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A portable electrochemical immunosensor for highly sensitive point-of-care testing of genetically modified crops

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ABSTRACT

The wide cultivation of genetically modified (GM) crops has raised concerns on the risks to humans and the environment. 5-enolpyruvylshikimate-3-phosphate synthase isolated from *Agrobacterium* species strain CP4 (CP4-EPSPS) protein is most widely present in these crops. Therefore the measurement of CP4-EPSPS sensitively in a point-of-care testing (POCT) manner for the screening of transgenic plants is demanded. To date the development of quantitative POCT system has not yet been reported. In presented study, an electrochemical immunosensor towards CP4-EPSPS has been fabricated by integrating a portable bioanalytical device with a disposable screen-printed carbon electrode (SPCE) for POCT of GM crops. The dual-functionalized AuNPs were used as nanoprobes and prepared by simultaneously tagging horseradish peroxidase (HRP) and antibody on AuNPs with an exceptionally simple protocol. The sensitivity of the developed nanoprobe-based immunosensor was 62.5-fold higher than that using HRP-labeled antibody. As a result, the proposed immunosensor using SPCE could detect CP4-EPSPS down to 0.050 ng mL^{-1} with the linear range of 0.10-10 ng mL^{-1} within 65 min. In addition, the developed method has been validated with genuine GM crops and the results show a good correlation coefficient of 0.9909 compared with those of a commercial ELISA kit. Therefore, this portable electrochemical immunosensor is suitable for rapid and sensitive detection and provides a convenient and reliable platform for POCT assay.

1. Introduction

Genetically modified (GM) crops possess improved characteristics, for instance the resistance to herbicides and pests, enabling more effective weed control and less utilization of pesticide. As a result, GM crops have been widely cultivated over last 22 years. In 2017, GM crops have reached 189.8 million hectares globally. Herbicide-tolerant crops have consistently dominated 47% of the global hectarage (James, 2018). Among herbicide-tolerant crops, glyphosate resistance crops with an inserting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium tumefaciens* CP4 (CP4-EPSPS) is the most widely planted. Despite the improved traits offered by GM crops, there are public concerns about the impact of GM products on humans and environments. The labeling of GM organisms has been mandatory in many countries for safety control of agricultural GM crops based on a low labeling threshold, such as 0.90% in EU. With the increase of planting area of GM crops, there is a high demand to measure GM ingredients sensitively in a simple, rapid and point-of-care testing (POCT) manner.

Plenty of exogenous-DNA-based methods including real-time quantitative polymerase chain reaction (PCR) (Bonfini et al., 2012; Mano et al., 2017; Niu et al., 2018), quartz crystal microbalance biosensor (Mannelli et al., 2003), surface plasma resonance biosensor (Mariotti et al., 2002), Lateral flow nucleic acid biosensor (Cheng et al., 2017), electrochemical biosensor (Fortunati et al., 2019; Freitas et al., 2016) and electrochemiluminescent biosensors (Guo et al., 2009) have been widely used to detect GM ingredients in plants. However, they do not achieve the goal of POCT due to the fact that these methods involve laborious sample pretreatments or expensive instrumentations. Alternative methods for GM plant detection are target-protein-based

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immunoassays. Especially, the immunochromatographic assay is fit for POCT owing to the its great intrinsic properties of simple operation, rapid response and limited demands on equipment and staff resources (Emslie et al., 2007). However, so far a visible colorimetric readout strategy was adopted in the reported immunochromatographic assay for the detection of GM crops, which only provided a yes/no response and often suffered from insufficiently sensitivity (Emslie et al., 2007; Santos et al., 2015; Zhou et al., 2015). The management of GM organisms usually demands a very low labeling threshold and thresholds varies in different countries. Therefore, highly sensitive and quantitative detection of GM ingredients can meet the requirements of the implementation of GM products labeling policy.

Recently, electrochemical immunosensors have drawn extensive attentions due to its inherent benefits over other detection technologies, such as high sensitivity, simplicity of manipulate, easy integration in compact analytical devices (Quesada-Gonzalez and Merkoci, 2018; Wan et al., 2013; D.M. Wang et al., 2018). Therefore, electrochemical immunosensors provide a promising pathway for POCT of GM crops. Several efforts have been made to improve the sensitivity of electrochemical immunosensors. Firstly, a number of film matrices have been developed to increase loading capacity of antibody on electrode, thereby enhancing the detection sensitivity (Wang et al., 2012). For instance, due to its ion-exchange and membrane building feature, nafion has been successfully reported for combining of carbon nanodots to form a novel nanocomposite film that can easily entrap antibody by the electrostatic and physical adsorption, greatly enhancing the immobilization efficiency (Dai et al., 2012). Secondly, the development of amplification strategy was another way to achieve high assay sensitivity. Nanomaterials have been widely utilized to label biomolecules for signal amplification (Hou et al., 2018; Liang et al., 2017). Among various nanoparicles, AuNPs are highlighted owing to the advantages in facile synthesis, large specific surface area, and biocompatibility. As reported by Kim et al. AuNPs have been used as nanocarriers to load large amount of secondary antibodies to perform high sensitive detection of targets (Kim et al., 2010). So far recent researches have concentrated on the methodology development of electrochemical immunosensor while the application of it has rarely been reported. The development of POCT systems with desirable features of integration, miniaturization and automation is highly demand.

Herein, we developed a highly sensitive electrochemical immunosensor that allows for POCT of GM crops using a disposable screen-printed carbon electrode (SPCE) as the substrate. In this study, nafion or chitosan was first combined with AuNPs to form a nanocomposite film to entrap antibody. An enhanced sensitivity was achieved by using AuNPs dually functionalized with antibody and horseradish peroxidase (HRP) as signal amplification nanoprobes. In addition we developed a portable eletrochemical analyzer where immunosensor was inserted and quantitative analysis was performed. The integration of the portable bioanalytical device with a disposable electrochemical immunosensor allows for improved POCT.

2. Experimental

2.1. Materials and instruments

Nafion and streptavidin-HRP were supplied by Sigma-Aldrich Chemical Co., Ltd. (USA). Chitosan was purchased from Aladdin Chemistry Co., Ltd (China). Cry1Ab was obtained from Case Western Reserve University. PAT/*bar* protein and PAT/*pat* protein were all purchased from YouLong Biotechnology Co., Ltd. (China). CP4-EPSPS protein and monoclonal and polyclonal antibodies for CP4-EPSPS were all provided by Riogene Inc. (China). An ELISA kit for CP4-EPSPS was bought from Envirologix Inc. (USA). Bovine serum albumin (BSA) was provided by Biosharp Co., Ltd (China). CP4-EPSPS was diluted with phosphate buffer (PB) at 0.010 M and pH 7.4 containing 1.0% BSA. PB containing 2.0% BSA and 0.050% Tween-20 was utilized as the blocking buffer. Other reagents and solvents were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). All aqueous solutions were prepared using 18.2 MΩ water obtained by a Millipore-XQ system.

Genuine GM rapeseed samples carrying a *CP4-EPSPS* gene were collected and identified by the Supervision and Test Center (Wuhan) for Environmental Safety of Genetically Modified Plants of the Chinese Ministry of Agriculture.

Particle size distributions were measured by a Zetasizer Nano ZSP (Malvern Panalytical, UK). The ultraviolet visible (UV-vis) absorption spectra were obtained from a SPECORD 205 UV-vis spectrophotometer (Analytikjena, Germany). Scanning electron microscopy (SEM) was examined on a VEGA 3 LMU scanning electron microscope (Tescan Ltd., Czech Republic). Electrochemical impedance spectroscopy (EIS) was conducted by a Corrtest CS350H electrochemical workstation (Corrtest Instruments Corp., Ltd., China).

2.2. Preparation of genuine samples

GM crop seed and leaf samples were first ground into a power. Then 0.20 g of GM crop powder was suspended in 1.0 mL of ultrapure water, followed by shaking for 10 min. After centrifugation at 8000 rpm for 5 min to discard the precipitates, the resultant solution was stored at 4 °C for further use.

2.3. Preparation of dual-functionalized AuNPs

AuNPs were prepared as previously described (Brown et al., 1996). Briefly, 100 mL of 0.010% $HAuCl_4$ solution was boiled under stirring. Afterwards 2.0 mL of 1.0% trisodium citrate solution was added. The mixture was constantly heated for 10 min after the color stay unchanged and AuNPs was formed in the solution. As-prepared AuNPs solution was cooled down to room temperature (RT) and stored at 4 °C for further experiments.

To prepare the dual-functionalized AuNPs composite, 1.0 mL of the AuNPs solution was adjusted to pH 8.5 using 0.20 M K_2CO_3 solution, and then centrifugated at 9000 rpm for 30 min to discard the supernatant.. Afterwards, 230 μL of PB buffer, 50 μL of 1.0 mg mL^{-1} streptavidin-HRP and 60 μL of 1.1 mg mL^{-1} monoclonal antibody for CP4-EPSPS (mAb) were added into the centrifuge tube containing the AuNPs pellet. The resultant solution was then stirred for 1 h at 25 °C afterwards standing still for 1 h at RT. At last 30 μL of 5.0% PEG20000 was added into the resultant solution and stored at 4 °C overnight under stirring. Next day as-prepared dual-functionalized AuNPs was collected via centrifugation at 9000 rpm and 4 °C for 30 min and washed with 2.0 mL of 0.010 M Tris-HCl buffer (pH 7.8) containing 10% sucrose, 2.0% BSA, 10% polyvinyl pyrrolidone (PVP), 10% polyethylene glycol (PEG)-8000 and 0.15% Tween-20 for three times. The purified dual-functionalized AuNPs were dispersed in 1.0 mL of Tris-HCl buffer mixture as stated above and stored at 4 °C for further studies.

2.4. Preparation of electrochemical immunosensor

At first a glassy carbon working electrode (GCE) was polished with 0.050-μm alumina powder. Polished GCE was then sonicated in 75% ethanol for 5 min and afterwards ultrapure water for another 5 min. After that, 3.0 μL of immobilization solution containing 0.5% (v/v) nafion solution, AuNPs and 5.0 mg mL^{-1} of polyclonal antibody for CP4-EPSPS (pAb) with a ratio of 2:1:1 (v/v/v) was added onto the surface of the working electrode and dried at RT to form a nanocomposite film. The modified electrode was then treated with 2.0% BSA for 40 min at RT to block the nonspecific adsorption. At last electrochemical immunosensor was prepared and rinsed with PB buffer for further studies.

2.5. Electrochemical immunosensor for POCT of GM crops

To achieve POCT of GM crops, a disposable SPCE substrate (3.8 cm × 1.2 cm) was assembled using one Ag reference electrode, one carbon counter electrode and one carbon working electrode (3.0 mm in diameter). The working electrode was girdled around by the reference electrode and the counter electrode. After being cleaned with ultrapure water, the SPCE substrate was activated in 0.10 M PB buffer at pH 7.0 by a cyclic voltammetry measurement between 0.8 V and 1.3 V at a scan rate of 100 mV s^{-1} for 5 circles. Five microliter of immobilization solution containing chitosan, AuNPs and 5.0 mg mL^{-1} of pAb with a ratio of 1:1.5:1.5 was then dropped onto the working electrode of SPCE and dried at RT to prepare the immunosensor. Afterwards immunosensor was blocked with BSA as GCE stated above.

To perform the electrochemical immunosensor targeting CP4-EPSPS for GM crop detection, 30 μL of mixture solution containing CP4-EPSPS or GM crop samples and the dual-functionalized AuNP nanoprobes was deposited on the fabricated immunosensors, followed by incubation at 37 °C for 1 h. Afterwards the immunosensor was thoroughly washed with PB buffer and inserted into the portable electrochemical analyzer (19 cm × 11 cm × 4.0 cm). In the analyzer, the immunosensor was immersed in 0.20 M PB buffer with pH value of 6.0 containing 20 mM *o*-PD and 20 mM H_2O_2. With the application of a differential pulse voltammetric (DPV) in the sweeping range from -0.3 to -0.7 V, the elecrochemical signal was triggered and collected after 60 s.

3. Results and discussion

3.1. Characterization of electrochemical immunosensor

The dual-functionalized AuNP nanoprobes were prepared through a facile method of directly incubating AuNPs with mAb and HRP via the electrostatic interactions. The particle size distributions of the AuNPs were investigated and shown in Fig. 1A. It was observed that the as-synthesized AuNPs possess an average diameter of 22 nm. The modification of proteins on the surface of AuNPs was studied with UV-vis absorption spectroscopy. As presented in Fig. 1B, the maximal absorption peak of the dual-functionalized AuNPs shifted form 522 nm to 530 nm in comparison with that of bare AuNPs, indicating the presence of protein on the surface of AuNPs. To further verify the coexistence of HRP and mAb on the dual-functionalized AuNPs, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out. As shown in. Fig. 1C after AuNPs conjugate reacted with SDS reducing buffer, The proteins on its surface were denatured and separated from the nanoparticles. Distinct bands corresponding to mAb and streptavidin-HRP were then observed. The other bright band was BSA due to the addition of abundant BSA as blocking agents during the preparation process of the AuNP probes. Consequently, the two kinds of proteins were simultaneously functionalized on the surface of AuNPs.

The morphologies of bare, AuNPs/chitosan film modified and pAb/AuNPs/chitosan modified SPCEs were characterized using SEM. Compared with the inhomogeneous and uneven surface of bare electrode (Fig. 1D), AuNPs/chitosan film modified electrode showed a three-dimensional porous structure (Fig. 1E), which facilitated electrical conductivity and loading of pAb. As pAb was embedded in the chitosan film (Fig. 1F), the bright particles in the porous structure were clearly observed, demonstrating the effective immobilization of the abundant antibodies onto immunosensor.

The stepwise fabrication of the proposed immunosensor was confirmed by monitoring the change of electric resistance (*R*et) at each assembly step with EIS conducted in 5.0 mM $K_3[Fe(CN_6)]/K_4[Fe(CN_6)]$ solution containing 0.10 M KCl. As shown in Fig. S1A, when doped with AuNPs (curve b), GCE modified with nafion film (curve a) displayed a obviously decreased *R*et value due to the acceleration of interfacial electron transference by AuNPs. With the sequential assembled with pAb (curve c), BSA (curve d), CP4-EPSPS (curve e) and dual-functionalized AuNP probes (curve f), the *R*et values increased steadily, because the deposition of protein layers hinder the access of the redox probes of $[Fe(CN)_6]^{3-4-}$ to electrode. These results were in good agreement with those obtained from EIS measurements of chitosan coated SPCEs (Fig. S1B). Therefore, the proposed immunosensor was successfully assembled.

3.2. Principle of electrochemical immunosensor

The principle of the developed electrochemical immunosensor is illustrated in Fig. 2. The proposed electrochemical immunosensor was a POCT system by integrating a portable eletrochemical analyzer (F) with a SPCE in the size of a coin (A). In this study, as shown in Fig. 2B, mAb and HRP were functionalized on the surface of the AuNPs via a one-step process to prepared signal amplification nanoprobes. Nanocomposite films of nafion/AuNPs or chitosan/AuNPs were used to immobilize pAb on electrode (Fig. 2C and D), which increased the loading amount of antibody and facilitated the electron transfer. As seen in Fig. 2E, In the presence of CP4-EPSPS, AuNP nanoprobes could anchor on the electrode surface by a sandwich immunocomplex resulting from the specific

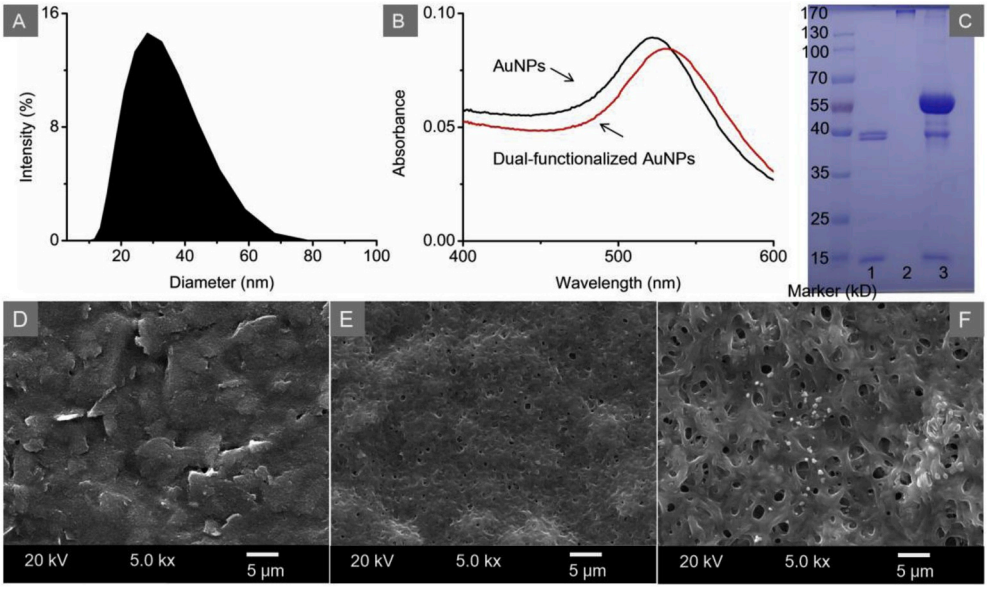

Fig. 1. (A) Particle size distributions of AuNPs. (B) UV-vis absorption spectra of bare AuNPs and dual-functionalized AuNPs. (C) SDS-PAGE image; samples from left to right are as follows: marker, mAb, streptavidin-HRP and dual-functionalized AuNP nanoprobes. SEM images of (D) bare, (E) AuNPs/chitosan film modified and (F) pAb/AuNPs/chitosan modified SPCEs.

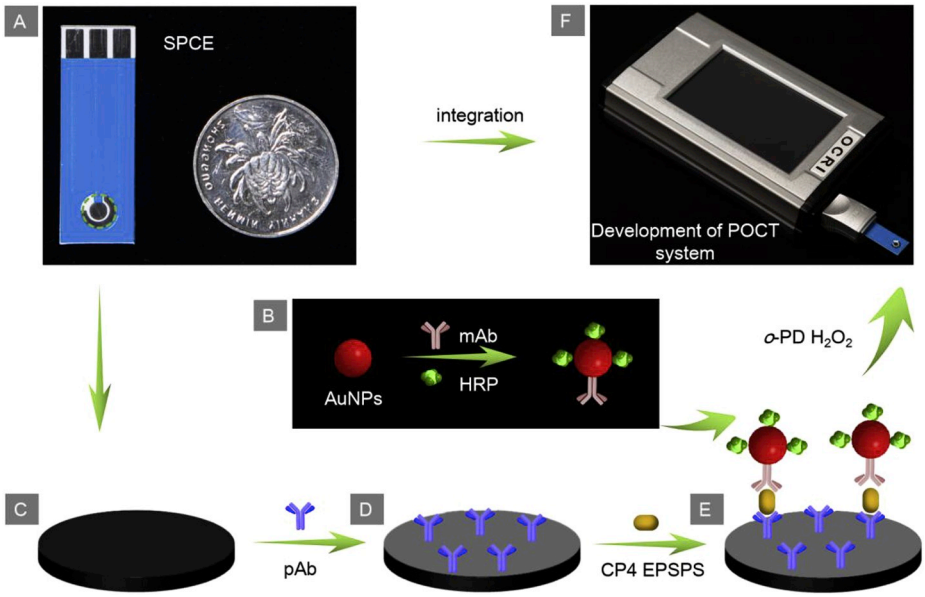

Fig. 2. Schematic illustration of the proposed dual-functionalized AuNP nanoprobes-based electrochemical immunosensor for POCT of GM crops.

recognition between pAb, CP4-EPSPS and mAb. Upon addition of *o*-PD and H_2O_2, the DPV curves of probe-anchored electrode displayed a reduction peak resulting from the electrochemical reduction of the oxidation product of *o*-PD and H_2O_2 in presence of HRP. The DPV current showed a good relationship with the amount of CP4-EPSPS captured on electrode. By utilization of the application of SPCE substrate and portable eletrochemical analyzer, the proposed approach can achieve a POCT of GM crops.

3.3. Optimization of detection conditions

The performance of the electrochemical immunosensor was influenced by key parameters including reaction conditions, the amounts of pAb and nanoprobe. The effect of pAb concentrationon on the DPV response of the immunosensor was investigated utilizing CP4-EPSPS at 10 ng mL^{-1} (as a signal) and a dilution PB buffer containing 1.0% BSA (as a blank) in parallel. As seen in Fig. S2, the signal-to-blank ratio reached the maximum value at 5.0 mg mL^{-1} of pAb, implying that the immunoreaction was saturated. Therefore, 5.0 mg mL^{-1} of pAb was selected for the following experiments. Similar results were observed for the dual-functionalized AuNPs at a dilution ratio of 1:10, thus AuNP nanoprobes at this dilution ratio were chosen (Fig. S3). The influence of incubation time was also investigated using CP4-EPSPS at 10 ng mL^{-1} (Fig. S4). The DPV response increased gradually with the increased incubation time and reached the plateau after 60 min, thus incubation time was set at 60 min in this study. For this HRP-based electrochemical reaction, *o*-PD and H_2O_2 were used as the substrate. The concentrations of *o*-PD and H_2O_2 were the important factors in the signal response. After a careful investigation (Fig. S5), 20 mM of *o*-PD and 20 of mM H_2O_2 were used for GM crop detection.

3.4. Specificity, repeatability and stability of electrochemical immunosensor

Three interfering proteins widely found in GM crops including Cry 1Ab, PAT/*bar* and PAT/*pat* were investigated to evaluate the specificity of the proposed immunosensor. As seen in Fig. 3, it was found that DPV response from the interfering proteins at 10 ng mL^{-1} exhibited negligible differences in comparison with the blank signal obtained from a dilution PB buffer. However, obvious increase of signal was observed from CP4-EPSPS at the same concentration. The degree of interference (DI) value for these interferents can be calculated from the following

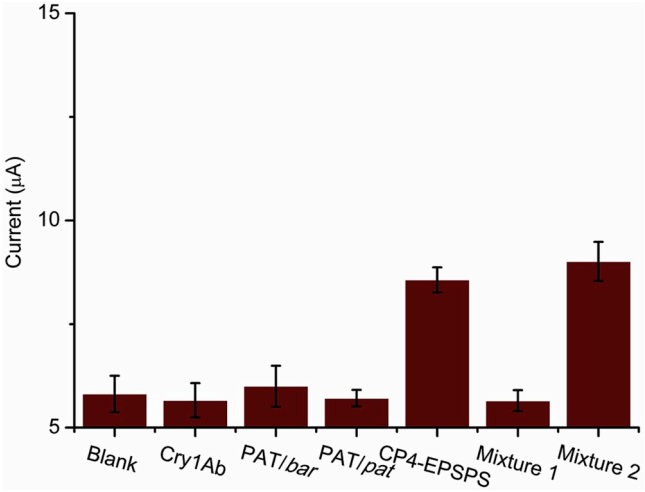

Fig. 3. Specificity of the proposed electrochemical immunosensor.

equation:

$$DI = \frac{A - C}{B - C} \times 100\% \quad (1)$$

Where A, B and C are the DPV responses of the interferences, CP4-EPSPS and the blank, respectively. According to equation (1), DI values for Cry 1Ab, PAT/*bar* and PAT/*pat* were 5.5%, 6.8% and 3.7%, respectively. Additionally, mixture 1 consisting of three interfering proteins (10 ng mL^{-1} for each), and mixture 2 consisting of the three interferents and CP4-EPSPS (10 ng mL^{-1} for each) were prepared, and then detected with the proposed method. The DI value for mixture 1 was 5.9%. Compared with signal of CP4-EPSPS, only a slight change of 5.2% was observed from that of mixture 2. As a result, the detection of CP4 EPSPS with immunosensor stated above was not interfered by these common GM proteins, and showed ideal specificity.

CP4-EPSPS at concentrations of 0.10, 1.0 and 10 ng mL^{-1} was repeatedly measured for 5 times to investigate the repeatability of the proposed method. The relative standard deviations were 4.8%, 7.1% and 6.5%, respectively. Moreover, the stability of the fabricated immunosensor was estimated using CP4-EPSPS at 1.0 ng mL^{-1}. The initial DPV response reduced only 4.8% after storage at 4 °C for two weeks.

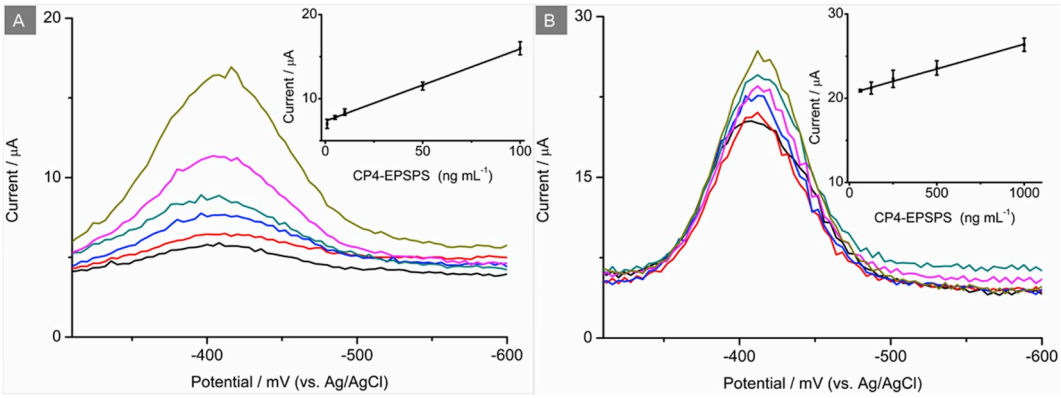

Fig. 4. DPV responses of CP4-EPSPS using GCE substrate and (A) AuNP nanoprobes at the concentrations of 0, 1.0, 5.0, 10, 50, 100 ng mL^{-1}; (B) HRP-mAb probe at the concentrations of 0, 62.5, 125, 250, 500, 1000 ng mL^{-1}; from bottom to top. Inset: calibration curves, where n = 5 for each point.

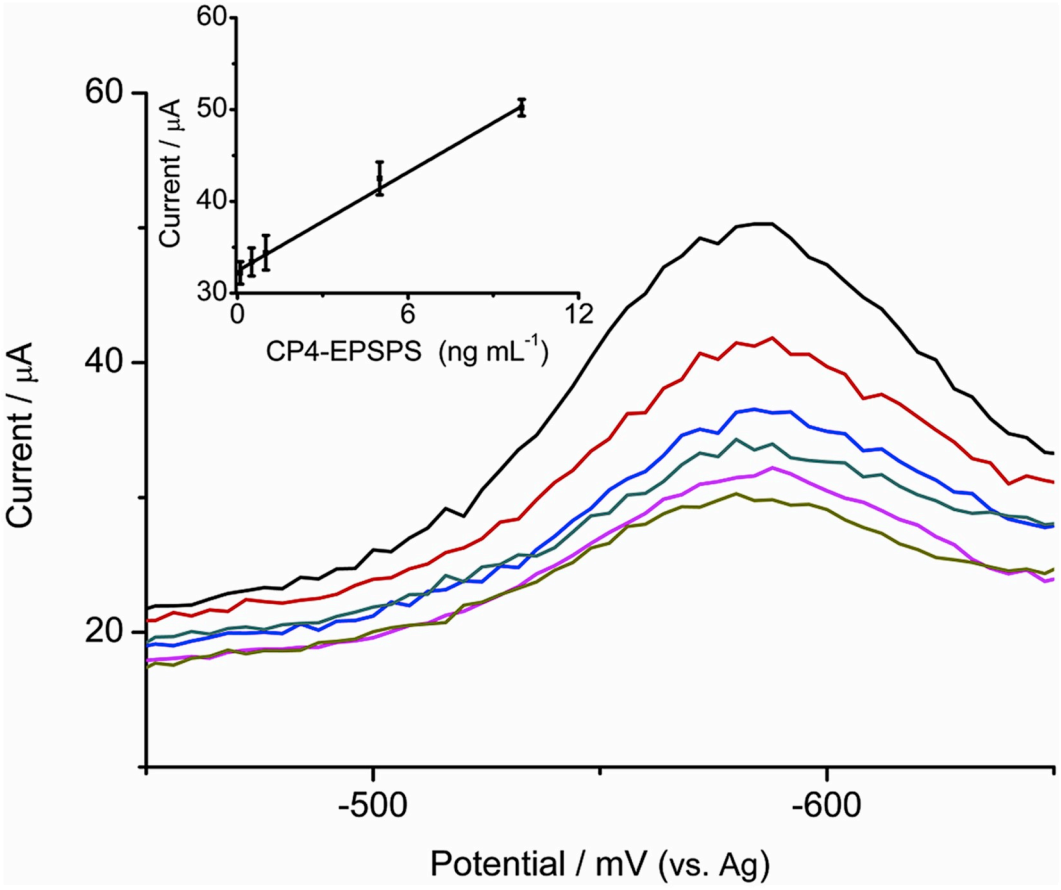

Fig. 5. DPV responses of CP4-EPSPS using SPCE substrate and AuNP nanoprobes at the concentrations of 0, 0.10, 0.50, 1.0, 5.0, 10 ng mL^{-1}; from bottom to top. Inset: calibration curves, where n = 5 for each point.

Table 1
An overview on recently reported immunoassays for determination of CP4-EPSPS.

method	material used	LOD (ng mL^{-1})	specificity	references
commercial ELISA kit		0.23	data no shown	Bookout et al. (2000)
time-resolved fluorescent immunoassay	dual label with Eu^{3+}/Sm^{3+}	0.17	data no shown	Bookout et al. (2000)
fluorescent immunosensor	Iron-polymer-graphene	0.34	specificity in the presence of interferences including HCG, PSA, AFP, NDKA and Bt proteins	Yin et al. (2017)
electrochemical immunosensor	ordered mesoporous carbon	0.00072	specificity in the presence of interferences including Cry 1Aa, Cry 1B and Cry 1F	Zhang et al. (2018)
the proposed immunosensor	dual-functionalized AuNPs	0.050	specificity in the presence of interferences including Cry 1Ab, PAT/*bar* and PAT/*pat*	this work

Table 2
Detection of GM crop samples using the proposed method and the ELISA kit (n = 3).

	GM rapeseed			GM rape leaf	GM soybean seed
GM crop samples	GT73	1	2	3	4
Detected by proposed method (ng mL^{-1})	5209.8 ± 471.8	602.8 ± 53.6	3896.5 ± 380.6	3614.1 ± 532.5	24956.9 ± 981.3
Detected by ELISA kit (ng mL^{-1})	4733.6 ± 11.0	593.7 ± 4.1	3146.8 ± 60.6	4273.5 ± 49.1	23978.2 ± 193.0
Added (ng mL^{-1})	4000.0	2000.0	4000.0	4000.0	2000.0
Found (ng mL^{-1})	8415.9	2688.9	7737.6	7860.4	26970.8
RSD (%)	6.4	7.3	6.2	8.1	4.9
Recovery (%)	80.2	104.3	96.0	106.2	100.7

Therefore the repeatability and stability of the developed immunosensor was acceptable.

3.5. Analytical performance for CP4-EPSPS

Under the optimized detection conditions, the DPV response increased with the increase of CP4-EPSPS concentration. As shown in Fig. 4A, a dose-response calibration curve for CP4-EPSPS detection using GCE and an Ag/AgCl reference electrode was observed in the concentration range from 1.0 ng mL^{-1} to 100 ng mL^{-1}. The linear regression equation was I (a. u.) = 0.08 C (ng mL^{-1}) + 7.33 (I and C are the DPV response and the CP4-EPSP concentration, respectively) with a correlation coefficient (R^2) of 0.9914. The limit of detection (LOD) defined as the target concentration generating a signal-to-noise ratio of 3 (Hong et al., 2016) was 0.50 ng mL^{-1}. In order to verify the signal amplification of the dual-functionalized AuNP nanoprobes, the commercial HRP-labeled mAb was utilized to replace the nanoprobe in the same electrochemical assay. Under optimal concentration of HRP-mAb (2.0 μg mL^{-1}), the DPV response increased linearly with the increase of CP4-EPSPS concentration from 62.5 ng mL^{-1} to 1.0 μg mL^{-1} (Fig. 4B). The linear regression equation was I (a. u.) = 0.01 C (ng mL^{-1}) + 20.54 (I and C are DPV response and the CP4-EPSP concentration, respectively) with a correlation coefficient (R^2) of 0.9972. The LOD was 31.25 ng mL^{-1} at a signal to noise ratio of 3. By comparison of two limits of quantitation, the detection sensitivity of the developed approach was 62.5 times higher than that using HRP-mAb as signal probe, demonstrating an enhanced sensitivity.

Subsequently, the developed electrochemical immunosensor targeting CP4-EPSPS was applied for POCT of GM crop using SPCE as substrate. An Ag reference electrode was applied in SPCE substrate. As seen in Fig. 5, the DPV response increased linearly with increasing concentrations of CP4-EPSPS in the range of 0.10 - 10 ng mL^{-1}. The linear regression equation was I (a. u.) = 1.79 C (ng mL^{-1}) + 32.41 (I and C represent the DPV response and the CP4-EPSPS concentration, respectively) with a R^2 of 0.9963 and a LOD of 0.050 ng mL^{-1} at S/N = 3. As shown in Table 1, the LOD of this method is lower than those of most reported immunoassays for the determination of CP4-EPSPS.

3.6. POCT of GM crops

In order to validate the reliability of the fabricated immunosensor for POCT of GM crops, three GM rapeseed samples, a GM soybean seed sample and a GM rape leaf sample carrying a *cp4-epsps* gene were tested. These genuine samples were diluted appropriately prior to assay to ensure that the levels of CP4-EPSPS were in the linear ranges. As seen in Table 2, the concentrations of CP4-EPSPS in these GM crops were detected to be 5209.8 ± 471.8, 602.8 ± 53.6, 3896.5 ± 380.6, 3614.1 ± 532.5, 24956.9 ± 981.3 ng mL^{-1}. Furthermore, the results were verified with a commercial ELISA kit. The R^2 of the results obtained from two methods was 0.9909, demonstrating that the proposed method could be utilized for GM crops detection. In addition, known amounts of CP4-EPSPS were spiked into the genuine GM rape and soybean samples to conduct the recovery assay using the proposed method. As presented in Table 2, the recoveries were all between 80.2% and 106.2%, and all the relative standard deviation (RSD) values were less than 8.1%, implying acceptable reliability of the developed method.

Recently several promising electrochemical nanosensors have been reported and displayed advantages in small dimension, high sensitivity and fast response for real samples analysis including single Au nanowire electrodes (Tang et al., 2019; Y. Wang et al., 2018) and single Pt@Au nanowire electrodes (Zhang et al., 2017). The miniature SPCE displays merits in the simple and economic manufacture as well as advantages mentioned above. Furthermore SPCE is equipped in the portable electrochemical analyzer which is capable for the field detection.

4. Conclusions

In summary, a highly sensitive electrochemical immunosensor targeting CP4-EPSPS has been developed based on the dual-functionalized AuNPs for rapid POCT of GM crops. AuNPs were used as nanovehicles to simultaneously label HRP and mAb for amplifying enzymatic catalysis signal resulting in a enhanced sensitivity. The proposed immunosensor was easy-to-use POCT device owing to its facile fabrication by inserting a SPCE into the portable electrochemical analyzer. The proposed immunosensor device had advantages in convenient operation, small size, easy portability and direct reading concentration in the absence of computer connections or data analyses. Thus it is exceptionally appropriate for offline detection of GM crops in the field and can be used as simple as test strips by nonprofessionals. At last the designed immunosensor open a new vista for POCT of GM organisms detection, and can be extended to measure other analytes. With continuous increasing of exogenous proteins in GM organisms, the developed method towards one target may reduce the detection efficiency. In the future, we will fabricate immunosensor array for high-throughput quantitative POCT of GM organisms based on the obtained results.

Data statement

All data included in this study are available upon request by contact with the corresponding author.

Declaration of competing interest

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:

CRediT authorship contribution statement

Hongfei Gao: Formal analysis, Writing - original draft. **Luke Wen:** Data curation. **Jing Tian:** Investigation. **Yuhua Wu:** Validation. **Fang Liu:** Resources. **Yongjun Lin:** Writing - review & editing. **Wei Hua:** Writing - review & editing. **Gang Wu:** Writing - review & editing.

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Appendix A. Supplementary data

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