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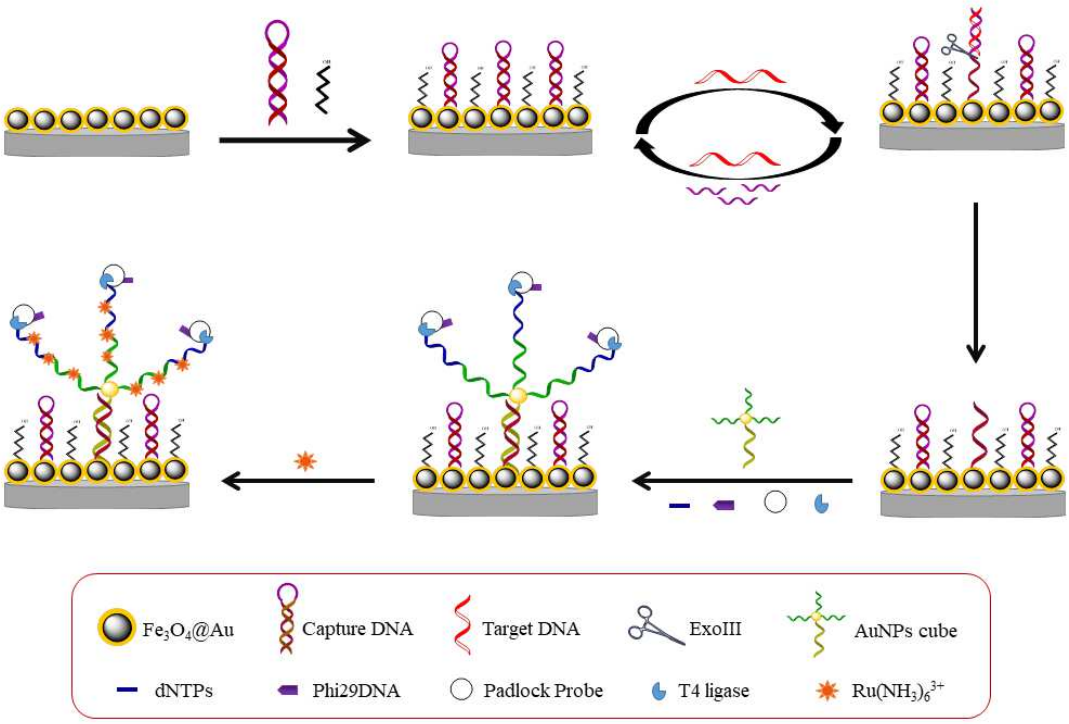
Graphic abstract

A novel electrochemical biosensor, realized high sensitivity based on triple signal amplification of shearing recycling target DNA, rolling circle reaction, and AuNPs cube labeled multiple probe, was developed for transgenic soybean DNA detection.



Detection of transgenic soybean based on triple signal amplification by electrochemical DNA biosensor

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A novel electrochemical DNA biosensor for transgenic soybean detection based on triple signal amplification

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Abstract A novel electrochemical DNA biosensor was developed and MON89788 of soybean transgenic gene sequence was detected based on a strategy of rolling circle amplification (RCA) and gold nanoparticle cube (AuNPC)-labeled multiple probes. First, the mercapto-modified capture DNA was immobilized on the surface of the Fe_3O_4@Au magnetic nanoparticles via an Au-S bond, and the capture DNA was opened and complementarily hybridized with the target DNA to form a double-stranded DNA. In the 10× reaction buffer, Exonuclease III (ExoIII) specifically recognized and sheared the double-stranded DNA to release the target DNA, which led to the next round of reaction. Afterward, AuNP cube-loaded ssDNA (AuNPC/DNA) was added with the rolling circle reaction with the help of Phi29 DNA polymerase and T4 ligase. Finally, $[Ru(NH_3)_6]^{3+}$ was attracted directly by the anionic phosphate of ssDNA via electrostatic interaction. The determination was carried out by using chronocoulometry (CC), and the CC signal was recorded. The mass amount of DNA strands extended infinitely on the AuNPs cube and numerous $[Ru(NH_3)_6]^{3+}$ were absorbed, thus the detected signal was highly amplified. The corresponding CC signal showed a good linear relationship with the logarithm of the target DNA concentration in the range of 1×10^{-16} to 1×10^{-7} mol L^{-1}, with a detection limit of 4.5×10^{-17} mol L^{-1}. Specific gene sequence of MON89788 in soybean samples was determined, and the recoveries ranged from 97.3% to 102.0%. This sensor is one of the most sensitive sensors for genetic sequence assessment at present. Moreover, it demonstrates good selectivity, stability, and reproducibility.

Keywords: Electrochemical biosensor; Rolling circle amplification; AuNPs cube; Exonuclease III; Chronocoulometry; Signal amplification

1. Introduction

Genetically modified crops (GMC) contain exogenous DNA. With the help of genetic engineering, the exogenous DNA can be used to replace the unwanted genes or add to the original DNA chain to achieve desired gene expressions in the expected crops [1]. With the increasing variety of transgenic crops, the biological safety of GMC has become a hot topic worldwide. Most countries have regulations that genetically modified foods must be labeled; therefore, detecting GMC is highly important for food safety [2, 3]. Current detection methods of genetically modified genes include enzyme-linked immunosorbent assay (ELISA) [4], polymerase chain reaction (PCR) [5, 6], and loop-mediated isothermal amplification (LAMP) [7]. However, ELISA is often suffered from interference factors which are prone to false positive results, and the reagents used are expensive. While PCR is not suitable for rapid detection since it requires repeated thermal denaturation, which is time-consuming and high depends on equipment and operator. Likewise, LAMP reaction needs to design a large number of specific primers and easily contaminated, requires a lot of work. Furthermore, the sensitivity of these methods need to be further improved. Nowadays, the latest developments in nucleotide sequence specificity detection are relevant to many research areas, including cancer diagnostics and GMC detection [8-10]. Among GMC, MON89788 is a genetically modified herbicide-tolerant soybean developed by Monsanto Corporation from the United States, and has been commercially grown in several countries. Qualitative detection of the transgenic soybean gene sequence of MON89788 is crucial in early food safety testing [11-13].

To improve detection sensitivity, researchers have developed many nucleic acid-based signal amplification methods, such as catalytic hairpin assembly (CHA) [14], rolling circle amplification (RCA) [15], and hybrid chain reaction (HCR) [16]. Among them, RCA is a powerful nucleic acid amplification method, and can be driven by DNA polymerase and replicate circularized oligonucleotide probes with either linear or geometric kinetics under isothermal conditions. RCA has attracted considerable attention due to its low background, wide usage, and fast and effective analysis [17-19].

Electrochemical DNA biosensors offer high accuracy, good selectivity, economical operation, and efficient detection of selected DNA sequences with small sample volumes, and have gathered

interest in the field of DNA detection [20-24]. However, the application of the electrochemical DNA biosensors in GMC is still limited due to the restriction of the sensitivity. At present, it is one of the hot spots to develop a method to further improve the sensitivity of electrochemical DNA biosensors.

In this paper, a novel electrochemical DNA biosensor is constructed for the ultrasensitive detection of the transgenic soybean gene sequence of MON89788 based on the signal amplification derived from shearing recycling target DNA, rolling circle reaction, and gold nanoparticle cube-labeled multiple probes. Moreover, chronocoulometry [25, 26] is used in quantitative determination by recording the chronocoulometric charge produced by the probes $[Ru(NH_3)_6]^{3+}$ absorbed on AuNPC/DNA. The mass amount of DNA strands that extended unceasingly on the AuNPs cube and numerous $[Ru(NH_3)_6]^{3+}$ were absorbed on DNA strands, thus the detected signal was highly amplified. This new method has high sensitivity and good selectivity for target DNA sequence detection and has been successfully applied in soybean samples detection. Moreover, this method can provide a platform for analyzing specific genetic sequences.

2. Experimental

2.1. Apparatus and Reagents

The CC and CV were carried out using a CHI660D Electrochemical Workstation (Shanghai Chenhua Instrument Co., China); the electrochemical Impedance spectroscopy (EIS) was performed on an Autolab Electrochemical Workstation (PGSTAT128N, Metrohm Co., China); the transmimssion electron microscope (TEM) images were obtained from a field transmimssion electron microscope (JEM-2100F, Electronics Co., Japan); the DF-101S collector temperature heating magnetic stirrer (Jiangsu Jinxiang Long Electronics Co., China) and the Digital display constant temperature water bath (Changzhou Guohua Electric Appliance Co., China) were used for the preparation of AuNPs and Fe_3O_4@Au magnetic nanoparticles.

$FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, KCl, NaOH, NaCl, Tris-HCl buffer, tris (2-carboxyethyl) phosphine (TCEP), ethylenediaminetetraacetic acid (EDTA), Mercaptoethanol (MCH), $NaBH_4$, and $HAuCl_4$ were purchased from Aladdin (Shanghai, China); $[Ru(NH_3)_6]^{3+}$ was purchased from

Zhengzhou Xidale Chemical Products Co., Ltd. $Na_2HPO_4 \cdot 12H_2O$ and $NaH_2PO_4 \cdot 2H_2O$ were purchased from West Long Chemical Co., Ltd. Reagents used were of analytical grade. PBS solution ($Na_2HPO_4 \cdot 12H_2O$ 11.6037 g, $NaH_2PO_4 \cdot 2H_2O$ 1.1850 g) and TE buffer (10 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} EDTA, 100 mmol L^{-1} NaCl, pH 7.8) were bought from Aladdin. Exonuclease III (2.0 U μL^{-1}), T4 ligase (2.0 U μL^{-1}), Phi29 DNA polymerase (2.0 U μL^{-1}), 10× enzyme reaction buffer, and deoxyribonucleic acid mixture (dNTP Mix) were purchased from Shanghai Bioengineering Engineering Company. The sequences of the oligoes are listed in Table 1. Double-distilled water was used as experimental water.

Table 1

2.2. Preparation of AuNPs

The preparation of AuNPs followed the method used in a previous study [27]. All glassware was cleaned with freshly prepared aqua regia before testing and repeatedly washed with double-distilled water followed by natural drying. Afterward, 1 mL $HAuCl_4 \cdot 3H_2O$ (1%) solution and 99 mL double-distilled water were added to a 250 mL three-neck flask. After adding 3 mL of sodium citrate buffer (1%) along with vigorous mechanical stirring, the color gradually changed into wine-red. The solution was boiled for 30 min, cooled to room temperature, and stored at 4 °C. As shown in Fig. S1, the AuNPs was well prepared showed even dispersion.

2.3. Preparation of Magnetic Nanoparticles (Fe_3O_4@Au)

The synthesis of Fe_3O_4@Au magnetic nanoparticles followed the method from a previous report [28] with modifications. First, 0.67 g $FeSO_4 \cdot 7H_2O$ and 1.14 g $FeCl_3 \cdot 6H_2O$ were weighed and dissolved in 250 mL double-distilled water. The mixture was poured into a three-necked flask protected by a constant supply of nitrogen during the experiment. Afterward, 2 mol L^{-1} NaOH was quickly added to adjust the pH of the solution to maintain pH 10 under stirring and it was kept in a 50 °C hot water bath provided by N_2 for 1.5 h. Subsequently, the solution temperature was adjusted to 80 °C and reacted at about 30 min, and the pH level was measured every 5 min to ensure that the pH value was maintained. After being cooled to room temperature, the solution was

magnetically separated and washed several times with double-distilled water and absolute ethyl alcohol every 30 min until the solution became neutral. Finally, 5 mg L^{-1} Fe_3O_4 solution was obtained and refrigerated at 4 ℃ until use.

A total of 0.229 g sodium citrate was weighed and dissolved in 100 mL double-distilled water. The solution was poured into a 250 mL three-necked flask and heated to 99 ℃ under mechanical stirring. Afterward, 1 mL 1 g mL^{-1} $HAuCl_4$ and 1 mL of ready-to-use Fe_3O_4 magnetic nanoparticles were quickly added to the solution. Stirring was maintained for 15 min after the heat source was removed. The magnetic nanoparticles were magnetically separated after the wine-red solution was cooled to room temperature. The solution was washed several times with double-distilled water until the pH value went back to neutral. A stock of 2.5 mg mL^{-1} Fe_3O_4@Au solution (20 mL) was prepared and stored at 4 ℃ for later use. Fig. S2 shows the TEM images of Fe_3O_4@Au magnetic nanoparticles. The figure illustrates the uniform dispersion of the nanoparticles and a gold nanoparticle coat on the outer layer of Fe_3O_4 with a diameter of about 35 nm.

2.4. Preparation of AuNPC/DNA and the RCA procedure

A total of 10 μL of 100 μmol L^{-1} Probe DNA, 10 μL of 100 μmol L^{-1} Assistant DNA, and 10 μL of 10 mmol L^{-1} TCEP were added to 20 μL AuNP solution, mixed completely under ultrasound conditions, and incubated for 2 h in the dark. A total of 20 μL of 2.0 U μL^{-1} Padlock probe and 15 μL of 2.0 U μL^{-1} T4 ligase (including 1 x ligase buffer) were introduced to the mixture and reacted for 1 h at room temperature. Finally, 20 μL Phi29 DNA polymerase, 10× enzyme reaction buffer, and 20 μL bases were added as the RCA reaction solution for DNA extending. The solution was kept at 36.8±0.5 ℃ for 1.5 h to obtain the AuNPC/DNA solution and stored at 4 ℃ for later use.

2.5. Pretreatment of magnet-controlled glassy carbon electrode

The magnet-controlled glassy carbon electrode (MGCE, d = 3 mm) was polished with 0.03 μm alumina and was used as the working electrode; the Ag/AgCl electrode was the reference electrode and the Pt wire was used as the counter electrode. The electrode potential of the electrode was measured by using the electrochemical workstation in the electrolyte solution containing 0.1 mol L^{-1} KCl and 0.05 mol L^{-1} $[Fe(CN)_6]^{3-/4-}$ working solution; moreover, the

scanning potential was from -0.2 V to +0.6 V and the scanning speed was 0.1 V s^{-1}. The bare electrode was scanned until the potential difference (ΔEp) of $[Fe(CN)_6]^{3-/4-}$ redox peaks was lower than 0.10 V and then washed with distilled water and ethanol to fully prepare the electrode for use.

2.6. Procedure of assay

A total of 0.1 mL of 1 μmol L^{-1} capture DNA and 40 μL of 10 mmol L^{-1} TCEP were incubated for 2 h to activate the thiol-group of capture DNA. The activated capture DNA was mixed with 1 mL Fe_3O_4@Au solution and incubated it for 2 h at 36.8±0.5 °C, and the capture DNA was self-assembled on the surface of magnetic nanoparticles via the Au-S bond. Afterward, 100 μL of mercaptohexanol (MCH, 1 mmol L^{-1}) solution was added to the above solution and incubated for 1 h to prevent non-specific adsorption. After centrifuging at 12000 r min^{-1} for 20 min, the supernatant was discarded and the pellet was resuspended in TE buffer to obtain Fe_3O_4@Au/capture DNA solution. The pretreated MGCE was immersed in a mixture of 20 μL of Fe_3O_4@Au/capture DNA solution and 40 μL of 1 mol L^{-1} TE buffer solution, then incubated at 36.8±0.5 °C for 12 h. To further reduce the nonspecific adsorption of capture DNA onto the electrode surface, the electrode was immersed in 1 mmol L^{-1} of mercaptohexanol at 36.8±0.5 °C for 1 h.

Subsequently, different concentrations of the target DNA were added and incubated at 36.8±0.5 °C for 1 h. In the presence of the target DNA, the capture DNA and target DNA were complementarily hybridized, and the hairpin structure was opened. The above electrode was placed within 15 μL of 2.0 U μL^{-1} ExoIII containing a 10× enzyme reaction buffer solution reacted for 1 h at 36.8±0.5 °C. The ExoIII obtained a specific recognition and sheared recycling of double-stranded DNA from 3' to 5', thus leading to the release of target DNA and starting the next cyclic shearing. Afterward, the electrode was moved into the hot water bath and then kept in constant 70 °C for 20 min to inactivate the ExoIII and suspend ExoIII-assisted circulation. The electrode was then immersed in the proposed AuNPC/DNA/RuNH (20 μL) and reacted for 2 h at 36.8±0.5 °C to achieve the designed electrode. Finally, the modified electrode was washed thrice with TE buffer solution and double-distilled water for further measurements. The CC signal was recorded in a 10 mmol L^{-1} Tris-HCl solution containing 50 μmol L^{-1} $[Ru(NH_3)_6]^{3+}$ (PH = 7.4). The schematic diagram is shown in Fig. 1.

Fig. 1.

2.7. Electrochemical measurements

The modified electrode was used as the working electrode, the platinum wire electrode was used as the counter electrode, and the Ag/AgCl (saturated KCl) electrode was used as the reference electrode; the chronocoulometry signal was detected by using CHI660D electrochemical workstation in 10 mmol L^{-1} Tris-HCl solution containing 50 μmol L^{-1} $[Ru(NH_3)_6]^{3+}$ (pH = 7.4), and the pulse period was 250 ms. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) experiments were performed to identify how the electrode surface modification affected the probe redox and their reversibility/irreversibility behavior. The CV measurement was performed in 0.05 mol L^{-1} $[Fe(CN)_6]^{3-/4-}$ containing 0.1 mol L^{-1} KCl with a potential range of -0.2 to +0.6 V at a scan speed of 0.1 V s^{-1}. EIS experiments was performed in the same solution of CV with 0.19 V impedance potential, 5 mV amplitude, and 0.1−100000 Hz frequency range.

3. Results and discussions

3.1. Characterization of electrode modification process

3.1.1 Cyclic voltammetry and Electrochemical impedance spectroscopy characterization

Cyclic voltammetry is an effective tool to probe the electrochemical properties of modified electrodes. Fig. 2A illustrates the CV changes of MGCE in 0.05 mol L^{-1} $[Fe(CN)_6]^{3-/4-}$ solution during modification with different materials. a shows the CV curve of the bare electrode, with the current signal being shown as the highest. b shows the decreasing current when Fe_3O_4@Au was immobilized on the bare electrode. c shows the curve of the electrode modified with capture DNA and MCH. The modification resulted in the decreased current signal because the electrode surface was self-assembled by the capture DNA by Au-S bond, and the uncombined sections were closed by MCH. After adding the target DNA, a considerable increase was observed in the coverage of the DNA phosphoric acid skeleton on the electrode surface, thereby leading to an apparent current decrease (d). When ExoIII and enzyme reaction buffer was added to the system, ExoIII detected

the double-stranded DNA binding sites and sheared them from 3' to 5', and then released the target DNA into the next cycle. This step resulted in a higher current signal (e) than the current signal showed in curve d. f shows the CV after adding the designed AuNPC/DNA (20 μL). The redox current decrease suggests that the AuNP cube-labeled multiple probes obtained from RCA achieved successful immobilization and adsorption on the modified electrode surface.

Electrochemical impedance spectroscopy is a common-used electrochemical method for evaluating the electrode modification process of the prepared biosensor. Fig. 2B shows the variations in the EIS of electrode modification. The bare magnet-controlled glassy carbon electrode (a) shows minimum resistance that almost reached 0, and the resistance of the Fe_3O_4@Au (b) was higher than that of the bare electrode (a). After adding MCH and capture DNA (c), the electron transfer rate was further decreased due to the capture DNA self-assembly via the Au-S bond, and the electrode surface sites were closed. When the target DNA was introduced (d), the resistance increased again; meanwhile, the electrode resistance (curve d) was higher than that of the ExoIII shears recycling on the modified electrode (e). Finally, upon the addition of 20 μL AuNPC/DNA, the largest resistance was reached and was due to the hinderance of electron transfer (f).

Fig. 2.

3.1.2 Chronocoulometry signal characterization of modified electrode

The signal amplification of this method was characterized and verified by chronocoulometry. Chronocoulometry curves for the original activated capture probe (curve a), with the signal AuNP/DNA added probe (curve b), and with both signal AuNP/DNA and RCA reaction helped probe (curve c) were compared in Fig. 3. The localization of the AuNP/DNA signal without RCA contributed only to a limited increase in charge (Fig. 3b). However, the AuNPC/DNA with RCA amplification led to a considerable increase in charge value (Fig. 3c), and the activated capture DNA showed minimal value (Fig. 3a). These results demonstrate that the proposed AuNPC/DNA with RCA technology is more beneficial for signal enhancement than for single AuNPs-DNA-mediated probes. The DNA chains of AuNPC/DNA in this work were extended

constantly by the RCA, and led to high level of adsorption of $[Ru(NH_3)_6]^{3+}$ molecules and greatly enhanced electrical signals.

Fig. 3.

3.2. Optimization of experimental conditions

3.2.1 Optimization of Fe_3O_4@Au nanoparticle amount and target DNA hybridization time

The amount of Fe_3O_4@Au absorbed on the electrode surface directly affected the measurement of the experiment. Fig. S3a shows that the CC signal considerable increased and became steady as the amount of Fe_3O_4@Au increased to 20 μL. Therefore, the optimal amount of Fe_3O_4@Au was 20 μL. Hybridization time is an important factor that influences the efficiency of hybridization. To improve the detection performance of the sensor, the hybridization time of the target DNA and capture DNA was optimized. Fig. S3b shows that the CC signal gradually increased and peaked at 60 min; therefore, the sample hybridization time was 60 min.

3.2.2 Optimization of ExoIII amount and reaction time

We used ExoIII to recognize the binding site of double-stranded DNA and shears recycling target DNA to enhance the detection signal, which was one of the most important influencing factors of the experiment. When 15 μL enzyme was used, the CC signal reached its maximum and was mostly stable (Fig. S4a). Therefore, the amount of ExoIII used in this experiment was 15 μL. We focused on the reaction time of ExoIII. Fig. S4b shows that the CC signal gradually increased with enzymatic react time, thus indicating that the enzyme was involved in the circulation of the target DNA hybridization. The CC signal gradually increased and peaked at the reaction time of 60 min.

3.2.3 Optimization of Phi29 DNA polymerase amount and reaction time

Phi29 DNA polymerase was another key factor in the experiment. The polymerase directly affected the sensitivity of the method. Fig. S5a shows that the amount of Phi29 DNA polymerase ranged from 0–30 μL. The CC signal gradually increased as the polymerase amount increased, and

finally reached a plateau. Therefore, the amount of Phi29 DNA polymerase was selected as 20 μL. Moreover, the reaction time of polymerase directly affected the signal magnitude. Fig. S5b shows that when the reaction time was less than 1.5 h, the intensity of the CC signal significantly increased, and it was mostly stable after 1.5 h. Therefore, the optimum reaction time of Phi29 DNA polymerase was 1.5 h.

3.2.4 Optimization of T4 ligase amount and reaction time

The amount of T4 ligase to the biosensor directly affected the signal amplification. Fig. S6a shows that CC signal increased as the T4 ligase increased, and the CC signal peaked when the enzyme amount was 15 μL. Therefore, the amount of T4 ligase selected in this experiment was 15 μL. In addition to controlling the amount of T4 ligase in this experiment, we focused on the reaction time. Fig. S6b shows that when the T4 ligase reaction time was less than 60 min, the intensity of CC signal was considerably different, and it was mostly stable after 60 min. Therefore, the reaction time of T4 ligase was selected as 60 min.

3.3. Calibration curve

Fig. 4A shows the chronocoulometry curve corresponding to different concentrations of target DNA hybridized with the proposed AuNPC/DNA-labeled capture DNA for 2 h. As the DNA concentration increased, Au NPC-labeled probes became increasingly connected; thus, the chronocoulometry curves increased. Fig. 4B illustrates the calibration curve. The chronocoulometric intercept (charge versus $t^{1/2}$) is defined as Q according to the definition of the time coulomb method [29, 30]. The logarithm of concentration (Log*C*) of the DNA in the concentration ranged from 1.0×10^{-16} mol L^{-1} to 1.0×10^{-7} mol L^{-1}, and showed a linear relationship with the CC intensity (ΔQ). The linear regression equation was ΔQ (μC) = 3.323 + 0.791 lgC (10^{-16} mol L^{-1}) (Fig. 4B), the linear correlation coefficient was r = 0.9985, and the detection limit was 4.5×10^{-17} mol L^{-1} (S/N = 3), and this result was lower than in most methods that were previously reported (Table 2). The low detection limit benefited from the new strategy based on triple amplification of shearing recycling target DNA, rolling circle reaction, and AuNP cube-labeled multiple probes.

Fig. 4.

Table 2

3.4. Reproducibility and stability

The reproducibility and stability of the new sensor were tested by sealing and storing the sensor at 4 °C for 14 days. The CC signal was only reduced by 2.42%, thus indicating that the sensor had good stability. Under the same conditions, five sensors were prepared to detect the solutions of 1.0×10^{-7} mol L^{-1} and 1.0×10^{-12} mol L^{-1} DNA, respectively. The relative standard deviation of determinations was 2.89% for 1.0×10^{-7} mol L^{-1} and 3.46% for 1.0×10^{-12} mol L^{-1} DNA. It indicated that the sensor had good reproducibility.

3.5. Selective study

The selectivity of the novel biosensor was evaluated by CC signal responses to (a) 1.0×10^{-9} mol L^{-1} target DNA, (b) 1.0×10^{-7} mol L^{-1} single-base mismatch of DNA, (c) 1.0×10^{-7} mol L^{-1} non-complementary strand of DNA, (d) 1.0×10^{-7} mol L^{-1} three-base mismatch of DNA, and (e) blank. Fig. 5 shows that CC intensity increased in response to target DNA (a). Hence, the single-base mismatch DNA (b), three-base mismatch mRNA (d), non-complementary mRNA (c), and blank (e) rarely had interference during target DNA detection, and this finding accounts for the fully complementary sequence DNA CC signals of 13.26%, 4.17%, 8.16%, and 2.56%, respectively. Therefore, the biosensor can effectively detect target DNA with high selectivity.

Fig. 5.

3.6. Samples Analysis and Results

The genetically modified (GM) soybean and non-GM soybean purchased from Shenzhen Hengtai Kangyuan technology Co. Ltd was used to demonstrate the practical applications of the biosensor. The samples were extracted from the soybean powder according to the instructions of the Plant Genomic DNA Kit (Sangon Biotech Co., Ltd. Shanghai, China). The purity and

concentration of the extracted DNA were measured by Nano-Drop 1000 UV-vis spectrophotometer (NanoDrop, Wilmington, DE, USA). Then the DNA solutions were separately diluted with 50.0 mmol L^{-1} pH 7.0 PBS. Three known-amount target DNA solutions were added to the extracted non-GM soybean sample solutions and marked for recovery experiments, and the solutions were assayed by this proposed method. Table 3 shows the results. The recoveries ranged from 97.3% to 102.0%, and the RSD (n = 5) was 2.6% ~ 4.4%, which were all lower than 5%; this finding indicates that the sensor has good recovery and applicability.

Then 10 μL gene sample solution from GM soybean was dropped directly on the surface of CP/Fe_3O_4@Au/MGCE for hybridization and was detected by this electrochemical DNA biosensor under the optimum condition. The results were presented in Fig. 6. Compared with the signal obtained with CP/Fe_3O_4@Au/MGCE to non-GM soybean gene (curve a), blank solution (curve b), and GM soybean MON89788 gene (curve c), it is apparently that the CC signal of non-GM soybean gene is slightly higher than that of blank solution since the adsorption amount of $[Ru(NH_3)_6]^{3+}$ on DNA increased, while the CC signal increased significantly after hybridization with the extracted MON89788 gene in real (curve c), which indicated that this electrochemical DNA biosensor could effectively detect the GM soybean sample and has good applicability.

Table 3

Fig. 6

4. Conclusion

In conclusion, we developed an electrochemical biosensor, and propose a novel strategy for the ultrasensitive detection of transgenic soybean DNA. The sensitivity was significantly improved due to the triple signal amplification of shearing recycling target DNA, rolling circle reaction, and AuNPC/DNA-labeled multiple probes. The detection limit of target DNA was obtained at 4.5×10^{-17} mol L^{-1}. This novel technique was successfully applied in soybean samples detection, and it offers a promising strategy for detecting other transgenic crops.

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Figures

Fig. 1 Schematic representation of electrochemical DNA biosensor based on triple signal amplification of shearing recycling target DNA, rolling circle reaction, and AuNPC/DNA-labeled multiple probes for the detection of transgenic soybean gene sequence of MON89788

Fig. 2 (A) CV and (B) EIS characterization of different electrodes in 0.05 mol L^{-1} $[Fe(CN)_6]^{3-/4-}$ containing 0.1 mol L^{-1} KCl. (a) bare electrode; (b) Fe_3O_4@Au/(a); (c) capture DNA/MCH/(b); (d) target DNA/(c); (e) ExoIII/(d); (f) AuNPC/DNA/(e). Scan rate: 0.1V s^{-1}

Fig. 3 Chronocoulometry curves for (a) the activated capture probe after ExoIII shearing cyclizing, (b) the activated capture probe after being connected with the signal AuNP/DNA but without RCA, (c) the activated capture probe after being connected with the AuNPC/DNA and with RCA

Fig. 4 (A) Chronocoulometry intensity and (B) calibration curve of DNA biosensor at different target DNA concentrations, from a to k: 0, 1.0×10^{-16}, 1.0×10^{-15}, 1.0×10^{-14}, 1.0×10^{-13}, 1.0×10^{-12}, 1.0×10^{-11}, 1.0×10^{-10}, 1.0×10^{-9}, 1.0×10^{-8}, 1.0×10^{-7} mol L^{-1}.

Fig. 5 Selectivity of fabricated biosensor

Fig. 6 Chronocoulometry curves of (a) CP/Fe_3O_4@Au/MGCE to non-GM soybean gene; (b) CP/Fe_3O_4@Au/MGCE to blank solution; (c) CP/Fe_3O_4@Au/MGCE to GM soybean MON89788 gene.

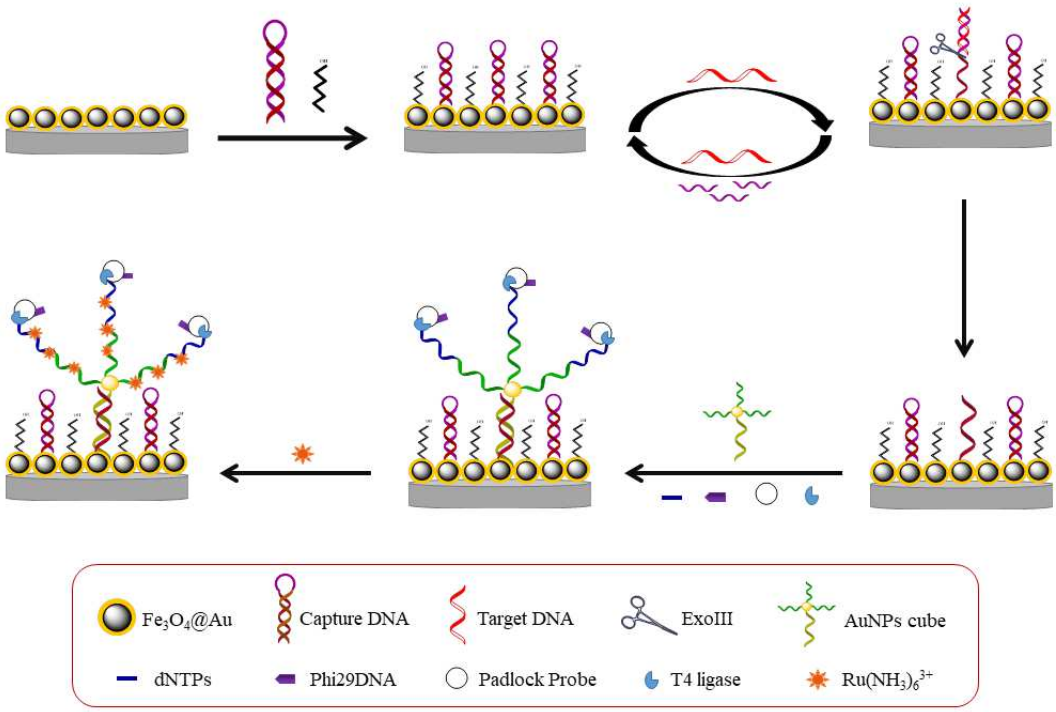

Fig. 1.

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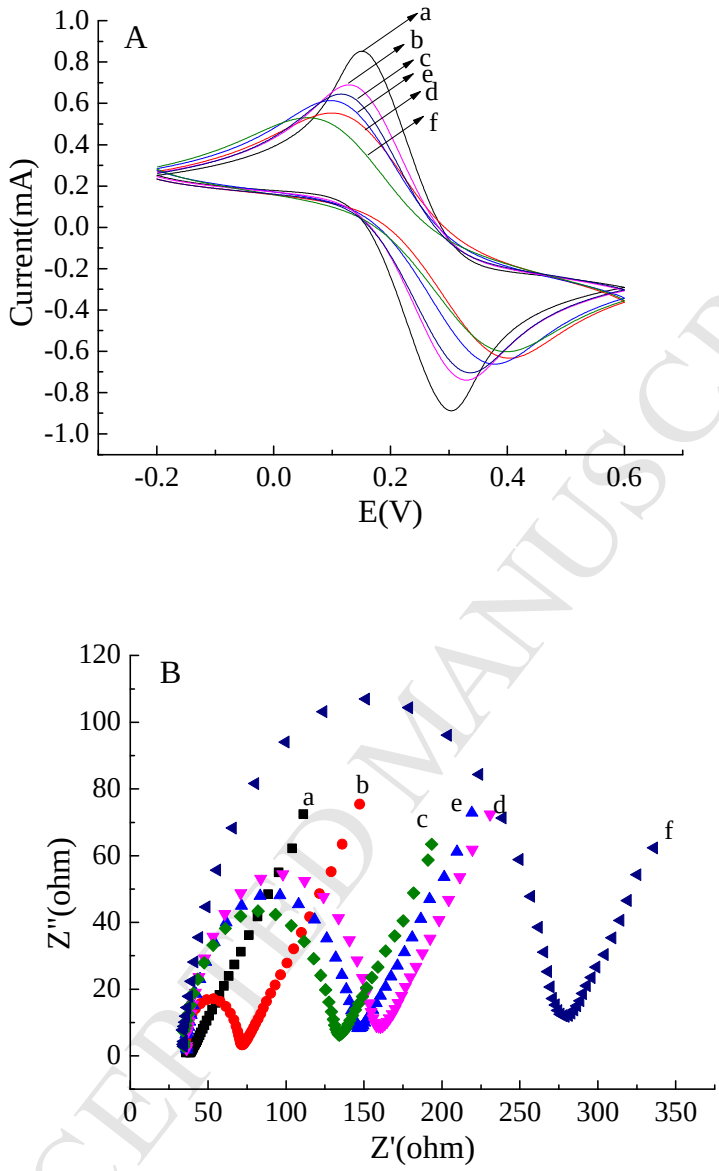

Fig. 2.

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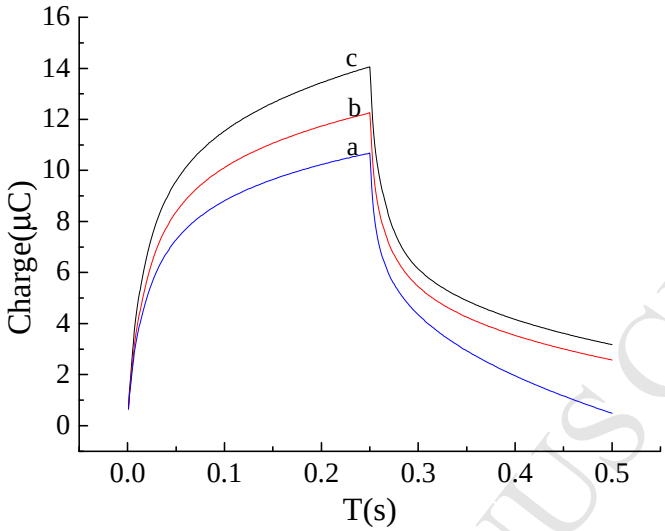

Fig. 3.

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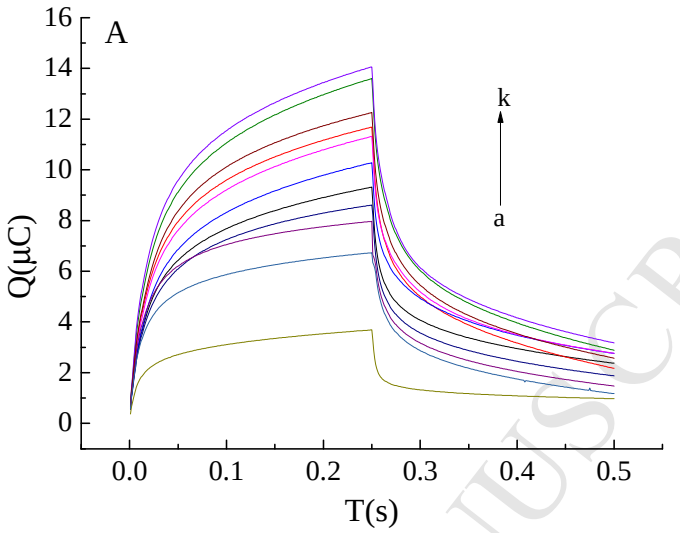

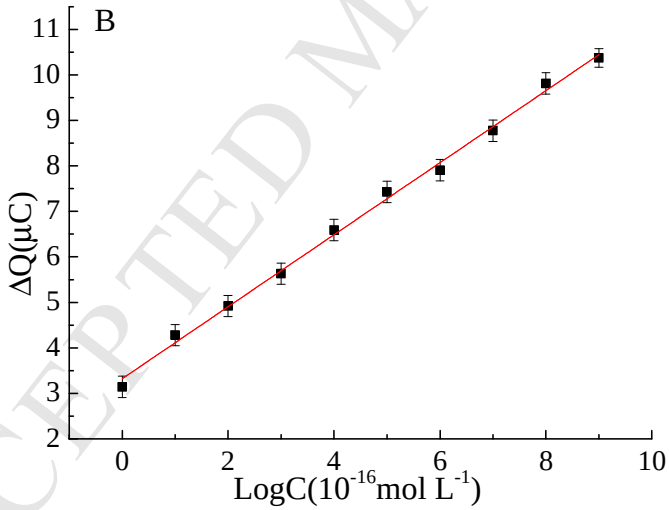

Fig. 4

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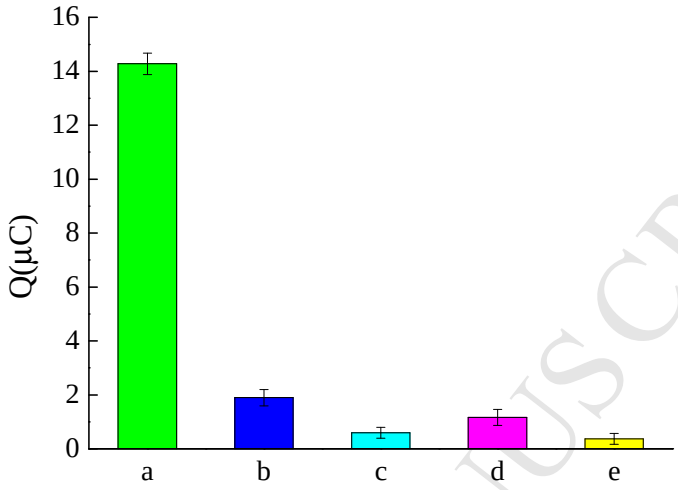

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5 Fig. 5.

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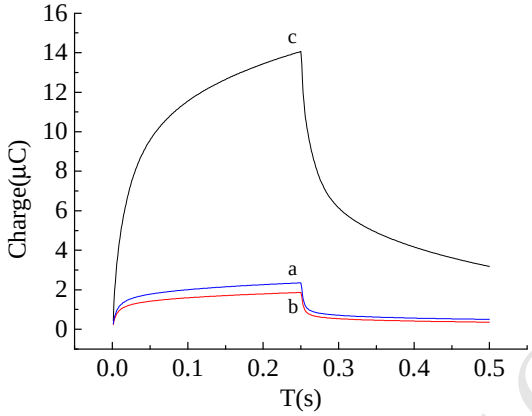

Fig. 6.

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Tables

Table 1 Experimental materials

Name	Sequence
Capture probe DNA (1-DNA)	5’-HS-GAC TGA CGC CTG AAG GCG GGA AAC GAC AAT-3’
Target DNA (2-DNA)	5’-ATT GTC GTT TCC CGC CTT CAG-3’
Probe DNA (3-DNA)	5’-HS-GCG TCA GTC -3’
Assistant DNA (4-DNA)	5’-HS-TCA GGT ACG TCG -3
Padlock DNA (5-DNA)	5′-CGATCTACCCAACCCGCCCTACCCAAAACCCAACCCGCCCT ACCCAAAACCCAACCCGCCCTACCCAAGGACGT-3′
One-base mismatched target DNA (1MT)	5’-ATT GTC GTT TCA CGC CTT CAG-3’
Three-base mismatched target DNA (3MT)	5’-ATG GTC GTT TCA CGC CTT CTG-3’
Non-complimentary target DNA (NCT)	5’-GAC AGT GCC GTA ATA AGG TCC-3’

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4 Table 2 Comparison of the present method with other reported methods.

Detection methods	Linear range (mol L^{-1})	Detection limit (mol L^{-1})	Reference
Polymerase chain reaction (PCR)	1.0×10^{-9} ~ 5.0×10^{-6}	3.0×10^{-10}	[31]
Electrochemical luminescence method (ECL)	5.0×10^{-16} ~ 5.0×10^{-13}	1.2×10^{-16}	[32]
Recombinase polymerase amplification (RPA)	1.0×10^{-14} ~ 1.0×10^{-8}	1.3×10^{-13}	[33]
Fluorescence	1.0×10^{-15} ~ 1.0×10^{-8}	4.0×10^{-16}	[34]
Chronopotentiometry	1.0×10^{-16} ~ 1.0×10^{-7}	4.5×10^{-17}	This work

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Table 3 Analytical results of soybean samples

Samples	Found (mol L^{-1})	Added (mol L^{-1})	Found (mol L^{-1})	RSD (n = 5, %)	Recovery (%)
1	Not Detected	1.00×10^{-13}	1.02×10^{-13}	2.6	102
2	Not Detected	1.00×10^{-12}	9.73×10^{-13}	3.8	97.3
3	Not Detected	3.00×10^{-12}	2.97×10^{-12}	4.4	99.0

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Highlights

1. A novel electrochemical biosensor was fabricated for the ultrasensitive detection of transgenic soybean (MON89788) DNA based on triple signal amplification.

2. ExoIII can recognize the binding site of double-stranded DNA and shears recycling target DNA to enhance the detection signal.

3. Au NPs cube increases the amount of DNA strands loaded.

4. Rolling circle reaction extends the DNA strands used for $[Ru(NH_3)_6]^{3+}$ absorption.

5. The detected signal is highly amplified since lots of DNA strands were extended on the AuNPs cube and numerous $[Ru(NH_3)_6]^{3+}$ were absorbed.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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