptasensor, J. Am. Chem. Soc. 128 (2005) 117–124.

[30] H. Cai, X. Cao, Y. Jiang, P. He, Y. Fang, Carbon nanotube-enhanced electrochemical DNA biosensor for DNA hybridization detection, Anal. Bioanal. Chem. 375 (2003) 287-293.

[31] Y. Zhang, K. Zhang, H. Ma, Electrochemical DNA biosensors based on gold nanoparticles / Cysteamine/ Poly(glutamic acid) modified electrode, Am. J. Biomed. Sci. 1 (2009) 115-125.

[32] N. N. Zhu, Z. Chang, P. G. He, Y. Z. Fang, Electrochemically fabricated polyaniline nanowire-modified electrode for voltammetric detection of DNA hybridization, Electrochim. Acta 51 (2006) 3758–3762.

Table 1 Comparison of analytical performances of several electrochemical DNA sensors

DNA sensor	Detection technique	Linear range (mol L^{-1})	Detection Limit (mol L^{-1})	Reference
DNA/PICA/GCE	CV	3.34×10^{-9}-10.6×10^{-9}	1.0×10^{-9}	[21]
GNs/GCE	EIS	1.0×10^{-20}-1.0×10^{-14}	7.1×10^{-21}	[25]
GR–PBA/Au	SPR	1.0×10^{-15}-1.0×10^{-12}	3.8×10^{-16}	[26]
MWCNTs/GCE	CV	2.0×10^{-10}-5.0×10^{-8}	1.0×10^{-10}	[30]
DNA/AuNP/cys/PGA	DPV	0.09×10^{-9}- 4.8×10^{-9}	0.042×10^{-9}	[31]
DNA/polyaniline/GCE	DPV	22.5×10^{-9}-2.25×10^{-12}	1.0×10^{-12}	[32]
DNA/PANHS/RGO/GCE	EIS	1.0×10^{-18}-1.0×10^{-10}	3.5×10^{-19}	This work

(PICA): poly (indole-5-carboxylic acid)

GNs: graphene nano sheets

(GR): graphene

(PBA): pyrenebutyric acid

(AuNP/cys/PGA): Gold nanoparticles / Cysteamine/ Poly glutamic acid)

Table 2 Comparison of ΔRct values of hybridization process for complementary and noncomplementary sequences at different fabricated electrodes (n=5)

The name of electrode	Complementary $\Delta R_{ct}(K\Omega)$	Noncomplementary $\Delta R_{ct}(K\Omega)$
ssDNA/PANHS/RGO/GCE	6.10 (±0.22)	0.45(±0.10)
ssDNA/PANHS/MWCNT/GCE	4.80 (±0.20)	0.48 (±0.13)
ssDNA/PANHS /GCE	4.55 (±0.11)	0.40 (±0.12)

Caption of figures:

Schem. 1 Schematic representation of the modified electrochemical biosensor based on PANHS, nanomaterials of RGO and MWCNTs and GCE as a platform.

Fig. 1 SEM images of (A) bare GCE, (B) CNTs/GCE, (C) RGO/GCE

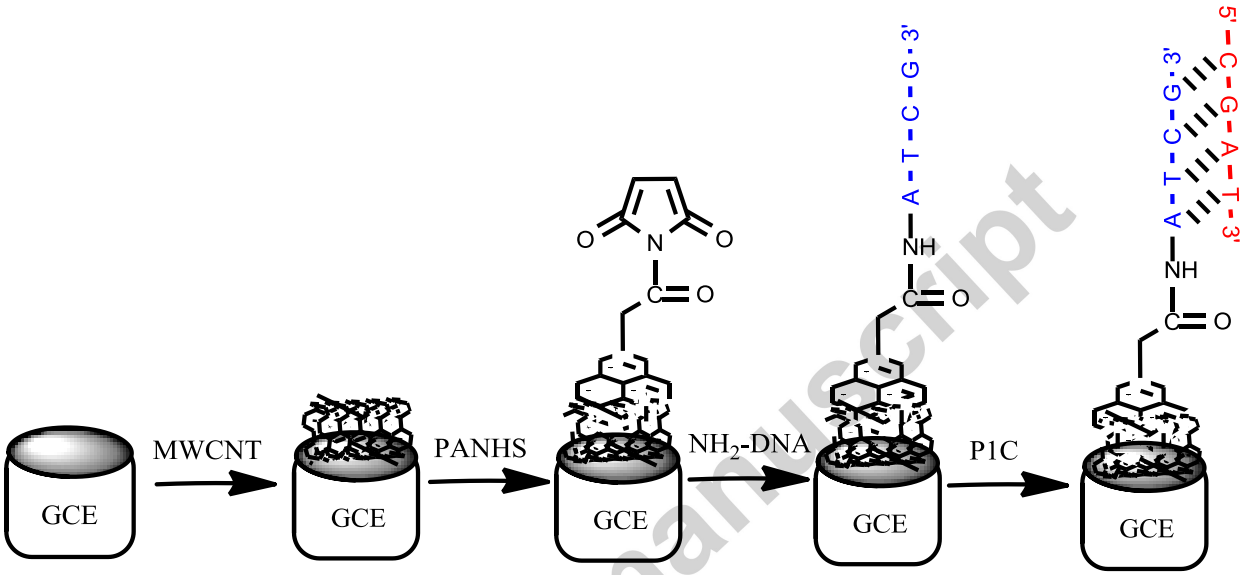
Fig. 2 Cyclic voltammograms of electrode in 0.5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol L^{-1} KCl for the modification steps of (a) GCE, (b) PANHS/GCE, (c) RGO/PANHS/GCE, (d) ssDNA/RGO/PANHS/GCE (e) dsDNA/RGO/PANHS/GCE. (p1)=ssDNA, (p1C) = dsDNA

Fig. 3 Electrochemical Impedence Spectroscopy (EIS) obtained for 0.5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ in a 0.1 mol L^{-1} PBS at (a) GCE, (b) RGO/GCE, (c) PANHS/RGO/ GCE, (d)) ssDNA/PANHS /RGO /GCE (e) Tween/ssDNA/PANHS/RGO/GCE (f) dsDNA/Tween/PANHS/RGO/GCE (g) Noncom-DNA/Tween/PANHS/RGO/GCE. EIS conditions: ac potential 5 mV, frequency range 10 kHz to 0.1 Hz and (p1) =ssDNA, (p1c) = dsDNA, (p1nC) = noncomplementary dsDNA

Fig. 4 The optimization of (A) immobilization time (B) Tween incubation time (C) the time of hybridization (D) the temperature of hybridization

Fig. 5 Nyquist diagrams recorded at ssDNA immobilized and after hybridization with its complementary with different concentrations, Inset A: The applied equivalent circuit for impedance data in the presence of redox couples

Fig. 6 Plots of the dependence of ΔR_{ct} versus the different concentrations of target for (A) ssDNA /PANHS/GCE, (B) ssDNA/MWCNTs/PANHS/GCE, (C) ssDNA/RGO/PANHS/GCE



Scheme. 1

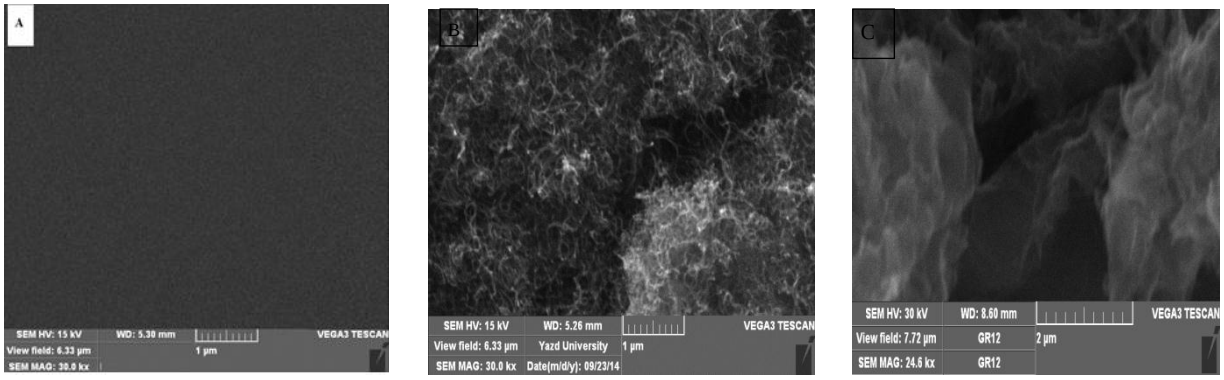


Fig. 1

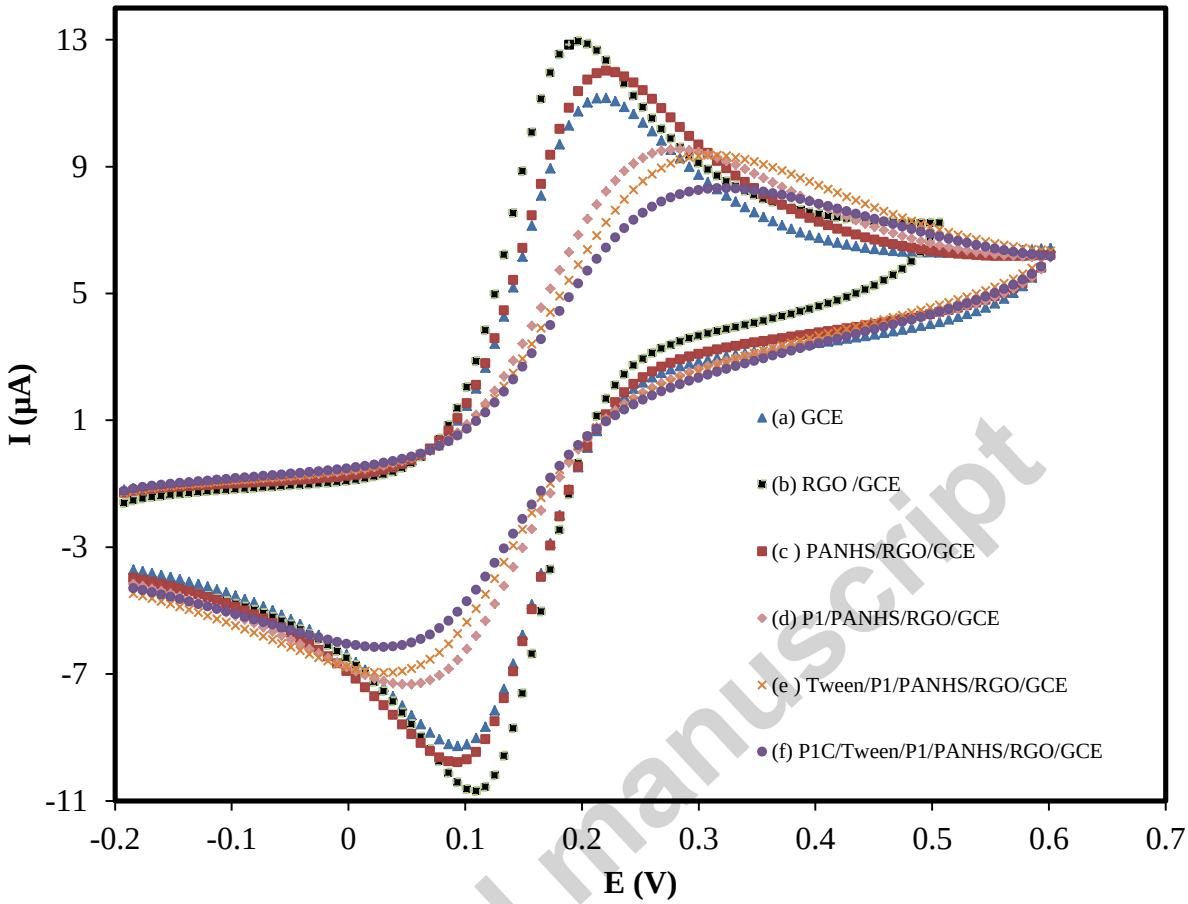


Fig. 2

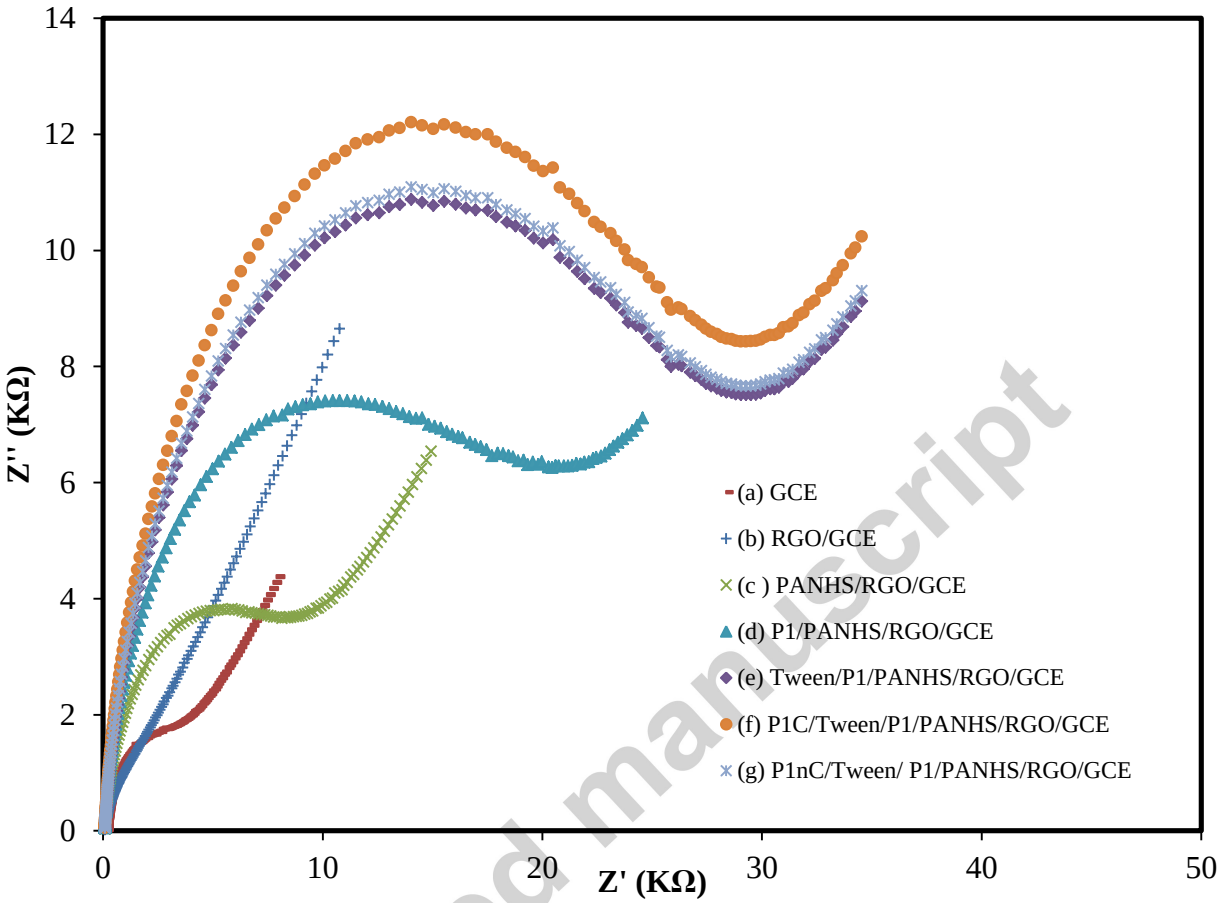


Fig. 3

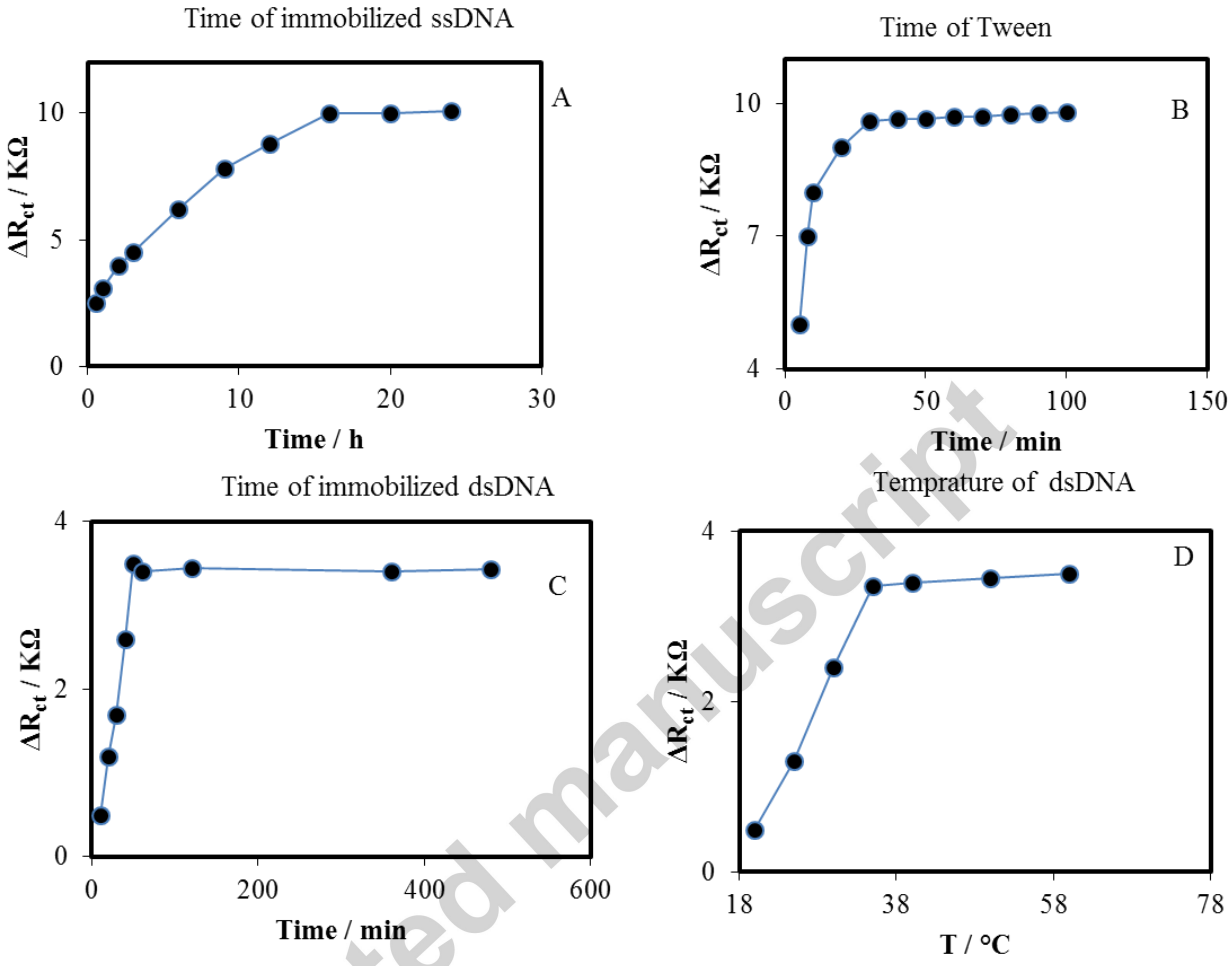


Fig. 4

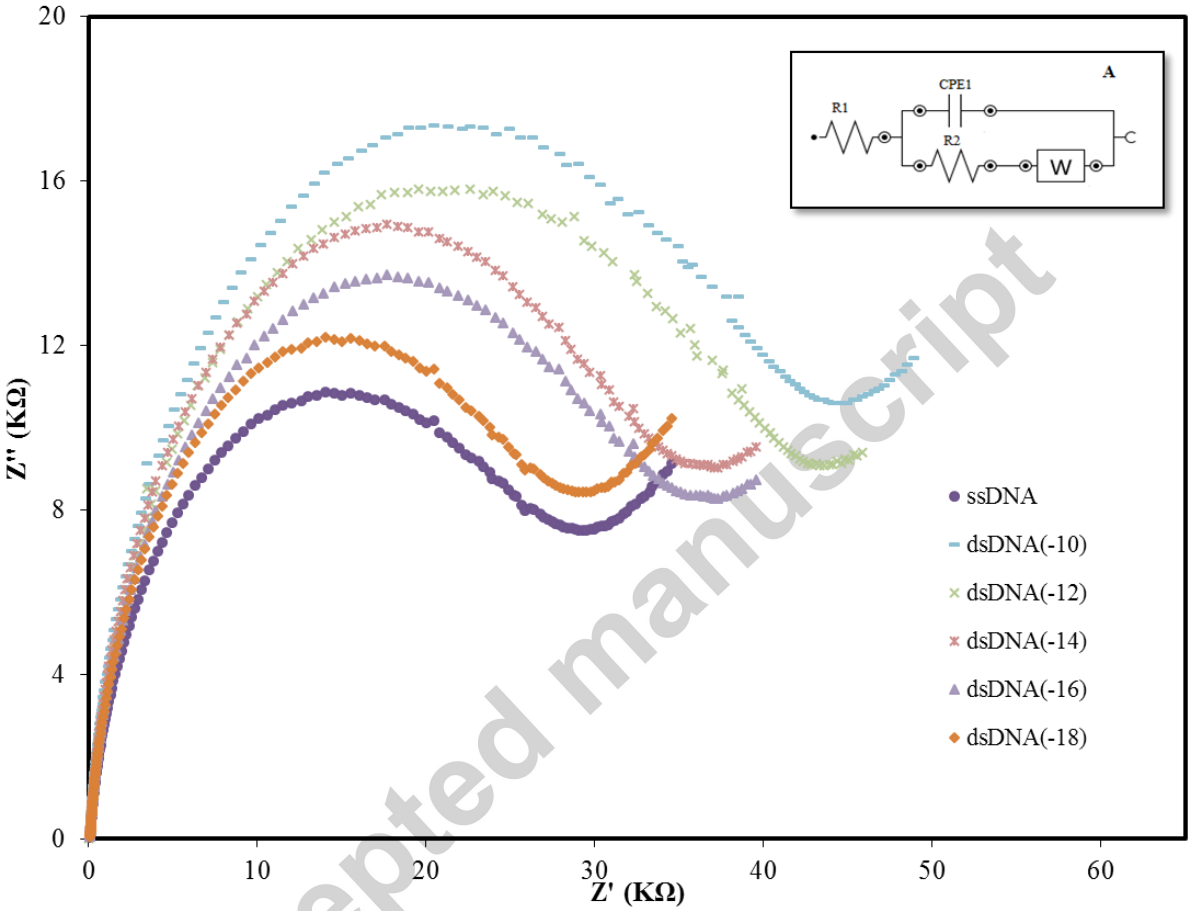


Fig. 5

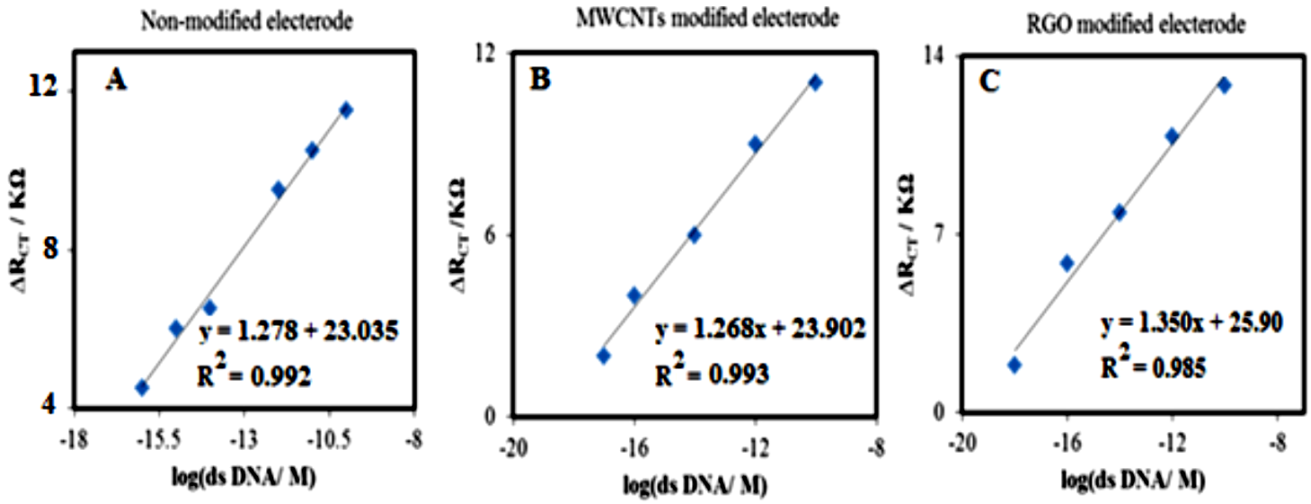
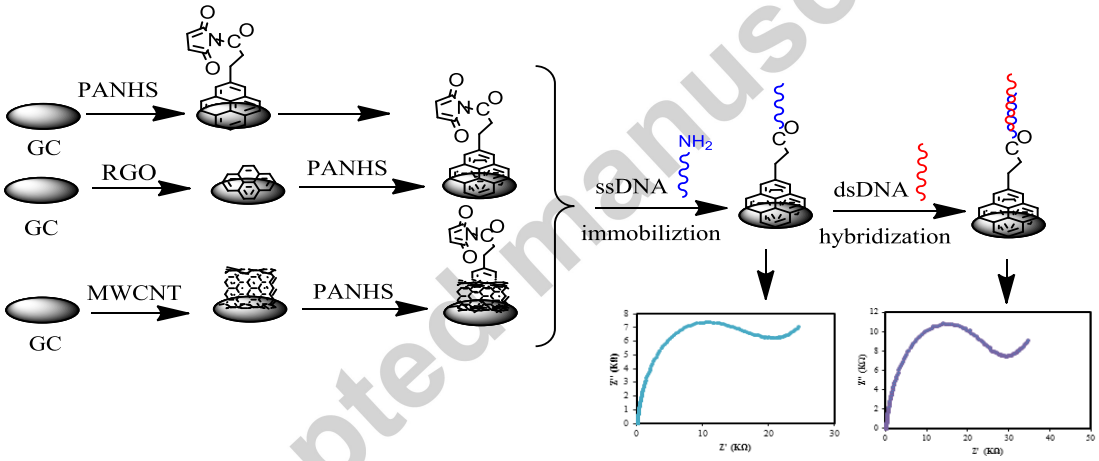


Fig. 6



Highlights:

We have designed and characterized a new electrochemical biosensor based on glassy carbon electrode (GCE) with these advantages:

1-Using RGO and MWCNTs as modifiers with their unique properties

2- Decreasing electrode preparation by using PANHS without need to use EDC/NHS for activation of carboxylic acid groups

3- A low detection limit of 3.5×10^{-19} M was obtained for target DNA at ssDNA/PANHS/RGO/GCE

4- Comparison of linear range and detection limit for non-modified and modified biosensor with RGO and MWCNTs

5- High sensitivity and selectivity for detection of complementary and non-complementary sequences of BRCA1 5382

6- High stability and repeatability of the designed electrochemical biosensor