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Photoelectrochemical CaMV35S biosensor for discriminating transgenic from non-transgenic soybean based on SiO_2@CdTe quantum dots core-shell nanoparticles as signal indicators

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Abstract

A methodology for detection of the Cauliflower Mosaic Virus 35S (CaMV35S) promoter was developed to distinguish transgenic from non-transgenic soybean samples by using photoelectrochemical (PEC) biosensor. In this PEC biosensing system, the as-prepared gold nanoparticles-reduced graphene oxide acted as a nanocarrier to immobilize the thiol-functional probe (probe1), and the SiO_2@CdTe quantum dots (QDs) core-shell nanoparticles tagged with the amino-functional probe (probe2) acted as signal indicators, respectively. In the presence of target DNA (tDNA) of CaMV35S, the binding of tDNA with probe1 and probe2 through the high specific

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DNA hybridization led to the fabrication of sandwich structure, and thus the high loading of the signal indicators SiO_2@CdTe QDs at the electrode surface, which increased the PEC signal. The increased PEC signal depended on the concentration of tDNA, and a wide linear range from 0.1 pM to 0.5 nM with low detection limit of 0.05 pM was obtained. In addition, the PEC biosensor has been successfully used for discriminating transgenic soybean from non-transgenic samples, which was consistent with the polymerase chain reaction (PCR) results, suggesting the proposed PEC biosensor is a feasible tool for the further daily genetically modified organism detection.

Keywords: Transgenic soybean; Photoelectrochemical biosensor; Silica coated CdTe quantum dot; Gold nanoparticles-reduced graphene oxide

1. Introduction

The transgenic soybean is a most important genetically modified crop which was modified by inserting the exogenous genes including the promoter element, the structural gene and the terminator element to get new characteristics, corresponding to 50% of the global biotech area in 2014[1,2]. Transgenic soybean is one of the most important protein sources used in feed and food, and it plays a very important role in reducing the environmental footprint of agriculture and greenhouse gases, contributing to the alleviation of poverty and hunger, and bringing great benefits such as increased soybean yields, reduced need for pesticides, enhanced nutrient composition and food quality[3,4]. Meanwhile, the security of transgenic soybean and its impact towards environment and health is still controversial. In many countries, transgenic soybean is required to be analyzed before being marketed and sold to

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consumers, and the law pertaining to this has been put into effect to ensure strict adherence to the labeling regulation[5]. In connection with this, it is especially significant to detection and discrimination of transgenic soybean[6,7].

There are two kinds of strategies employed in detecting of transgenic soybean. The first is based on the detection of the proteins expressed by the exogenous gene, which include Western blotting, enzyme linked immunosorbent assays, and lateral flow strips[8]. These methods for protein detection need equipment and well-trained personnel to perform field tests, and the protein were probably destroyed before the tests[9]. Therefore, methods based on detecting the exogenous gene have arisen much attention due to the good stability of gene. Because of the Cauliflwer Mosaic Virus 35S promoter (CaMV35S) is frequently present in the genetically modified organisms (GMOs), it became a widely used target[10,11]. The traditional method commonly used for GMOs DNA detection is based on polymerase chain reaction (PCR) method combined with gel electrophoresis. However, its major drawbacks are time consuming, laborious and poisonous[5]. Thus, it is necessary to fabricate the simple, rapid and low cost DNA biosensors for detecting transgenic soybean.

Photoelectrochemical (PEC) detection is a vibrantly developing electrochemical analytical method for its desirable sensitivity and good analytical performance. Firstly, the PEC method is very sensitive due to the separation of excitation signal (light) and detection signal (current). Secondly, compared to optical detection methods such as fluorescence (FL) and electrochemiluminescence (ECL), the instrument of PEC is much simpler and lower cost, and it has been used to detect specific target analytes in a wide range of areas[12-15]. Wang *et al.*[16] utilizing molecularly imprinted polymers as recognition element and CdTe quantum dots (QDs) as photoactive materials have successfully developed a favorable microfluidic origami PEC

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biosensor of highly selective and sensitive detection for the S-fenvalerate. Liu *et al.*[17] used the CdTe QDs as negatively charged PEC material to fabricate the PEC platform with a low limits detection (LOD) for protease. All these results above indicated that the PEC biosensor with the merits of simple devices, low cost and easy miniaturization exhibited potential applications in the fields from environmental monitoring to bioanalysis, which may pave the way for the practical applications of PEC biosensor in the detection of transgenic soybean, which has not been reported up to date.

The photoactive materials CdTe QDs have attracted extensive attention, because of their desirable features such as broad excitation, symmetric tunable emission spectra, photochemical stability, and binding compatibility with biomolecules[18-21]. In order to enhance the sensitivity of the PEC biosensor with CdTe QDs as indicators, increased the amount of CdTe QDs on the carrier was a commonly strategy[22]. Silica nanospheres with good monodispersity and uniform structure were employed as the carrier for CdTe QDs to achieve the SiO_2@CdTe core-shell nanoparticles (NPs). In this paper, a rapid and simple PEC biosensor was fabricated using the SiO_2@CdTe core-shell NPs as a signal indicator for detection the CaMV35S to discriminate the transgenic soybeans from non-transgenic soybeans. Due to the signal amplification from the high loading of CdTe QDs, 2.5-fold photocurrent enhancement of SiO_2@CdTe core-shell NPs was achieved in comparison with that under the unamplified method. Then, the core-shell SiO_2@CdTe as an indicator was combined with the H_2N-probes (probe2) by the amidation of the amino groups and carboxyl groups. Meanwhile, gold nanoparticles-reduced graphene oxide (AuNPs-rGO) was employed as an excellent nanocarrier to fix abundant SH-probes (probe1). The sandwich structure was achieved by assembly of the core-shell SiO_2@CdTe-probe2

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onto the surface of AuNPs-rGO-probe1 through the high specific DNA hybridization between probes and target DNA (tDNA). The fabricated PEC biosensor was utilized for the sensitive and selective detection of the specific CaMV35S with a wide linear range and a low detection limit, and had been successfully applied in detection of CaMV35S promoter in real transgenic soybean samples, showing the potential applications in the fields of GMOs discriminating.

2. Experimental

2.1. Materials and reagents

Tetraethylorthosilicate (TEOS), 3-aminopropyltriethoxysilane (APTS), 3-mercaptopropionic acid (MPA), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxysuccinimide (NHS) and Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$) were purchased from Sigma-Aldrich. 6-mercapto-1-hexanol (MCH), tris (hydroxymethyl) aminomethane (Tris) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Aladdin (Shanghai, China), graphite was purchased from Qingdao Tianhe Graphite Co., Ltd. GO was synthesized from graphite powder by the modified Hummers' method[23], and the AuNPs-rGO was synthesized as our previous works[24]. Tellurium powder (99%), $CdCl_2 \cdot 2.5H_2O$, ammonium hydroxide ($NH_3 \cdot H_2O$, 25%), $NaBH_4$ (99%), tris (hydroxymethyl) aminomethane, ethanol, and phenol were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). CdTe QDs capped with MPA containing carboxylic group were acquired according to a previous report[25].

All synthetic oligonucleotides were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). The sequences of oligonucleotides used in this work are given as below (5' to 3'):

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Target CaMV35S sequence: GGCCATCGTTGAAGATGCCTCTGCC

SH-probe (probe1): HS-GGCCATCGTTGAA

H_2N-probe (probe2): GATGCCTCTGCC-NH_2

Two-base mismatched: GGCAGAGGCAACTTCAAGGATGGCC

Five-base mismatched: GCCAGAGGCAACTTCAAGGATGCCG

Non-complementary: CCTGCTCCGTAGCCTGCGTCATCTG

The DNA sample for PCR amplification was extracted from transgenic soybean. The PCR reaction was performed using oligonucleotide primers for soybean CaMV35S gene with the following sequences (5' to 3'):

Primer F: AGGAAGGGTCTTGCGAAGGATA

Primer R: GGACCTAACAGAACTCGCCGTA

2.2. Apparatus

The transmission electron microscopy (TEM) images were taken with a JEOL 2100 TEM (JEOL, Japan) at 200 kV. UV-vis absorption spectra were recorded by UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were obtained on a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan). All the photographs were taken using a Canon digital camera (IXUS 230 HS, China) under the illumination of a 365 nm UV lamp. The X-ray photoelectron spectroscopy (XPS) data were recorded on an Escalab-MKII spectrometer with Mo Kα X-rays as the excitation source. All the electrochemical and PEC measurements were conducted using CHI660B electrochemical analyzer (ChenHua Instruments, Shanghai, China). All experiments were carried out at room temperature using a conventional three-electrode system with a modified indium tinoxide (ITO) electrode as a working electrode, a platinum wire as an auxiliary electrode, and a saturated calomel electrode (SCE) as a reference electrode. The geometrical area of the working

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electrode was 0.5 ± 0.05 cm^2. All the PEC measurements were performed in 0.1 M PBS (pH 7.4) containing 0.01M ascorbic acid (AA) at −0.2 V, and a 250 W Xe lamp (Beijing Trusttech Co. Ltd.) was used as the visible-light source with an intensity (passing through a 400 nm UV-cut filter) of 100 mW cm^{-2}, which was deaerated with high purity nitrogen for 15 min before PEC experiments and then kept over a N_2 atmosphere for the entire experimental process. All the Electrochemical Impedance Spectroscopy (EIS) measurements carried out by Zennium electrochemical workstation (Zahner, Germany) were performed under an oscillation potential of 5 mV with the frequency range of 0.1~10000 Hz, in a solution of 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl at room temperature.

2.3. Preparation of the core-shell SiO_2@CdTe-probe2

Monodispersed silica NPs synthesized with seed-growth method, were used as carriers for CdTe QDs and probe-NH_2[26]. The diameter of the as-prepared silica NPs was 110 ± 3.0 nm, recorded by TEM microscopy. The CdTe QDs (average size of 5 nm) were synthesized as our previous works, and the core-shell SiO_2@CdTe were prepared according to the literature[27]. Briefly, silica NPs (0.02 g) was first dispersed in 2 mL of ethanol and treated with 0.4 mL of APTS. After stirring for 6 h, the suspension was centrifuged and washed with ethanol repeatedly for four times, and the amino-functionalized NPs were obtained. Then, the amino-functionalized silica NPs were dispersed in a mixture of 1 mL of CdTe QDs (5 mg mL^{-1}) stabilized with 3-mercaptopropionic acid and 1 mL of EDC (20 mg mL^{-1}). The mixed suspension was stirred at 4 °C for 12 h. Unbound CdTe QDs were removed by successive centrifugation and washing with water several times. Finally, the as-prepared core-shell SiO_2@CdTe, which had the same red color as CdTe QDs itself, were obtained and dispersed in water to obtain a final volume of 1 mL.

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To generate core-shell SiO_2@CdTe-probe2 conjugates, the probe2 modified with amino at their 5' end were used. In detail, different volumes of 1 μM probe2 were added to 100 mL of core-shell SiO_2@CdTe, after shaking gently for 12 h, they covalently attached through the binding reaction of activated carboxylic acid group and amino group. Then centrifuged at 10,000 rpm for 30 min, the supernatant was taken for XPS detection.

2.4. Fabrication of the PEC biosensor

The ITO slices were used as the working electrode, which were washed by immersing in acetone, ethanol/NaOH mixed solution (v/v, 1:1), and water, respectively, followed by drying at 60 °C for 2 h. Then, 10 μL of the chitosan was dropped onto the surface of the electrode and 10 μL of the AuNPs-rGO was added onto the modified electrode surface and dried at room temperature and then incubated with 20 μL of 1 mM MCH at room temperature for 1 h to block nonspecific binding sites. After that, 20 μL of 5 μM probe-SH pretreated by TCEP (0.6 μL, 10 mM) for 1 h was dropped on the surface of the electrode to combine with AuNPs-rGO nanocomposites for 1 h. And then 20 μL of the tDNA at different concentrations was added onto the modified electrode surface and incubated for 40 min at room temperature. The Tris-HCl buffer was used to remove the excessive tDNA. And then 20 μL of the core-shell SiO_2@CdTe-probe2 was dropped onto the modified electrode surface and incubated for 40 min at room temperature. The electrode was washed three times with Tris-HCl buffer and blow-dried with nitrogen after each step of the modification process. Scheme 1 shows the steps of hybridization and immobilization. The core-shell SiO_2@CdTe combined with the amino-functional probe2 acted as a signal indicator, and the AuNPs-rGO acted as a nanocarrier to immobilize the thiol-functional probe1. In the presence of tDNA, the binding of tDNA with probe1

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and probe2 respectively through the high specific DNA hybridization led to the fabrication of sandwich structure to generate concentration-dependent PEC response signals.

2.5. DNA Extraction from transgenic soybean and PCR procedure

The transgenic soybeans were supplied by Sinograin Zhenjiang Grains and Oils Quality Testing Center Co., Ltd. Zhenjiang, China. The DNA extraction steps were performed from a 100 mg sample of transgenic and non-transgenic soybean by means of the Plant Genomic DNA Kit (Sangon Biotech Co., Ltd. Shanghai, China) according to the manufacturer's instructions. The extracted DNA was used as the template DNA for the PCR in a final volume of 25μL. And then 5μL each of the PCR products was analyzed by electrophoresis separation (5 V/cm, 40 min) on a 2.0 % agarose gel and the result could be visualized with UV transilluminator.

2.6. PEC Measurement

PEC detection was carried out at room temperature in PBS buffer (0.1 M, pH 7.4) containing 0.01 M AA which served as a sacrificial electron donor during the photocurrent measurement[28]. White light, with a spectral range of 200~2500 nm, was utilized as excitation light and was switched on and off every 20 s. The applied potential was −0.2 V. The AA electrolyte was deaerated by pure nitrogen for 10 min before photocurrent measurement.

2.7. Detection of real soybean samples

Samples were extracted from soybeans were detected by the PEC biosensor, and amplified by PCR method. The extracted samples were diluted with 50 mM PBS, denatured by heating in a boiling water bath for 10 min and then frozen in an ice bath for 2 min to obtain the ssDNA solution. Then 10 μL of sample solution was dropped directly onto the surface of modified electrode.

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3. Results and discussion

3.1. Characterization of GO and AuNPs-rGO composites

Because of the abundant surface oxygen-containing groups such as carboxyl and hydroxyl, GO was easily suspended in water to form a stable colloidal solution[29]. AuNPs-rGO hybrid was synthesized by a one-step reduction of the mixture of GO-SH and $HAuCl_4$. During this process, AuNPs were in situ attached on the surface of GO through the well-developed Au-S chemistry, meanwhile GO was also reduced into rGO by sodium citrate at the same time. The direct evidence for the successful immobilization of AuNPs onto the rGO surface was given by the TEM images of GO-SH (Figure 1A) and AuNPs-rGO (Figure 1B). As can be seen from Figure 1B, a large number of AuNPs with an average size of 15 nm were homogeneously and densely planted on the rGO surface, which would provide tremendous binding sites for DNA strands[24]. Furthermore, no free AuNPs were observed outside of the rGO surface, indicating that AuNPs were well bound to rGO sheets via strong covalent binding.

3.2. Characterization of SiO_2, CdTe QDs and core-shell SiO_2@ CdTe

Photographs of aqueous dispersed (a) SiO_2, (b) CdTe QDs, (c) core-shell SiO_2@CdTe, (d) core-shell SiO_2@CdTe-probe2 under UV lamp (λ_{ex}=365 nm) are shown in Figure 2A. The silica nanospheres were synthesized with our previously reported seed growth method[25]. Fluorescence spectra (Figure 2B) showed that the prepared SiO_2 had no obvious fluorescence intensity at about 653 nm. CdTe QDs emitted strong fluorescence at 653 nm. The core-shell SiO_2@CdTe and core-shell SiO_2@CdTe-probe2 retained their strong fluorescence although it decreased[30]. As shown in Figure 2C the prepared silica NPs had a chemically clean and homogenized

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structure with a diameter of 110 ± 5.0 nm. The coating of CdTe QDs on silica nanospheres was confirmed by TEM image (Figure 2D), which confirmed the deposition of CdTe QDs on the surface of silica nanospheres within a uniform distribution. Important information on the chemical component of the core-shell SiO_2@CdTe-probe2 can be provided by XPS measurements. As shown in Figure 2E, the wide scan XPS spectrum of the core-shell SiO_2@CdTe-probe2 clearly indicated that the sample was composed of Si, Cd, Te, C, P, N and O elements. A maximum P 2p signal at 133.1 eV was observed for the core-shell SiO_2@CdTe-Probe2 (Figure 2F), which indicated that the characterized P 2p line was from the phosphate backbones of probe2, confirming probe2 was immobilized onto the surface of the core-shell SiO_2@CdTe successfully.

3.3. EIS characterization of the biosensor construction.

EIS measurement is a powerful tool to monitor the whole procedure in preparing modified electrodes[31,31], which was carried out using 5 mM $[Fe(CN)_6]^{3-/4-}$ solution in a three-electrode system. Figure 3A shows that the EIS spectra at different stages were used to test the immobilization of the probe DNA on the electrode. The R_{ct} value of ITO was 375 Ω (curve a). When the chitosan was dropped onto the surfaces of ITO as protector and disperser to ensure AuNPs-rGO stably and uniformly dispersed in water, the R_{ct} value of ITO/Chit increased to 708 Ω. After the AuNPs-rGO was dispersed in the chitosan, the R_{ct} value of ITO/Chit/AuNPs-rGO decreased to 476 Ω, which could be attributed that both rGO and AuNPs could act as a good electron-transfer interface[33]. When the thiolated capture probe1 was immobilized on the ITO/Chit/AuNPs-rGO through the formation of Au-S covalent binding with Au NPs and noncovalent π-π stacking interactions between the nucleobases and graphene sheets and then treated with MCH, the R_{ct} value of ITO/Chit/AuNPs-rGO-probe1

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increased to 779 Ω, due to the presence of negatively charged backbones of DNA strands and the blocking effect of MCH which retarded the interfacial electron-transfer kinetics of the probes. After the ITO/Chit/AuNPs-rGO-Probe1 were partially hybridized with tDNA, a part-duplex structure was formed and more negatively charged phosphate backbones could repel the negatively charged redox probe, leading to a further enhancement of R_{ct} value, which demonstrated the tDNA had been successfully immobilized on the electrode. When the core-shell SiO_2@CdTe-probe2 was dropped onto the surface of the modified electrode, the impedance increased to 3785 Ω, which could be attributed that the core-shell SiO_2@CdTe-probe2 was immobilized on the electrode through the hybridized tDNA, so that more negatively charged phosphate backbones repelled the negatively charged redox probe and the introduction of the core-shell SiO_2@CdTe. These results obtained from the Nyquist plots in the whole impedimetric experiment demonstrated that the sensor was successfully fabricated according to the described procedures.

3.4. PEC behavior of the biosensor

Figure 3B reveals the feasibility of this PEC biosensor used in detection of tDNA. The ITO/AuNPs-rGO (curve a) and ITO/AuNPs-rGO-probe1 (curve b) showed weak PEC response, due to the surface plasmon resonance of the AuNPs could be excited with light, which facilitated the excitation of the surface electron and interfacial electron transfer[24]. When tDNA was absent, the ITO/AuNPs-rGO-probe1/probe2-CdTe (curve c) showed low PEC response, which could be attributed that parts of CdTe-probe2 was adsorbed on the graphene sheets, with the nucleobases "lying" nearly flat on the surface due to π-π stacking attraction. The electrode was modified by the ITO/Chit/AuNPs-rGO-probe1/tDNA/probe2-CdTeQDs (curve d), CdTe QDs as indicators combined tDNA by the high specific hybridization to the

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surface of the ITO to generate the appropriate photocurrent. When the core-shell SiO_2@CdTe took place of the CdTe QDs as the indicators, the photocurrent of the ITO/Chit/AuNPs-rGO-probe1/tDNA/probe2-SiO_2@CdTe (curve e) enhanced strongly. This indicated that the core-shell SiO_2@CdTe nanoprobes could amplify the PEC signals effectively. When compared to CdTe QDs, it was about 2.5-fold than that of the CdTe QDs labeled probe2 modified electrode. All these characteristics had lead to an excellent PEC biosensor.

3.5. Optimization of experiment parameters

To obtain a sensitive and stable PEC biosensor with a low LOD for CaMV35S, it is significant to optimize important parameters during the sensor fabrication process. As shown in Figure 4A, the modification of Probe1 had a saturation value for the amount of Probe1 on the ITO/Chit/AuNPs-rGO electrode surface. Before saturation, the photocurrent responses of ITO/Chit/AuNPs- rGO-probe1 increased with the increasing amounts of probe1, and the photocurrent value tended to be stable when the concentration of probe1 was above 5 μM. Therefore, 5 μM was selected to be the optimal concentration of probe1. Because DNA hybridization is a time-dependent process, the hybridization time between probes and tDNA was also optimized. The hybridization time from 20 to 60 min was studied as shown in Figure 4B. It was apparent that photocurrent responses obviously increased with the increasing interaction time from 20 to 40 min and then reached a plateau in 40 min. This suggested that 40 min was enough and thus chosen as the hybridization time in the following research.

3.6. CaMV35S promoter Detection based on the PEC biosensor

Based on the optimized condition, the developed PEC biosensor was used to detect series of different concentrations of CaMV35S. As shown in Figure 5A the

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PEC intensity increased with the increasing concentration of CaMV35S. The PEC signal (Figure 5B) showed a wide linear range, low LOD and low limit of quantity (LOQ). The relevant numerical values were listed in Table 1.

Furthermore, the performance of the proposed PEC biosensor was compared with other CaMV35S detection methods reported in the literatures (Table 2). It could be discovered that the proposed biosensor had a comparative or relative wide linear range and low detection limit, which might be attributed to the effectively amplified PEC response using the high loading of the signal indicators SiO_2@CdTe QDs. Obviously, the proposed ITO/AuNPs-rGO-probe1/tDNA/probe2-SiO_2@CdTe PEC biosensor showed promise for application in the simple monitoring of CaMV35S.

3.7. Selectivity, reproducibility and stability of the biosensor

Selectivity, reproducibility and stability are important factors for evaluating the performance of the PEC biosensor. In order to prove the selectivity of the method, two bases mismatch DNA (2mDNA), five bases mismatch DNA (5mDNA), non-complementary bases and complementary tDNA were detected. As shown in Figure 5C, compared to complementary tDNA, the PEC intensities for two bases 2mDNA, 5mDNA, non-complementary bases decreased abruptly. The reason was that mismatch DNA could not hybridize efficiently with probes to form a stable duplex complex, resulting in few core-shell SiO_2@CdTe was proximate to the electrode surface. Therefore, this biosensor was proved to be excellently selective to detect the specific sequence of tDNA. Different electrodes constructed by the same procedure showed a relative standard deviation (RSD) of 6.5%. Meanwhile, five measurements for a single electrode were completed upon by addition of 100 pM CaMV35S with RSD of 5.8%, demonstrating excellent reproducibility of the PEC biosensor. The stability was an important element for the developed PEC biosensor, therefore it was

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also evaluated by performing 380 s repeated measurements. As shown in Figure 5D, the photocurrent was very stable over time without any noticeable decrease. When the modified electrode was stored in dark under nitrogen environment one week at 4 °C, no apparent change in photocurrent response was found, indicating its good storage stability.

3.8. Real soybean samples analysis

Four transgenic soybean samples from different countries, non-transgenic soybean sample and blank sample were detected by the developed PEC biosensor method. The results are shown in Figure 6A, after hybridization with the four kinds of transgenic sample solution (curve 1, 2, 3 and 4), the PEC signal increased strongly. But the PEC signal of non-transgenic soybean sample and blank sample (curve 5 and 6) were very weak. The significant different signals proved that this PEC DNA biosensor could effectively discriminate the transgenic components from non-transgenic soybeans. To verify the accuracy of the fabricated PEC biosensor method, transgenic soybeans tDNA (PCR amplified CaMV35S (469-bp)) were analyzed by 2% agarose gel electrophoresis and EB staining for comparison. As shown in Figure 6B, bands of 469 bp appeared in the lanes of transgenic soybean clearly, while no bands were found in non-transgenic soybean or blank sample. This result is consistent with the result detected by the PEC biosensor, demonstrating a remarkable accuracy of the PEC biosensor method.

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4. Conclusions

A novel PEC biosensor based on multifunctional probes of core-shell SiO_2@CdTe and AuNPs-rGO for discriminating the transgenic soybean from non-transgenic soybeans by the CaMV35S gene sequence detection has been successfully fabricated.

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This paper demonstrated that the loading of CdTe QDs on the surface of the silica nanospheres with good monodispersion and similar surface morphology was vital for the fabrication of this sensor. The core-shell SiO_2@CdTe as an indicator for PEC signal generation, combined with NH_2-probe to accomplish the fabrication of multifunctional probes. The PEC biosensors were also tested with real transgenic soybean samples, and their results were consistent with those obtained by using traditional PCR. The developed biosensors showed great advantages including simple preparation procedure, good selectivity, wide linear range and high sensitivity. It can potentially become a convenient tool for daily GMOs discriminating, as well as of other specific oligonucleotides for medical, pabular, and environmental purposes.

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Figure captions

Scheme 1. Schematic Illustration of the fabrication procedure of the PEC biosensor and detection of the CaMV35S promoter.

Figure 1. The TEM images of (A) GO-SH and (B) AuNPs-rGO.

Figure 2. (A) The photographs of (a) SiO_2, (b) CdTe QDs, (c) SiO_2@CdTe, (d) SiO_2@CdTe-probe2 under UV lamp (λ_{ex} = 365 nm); (B) The corresponding fluorescence spectra of (a) SiO_2, (b) CdTe QDs, (c) SiO_2@CdTe, (d) SiO_2@CdTe-probe2; (C) The TEM image of the SiO_2; (D) The TEM image of the SiO_2@CdTe; (E) XPS spectrum of SiO_2@CdTe-probe2; (F) The XPS of P 2p of SiO_2@CdTe-probe2.

Figure 3. (A) EIS spectra of (a) bare ITO electrode, (b) ITO/Chit, (c) ITO/Chit/AuNPs-rGO, (d) ITO/Chit/AuNPs-rGO/probe1, (e) ITO/Chit/AuNPs-rGO/ probe1-tDNA, (f) ITO/Chit/AuNPs-rGO/probe1-tDNA/probe2-SiO_2@CdTe; (B) PEC

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responses of (a) ITO/Chit/AuNPs-rGO-probe1, (b) ITO/Chit/AuNPs-rGO-probe1/tDNA, (c) ITO/Chit/AuNPs-rGO-probe1/probe2-CdTe QDs, (d) ITO/Chit/AuNPs-rGO-probe1/tDNA/probe2-CdTeQDs, (e) ITO/Chit/AuNPs-rGO-probe1/tDNA/probe2-SiO_2@CdTe in 0.1 M pH 7.4 PBS containing 0.01 M ascorbic acid at –0.2 V.

Figure 4. (A) PEC responses by different concentrations of probes; (B) The different hybridization time of probes with tDNA.

Figure 5. (A) PEC responses of the biosensor to 0, 0.1, 0.5, 1.0 ,10, 50 ,100, and 500 pM of tDNA (from a to g); (B) linear relationship for tDNA detection under visible irradiation repeated every 20 s; (C) PEC responses of (a) blank, (b) tDNA, (c) 2-base-mismatch, (d) 5-base-mismatch, (e) non-complementary; (D) Time-based PEC responses of ITO/AuNPs-rGO-probe1/tDNA/probe2- SiO_2@CdTe;

Figure 6. (A) PEC responses of (1) transgenic soybean 1, (2) transgenic soybean 2, (3) transgenic soybean3, (4) transgenic soybean 4. (5) blank, (6) non-transgenic soybean; (B) Agarose gel electrophoresis of DNA extracted from different soybean samples, (M) DNA markers, (1) transgenic soybean 1, (2) transgenic soybean 2, (3) transgenic soybean 3, (4) transgenic soybean 4, (5) blank, (6) non-transgenic soybean.

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Scheme 1

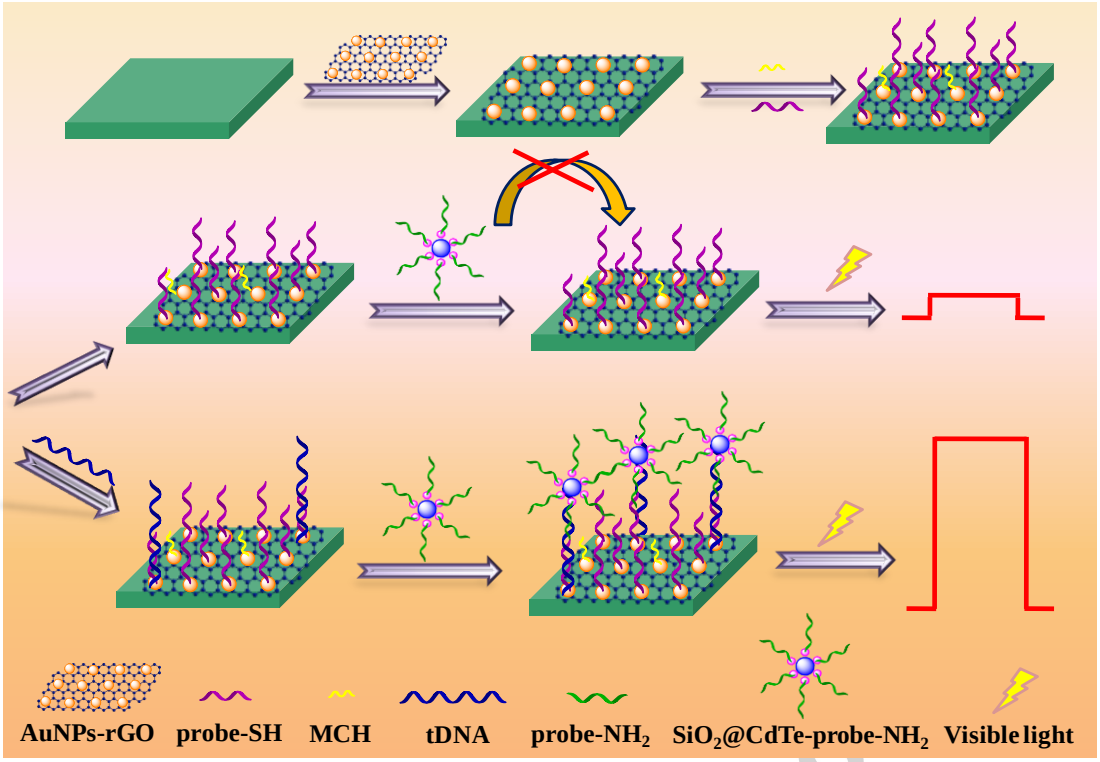

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Figure 1

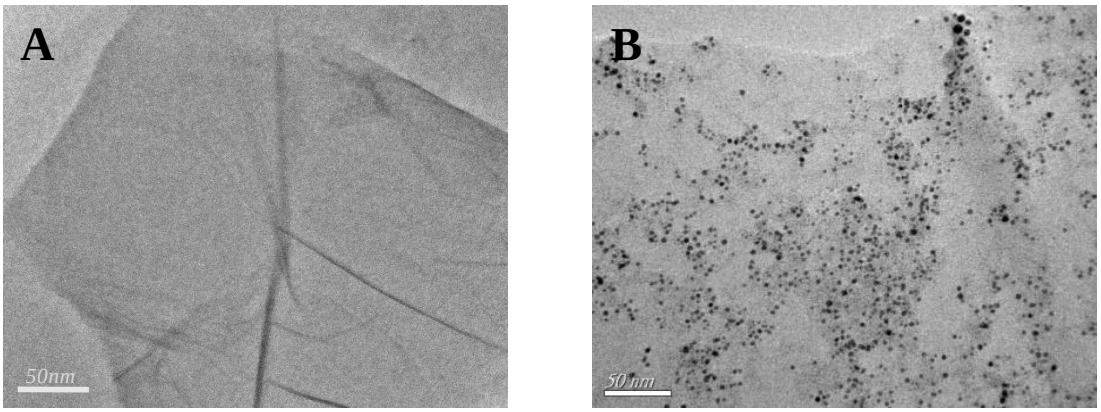

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Figure 2

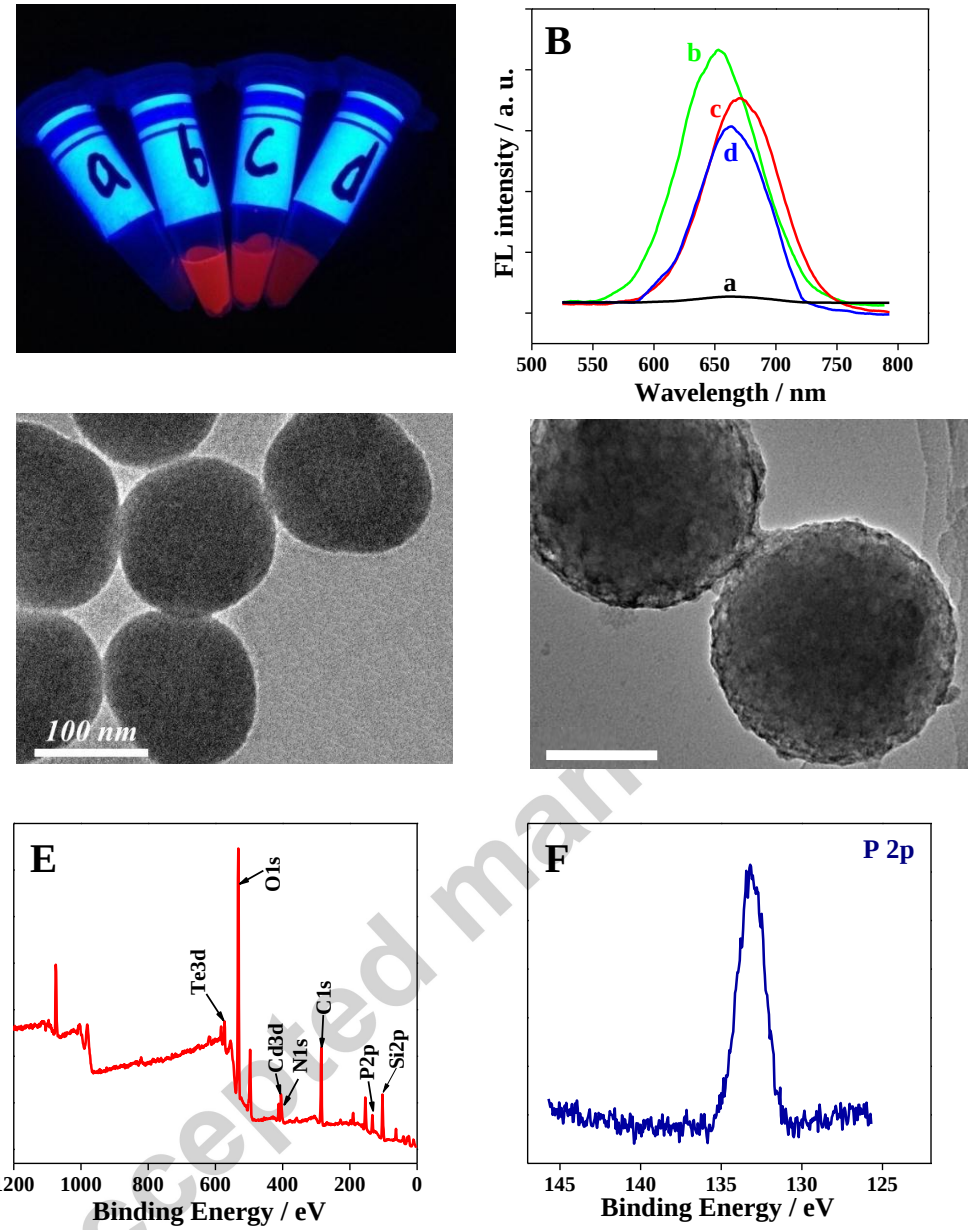

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Figure 3

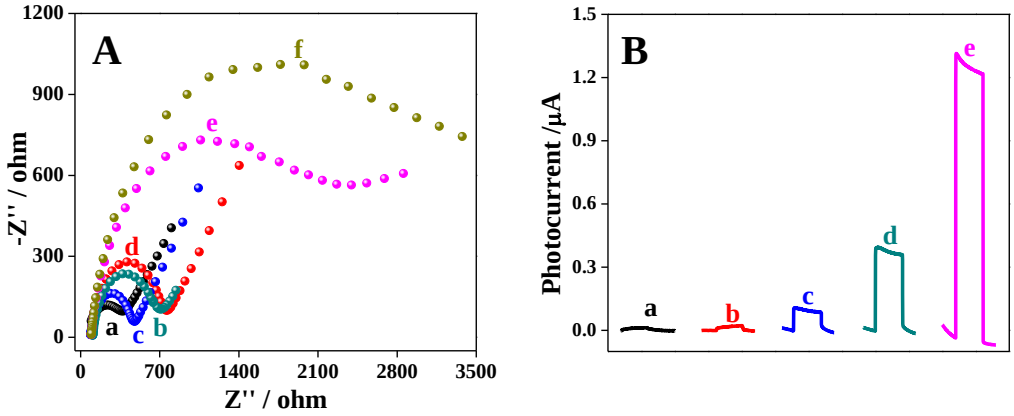

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Figure 4

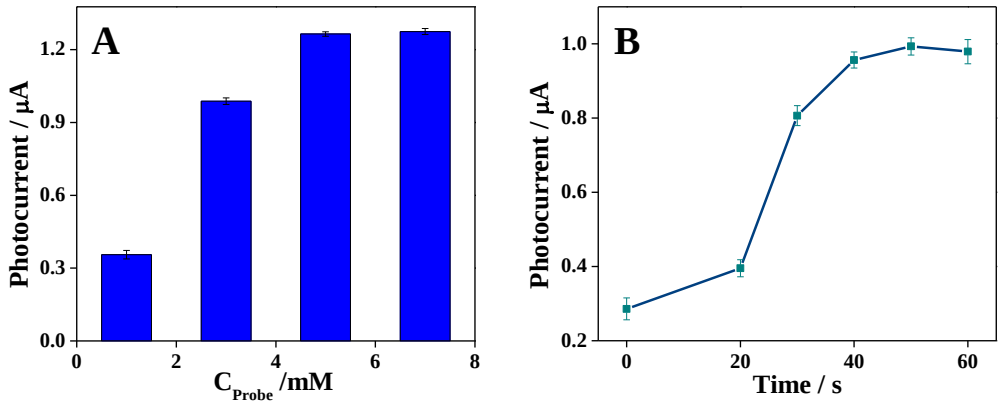

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Figure 5

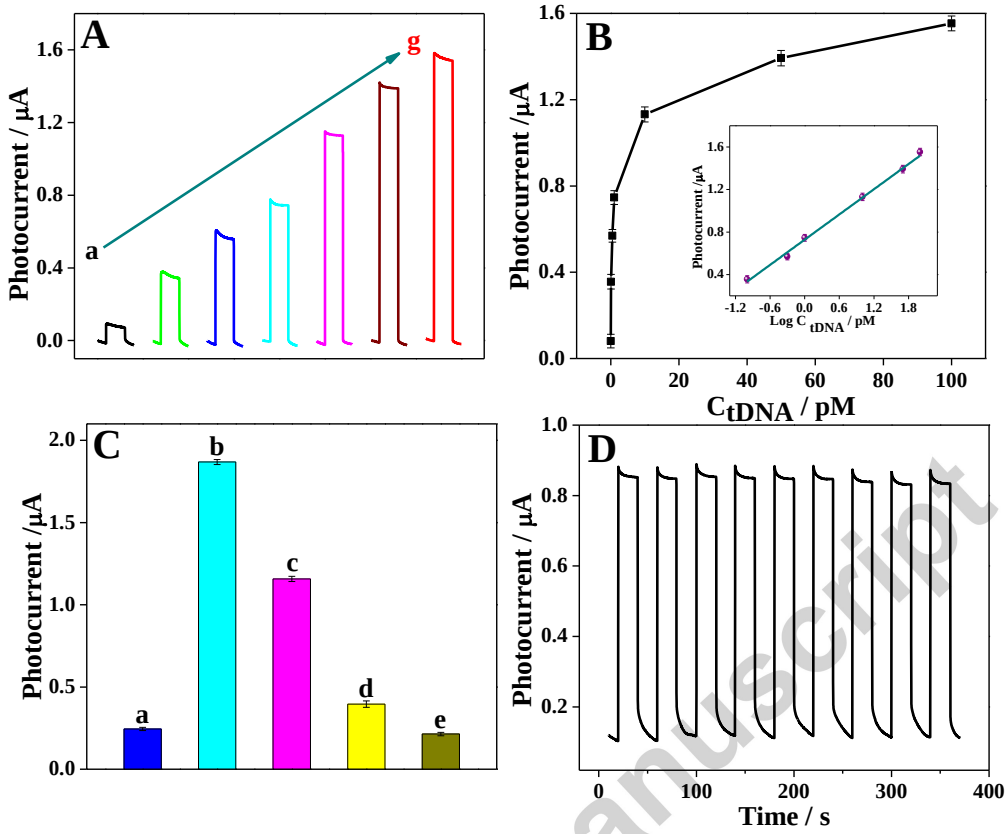

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Figure 6

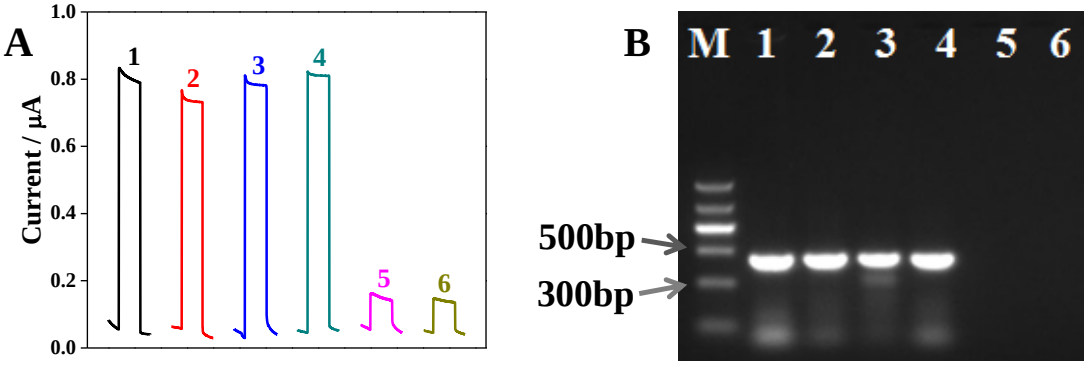

Table 1

Table 1 The linear equation, LOD and LOQ of this PEC biosensor

Linear equation	LOD (pM)	LOQ (pM)
I = 0.4 lg (c/pM) – 0.72 (R^2 = 0.9956)	0.05	0.1

Table 2

Table 2 Comparison of the analytical performance of different methods for determination of CaMV35S.

Methods	Linear range (M)	LOD (M)	Reference
SPR[a]	2.5×10^{-6}~25.0×10^{-5}	2.5×10^{-6}	[34]
ECL	5.0×10^{-6}~5.0×10^{-3}	5.0×10^{-6}	[35]
FL	5.0×10^{-9}~9.0×10^{-7}	2.0×10^{-9}	[36]
QCM[b]	1.0×10^{-9}~3.0×10^{-7}	4.0×10^{-9}	[37]
EIS	1.0×10^{-6}~1.5×10^{-5}	1.0×10^{-6}	[38]

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PEC	1.0×10^{-10} ~ 5×10^{-7}	5.0×10^{-11}	This work

[a]SPR, surface plasmon resonance

[b]QCM, quartz crystal microbalance

Highlights

- Photoelectrochemical CaMV35S biosensor as a new method was fabricated.
- SiO_2@CdTe quantum dots core-shell nanoparticles acted as signal indicators.
- The biosensors could discriminate the transgenic from non-transgenic soybean.
- Simple preparation, good selectivity and high sensitivity were obtained.

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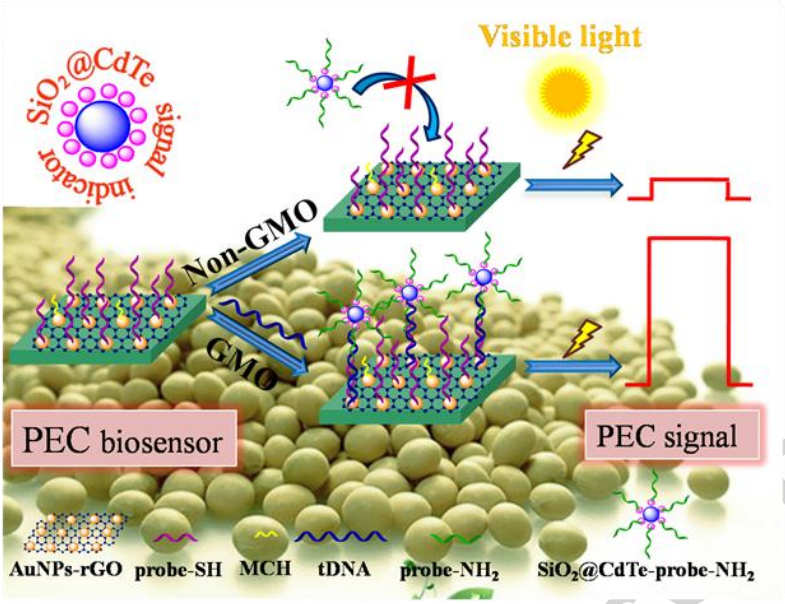
Graphical abstract



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