



# A label-free electrochemical impedimetric DNA biosensor for genetically modified soybean detection based on gold carbon dots

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## Abstract

A label-free electrochemical impedimetric biosensor was constructed based on gold carbon dots (GCDs) modified screen-printed carbon electrode for the detection of genetic modified (GM) soybean. The structure and property of GCDs were investigated. The GCDs can directly bind to single-stranded DNA probes through Au-thiol interaction and boost electric conductivity for the DNA sensor construction. The quantification of target DNA was monitored by the change of electron-transfer resistance ( $R_{et}$ ) upon the DNA hybridization on sensor surface. Under the optimal conditions, the  $R_{et}$  response (vs. Ag reference electrode) increased with the logarithm of target DNA concentrations in a wide linear range of  $1.0 \times 10^{-7} - 1.0 \times 10^{-13}$  M with a detection limit of  $3.1 \times 10^{-14}$  M ( $S/N=3$ ). It was also demonstrated that the proposed DNA sensor possessed high specificity for discriminating target DNA from mismatched sequences. Moreover, the developed biosensor was applied to detect SHZD32-1 in actual samples, and the results showed a good consistency with those obtained from the gel electrophoresis method. Compared with the previous reports for DNA detection, the label-free biosensor showed a comparatively simple platform due to elimination of complicated DNA labeling. Therefore, the proposed method showed great potential to be an alternative device for simple, sensitive, specific, and portable DNA sensor.

**Keywords** Electrochemical impedance · Biosensor · Gold carbon dots · Label-free · Event-specific detection · Genetically modified organism

## Introduction

Over the last two decades, genetically modified (GM) technology has become the fastest adopted crop biotechnology in the world. Statistically, GM soybean with 91.9 million hectares cultivation occupied almost half of the global GM crops area in 2019 [1]. There are still many new GM soybean events approved for environmental release or cultivation. For example, herbicide-tolerance soybean SHZD32-1 was developed by translating glyphosate resistant gene *G10-EPSPS* into cultivated variety of Zhongdou 32. Soybean SHZD32-1 has obtained a safety certificate in China in 2019, exhibiting

a broad prospect for commercial cultivation. In order to manage the safety control of GM crops, many countries have implemented a mandatory labeling policy of products containing approved GM traits, and cultivation of unauthorized GM varieties is strictly prohibited from commercial cultivation and entering the market. Therefore, there is a necessity to develop reliable detection methods for the identification and quantification of GM soybean varieties.

So far, conventional PCR and quantitative real-time PCR methods are widely used in GM crops identification to verify compliance with legislation [2–5]. However, PCR methods are limited to complicated operations and require strict environment, expensive instruments, and well-trained technicians. DNA biosensor mainly relying on specific hybridization of nucleic acid strands has the superiority of high sensitivity, simple manipulation, low cost, and possibility of miniaturization and has the potential in resource-limited settings. Various types of DNA sensors including quartz crystal microbalance biosensor [6], surface plasma resonance biosensor [7], electrochemical biosensor [8–11], photoelectrochemical biosensor [12], and electrochemiluminescence

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biosensor [13] have been established for GM ingredients detection. Despite these methods are powerful, they usually require hybridization indicators or labeled of probes. A label-free electrochemical impedimetric (EI) DNA biosensor that relies on the change of impedimetric signals from the DNA hybridization on electrode has been developed to overcome the above limitations [14–17]. EI biosensor is simply structured, easily operated and integrated into a portable sensor, thus becoming a more practical approach for the identification of GM crops.

Recently, a variety of nanomaterials have been used to enhance the sensor analysis performance due to the improvement of surface area and conductivity of the electrode. Due to the merit of facile binding thiol-functionalized DNA probes through Au–S bonds, gold nanoparticles (AuNPs) have extensively employed as nanocarriers for the fabrication of biosensor [18, 19]. In addition, a plenty of AuNPs composite materials were also prepared for the immobilization of DNA probes on electrode, such as AuNPs/graphene oxide nanocomposites [20–22], AuNPs/carbon dots/metal organic frameworks [23], and AuNPs/other nanoparticles mixture [24, 25]. These methods require complicated and time-consuming steps to prepare nanocomposites. Compared with these AuNPs composites, gold carbon dots (GCDs) integrated with carbon skeleton and gold nanoclusters were prepared from glucose and  $\text{HAuCl}_4$  with a very simple microwave heating method and applied for the real-time fluorescent imaging in living cells [26, 27]. GCDs as an economical nanomaterial are ideal for constructing DNA

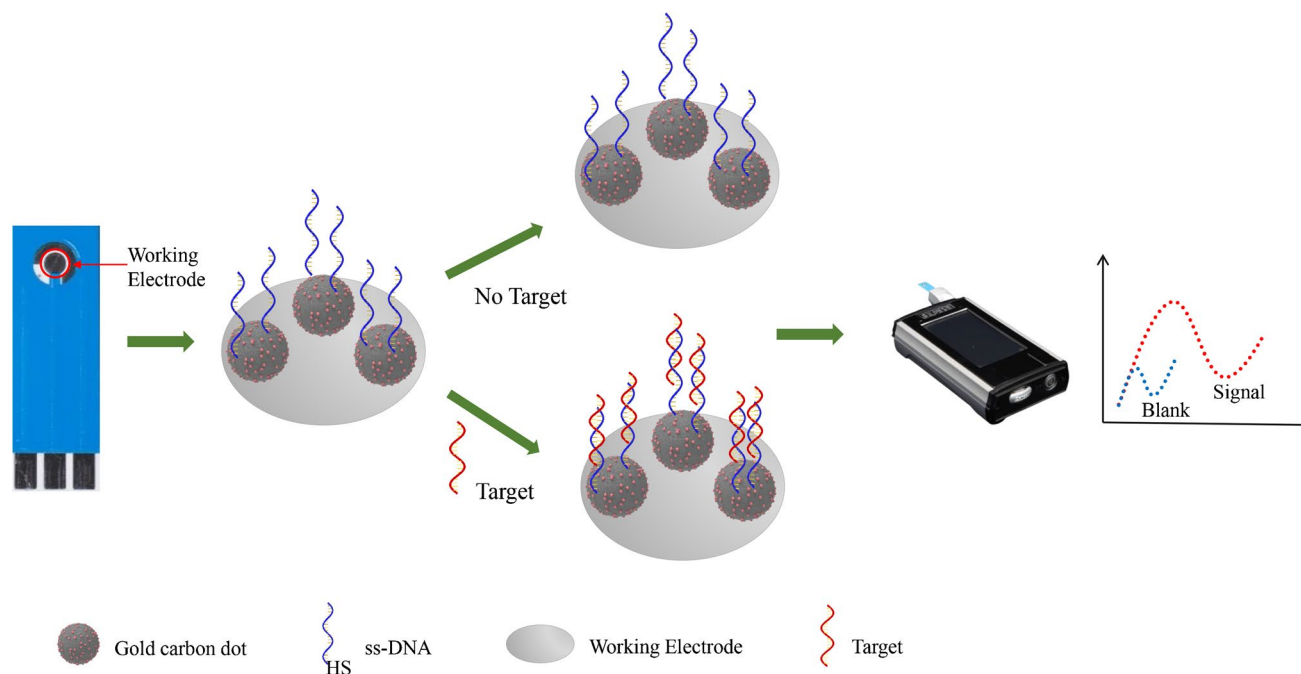
sensor because of their simple synthesis and facile binding to DNA probes.

In this work, we developed a label-free EI biosensor constructed by GCDs-modified disposable screen-printed carbon electrode (SPCE) for the detection of GM soybean SHZD32-1 event-specific sequence. The detection mechanism was illustrated in Fig. 1; the GCDs were used as the sensing platform to immobilize the thiol-functional DNA (HS-DNA) probe and modified the working electrode in SPCE. The blank response was obtained in the absence of target. In the presence of target DNA, it hybridized with probe to form a double-stranded biocomplex on electrode, thus restrained the interfacial electron transfer and increased the EI signal. The proposed label-free DNA sensor can be applied to measure target DNA just by monitoring the change of EI signal, possessing an extremely simple manipulation. To our knowledge, there has been no previous related report to date.

## Materials and methods

### Materials

Chloroauric acid ( $\text{HAuCl}_4$ , purity of 99%) and 6-mercapto-1-hexanol (MCH, purity of 97%) were provided by Sigma-Aldrich Chemical Co., Ltd. (USA). Chitosan and glutathione (GSH, purity of 98%) were supplied by Aladdin Chemistry Co., Ltd. (China). Glucose,  $\text{K}_3[\text{Fe}(\text{CN})_6]$ ,



**Fig. 1** Schematic illustration of label-free EI biosensor for DNA detection based on GCDs

and  $K_4[Fe(CN)_6]$  were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Phosphate buffer (PB) at 0.010 M and pH 7.4 containing 0.010 M of  $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$  was applied for EI detection substrate. The chitosan solution at 0.20% (m/v) was prepared and utilized for the experiment. All of the oligonucleotide sequences and primers were synthesized by Sangon Biotech Co., Ltd. (China), and the sequences were listed in Table 1. DNA oligonucleotide solution was diluted with Tris-ethylenediaminetetraacetic acid (TE) buffer at pH 8.0 consisting of 0.010 M Tris-HCl (purity of 99%) and 1.0 mM EDTA (analytical grade). Other reagents used in this work were of analytical grade. All aqueous solvents were prepared using ultrapure water produced by a Millipore-XQ system. The GM soybean SHZD32-1 was kindly offered by the developer.

## Apparatus

Transmission electron microscopy (TEM) was conducted with a TECNAI-G20 transmission electron microscope (FEI Company, USA). Scanning electron microscopy (SEM) was performed with a VEGA 3 LMU scanning electron microscope (Tescan Ltd., Czech Republic). X-ray photoelectron spectroscopy (XPS) was examined on an Escalab 250Xi X-ray photoelectron spectroscope. Scanning transmission electron microscopy was measured with JEM-ARM300F scanning transmission electron microscope equipped with high-angle annular dark field (HAADF) and annular bright field (ABF) (JEOL Company, Japan). Cyclic voltammetry (CV) and EI measurements were performed using a portable ACIP100 impedance analyzer (Zensor co., Ltd., China). A disposable SPCE was supplied by Zensor co., Ltd. (China). Polymerase chain reaction (PCR) was carried out with a C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Inc., USA).

## Preparation of GCDs

Firstly, 1.5 mL of 0.10 M GSH solution was added into 5.0 mL of 0.020 M  $H AuCl_4$  solution with gently stirring at 300 rpm in a conical flask, followed by dropping into

43.5 mL ultrapure water. After that, 2.0 g of glucose was added into the above mixture under stirring at 300 rpm to achieve a clarified solution. The resultant solution was then heated by a household microwave oven with 700 W for 6 min until it was evaporated and caramel-colored bubbles appeared. The solid pellet was thoroughly dissolved into 50 mL of ultrapure water with vigorous stirring at 500 rpm. Afterwards, the mixture was separated by centrifugation at 10,000 rpm for 10 min, and a brown supernatant was collected. At last, the brown supernatant was dialyzed using a dialysis tube with molecular weight of 8000–14,000 against water for 48 h to obtain GCDs.

## Fabrication of label-free DNA sensor

A disposable SPCE (3.8 cm × 1.2 cm) assembled using one Ag reference electrode, one carbon counter electrode, and one carbon working electrode was adopted as the working electrode for the development of the label-free DNA sensor. The SPCE was cleaned with ultrapure water to remove surface impurities, and then activated in PB buffer at 0.10 M and pH 7.0 with a CV scanning for 5 circles from 0.8 to 1.3 V at a scan rate of 100 mV/s. GCDs at 6.0 mg/mL was mixed with 0.20% chitosan solution with a volume ratio of 1/1, followed by a sonication for 40 min. Afterwards, 5.0  $\mu$ L of the above immobilization mixture was deposited onto the surface of the SPCE and dried at room temperature (RT) to obtain a GCDs-modified electrode. The thiol-functionalized ssDNA used as capture probe (CP) was covalently anchored on the surface of GCDs-modified electrode via the thiol-Au interaction. In brief, 5  $\mu$ L of 10  $\mu$ M CP was dripped onto the modified electrode, followed by incubation at RT for 1.5 h. After that, the self-assembled sensor was washed with ultrapure water to eliminate excess CP and incubated with 10  $\mu$ L of 1.0 mM MCH at RT for 1 h to avoid nonspecific adsorption of nucleic acids.

## Hybridization and electrochemical measurements

Hybridization reaction was performed by addition of 10  $\mu$ L of TE buffer at pH 8.0 containing various concentrations of target DNA (TD) on the surface of CP/GCDs/SPCE at

**Table 1** The sequences of the oligonucleotides used in this work

Oligonucleotides	Sequences (from 5' to 3')
Capture probe (CP)	HS-C6-GTTGTATATGGCTAGTACTATGGCG
Complementary sequence (TD)	CGCCATAGTACTAGCCATATACAAC
One-base mismatch sequences (1MT)	CGCCATAG $\Delta$ ACTAGCCATATACAAC
Three-base mismatch sequences (3MT)	CCCATAG $\Delta$ ACTAGCCATATACAAG
Noncomplementary sequences (NC)	ACTTCGTA $\Delta$ ACTGCTATTCTGCTGTG
PCR primer 1	TCGTTTCCCGCCATAAGG
PCR primer 2	CATCAACCAAGAGCAACAGCAT

45 °C for 45 min. After that, the TD-captured electrode was rinsed with 0.010 M PB at pH 7.4 to remove nonhybridized sequences. In the EI detection, the DNA sensor was immersed into the EI substrate containing  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . EI measurements were recorded in a frequency range from 1000 to 0.1 Hz with the potential at 100 mV using a portable EI analyzer (19.3 cm × 11.2 cm × 4.3 cm). EI spectra were plotted in the form of Nyquist diagrams, where the diameter of the semi-circle reflects the electron-transfer resistance ( $R_{\text{et}}$ ) of the redox conversion of the electroactive marker  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  on electrode. The concentration of TD was quantified by the increase of  $\Delta R_{\text{et}}$  ( $\Delta R_{\text{et}} = R_{\text{et,TD}} - R_{\text{et,blank}}$ ), where  $R_{\text{et,TD}}$  is the  $R_{\text{et}}$  in the presence of TD and  $R_{\text{et,blank}}$  is  $R_{\text{et}}$  in the absence of TD.

### Detection in actual soybean sample

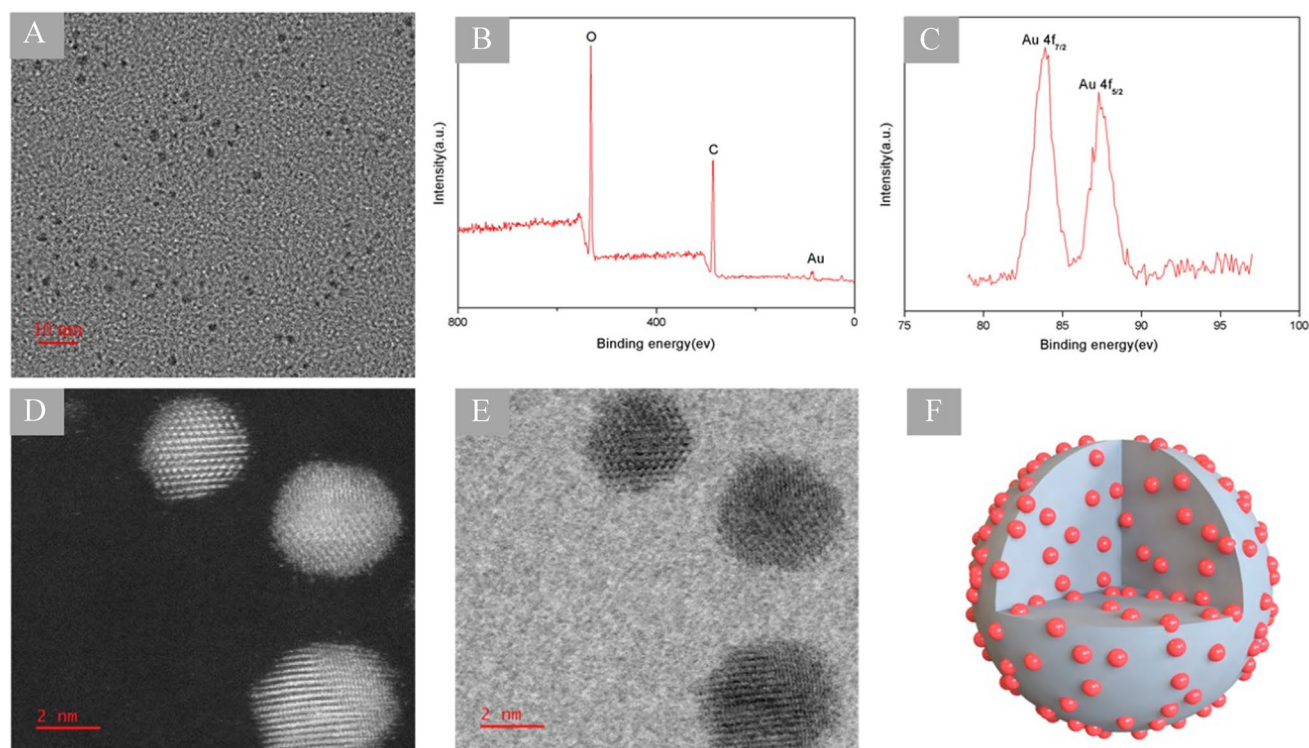
Soybean SHZD32-1 seed was ground into a power. Genomic DNA was then extracted by CTAB-based method [28]. Subsequently, the PCR primer 1 and PCR primer 2 were used as specific primer for PCR amplification. The thermal program for PCR was used as follows: 98 °C for 1 min; 35 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. PCR products were examined by electrophoresis (110 V, 40 min) in a 1.5% agarose gel containing ethidium bromide under ultraviolet light. To achieve the actual sample assay, the process was

as follows: the PCR amplicons were thermally denatured at 95 °C for 5 min, and then frozen on ice for 3 min. After that, the hybridization procedure was processed as stated above.

## Results and discussion

### Characterization of GCDs

The morphology of as-prepared GCDs was observed with TEM characterization. Figure 2A depicted that GCDs were monodisperse and spherical particles with an average size of 2 nm, showing a similar morphology to typical carbon dots [29, 30]. To study the surface chemical compositions and status of the GCDs, XPS was carried out. As shown in Fig. 2B, the main elements of C, O, and Au were found on the surface of GCDs. The peaks at 287 eV and 532 eV were attributed to C and O, respectively. Figure 2C exhibited that peaks at 84 eV and 87 eV were corresponded to the binding energies of Au 4f<sub>7/2</sub> and 4f<sub>5/2</sub>, verifying the presence of Au elemental on GCDs surface. The further structure of GCDs was characterized with STEM equipped with HAADF and ABF. HAADF-STEM is an excellent technique to distinguish heavy (such as gold, Z=79) from light atoms (such as carbon, Z=6) because it is difficult to observe light atoms with this characterization. HAADF-STEM images exhibited in Fig. 2D indicated that Au atoms were orderly arranged in



**Fig. 2** A TEM image, B and C XPS spectra, D HAADF-STEM and E ABF-STEM images of GCDs, and F schematic structure of GCDs

crystal form in some GCDs, whereas others showed amorphous nature presented in the microstructure of GCDs. HAADF-STEM and ABF-STEM presented in Fig. 2D and E indicated Au and carbon atoms were alternately arranged in GCDs. The results were consistent with the previous work [31], demonstrating GCDs were a type of composite nanoparticles integrated with carbon skeleton and gold nanoclusters (Fig. 2F). The surface of GCDs dispersed with Au nanoclusters facilitated the conjugation with DNA molecules via Au–S bonds.

### Electrochemical characterization

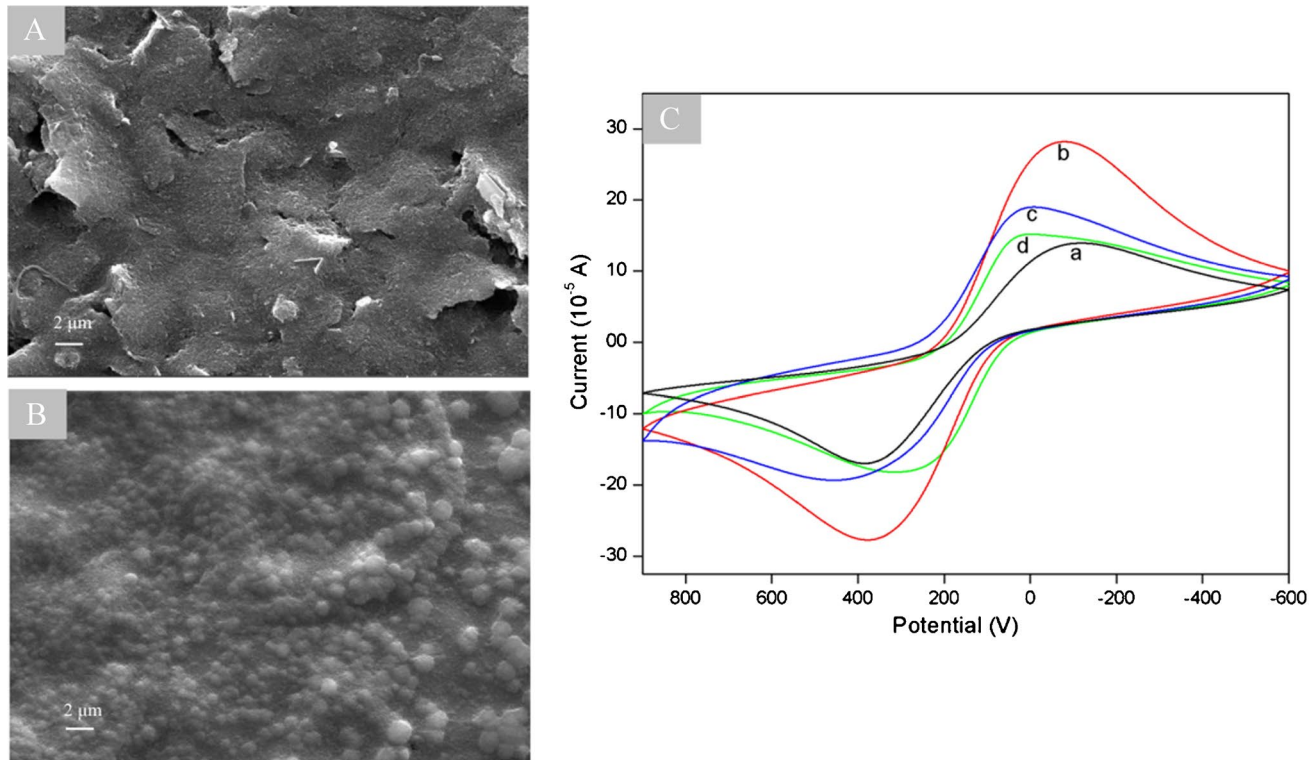
The morphologies of bare and GCDs-doped electrodes were characterized with SEM. Compared with an uneven and inhomogeneous surface of bare sensor (Fig. 3A), thousands of GCDs were aggregated on the sensor surface when GCDs were doped on electrode (Fig. 3B). GCDs-assembled sensing platform can contribute to an enhanced loading capacity of oligonucleotides.

CV was measured in 0.010 M  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  solution containing 0.10 M KCl to monitor the assembly process of the developed biosensor. As shown in Fig. 3C, it was observed that when GCD was assembled on electrode, the peak current (curve b) obtained was significantly higher than

the bare sensor (curve a), which can be attributed to the accelerated electron transfer activity of GCDs. After assembling the CP, the peak current (curve c) decreased obviously in comparison with GCD-modified electrode because the immobilization of negatively charged CP ssDNA on electrode hindered the access of  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . After TD was hybridized with CP immobilized on the sensor, the peak current decreased further, demonstrating that the increase of negative charge by introduction of TD further enhanced the repellence of redox species. Therefore, these results confirmed that the label-free biosensor was successfully assembled.

### Optimization of experimental conditions

High analytical performance of DNA sensor can be achieved by optimizing the important experimental conditions including the immobilization of the capture probe and the hybridization reaction conditions. The influence of CP immobilization time on the EI response was investigated in Fig. S1. The  $\Delta R_{\text{et}}$  between the DNA hybridization response and the blank response was adopted to express detection results.  $\Delta R_{\text{et}}$  response increased significantly from 60 to 90 min and remained stable thereafter, indicating the immobilization of



**Fig. 3** SEM images of **A** bare and **B** GCDs-modified working electrodes, and **C** CV spectra of **A** bare, **B** GCDs, **C** CP/GCDs, and **D** TD/CP/GCD-modified electrodes

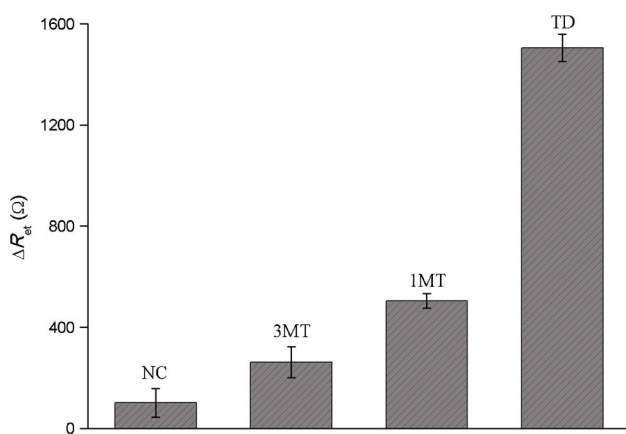
CP on sensor was completed within 90 min. Thus, 90 min was set as the optimal immobilization time in this work.

The effect of hybridization time and temperature of between CP and TD on the  $\Delta R_{et}$  response were also assessed in detail. As shown in Fig. S2,  $\Delta R_{et}$  response obviously increased with the increased hybridization time and reached a plateau after 45 min, implying the hybridization reaction can be completed within 45 min. As seen in Fig. S3, the effect of hybridization temperature on the  $\Delta R_{et}$  response was investigated in the range of 35–55 °C and the largest  $\Delta R_{et}$  response was achieved at 45 °C. Therefore, the hybridization time of 45 min and hybridization temperature of 45 °C were chosen for the further experiment.

### Specificity, repeatability, and stability of label-free biosensor

To evaluate the specificity of the proposed label-free biosensor, four types of oligonucleotide sequences (Table 1) including noncomplementary sequence (NC), three-base mismatch target (3MT), single-base mismatch target (1MT), and TD were tested. The concentrations of these DNA sequences were all  $1.0 \times 10^{-11}$  M. As displayed in Fig. 4, the  $\Delta R_{et}$  responses of NC, 3MT, and 1MT were gradually increased with the decreasing mismatch degree, demonstrating that the base mismatch restrained accomplishment of hybridization. Moreover, it was observed that the  $\Delta R_{et}$  of TD showed a significantly enhanced value that was 3-times higher than that of 1MT. Taken together, these results above demonstrated the ideal specificity of the proposed DNA sensor.

In addition, the repeatability of the DNA sensor was investigated by parallelly measuring TD for five times at the concentration of  $1.0 \times 10^{-11}$  M and  $1.0 \times 10^{-12}$  M, respectively. The results showed that the relative standard



**Fig. 4** Comparison of  $\Delta R_{et}$  responses of four different types of DNA sequences. *NC*, noncomplementary sequence; *3MT*, three-base mismatch target; *1MT*, single-base mismatch target; *TD*, target DNA

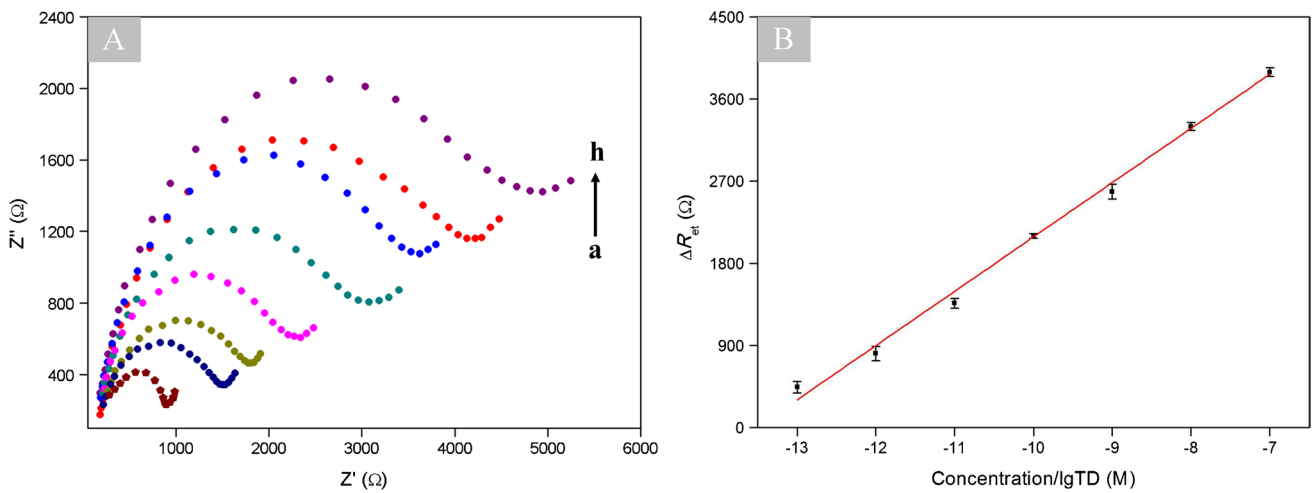
deviations (RSDs) of measurements were 4.6% and 4.0%, respectively, showing the high repeatability of the developed biosensor. Also, the stability of the developed DNA sensor was investigated by detecting TD at  $1.0 \times 10^{-9}$  M. After the ready-to-use DNA sensor was stored at 4 °C for 7 days, the initial  $\Delta R_{et}$  value decreased only by 9.5%. The satisfactory stability may be due to the fact that CP was attached firmly onto the surface of GCDs film.

### Analytical performance

Under the optimal conditions, the analytical performance of the label-free DNA biosensor based on SPCE was evaluated using various concentrations of TD for hybridization. An Ag reference electrode was applied in SPCE. As presented in Fig. 5A, the  $\Delta R_{et}$  increased with the increasing TD concentration. A seven-point calibration curve for  $\Delta R_{et}$  versus the logarithm of TD concentration was observed in the range of  $1.0 \times 10^{-7}$  to  $1.0 \times 10^{-13}$  M in Fig. 5B. The linear regression equation was expressed as  $Y (\Omega) = 594.36 \lg X (M) + 8032.97$  ( $Y$  and  $X$  are the  $\Delta R_{et}$  value and TD concentration, respectively) with a correlation coefficient of 0.9940. The limit of detection (LOD) was calculated to be  $3.1 \times 10^{-14}$  M at a signal to noise ratio of 3. Compared with the reported DNA biosensor based on AuNPs nanocomposite, the proposed method possessed a broader linear range of DNA concentration and a high sensitivity (Table 2). Furthermore, this GCD-based label-free DNA sensor showed facile structure, simple manipulation, and portable properties.

### Detection in actual soybean samples

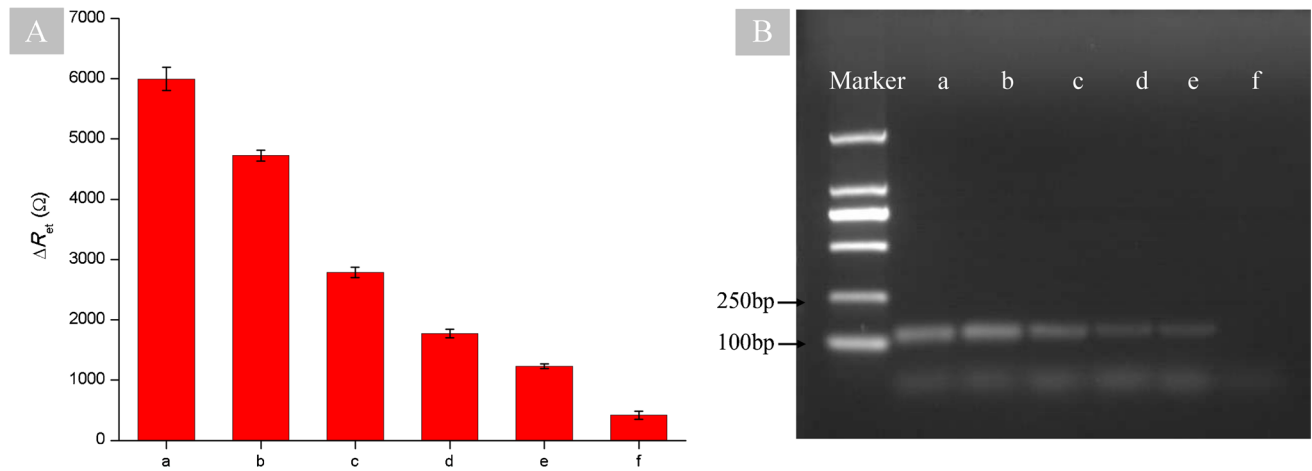
To verify the reliability of the developed method, the label-free biosensor was applied to measure GM soybean samples. Soybean SHZD32-1 at content of 50% was prepared by mixing homozygous SHZD32-1 seeds with non-GM soybean seeds. Genomic DNA was extracted from soybean SHZD32-1 standard sample and quantified to be 200 ng/mL by using a spectrophotometer. Extracted genomic DNA of SHZD32-1 was then at 1/10 ratio diluted into concentrations of 20, 2.0, 0.20, 0.020, and 0.0020 ng/mL. Afterwards, they were used as templates for PCR amplification. PCR products were finally detected utilizing the proposed biosensor. As shown in Fig. 6A, it was found that the  $\Delta R_{et}$  increased with the PCR products of the increasing concentrations of template DNA from SHZD32-1. The  $\Delta R_{et}$  response produced as low as 0.0020 ng/mL of initial template can be obviously distinguished from that of non-GM soybean, demonstrating the excellent applicability of the developed EI biosensor. In addition, these PCR products were also analyzed by 1.5% agarose gel electrophoresis for comparison. As seen in Fig. 6B, bands of 120 bp appeared in SHZD32-1 samples, while no band in non-GM soybean sample. These results



**Fig. 5** A  $R_{et}$  responses of TD at different concentrations of  $0, 1.0 \times 10^{-13}, 1.0 \times 10^{-12}, 1.0 \times 10^{-11}, 1.0 \times 10^{-10}, 1.0 \times 10^{-9}, 1.0 \times 10^{-8}, 1.0 \times 10^{-7}$  M, from a to h. B Calibration curves, where  $n = 5$  for each point

**Table 2** Comparison of linear ranges and detection limits of the different DNA biosensors based on AuNPs nanocomposite

Modified material and electrode	Detection technique	Linear range (M)	LOD (M)	References
$Fe_3O_4@Au$ MNPs	Chronoamperometric detection	$1.0 \times 10^{-10} - 1.0 \times 10^{-8}$	$2.0 \times 10^{-11}$	[10]
AuNPs-rGO	Photoelectrochemical detection	$1.0 \times 10^{-13} - 5.0 \times 10^{-10}$	$5.0 \times 10^{-14}$	[12]
AuNPs-ATPGO	EI	$1.0 \times 10^{-13} - 1.0 \times 10^{-9}$	$1.13 \times 10^{-14}$	[20]
AuNPs/TB-GO	DPV	$1.0 \times 10^{-11} - 1.0 \times 10^{-9}$	$2.9 \times 10^{-12}$	[21]
AuNRs-GO	DPV	$1.0 \times 10^{-14} - 1.0 \times 10^{-9}$	$3.5 \times 10^{-15}$	[22]
Au-PtNPs/Pty	EI	$1.0 \times 10^{-12} - 1.0 \times 10^{-7}$	$3.6 \times 10^{-13}$	[25]
GCDs	EI	$1.0 \times 10^{-13} - 1.0 \times 10^{-7}$	$3.1 \times 10^{-14}$	This work



**Fig. 6** A  $\Delta R_{et}$  responses and B agarose gel electrophoresis of different contents of SHZD32-1 soybean. Marker, DL2000. a-e, PCR products of extracted genomic DNA with the concentrations of 20, 2.0, 0.20, 0.020, and 0.0020 ng/mL; f, no-template

obtained from agarose gel electrophoresis were consistent with those obtained from the proposed method, verifying a reliability of the developed label-free DNA sensor in the field of GM crop assay.

## Conclusion

A novel label-free EI biosensor for the detection of GM soybean was developed by using GCDs for ssDNA immobilization. The proposed label-free sensor showed a simplicity of structure and manipulation without additional indicator reagents and tedious procedures. GCDs prepared with a simple method was used to facilitate bind DNA probe for assembling sensing platform, which can contribute to an enhanced biomolecule loading capacity and an accelerated interfacial electron transfer. As a result, the proposed method exhibited a wide linear range and high sensitivity. The proposed portable sensor was conveniently fabricated by inserting a SPCE into the handheld EI analyzer and friendly used by nonprofessionals. Therefore, the designed biosensor provides an alternative platform for simple, sensitive, and specific detection in the identification of GM products. In the future work, a rapid preamplification with isothermal amplification needs to be developed to improve the detection efficiency. The portable ready-to-use sensor coupled with facile pretreatment would be applied for the detection of GM products as well as other DNA targets in resource-limited laboratories.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00604-022-05223-7>.

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## Declarations

**Competing interests** The authors declare no competing of interests.

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