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A distinguished cancer-screening package containing a DNA sensor and an aptasensor for early and certain detection of acute lymphoblastic leukemia

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ABSTRACT

A disposable package of biosensors was developed along with the corresponding guidelines for early detection of the acute lymphoblastic leukemia cancer. This proposed cancer-screening package included a DNA sensor and an aptasensor as two main types of biosensors. The biosensors were used simultaneously. This combination of sensors can detect not only the presence of mutant genes but also the biomarkers of cancer. At current work, the combination of sensors were used to detect the presence of BCR-ABL1 as a mutant gene and CEA as a biomarkers of cancer, such a capability makes the package liable for early and certain detection of acute lymphoblastic leukemia.

To construct both the DNA sensor and the aptasensor, a nanocomposite consisting of electrosynthesis carbon quantum dots and biosynthesized gold nanoparticles was applied. The construction of these biosensors was characterized using four different electrochemical methods including DPV (Differential Pulse Voltammetry), EIS (Electrochemical Impedance Spectroscopy), CV (Cyclic Voltammetry) and chronoamperometry.

The peak current of a catechol solution that was used as an electroactive probe on the biosensor was linearly related to the logarithm of the concentrations of the target DNA and the target antigen in the range of 10 pM to 100 μM and 1 pg mL^{-1} to 0.001 g mL^{-1} with the detection limits of 1.5 pM and 0.26 pg mL^{-1} respectively, which are quite good results.

1. Introduction

There are more than 100 types of cancer, such as lung cancer, breast cancer, prostate cancer, ovarian cancer, hematologic cancer, and leukemia cancer, which some of them are among the deadliest forms of cancers.

Despite the fact that the early diagnosis of a cancer may increase the chance of successful treatment remarkably, most cancers are detected too late when they have metastasized all over the body.

Acute lymphoblastic leukemia (ALL) is a blood cancer that starts from lymphocyte cells. ALL develops rapidly in days or weeks. It usually spreads or metastasizes throughout human organs (e.g. lymph nodes, spleen, liver, central nervous system, and testicles) fairly quickly. Among the ALL patients, the philadelphia chromosome (BCR-ABL1)-positive (Ph^+) acute lymphoblastic leukemia is an uncommon disease, yet it is classified as a high risk cancer, because only 20–30% of (Ph^+) children with acute lymphoblastic leukemia are survived [1].

The concentrations of cancer biomarkers are usually high in cancerous blood samples. Evaluation of cancer biomarkers, which include different types of molecules such as proteins, has been the most widely used approach for the detection of cancers. These biomarkers can be found in tumor tissues or blood samples. Carcinoembryonic antigen (CEA, CD66) family is cancer biomarkers [2]. Despite being usually restricted to cells of myeloid or monocytic origin, CD66 antigen expression has also been reported in blasts from children acute lymphocytic leukemia [3]. Therefore, screening of the CEA biomarkers can serve as a way of diagnosing different cancers such as ALL.

Biosensing is a renowned clinical technique for monitoring and early diagnosis of different cancers. Biosensors, as accurate analytical devices that work based on converting biological signals into physicochemical signals, are used to measure specific analytes such as antigens and DNA.

CEA ELISA Kit and real-time quantitative PCR (Polymerase Chain Reaction), are two gold standard tests for detection of the CEA and BCR-ABL1.

PCR test and ELISA Kit are time-consuming, multi-steps and almost costly tests, while electrochemical DNA biosensors and aptasensors are two categories of biosensors with many advantages such as compact

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instrumentation, fast responding, cost-effectiveness, and high sensitivity and selectivity [4–6].

In this study, a disposable biosensor package was developed for early detection of acute lymphoblastic leukemia (ALL) at different cancer stages and in the linear range of the biosensors. The proposed cancer-screening package makes use of a DNA sensor and an aptasensor simultaneously.

In the present study, a patient with symptoms suspected of ALL cancer was treated according to the package guidelines. In the first step, the patient underwent a DNA test. In this package, the DNA sensor was applied to determine the presence of a certain mutation in the genome (i.e. the BCR-ABL fusion gene which is found in patients with an ALL cancer).

A negative test result of this DNA sensor would mean that the person actually had no ALL cancer. However, a positive test result of the DNA sensor would tell us that the person had a mutant gene and that he or she was at the risk of the cancer (though he was not a cancer victim yet).

If the patient's DNA test results emerged to be positive, in the second step, an aptamer test would be given to make sure that the positive cancer result was reliable. In this package, the aptasensor was applied to determine the concentration of the carcinoembryonic antigen (CEA) in blood samples. A positive cancer result would mean that cancer biomarkers were present in the blood. Also, a positive test result of the aptasensor would mean that the person definitely had the disease, and the cancer biomarkers were present in his or her blood. In this regard, it is to be noted that the concentration of biomarkers, which is associated with the number of tumor cells, should be determined in all the cancer stages [7–9]. A negative test result of the aptasensor would, mean that the patient did not have ALL; however, he or she was probably still at risk because of having the mutant gene responsible for ALL.

In our previous work, a DNA sensor was applied for the detection of ALL cancer, which has the advantages of good linear dynamic range and detection limits. It is noteworthy that DNA sensors have limitations of their own; that is to say, they can only determine whether a person has a mutant gene or not, but they cannot predict the cancer presence with certainty. As for the screening package proposed in the present study, a combination of a DNA sensor and an aptasensor can detect not only the presence of mutant genes but also the biomarkers of cancer. These advantages make the package capable of early and certain detection of acute lymphoblastic leukemia.

Studies show that nanomaterials demonstrate great capacities for developing reliable electrochemical biosensors with improved sensitivities and lower detection limits of several orders of magnitudes. This is by virtue of the unique characteristics of those materials, such as unmatched electrical properties and excellent biocompatibility [11]. Furthermore, the larger surface area of nanomaterials, as compared to other materials, leads to the immobilization of more bioreceptor units [12,13].

In this study, a nanocomposite consisting of electrosynthesis carbon quantum dots [14] and biosynthesized gold nanoparticles was used to construct both DNA sensors and an aptasensor.

According to the results, especially the ones which was concluded from the real-life sample detections, we anticipate this scheme have potential to develop the system of biosensors for screening other kinds of cancers too.

2. Experimental

2.1. Apparatus and reagents

Probes and antigens were obtained from the Gen Bank database. All the oligonucleotides were obtained from Macrogen (South Korea, 10F, 254 Beotkkot-ro Geumcheon-gu, Seoul 08511, Rep. of Korea).

The chemicals including ethanol (C_2H_5OH), Chloroauric acid ($HAuCl_4$), sodium hydroxide (NaOH), catechol ($C_6H_6O_2$) and bovine serum albumin were of analytical grades and were purchased from Sigma-Aldrich ((USA, http://www.sigmaaldrich.com/). The DNA sequences, aptamers and the CEA antigen were obtained from ABCOM Company (http://www.ABCOM.com). *Saccharomyces cerevisiae* yeast was taken from the microbial collection of Yazd University, Yazd, Iran. All the solutions were prepared freshly with double distilled water. All the materials were used as received, without further purification.

The electrochemical measurements were performed using an Autolab potentiostat/galvanostat (PGSTAT-302 N, Eco Chemie, The Netherlands). The experimental conditions were controlled with the General Purpose Electrochemical System (GPES) software (Kanaalweg 29/G, 3526 KM Utrecht, the Netherlands). To investigate the biosensors, an Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and a modified glassy carbon electrode (GCE) (Bio AuNP/CD/GCE) were employed as the reference, auxiliary and working electrodes respectively.

Another three-electrode system of a Pt (as the counter electrode), a graphite electrode (as the working electrode) and Ag/AgCl/KCl (3.0 M) (as the reference electrode) was employed to synthesize the carbon quantum dots.

The pH of the solutions was measured using a Metrohm 691 pH/ion Meter (Ionenstrasse 9101 Herisau Switzerland). A GFL1101 shaker (Schulze-Delitzsch-Strasse 4 30938 Burgwedel/Germany) was used to speed up the yeast growth by agitating the mixture. Biosensor assembly was carried out through several functionalizing steps by adding 2.5 μL of an appropriate solution to the surface of the working electrode (i.e. glassy carbon).

The DNA sequences were as follows:

Aptamer sequence (APTA):

5′-Thiol-TTT TTT TTT TTT ATA CCA GCT TAT TCA ATT-3′

The DNA probe sequence (ssDNA), which demonstrates the BCR/ABL fusion gene:

5′-Thiol-TTT TTT AGA GTT CAA AAG CCC TTC-3′

The complementary sequence:

5′-GAA GGG CTT TTG AAC TCT-3′

The non-complementary sequence:

5′-ACG TGG TCC CCA GCT CTC-3′

Stock solutions of the oligonucleotides (100.0 μM) were prepared in a 0.1 M phosphate buffer saline (PBS buffer, pH 7.4) and kept frozen at − 20 °C. The double distilled water and the buffers were sterilized using an autoclave.

2.2. Preparation of modified electrodes

2.2.1. Preparation of carbon dot nanoparticles

C-dots were synthesized electrochemically via one-step carbonization of ethanol. To do this, a conventional three-electrode system was used. In a typical process, 70 mL of ethanol was mixed with 5 mL of water, and then 0.5 g of NaOH was added under stirring. The reaction continued for 24 h at the potential of 4.5 V, and the solution turned brown. In the next step, 150 mL of ethanol was added in order to salt out NaOH overnight. The resulting mixture was then heated at 80 °C for 24 h until a yellow powder was achieved [14].

2.2.2. Preparation of gold nanoparticles

Au nanoparticles were manufactured via the biosynthesis technique using *Saccharomyces cerevisiae* yeast. The strains were grown in a liquid medium of a Yeast Nitrogen Base Agar with Glucose)YNBG(broth at 27 °C shaken for 72 h at 80 rpm. Then, the yeast biomass was separated from the culture liquid medium by centrifugation (5000 rpm, for 10 min). As little as 0.15 g of the obtained biomass was then added to the Au solutions (0.6 mM $HAuCl_4$) and mixed at 80 rpm and 27 °C for 48 h. To separate the Au nanoparticles, the mixture was centrifuged at10000 rpm for 10 min [7].

2.3. Development of biosensors

In order to develop biosensors, a GCE was polished with alumina powder (0.30 μm) and sonicated in an ethanol bath to remove any fine particulates. Then, 2.5 μL of a 0.008 g ml^{-1} CD solution (pH 7.4) was added onto the GCE drop by drop, and the electrode dried up at room temperature (CD/GCE). Next, 2.5 μL of 0.004 M biosynthesized gold nanoparticles was added onto the surface of the CD/GCE. In the third step, 2.5 μL of 5 μM 5′-SH probe DNA and 5 μM 5′-SH CEA aptamer were dropped onto two different Bio AuNP/CD/GCEs. The Au-thiol bond efficiently improved DNA and aptamer immobilization within an optimum immobilization time. In this study, the biosynthesized Au nanoparticles were bound to the thiol groups of the probe DNA and the aptamer. The developed biosensors were rinsed with a Tris-EDTA buffer solution to wash away the non-bound probe DNA and the aptamer strand. In fourth step, the mentioned modified electrode was incubated with 2.5 μL of a 1 mM solution of 6-mercapto-1-hexanol)MCH(for 1 h. In the fifth step, to modify the developed biosensor, 2.5 μL of the target DNA and CEA antigen solutions were dropped onto the ssDNA/Bio AuNP/CD/GCE and the aptamer/Bio AuNP/CD/GCE surfaces respectively. After the optimum hybridization time, the developed biosensors were washed with the Tris-EDTA buffer solution to remove the unreacted target DNA and CEA antigens (Scheme S1).

2.4. Electrochemical measurements

The developed DNA sensor and aptasensor were characterized using DPV (Differential Pulse Voltammetry), EIS (Electrochemical Impedance Spectroscopy), CV (Cyclic Voltammetry) and chronoamperometry in a redox probe solution. Cyclic voltammograms were recorded in a 2 mM catechol solution at the scan rate of 100 mV/s. Thereafter, the electrode was immediately detected in phosphate buffer saline (PBS) using DPV in the potential range of 0.1 to 0.8 V at the potential step of 5 mV. EIS recording was done in the frequency range of 0. 1 Hz to 1,000,000 Hz at the bias potential of 200 mV with the alternating voltage of 10 mV. Chronoamperometric measurements were carried out by setting the working electrode potential at 500 mV.

3. Results and discussion

3.1. Scanning electron microscopy (SEM) and UV–Visible spectroscopy characterization

The morphological characteristics of the synthesized carbon dots and the biosynthesized gold nanoparticles were determined by SEM. The typical SEM micrographs of the carbon dots and the biosynthesized gold nanoparticles are presented in Fig. 1 and Fig. 2 respectively. As it

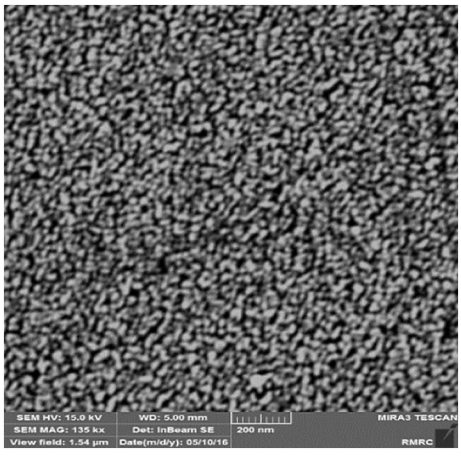

Fig. 1. The SEM micrograph of the carbon dot.

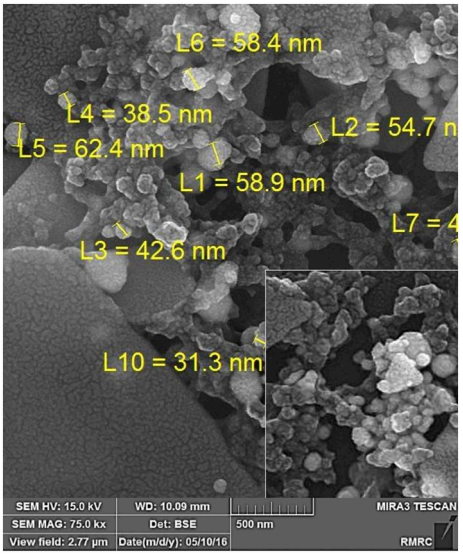

Fig. 2. The SEM micrograph of the gold nanoparticles.

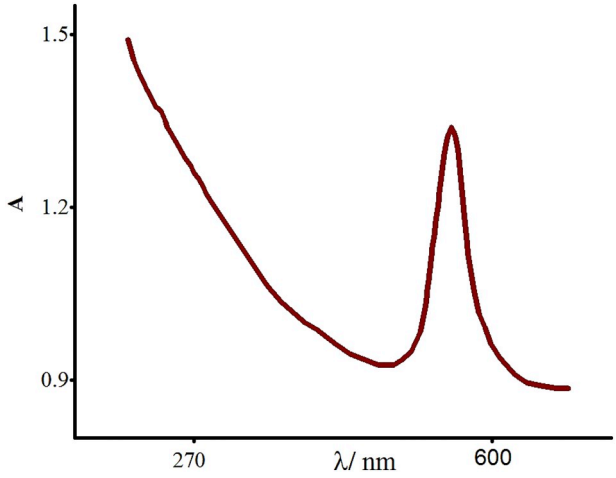

Fig. 3. UV–Visible spectroscopy of biosynthesized gold nanoparticles.

can be seen, the SEM micrographs in both figures consist of uniform mono-disperse spherical structures [7,14]. The UV–Visible spectroscopy of the biosynthesized gold nanoparticles showed a peak at 550 nm, which is due to the electron transitions among gold nanoparticles (Fig. 3).

3.2. Optimization of experimental parameters

The effective parameters in the preparation of the biosensors were optimized. The parameters included the DNA probe and the aptamer concentration, the incubation time of the DNA probe and the aptamer sequence and modification time. Also, different electrochemical techniques including DPV, EIS, CV, and chronoamperometry were applied to test the performance of the developed biosensors (i.e. surface-modified electrodes) [4,15–18].

Various concentrations of the DNA probe solution and the aptamer sequence (10^{-5} to 10^{-9} M) were tested to optimize the probe concentration. Measurements were also performed in a redox probe solution. The best choice of a redox probe solution was 2 mM of catechol in a PBS buffer with pH 7.4 (Figs. S1 and S2). According to the data (Fig. S3), 10^{-7} M was the optimum value for the DNA probe and the aptamer sequence concentrations, which suggests a good increment in this optimum value. However, according to the experimental results, due to

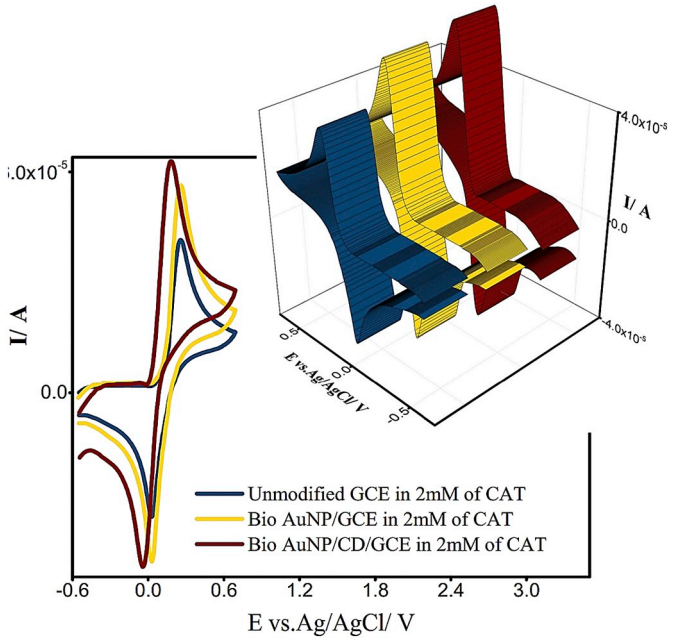

Fig. 4. Detection of the GCE, Bio AuNP/GCE and Bio AuNP/CD/GCE in 2 mM of catechol in PBS.

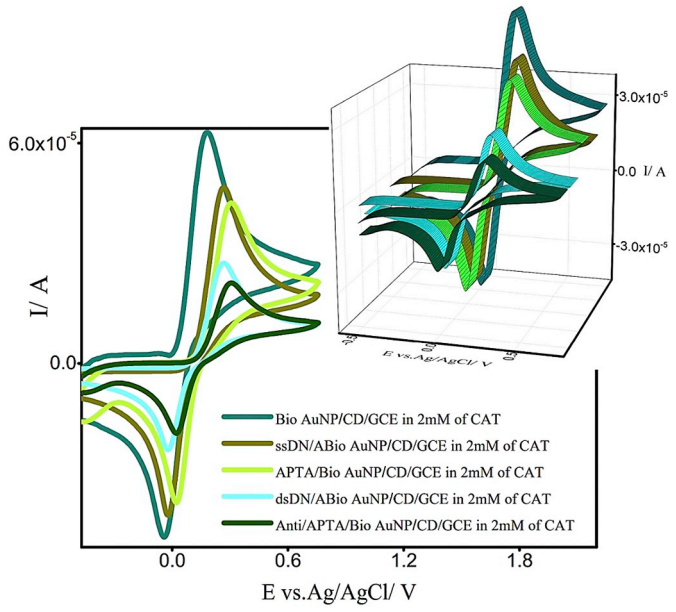

Fig. 5. Detection of the immobilization and hybridization processes at the Bio AuNP/CD/GCE, ss DNA/Bio AuNP/CD/GCE, and APTA/Bio AuNP/CD/GCE in 2 mM of catechol in PBS.

the saturation of the active sites, there was no considerably higher value than 10^{-7} M observed in the responses taken from different concentrations of the DNA probe or the aptamer sequence.

3.3. Electrochemical characterizations and detection of the hybridization process of the DNA biosensor

In order to confirm the progress of electrode modification in any step, the redox reaction was confirmed by applying the CV method to each modification step. The cyclic voltammograms were recorded in a 2 mM catechol solution at the scan rate of 100 mV/s.

Compared to the bare GCE, after modification of GCE with Bio AuNP, the oxidation peak current increased. After modification of GCE with Bio AuNP/CD, the oxidation peak current was obviously enhanced, and a shift to less positive potentials was observed. This is all because of the unique properties of CDs, namely their increased surface-to-volume ratio and quantum size effect (Fig. 4).

The active surface area of the modified electrode was estimated according to the slope of I_P versus $\nu^{1/2}$ plot for a known concentration of $[Fe(CN)_6]^{3-/4-}$, based on the Randles–Sevcik equation as follows:

$$I_{peak} = 2.69 \times 10^5 n^{3/2} A D^{1/2} C_{bulk} \nu^{1/2} \quad (1)$$

where I_{peak} is the anodic peak current, A is the surface area of the electrode, n represents the electron transfer number, D stands for the diffusion coefficient, C_{bulk} is the concentration of $[Fe(CN)_6]^{3-/4}$ and ν is the scan rate [19,20]. For 1.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ in a 1.0 mol L^{-1} KCl electrolyte, the microscopic area estimated on the basis of the slope of the I_{peak}– $\nu^{1/2}$ relationship was found to be 2.08 cm^2 for the Bio AuNP/CD/GCE (Fig. S4).

The cyclic voltammetry results of DNA and aptamer immobilization showed that the redox peak current reduced as a result of the covalent coupling thiol of the modified probe DNA and the aptamer sequence. Furthermore, it reduced to a lower value due to the hybridization of the probe DNA, the target DNA and the aptamer with the CEA antigen (Fig. 5). The DPV analyses confirmed the CV results (Fig. S5).

In the EIS technique, the linear part at low frequencies associates with the diffusion-limited process, and the semicircle portion at high frequencies relates to the electron transfer-limited process. Fig. 6 demonstrates the Nyquist plots of EIS associated with the construction of

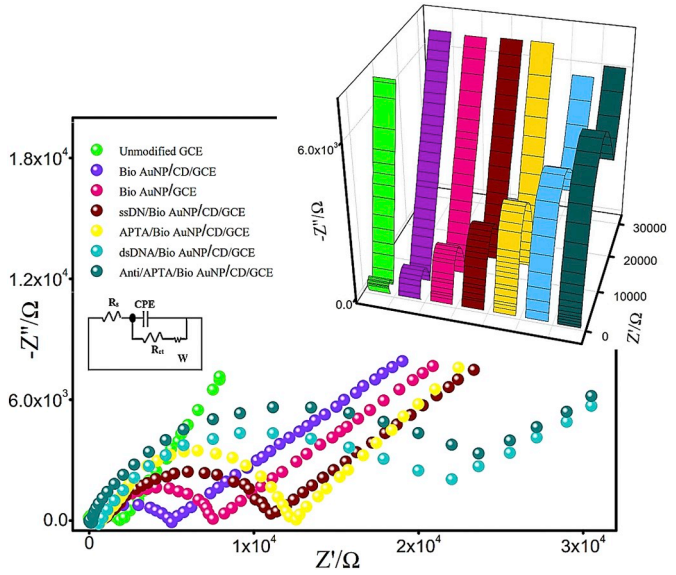

Fig. 6. Nyquist plots of the GCE, Bio AuNP/GCE, Bio AuNP/CD/GCE, ssDNA/Bio AuNP/CD/GCE and APTA/Bio AuNP/CD/GCE, dsDNA/Bio AuNP/CD/GCE and Anti/APTA/Bio AuNP/CD/GCE in 1 mM of $[Fe(CN)_6]^{3-/4-}$ in PBS. Inset: Equivalent circuit for fitting the plots.

the DNA sensor and the aptasensor after each step. $[Fe(CN)_6]^{3-/4-}$ was applied as a probe to demonstrate the changes in the behavior of the electrode after each surface modification step. As it can be seen, after pipetting the Bio AuNP on the GCE surface, the R_{ct} was enhanced in small amounts. This implied the prospering formation of a Bio AuNP film at the GCE surface. When the GCE electrode surface was modified with Bio AuNP/CD, the R_{ct} decreased, implying that the Bio AuNP/CD formed on the modified GCE was an excellent electron-conducting composite which facilitated the electron transfer.

Afterwards, when the probe DNA and the aptamer were covalently bound onto the Bio AuNP/CD/GCE surface, the electron-transfer resistance enhanced. This demonstrated that the probe DNA and the aptamer sequence had been immobilized on the surface of the Bio AuNP/

CD/GCE. Furthermore, due to the hybridization of the probe DNA, the target DNA and the aptamer sequence with the target antigen, the electron-transfer resistance increased the most. As it can be seen in Fig. 6, the EIS analyses also confirmed the CV and DPV results [21].

Through electrochemical methods, chronoamperometry made it possible to register quick and reproducible signals. Indeed, the registry was possible after the addition of the CD, Bio AuNP, aptamer sequence, antigen, probe DNA and target oligonucleotide. According to the data, the chronoamperometry results confirm the CV, DPV and EIS graphs (Fig. S6).

The incubation time of the DNA probe and the aptamer sequence was experimentally optimized. According to the results, 1 h and 2 h were the optimum values found for the DNA probe and the aptamer sequence respectively (Fig. S7). Generally, they are considered as short durations. Therefore, 2 h and 3 h were chosen as optimum hybridization times for the target DNA and the target antigen respectively (Fig. S8), which proved to be really good durations.

3.4. Repeatability and stability of the biosensor

To check the repeatability of the biosensor, three parallel immobilizations of the probe DNA and the aptamer were done on the modified GCE. Then, hybridization was carried out with the target DNA and the CEA antigen. Three DNA biosensors, which were made individually, showed a totally acceptable relative standard deviation of 0.014 and 0.012 for the DNA sensor and the aptasensor respectively (Fig. S9).

Generally, the stability of DNA sensors and aptasensors plays an important role in gaining an appropriate degree of sensitivity. To test the stability of the aptasensor and the DNA sensor in this study, cyclic voltammetry was repeated for three times, but no considerable change was observed in the I_{peak} values. Besides, when the modified electrode was stored at 4 °C for about a week, the results remained almost unchanged.

3.5. Evaluation of the selectivity of the DNA biosensor

To evaluate the selectivity of the aptasensor and the DNA sensor, an APTA/Bio AuNP/CD/GCE and an ssDNA/Bio AuNP/CD/GCE were used to appraise the hybridization process. In this regard, it has been found that there was a decrement in the current signal. This means that hybridization took place between the ssDNA and the complementary DNA as well as between the aptamer and the target antigen [22,23].

In our study, however, there was no obvious decrease in the peak current after hybridization with the mismatch target (bovine serum albumin) and the non-complementary DNA sequence. This demonstrates that there occurs no hybridization and that the slight reduction of the peak current might be due to the non-specific adsorption (Fig. S10).

3.6. Sensitivity of the electrochemical DNA biosensor

Biosensor sensitivity was tested by applying the modified probe DNA electrode and an aptamer sequence hybridized with various concentrations of the complementary DNA strand and the target antigen respectively. As it can be seen in Fig. 7, the DPV peak current of catechol on the mentioned biosensors decreased, along with the enhancement in the concentration of either the complementary DNA strand or the target antigen in each biosensor.

Also, as presented in Fig. 7, the peak currents of catechol on the biosensor are linearly related to the logarithm of the concentrations of the target DNA and the CEA antigen in the ranges of 10 pM to 100 μM and 1 pg mL^{-1} to 0.001 g mL^{-1} with the detection limits of 1.5 pM and 0.26 pg mL^{-1} respectively.

This result (detection limits of 0.26 pg mL^{-1} for the CEA antigen) is almost a good result, versus the Cell Biolabs ELISA kits (Catalog Numbers PRB- 5059) result with the detection sensitivity of 150 pg mL^{-1} for CEA, and the Thermo Scientific™ Human CEA ELISA Kit (EHCEA) result with the analytical sensitivity of 200 pg mL^{-1} for CEA.

3.7. Performance with patient samples

To investigate the simultaneous presence of BCR/ABL fusion gene and CEA in real-life samples of acute lymphoblastic leukemia patients, real-life samples were extracted from the human blood

(the sample collection is offered in partnership with the Khatam Al-Anbia Super Speciality Clinic).

At current work, a PCR targeting the BCR/ABL1 fusion gene in ALL patients was developed, hence, random blood samples were collected from 30 patients with acute lymphoblastic leukemia (the DNA sequence was collected from 500 μL blood samples of patients with ALL cancer).

PCR amplifications of the BCR/ABL fusion gene, were done through a pair of primers (forward primer: TTCAGAAGCTTCTCCCTGACAT; nucleotide position: 196–217 and Reverse primer: TGTTGACTGGCGT GATGTAGTTGCTTGG; nucleotide position: 655–628; with a product (amplicon) length of 460 bp). In order to do this, 0.5 μL of each primer (10 pmol), 0.8 μL of $MgCl_2$ (50 mM), 0.5 μL of dNTP (10 mM), 2.5 μL of 10× PCR and 1.5 Units of Taq DNA polymerase gave a total volume of 25 μL, containing 100 ng total DNA as a template (and the thermal cycle program was 94 °C for 1 min, 94 °C for 1 min 64 °C for 1 min, 72 °C for 1 min and 72 °C for 10 min).

Subsequently, patient samples were denatured by heating at 95 °C for 7 min and then cooled down in an ice bath for 3 min. After that, 1 μL of a sample was diluted with 9 μL of a hybridization buffer [10]. Then, 2.5 μL of that sample was dropped on the surface of the modified electrode. The hybridization reaction was allowed to proceed for an optimum time, and then the biosensor was washed with the Tris-EDTA buffer solution.

According to the results, there was an agreement in results of PCR investigations and the data recorded by biosensors.

To study the presence of the carcinoembryonic antigen in samples of acute lymphoblastic leukemia patients, real-life samples were extracted from the human blood. After that, 1 μL of a sample was diluted with 9 μL of a hybridization buffer. Then, 2.5 μL of that sample was dropped on the surface of the modified electrode. Then this reaction was allowed to proceed for an optimum time, and then the biosensor was washed with the Tris-EDTA buffer solution.

A DPV analysis was done in 2 mM of a catechol solution to study the hybridization process of the DNA strands and the attachment of the CEA to the aptamers, in patient samples. According to the results, there was a decrement in the DPV signal for the blood samples which were collected from ALL patients. This means that the attachment took place between the ssDNA and complementary real-life sample (patient sample) DNA as well as between the aptamer and the target antigen in real-life sample (patient sample) of acute lymphoblastic leukemia patients (Fig. S11).

4. Conclusion

The purpose of this paper is to introduce a developed package of biosensors (a combination of a DNA sensor and an aptasensor) which can detect not only the presence of mutant genes but also the biomarkers of cancer. According to the results, we anticipate this scheme have potential to develop the system of biosensors for screening other kinds of cancers too.

At current work, a positive test result of a DNA sensor generally means that a person has a mutant gene and that he or she is at the risk of a cancer; however, a positive test result of an aptasensor means that the person already has the disease (i.e. ALL cancer). In this regard, it should be noted that the concentration of biomarkers is used for the screening of cancer stages.

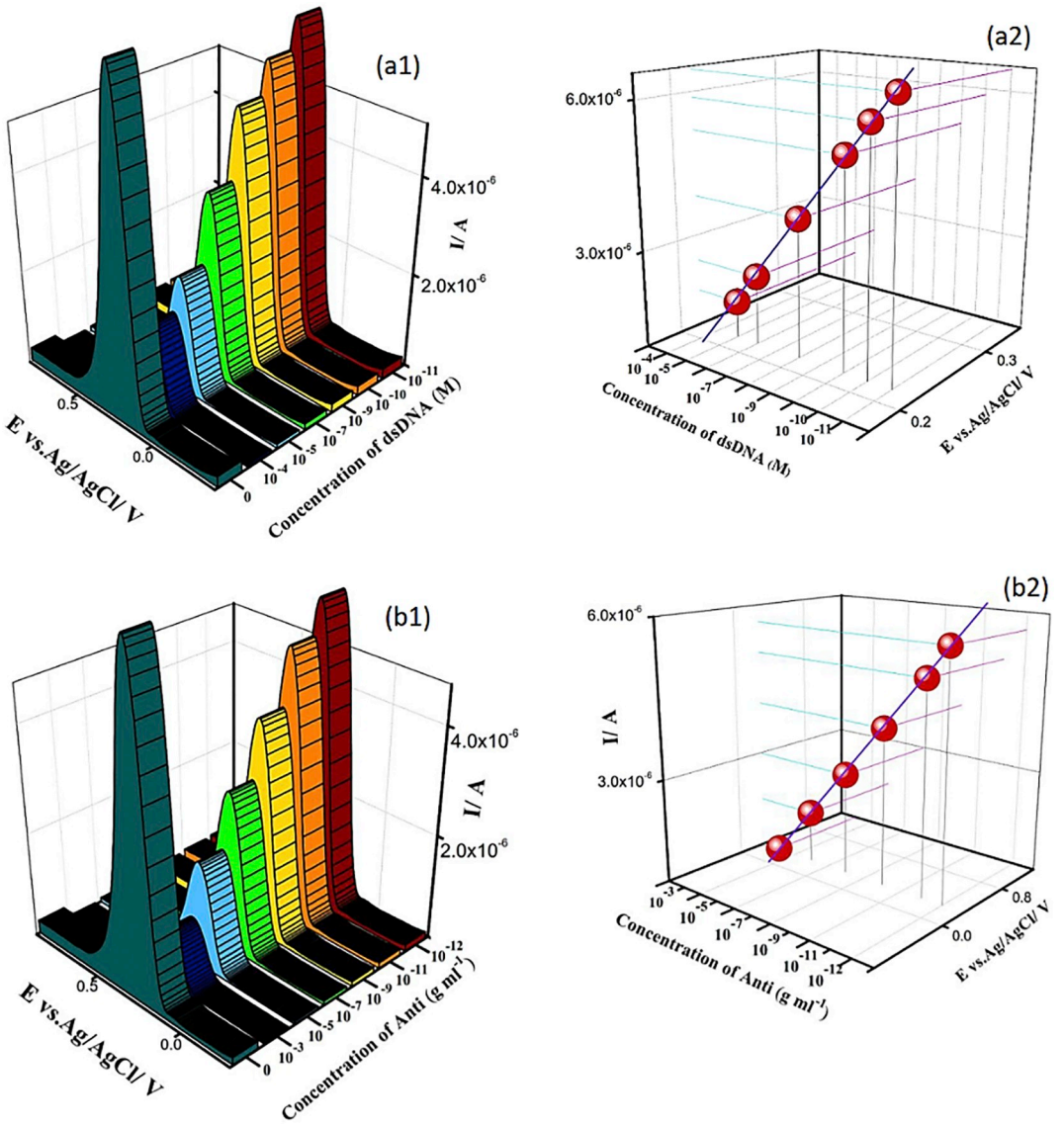

Fig. 7. (a_1) DPVs of the dsDNA/Bio AuNP/CD/GCE and (a_2) the calibration curve of the dsDNA/Bio AuNP/CD/GCE at different concentrations of the target DNA in 2 mM of catechol in PBS. (b_1) DPVs of the Anti/APTA/Bio AuNP/CD/GCE and (b_2) the calibration curve of the Anti/APTA/Bio AuNP/CD/GCE at different concentrations of the target DNA in 2 mM of catechol in PBS.

The surface areas of the biosensors were modified with a stable uniform nanocomposite that consisted of electrosynthesized carbon quantum dots and biosynthesized gold nanoparticles. This nanocomposite has good electrical properties and a quite extensive coupling area.

The peak currents of catechol on the biosensor was linearly related to the logarithm of the concentrations of the target DNA and the target antigen in the ranges of 10 pM to 100 μM and 1 pg mL^{-1} to 0.001 g mL^{-1} with the detection limits of 1.5 pM and 0.26 pg mL^{-1} respectively.

Sensitivity, selectivity, and reproducibility of the biosensors were evaluated and real samples detection was done through DPV, EIS, CV and chronoamperometry techniques, all of which provided totally acceptable results.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.07.009.

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