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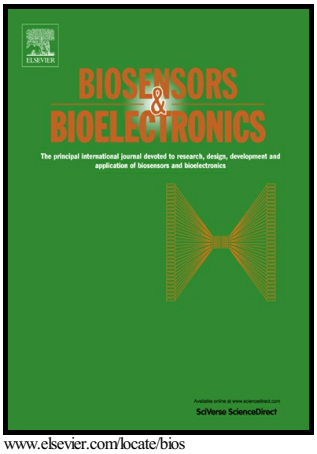

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A NanoBiosensor Composed of Exfoliated Graphene Oxide and Gold Nano-Urchins, for detection of GMO products

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Abstract

Genetically Modified Organisms, have been entered our food chain and detection of these organisms in market products are still the main challenge for scientists. Among several developed detection/quantification methods for detection of these organisms, the electrochemical nanobiosensors are the most attended which are combining the advantages of using nanomaterials, electrochemical methods and biosensors. In this research, a novel and sensitive electrochemical nanobiosensor for detection/quantification of these organisms have been developed using nanomaterials; Exfoliated Graphene Oxide and Gold Nano-Urchins for modification of the screen-printed carbon electrode, and also applying a specific DNA probe as well as hematoxylin for electrochemical indicator. Application time period and concentration of the components have been optimized and also several reliable methods have been used to assess the correct assembling of the nanobiosensor e.g. field emission scanning electron microscope, cyclic voltammetry and electrochemical impedance spectroscopy. The results shown the linear range of the sensor was 40.0-1100.0 femtomolar and the limit of detection calculated as 13.0 femtomolar. Besides, the biosensor had good selectivity towards the target DNA over the non-specific sequences and also it was cost and time-effective and possess ability to be used in real sample environment of extracted DNA of Genetically Modified Organism products. Therefore, the superiority of the aforementioned specification to the other previously published methods was proved adequate.

Keywords

GMOs; nanomaterial; nanobiosensor; electrochemical method; Gold Nano-Urchin

1. Introduction

By the development of the genetic engineering, breeding sciences and biotechnology in the food industry, some biosafety concerns are rising about the dramatic revolution of genetically modified organisms (GMOs) (1, 2). The GMOs are defined as transgenic organisms that their genetic material has been changed, in a way that does not occur naturally, for the production of desired biological products (3, 4). Although some regulations have been developed regarding obligation of labeling product containing GMOs in some developed countries, but the majority of the food products in markets around the world need to be analyzed for GMOs (2, 5). For example, the European Union determined the mandatory rules about the labeling of food products with a GMO content greater than 1%, in year 2000 (4, 6).

Day after day, new analytical methods have been developed for accurate detection and quantification of GMOs in the market products (3, 5). Successful designs and fabrications of new techniques and technologies have been introduced in this regard, so far, such as polymerase chain reaction (PCR) (7), real-time PCR (8), digital PCR (9), Next-generation sequencing (10), Microarray (11), Enzyme-linked immunosorbent assay (ELISA) (12), Surface Plasmon Resonance (SPR) biosensors (13), quartz crystal microbalance (QCM) biosensors (14), lateral-flow strip biosensor (15), electrochemical methods (16), etc.

Nanotechnology has been affected many fields of sciences and technologies, and there are fast growing list of applications of nanomaterials has tremendous impact on our life (17-22). A range of nanomaterials and nanostructures, from gold (23, 24) to carbon (25, 26), have been used to modify different kind of electrodes so far in biomedical applications such as biosensing (27-31). Several types of nanobiosensors have been developed and used to detect biological agents and biomarkers, from DNA to proteins, to detect GMOs (5, 32). Based on their transducer types, nanobiosensors are categorizing to electrochemical, colorimetric, fluorescence, piezoelectric, etc. (32-34). The electrochemical nanobiosensors have been used mostly, mostly because of their low cost, high sensitivity and selectivity, and taking advantage of nanomaterials to achieve lower detection limits, as well (33, 35).

Electrochemical biosensors/genosensors/nanobiosensors have been used so far for detection of different GMO organisms with different techniques, nanomaterials, nanostructures, electrochemical labels, e.g. (3, 5). Recently, Barroso and colleagues developed an electrochemical gensoensor based on the 3D-nanostructured Au electrodes for detection of a transgenic maize with limit of detection of 0.48 nM (36). In another study, the multiplexed

enzymatic labelling strategy were used by simultaneous magnetic entrapment via sandwich hybridization of two DNA sequences for detection of soybean GMOs with 0.11 ng of DNA (37). For the detection of transgenic maize, a screen-printed electrodes modified with carboxyl functionalized multi-walled carbon nanotubes were developed for an impedimetric genosensor with sensitivity of 22.0 fmol (38). Huang *et al* in 2015, presented an exonuclease enzyme catalytic reaction and gold nanoparticle-based bio-barcode related strategies to reach a 0.1 pM detection of DNA (39). Another research, attempted to determine the CaMV 35S promoter sequence using a printed electrode based on single-walled carbon nanotube field effect transistor which could detect 1.0 nM as lower detection limit (40). Through the application of loop-mediated isothermal amplification on a disposable electrochemical printed electrode, a group of researchers successfully achieved the sensitivity of 3.0 nM of GMO maize DNA (41).

Here, we developed an electrochemical nanobiosensor (or genosensor) based on the Exfoliated Graphene Oxide (EGO) and Gold Nano-Urchins (GNU) modified screen-printed carbon electrode (SPCE), in order to detect and quantify GMO organisms based on the detection of the "CaMV 35S promoter" sequence which is being used frequently in production of GMOs. For this purpose, a specific alkanethiol DNA probe is designed to be immobilized on the surface of the Gold Nano-Urchins (GNU) which are decorated on the Exfoliated Graphene Oxide (EGO) modified screen-printed carbon electrode (SPCE) as the proposed nanobiosensor.

2. Experimental

2.1. Materials and Oligonucleotides

Exfoliated Graphene Oxide (EGO) was synthesized according to the previously explained protocol (42) and Gold Nano-Urchins (GNU) were purchased from Aldrich (Cat number: 9797731) (St. Loius, MO, USA) with 70 nm diameter.

All the other chemicals used in this study were also taken from Sigma-Aldrich Company (St. Loius, MO, USA), were of analytical grade and were used as received without further purification. The DNA oligonucleotides were supplied (as lyophilized powder) from the TAG Copenhagen Company (Copenhagen, Denmark) with the following sequences:

Thiolated DNA probe: 5'-HS-$(CH_2)_6$-CCACGTCTTCAAAGCAAGTGG-3'. Complementary target DNA (CaMV 35S promoter): 5'-CCACTTGCTTTGAAGACGTGG-3'; Non–complementary DNA: 5'-GATGACCTATAGCGACTGCAT-3'; One base mismatch: 5'-CCACTTGCTTAGAAGACGTGG-3'; Three base mismatch: 5'-CCACTCGCTTAGAAGAAGTGG-3'.
The preparation protocol of the stock solution of the oligonucleotide and all the other used solutions, including immobilization, washing and hybridization solutions, were taken from the previous publication of Nasirizadeh and his colleagues (43).

2.2. Electrochemical instruments and studies

Electrochemical experiments, including Cyclic Voltammetry (CV), Electrochemical Impedance Spectroscopy (EIS) and Differential Pulse Voltammetry (DPV), were performed using an AutoLab potentiostat/galvanostat model PGSTAT 30 (Netherlands) and NOVA software at laboratory temperature (25±1 °C). A screen-printed carbon electrode (SPCE) from Dropsens Company (DRP-C110) (Spain), was utilized instead of the conventional three-electrode system. The working electrode (with 4.0 mm diameter) was made of carbon, where the counter electrode was made of platinum and the reference electrode was made of silver. Therefore, all the potentials in the text were reported with respect to this reference electrode (Ag).

2.3. Transmission electron microscopy (TEM) and Field Emission Scanning Electron Microscopy (FE-SEM) analysis

The TEM imaging was performed for the synthesized EGO and also for the purchased GNU, using a Zeiss-EM10C-80 KV transmission electron microscope (Germany). In addition, the SEM imaging was performed using a TESCAN MIRA3 instrument (Czech Republic) for assessment of the electrode surface modifications.

2.4. Nanobiosensor preparation

As it is mentioned before, the working electrode is the SPCE electrode which should be modified with a layer of the EGO and then GNU particles. Firstly, the working area of the SPCE was subjected to mild wash with double distilled water, before to use. The solution of nanomaterials (EGO and GNU) were sonicated for 5 minutes for better dispersing (in the sonication bath) before every application. Then the EGO solution was dropped carefully to the working electrode area and was kept in an isolated place to be completely dry, and then the GNU solution was also dropped to the modified electrode and also kept isolated in the container to be slowly dried. The Concentration and amount of EGO and GNU on the SPCE were achieved with a minor changes of previous protocols from previous papers (44) and (45), respectively. The correct modifications of electrode by GNU on EGO, was studied using FE-SEM imaging and EDX analysis.

After preparing the modified electrode with the layer of EGO and GNUs, the modified electrode was washed gently with double distilled water, and consequently, a 3.0 µl of the thiolated specific probes (22.0 nM) were dropped on the EGO-GNU modified electrode and kept for 100 min and in the isolated container to be slowly dried (self-assembly method). Next, a drop of 1.0 mM MCH solution was applied on the surface of the working electrode for 5 min and then washed up with the double distilled water. Afterward, a 3.0 µl of hybridization buffer containing the target DNA sequence, were dropped on the surface of the working electrode and kept for 120 min, and then washed using the washing solution. Finally, 4.0 µL of the 0.8 mM hematoxylin solution was dropped on the modified electrode and kept for 9 min and then was rinsed

thoroughly with double distilled water. In the end, the prepared modified electrode was connected to the potentiostat and the DPV measurement with an amplitude of 25 mV, a modulation time of 0.05 s, and a step potential of 50 mV was performed in a 0.1 M phosphate buffer solution (pH 7.0).

In addition to the FE-SEM imaging, two electrochemical methods, including cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), were used to assess the sensor preparation steps. The cyclic voltammetry (CV) was performed in 1.0 mM $K_3[Fe(CN)_6]$ in PBS buffer at the potential range of 0.025 to 0.33 V and a sweep rate of 0.02 V s^{-1}. The electrochemical impedance spectroscopy (EIS) experiments were performed on the biosensor in a solution of 5.0 mM $K_3[Fe(CN)_6]$ containing 1.0 M KCl, from 100 kHz to 0.01 Hz with an amplitude 5 mV and a potential of 0.27 V.

2.5. Docking studies

The 3D structure of primer was predicted using xxx web tool. The resulted structure were used as a receptor in molecular docking. The structural file of ligand, hematoxylin, downloaded from the ZINC database (zinc.docking.org) and accession number was “zinc_155803”. The receptor and ligand preparation were performed in AutoDocktools which was including adjust Hydrogen atoms, defined atom type and add potential charge. The search space was determined using the Grid box plugin of AutoDocktools. Lastly, molecular docking was done using vina-autodock and the results of docking were visualized in 2D representation using Ligplot software.

3. Result and Discussions

3.1 optimization of the parameters influencing nanobiosensor fabrication

The optimization of important parameters of a nanobiosensing system, plays a significant role in achieving the ideal performance of the proposed nanobiosensor. Therefore, the fundamental steps of the fabrication were carefully optimized in this study. The procedure of probe immobilization, probe concentration, incubation time of immobilization, the condition and duration of probe/target DNA hybridization, the hematoxylin concentration, and time span of its treatment were properly evaluated and the best conditions were selected for final application in electrochemical assessment.

3.1.1. Optimization of the ssDNA immobilization process

Generally, the procedure of single stranded probe immobilization is one of the critical steps in electrochemical nanobiosensor fabrication which considerably influences the sensitivity, stability, and reproducibility of the final results. Therefore, here, the thiolated single stranded capture probes were immobilized on the modified SPCE surface via two different common

methods, including droplet self-assembly and solution self-assembly, and their results were compared. The Fig. 1A is representing the DPVs of these two methods before and after the hybridization of thiolated probe with the target DNA. As it can be seen, the difference between current range of these two methods truly show that the use of droplet self-assembly method resulted higher current ranges before and also after the hybridization with the target DNA (curves b and d) than the solution self-assembly method (curves a and c). Therefore, the droplet self-assembly method was chosen to be the probe immobilization method of this project.

After choosing the immobilization method, the intensity of the DPV peak currents was considered for selecting the most appropriate concentration of the application of the capture probe. For that reason, nine different concentrations of the single stranded capture probe (from 4.0 to 28.0 nM) were used for modification of the modified working electrode. As the Fig. 1B and 1C shows, the concentration of 22.0 nM had higher current and was selected as the best concentration to be employed in the main study of the research.

Furthermore, the incubation time of immobilization step was also optimized for the capture probe study. As the Fig. 1D and 1E show, among the different tested incubation times (from 20 to 130 min), the incubation time of 100 min was chosen because of its the stronger final DPV peak.

3.1.2. Optimization of the DNA hybridization process

The hybridization step of probe with target sequences is intensely affected the analytical performance of hybridization-based oligonucleotide nanobiosensor. Hence, optimizing the parameters of this procedure such as the hybridization method and its incubation time was thoroughly carried out. In this study, three different hybridization methods were examined and their DPV peaks are shown in Fig. 2A. Based on the intensity of final DPV peak current, the solution hybridization method was selected as the best method and consequently employed in the following experiments.

In addition, after evaluating the results obtained from different hybridization time employment (from 60 to 160 min), a time span of 120 min was selected for hybridization of the target DNA with the immobilized probe on the modified SPCE surface (Fig 2B).

3.1.3. Optimization of the hematoxylin parameters

The hematoxylin was responsible for signal generation in the presented nanobiosensor which directly involved in the final results of biosensing analysis. Based on that reason, in order to optimize the concentration of this chemical material, the different pulse voltammograms were recorded (Fig 2C) while applying eight different concentrations of hematoxylin (from 0.2 to 1.2 mM). As it can be seen in the Fig 2D, the best concentration of the hematoxylin was 0.8 mM,

which had a higher current range. Moreover, the different time periods of hematoxylin accumulation (from 2 to 12 min) were additionally surveyed and as the Fig. 2E and 2F shows, the exposure time of 9 min was chosen due to its superior peak currents.

3.2. Evaluation of the nanobiosensor preparation

3.2.1. Results of TEM and SEM imaging

The TEM image, Fig 3A, is presenting the appropriate conditions of single layer EGO and its compatibility for nanobiosensor application. Also, the TEM image of the purchased GNUs (Fig 3B) is representing the correct shape and size and also lack of agglomeration.

Additionally, the FE-SEM imaging of the nanomaterials-modified working area of the SPCEs was shown the correct assemble and decoration of the nanomaterials. In FE-SME image 3C, a single layer of the EGO on the bare SPCE is shown while in the image of Fig 3D, the decoration of the GNUs on the EGO-modified SPCE are characterized. In addition, the Energy Dispersive Spectroscopy (EDS) analysis results are shown in the Fig 3E. As the graph depicting, the existence of the carbon and gold elements on the modified SPCE electrode is confirmed and also the lower rate of oxygen is verifying that the applied carbon material layer was essentially the reduced exfoliated Graphene Oxide (EGO). In conclusion, all of the TEM, SEM and EDS analysis were basically demonstrated the correct assembly of the different modification steps of the SPCE electrode to form the final biosensor.

3.2.2. Cyclic voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) studies

The CV and EIS are the most frequently used electrochemical methods for evaluating the process of biosensor fabrication step by step. In both of the methods, the $[Fe(CN)_6]^{3-/4-}$ solution is the preferred electrolyte which can also indicate the effects of each surface modification on the electrochemical behavior of electrode.

The Fig 3F shows the cyclic voltammograms of bare SPCE (curve c), SPCE/EGO (curve b), SPCE/EGO/GNU (curve a), SPCE/EGO/GNU/probe (d), SPCE/EGO/GNU/probe + MCH (e), and finally, SPCE/EGO/GNU/probe + target DNA (f). As it can be seen, the application of the nanomaterials such as EGO (curve b) and gold nano urchins (curve a) is significantly increasing the currents due to their higher electron conductivity. But, the probe immobilization step considerably decreased the resulted current (curve d) and this trend is repeated again after the hybridization of the target DNA with ssDNA probes (curve f). These observations can be reasonably explained based on the preventive effect of the immobilization layers against electron transfer to the surface of the electrode for $[Fe(CN)_6]^{3-/4-}$ ions.

Furthermore, the Fig. 3E contains the Nyquist plot of the EIS results which is included EIS study of bare SPCE (curve c), SPCE/EGO (curve b), SPCE/EGO/GNU (curve a),

SPCE/EGO/GNU/probe (d), SPCE/EGO/GNU/probe + MCH (e), and finally, SPCE/EGO/GNU/probe + target DNA (f). In this analysis, the changes in the recorded charge transfer resistance (R_{ct}) can be proportionally contributed to the effects of each formed layer on the electrode surface. It is clear that the R_{ct} value recorded for bare SPCE is nearly close to 0.065 Ω which then decreases even more after addition of EGO and consequently GNU, because of the higher conductivity of the EGO and GNU nanomaterials. But, after immobilization of the probe (curve d), the charge transfer resistance increases due to the space filling and also negative charge of the DNAs which blocks the $[Fe(CN)_6]^{3-/4-}$ ions to reach the electrode surface. Because of the same reason, after adding the target DNA and the hybridization process, the R_{ct} is increasing greatly (curve f). Finally, the similarity of the trends of CV results and EIS diagrams obviously approves the desirable and successful completion of each step in biosensor fabrication.

3.3. Analytical performance of the nanobiosensor

The fabricated biosensor was employed to detect targets in different concentration in a serial dilution of target DNA of GMOs. As explained before, the final electrochemical signal generation was occurring due to accumulation of hematoxylin. Fig. 4A shows the differential pulse voltammograms (DPVs) recorded in PBS (0.1 M, pH 7.0) in three replications. The mean current values were then used for plotting the standard curve which was linear in 40-1100 femtomolar (fM) of target concentration (Fig 4B). The limit of detection of this biosensor was assessed to be 13.0 femtomolar (fM) using $Cm=3s_{bl}/m$; where s_{bl} is the standard deviation of 18 repeated DPV of the blank sample (the signal of the Oracet blue on the non-hybridized probe), and *m* is the slope of the calibration plot.

With such a broad linear range and considerably low detection limit, the designed electrochemical nanobiosensor offers beneficial superiority comparing to recently-published GMO electrochemical DNA biosensors and nanobiosensors. With a brief overview of the previously published articles regarding the electrochemical genosensors and nanobiosensors, it can be concluded that the majority of them does have fairly lower detection limit and also most of them are more complicated and expensive that the nanobiosensor proposed here. This probably is based on the advantages of application of the screen printed electrodes and most importantly, the exfoliated graphene oxide and gold nano urchins which bring the ability of increasing the electrode surface area and also amplifying the signals through faster electron transfer which increases the sensitivity of the nanobiosensor. Such a combination of nanomaterials, and also the shape of the gold nano urchins is also an effective reason in this regard.

In addition, beside the good sensitivity and cost-effectivity of the proposed method, the good selectivity, easy handling, fast responses, and simple preparation, is making our nanobiosensor to

be considered as a promising system that helps in precise quantification of target DNA and consequently will be useful in different medical and biotechnological applications.

3.4. Selectivity of the nanobiosensor

The presented biosensor was subjected to a selectivity assessment via recording the DPV signals resulted from non-complementary and mismatched sequences instead of the target. The Fig. 5 contains the final DPV graphs obtained from the bare SPCE electrode (a), probe-modified SPCE/EGO/GNU (or as it is called the proposed nanobiosensor) (b), non-complementary sequence (c), three-base mismatched (d), single-base mismatched (e) and target DNA sequence (f). By comparing the DPV currents of these electrodes, it can obviously understand that the target DNA has the higher current than other non-specifics and/or partially non-specific oligonucleotides. In addition, the large difference between the target-hybridized biosensor (curve f) and the un-hybridized probe (curve b), represent the functionality of the hematoxylin as an electrochemical label in this nanobiosensor, which could make a great contrast between single strand oligos versus double strand oligos. This advantage may be because of the fact that the interaction mechanism of hematoxylin with DNA is intercalation. In final, these results can vividly confirm the satisfying selectivity of the nanobiosensor performance, which this ideal feature greatly elevates the value of an analytical method, especially in studying real samples in bio-microenvironments.

3.5. Real sample

The future development and application of an electrochemical nanobiosensing system and its large-scale practical application directly depends on its performance in the real biological environment. There are various types of biomolecules in these real samples that can interfere with the target detection process and result in false negative and/or positive responses. Therefore, here we developed a simulated extracted DNA solution by adding four potential interference molecules to the synthesized DNA solution. These four molecules, including CTAB (25%); glucose (25%); ethanol (25%) and SDS (25%); could be found in the final extracted DNA solutions in laboratory experiments. The known amounts of target DNA were spiked to the simulated extracted DNA samples, and the solution was investigated using regular nanobiosensing method, in the same day (an intraday study). Based on the added and found concentrations of target DNA, the relative recovery percentage and relative standard deviation (RSD) were calculated as it is shown in the Table 1.

In results, as can be seen in the Table 1, the relative recovery percentage of this biosensing method has been quite high whereas the obtained RSD has been low. These values indicate that the presented DNA biosensor has practical potential for being used for real laboratory tests with

the extracted DNA of GMOs and can be used in future in the food industry and/or detection laboratories to detect the GMOs containing products.

Table 1. The real sample assay of the nanobiosensor in the extracted DNA sample of GMO instead of synthetic environment.

added DNA	Detected DNA	Relative recovery percentage	Relative standard deviation percent
100.0 fM	98.3 fM	98.34 %	3.81 %
200.0 fM	203.7 fM	101.88 %	2.30 %
400.0 fM	397.8 fM	99.45 %	3.67 %

3.6. Docking study results

In our study, the docking study was performed to assess the interaction of hematoxylin with DNA. The results shown that the biding affinity of hematoxylin to the dsDNA for 9 cluster which was equal to -9.0 kcal/mol while for the single strand DNA (ssDNA Probe) the binding energy was about -5.7 kcal/mol. As it is obvious, the difference between single and double strand DNAs are very high. In addition, during the docking many different conformations were generated which grouped in 9 clusters based on the Root Mean Square Deviation (RMSD) values. The 2D demonstration of binding pocket is shown in Fig 9A which contains one hydrogenic bond and five non-polar binding with ligand. In addition, the three dimensional of interaction of hematoxylin with the single strand DNA and double strand DNA are shown in Fig 9B and Fig 9C, respectively, which represents that the binding pocket is located in major groove of DNA. These results, especially the difference between binding energy of the hematoxylin with ssDNA and dsDNA, are clearly depicting that the hematoxylin is more attracted to the dsDNA than the ssDNA. Then, as we discussed earlier in the selectivity of the nanobiosensor (section 3.4), the mechanism of intercalation for the hematoxylin-DNA interaction could be conclusively proved here for a second time by the results of docking study.

4. Conclusion

Application of nanomaterials for biosensing strategies have been used widely in different studies so far, especially in recent years. Electrochemical Nanobiosensors have known as one the best methods for DNA detection, including detection of the GMOs DNA in food products. Here, a novel electrochemical nanobiosensor for the detection of a genetically modified organism

(GMO) was developed based on using the SPCE electrode modified with nanomaterials such as Exfoliated Graphene Oxide and Gold Nano-Urchins as nanobiosensing platform and hematoxylin as signaling label. The results of the study showed the great performance of the proposed nanobiosensor in real sample environment, remarkable selectivity, wide linear range, significantly low limit of detection and finally easy, cost-effective and simple construction method. The presented nanobiosensor has offered desirable properties for functional detection of the GMO organism in a faster and easier way. Compared to the previous papers, it can be concluded that the presented nanobiosensor has great superiority not only in detection of the desired GMO organism but also in investigating other GMO organisms through little modification in using specific modifications in probe. The advantages of the results over the other methods and biosensors, is probably coming from the application of the nanomaterials, screen-printed electrode and also application of the appropriate electrochemical label, hematoxylin. Overall, the above-mentioned advantages of the proposed electrochemical nanobiosensor, seem to be noble reasons to use this method for the detection of the genetically modified organism (GMO) products, especially in food industries, import strategies of governments and also safety considerations.

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Figure Captions

Fig 1. Results of optimization of the probe conditions. The DPVs of the different applied methods of the probe immobilization (A). The DPVs (B) and peak currents (C) of the different probe concentrations. The DPVs (D) and peak currents (E) of the different incubation time of the probe. All the DPVs were measured in in 0.1 M phosphate buffer solution (pH 7.0).

Fig 2. Results of optimization of the hybridization and hematoxylin conditions. The DPVs of the different hybridization methods (A). The peak currents of the different hybridization time span (B). The DPVs (C) and peak currents (D) of the different concentrations of the hematoxylin. The DPVs (E) and peak current (F) of different incubation time of the hematoxylin. All the DPVs were measured in in 0.1 M phosphate buffer solution (pH 7.0).

Fig 3. Characterization of the modified electrodes in different fabrication steps. The TEM images of the Exfoliated Graphene Oxide (EGO) (A) and the Gold Nano-Urchins (GNUs) (B). The FE-SEM images of the EGO-modified SPCE electrode (C) and EGO/GNUs-modified SPCE electrode (D). The EDS graph of the EGO/GNUs-modified SPCE electrode (E). The cyclic voltammetry (CV) of the different modified electrode in the 1.0 mM $K_3[Fe(CN)_6]$ solution in phosphate buffer solution (F) and the Nyquist plot of the different electrodes in the solution of 5.0 mM $K_3[Fe(CN)_6]$ containing 1.0 M KCl (E).

Fig 4. Analytical specifications of the nanobiosensor. The DPVs of the nanobiosensor for measuring different nine concentrations of the target DNA in 0.1 M phosphate buffer solution (pH 7.0) (A). The calibration plot of peak currents versus concentrations of the target DNA in the linear range of the nanobiosensor (B).

Fig 5. Selectivity of the nanobiosensor. The DPVs of the different modified electrode in the in 0.1 M phosphate buffer solution (pH 7.0); the bare SPCE electrode (a), probe-modified SPCE/EGO/GNU (or as it is called the proposed nanobiosensor) (b), non-complementary

sequence (c), three-base mismatched (d), single-base mismatched (e) and target DNA sequence (f).

Fig 6. Results of the docking study. The 2D representation of binding pockets of the DNA with the hematoxylin (A). The 3D representation of the interaction between hematoxylin and dsDNA (B) and ssDNA (C).

Fig 1

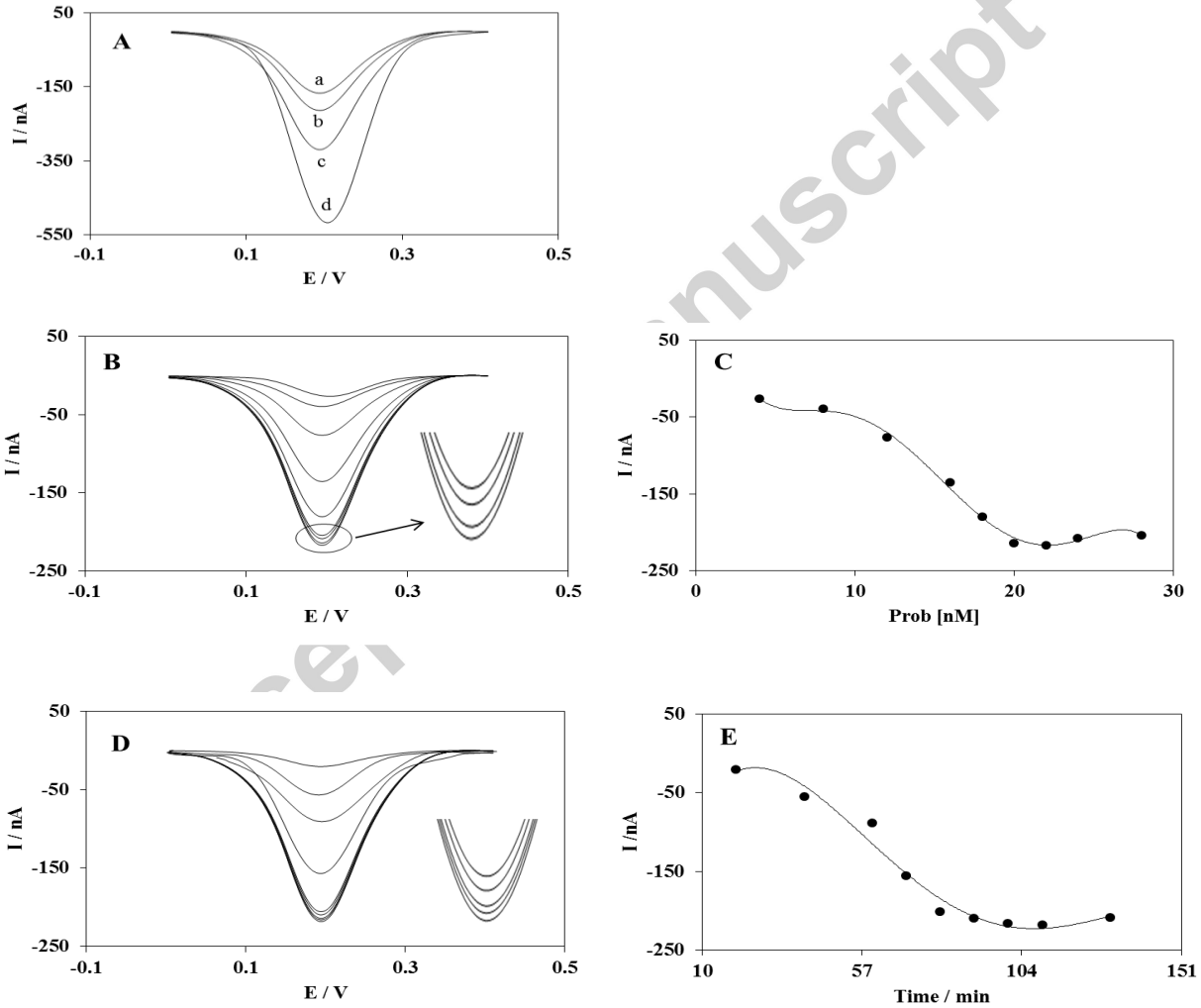


Fig 2

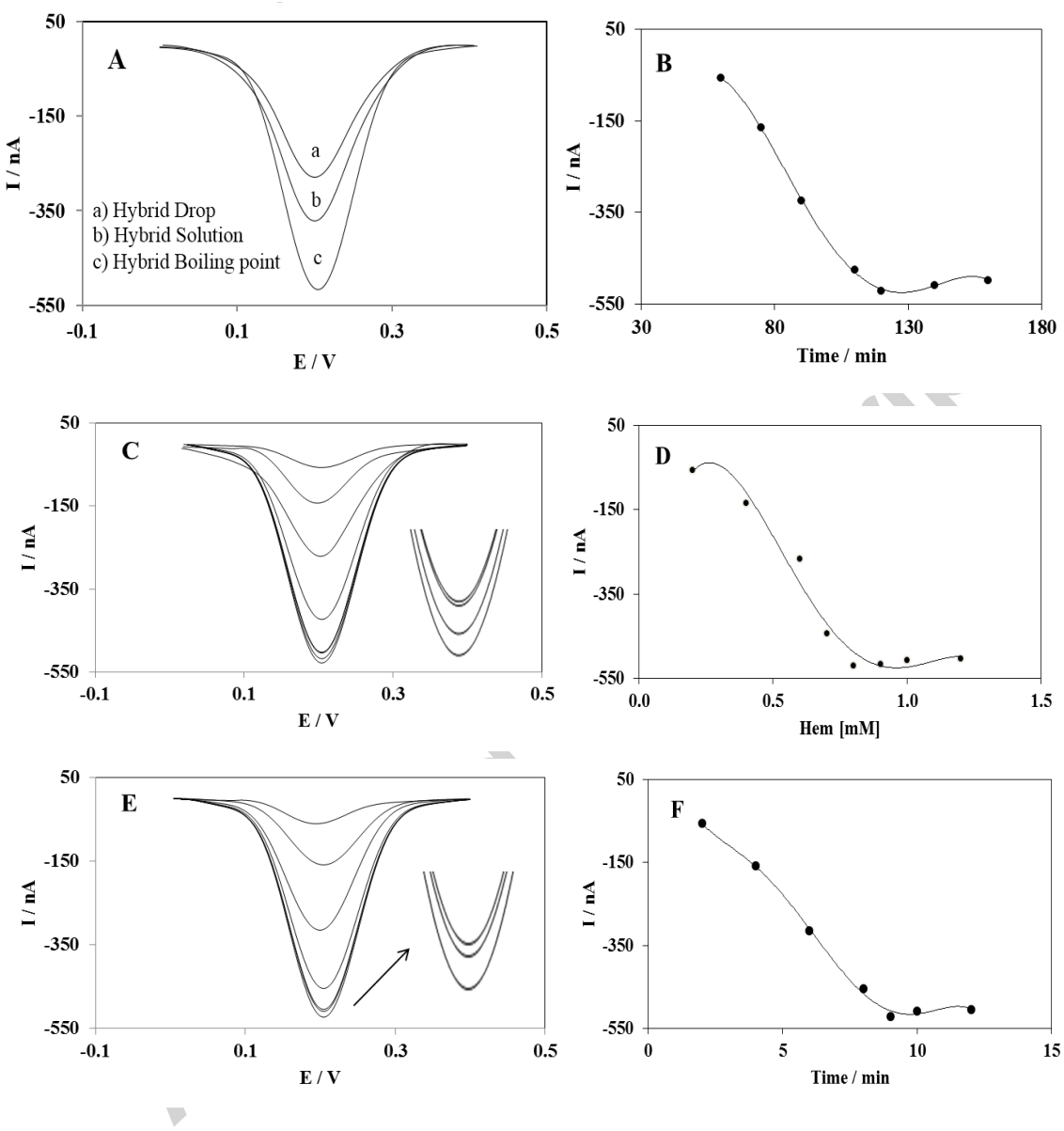


Fig 3

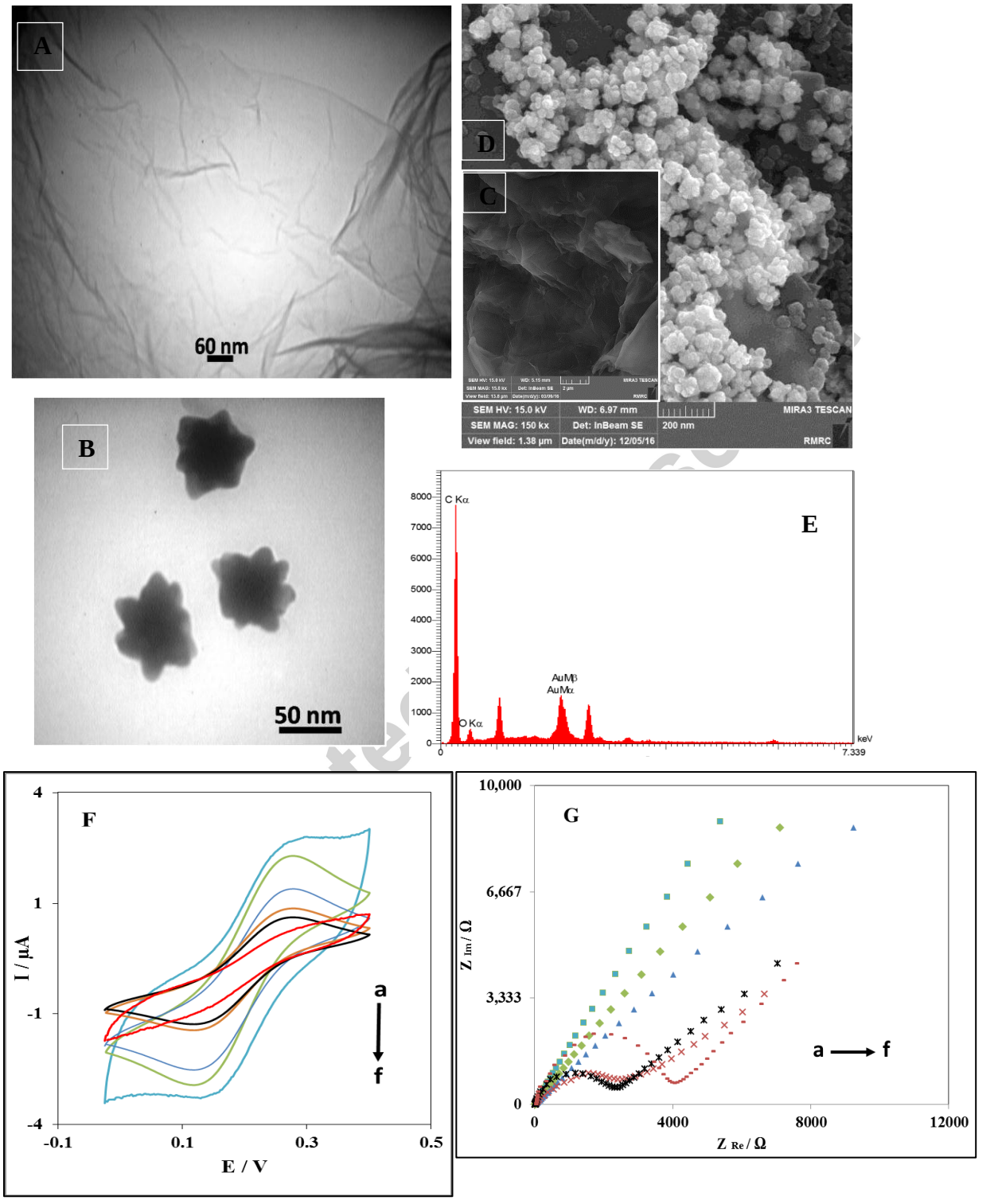

A
60 nm
B
50 nm
C
D
SEM HV: 15.0 kV
WD: 6.97 mm
MIRA3 TESCAN
SEM MAG: 150 kx
Det: InBeam SE
200 nm
View field: 1.38 µm
Date(m/d/y): 12/05/16
RMRC
E
C Kα
O Kα
AuMβ
AuMα
keV
7.339
F
I / µA
E / V
a
f
G
Z Im / Ω
Z Re / Ω
a → f

Fig 4

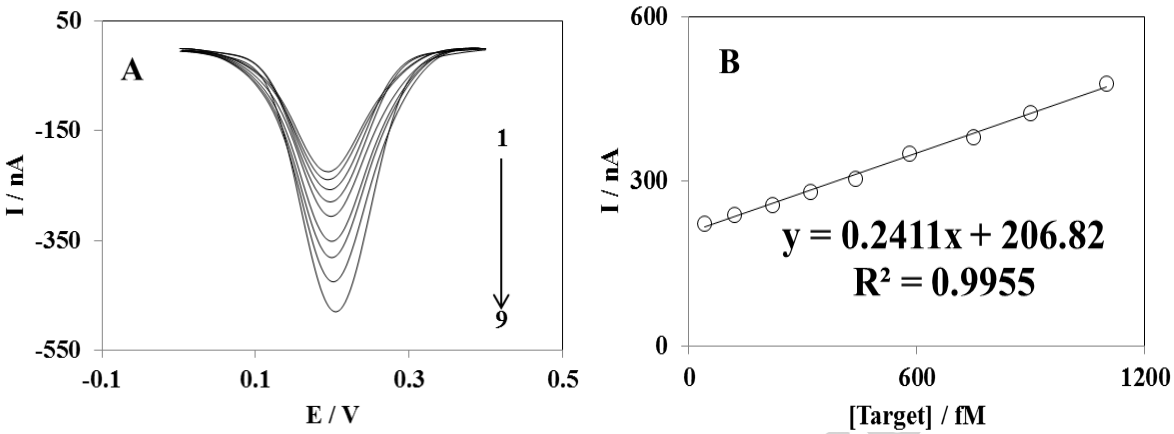


Fig 5

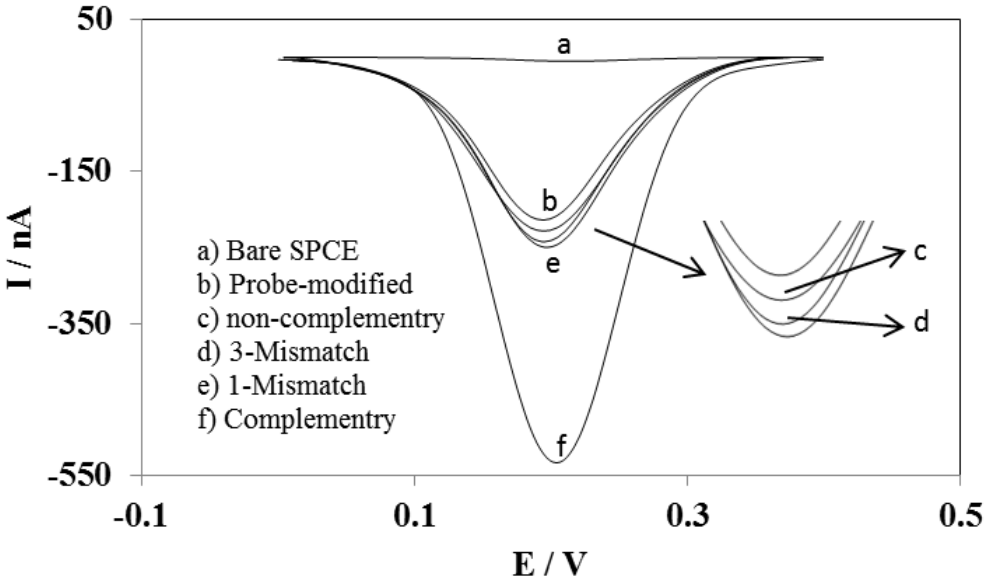
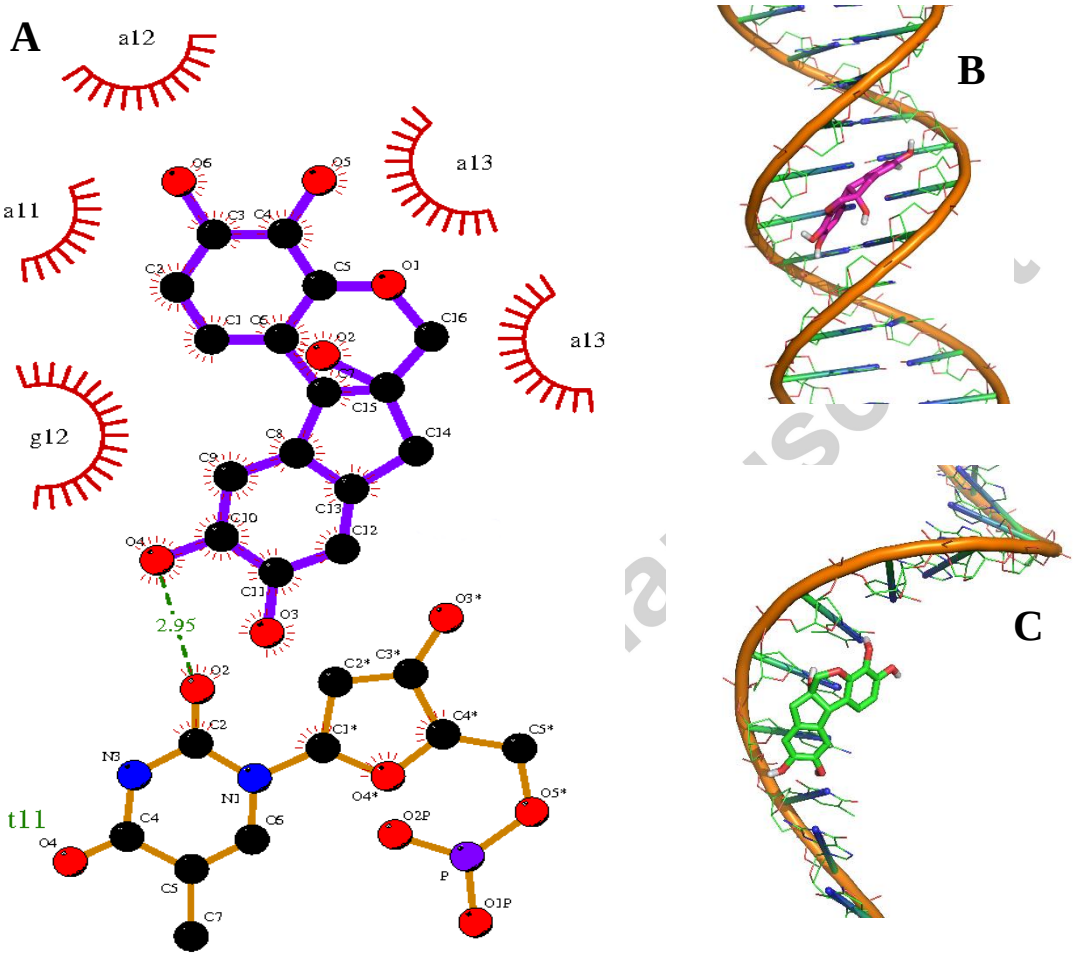


Fig 6



Highlights

- A novel electrochemical nanobiosensor was developed to detect Genetically Modified Organisms (GMOs) DNA.
- Exfoliated Graphene Oxide (EGO) and Gold Nano-Urchins (GNU) used for modification of the screen-printed carbon electrode (SPCE).
- The linear range of the sensor was 40.0-1100.0 fM and the limit of detection calculated as 13.0 fM.
- The sensor had good selectivity and was cost and time-effective
- The sensor was compatible for work in the real sample of simulated extracted DNA solution.