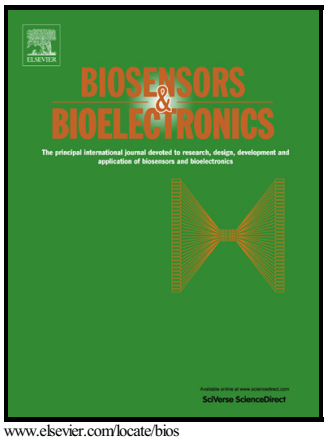

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A novel electrochemical DNA biosensor based on HRP-mimicking hemin/G-quadruplex wrapped GOx nanocomposites as tag for detection of *Escherichia coli* O157:H7

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Abstract: A novel sensitive electrochemical DNA biosensor was developed for amperometric detection of *Escherichia coli* O157:H7 (*E. coli* O157:H7). The graphene oxide (GOx) was utilized as nanocarrier to immobilize thionine (Thi) and the Au nanoparticles coated SiO_2 nanocomposites (Au-SiO_2) by electrostatic adsorption and the adsorption among nanomaterials. Then a large amounts of signal DNA (S2) and G-quadruplex were immobilized on the GOx-Thi-Au@SiO_2 nanocomposites. Finally, hemin was intercalated into the G-quadruplex to obtain the hemin/G-quadruplex structure as HRP-mimicking DNAzyme. On the basis of the signal amplification strategy of GOx-Thi-Au@SiO_2 nanocomposites and DNAzyme, the developed DNA biosensor could respond to 0.01 nM (S/N=3) with a linear calibration range from 0.02 to 50.0 nM *E. coli* O157:H7, which could be well accepted for early clinical detection. The studied system provides new opportunities, and might speed up disease diagnosis, treatment and prevention with pathogen.

Keywords: graphene oxide; G-quadruplex; DNAzyme; DNA biosensor; *Escherichia coli* O157:H7

1. Introduction

Among all pathogens, *Escherichia coli* O157:H7 (*E. coli* O157:H7) is one of the most dangerous pathogens. *E. coli* O157:H7 can cause serious food-borne illness (Tryland and Fiksdal, 1998) and result enormous economic loss in terms of medical cost (Varshney et al., 2007). Therefore, the development of new determination

methods for rapid and sensitive detection of *E. coli* O157:H7 is essential in clinical diagnostics, food safety inspection and environmental monitoring. With the advances of molecular biology, the polymerase chain reaction (PCR) and its derivative detection technique have been employed for the detection of *E. coli* O157:H7 (Holland et al., 2000; Wang et al., 2002; Bhagwat, 2003). However, there are several drawbacks, such as complexity, time consuming procedures, and narrow target. Recently, electrochemical DNA biosensor has attracted much interest for detection of bacteria, including *E. coli* O157:H7 pathogen, because of their convenience, high sensitivity and fast analysis time (Tak et al., 2014; Wang et al., 2009). With these methods, the sensitivity and analysis time was highly improved, but improvements are still needed.

To improve the sensitivity of electrochemical DNA biosensor, numerous signal amplification strategies based on various bionanocomposite probes have been designed by loading a large amount of electrochemical mediators and natural enzymes onto nanomaterials (Tang et al., 2011; Zhuo et al., 2009). Graphene oxide (GOx), with oxygen-containing functional groups, has recently attracted much attention in the area of electroanalysis for its high surface area, excellent conductivity and facile synthesis (Loh et al., 2010; Stoller et al., 2008). Owing to these advantageous structure and physicochemical properties, GOx-based nanocomposites have been mass reported to fabricate electrochemical sensors (Bai et al., 2012; Yuan et al., 2013; Han et al., 2013). Herein, the nanocomposite composed of graphene oxide (GOx)-thionine (Thi)-Au nanoparticles coated SiO_2 (Au@SiO_2) was prepared firstly and the resulted

GOx-Thi-Au@SiO_2 nanocomposites combined some advantages as followed: (1) GOx possesses fast electron transfer kinetics and large specific surface area; (2) Thi has good electrochemical redox active properties; (3) Au@SiO_2 can provide a microenvironment to retain the DNA tag conformation and make them free in orientation. In addition, the Au@SiO_2 could effectively promote electronic transfer which have been reported in our previous work (Li et al., 2013). The DNA biosensors based on the GOx-Thi-Au@SiO_2 nanocomposites may significantly enhance the sensitivity and greatly simplify the assay system.

To further improve the sensitivity of electrochemical DNA biosensor, enzymes are used as signal amplification strategies. However, the natural enzyme assembled on the nano-carriers has become a bottle-neck because of limited stability. Similar to natural enzymes, DNAzymes show high catalytic hydrolytic cleavage activities toward specific substrates, while they are more stable than natural enzymes, and can be denatured and renatured many times without losing their catalytic activities (Travascio et al., 1998; Witting et al., 2001). Thus DNAzyme based on binding a G-quadruplex DNA with a hemin molecule has been attempted to be used as a substitute in electrochemical catalysis (Golub et al., 2013; Yuan et al., 2013). As a promising HRP-mimicking enzyme, hemin/G-quadruplex can form a biocatalyst in the presence of H_2O_2 (Xiao et al., 2004; Zong et al., 2013; Zhou et al., 2014; Wang et al., 2014).

In this work, the mass of hemin/G-quadruplexes and S2 were united on the GOx-Thi-Au@SiO_2 formed nanocomposites to achieve the signal amplification and

the sensitive detection was realized by monitoring differential pulse voltammetry (DPV) signal. This employed method also provides potential applications for the ultrasensitive detection of other DNA sequences.

2. Experimental

2.1. Reagents and materials

Because of relevant and excellent genomic marker to *E. coli* O157:H7, *E. coli* attaching and effacing (eaeA) gene is selected as a target gene (Yu and Kaper, 1992; Kaper et al., 2004) in this study (S) (5'-GTC ACA GTT GCA GGC CTG GTT ACA ACA TTA TG-3'). The capture probe DNA (S1) (5'-TGC AAC TGT GAC AAA AAA AAA A-SH-3') was designed to hybridize with the target DNA near by the 5' end of sequence. The signal probe DNA (S2) (5'-SH-CAT AAT GTT GTA ACC AGG CC-3') was designed to hybridize the target DNA near by the 3' end of sequence. These target DNA, capture probe DNA, signal probe DNA, one-base mismatched ssDNA (S3) (5'-GTC ACA GTC GCA GGC CTG GTT ACA ACA TTA TG-3'), two-base mismatched ssDNA (S4) (5'-GTC ACA GTC GCA GGC CGG GTT ACA ACA TTA TG-3') and G-quadruplex DNA (5'-SH-TTT TTT GGG TTG GGC GGG ATG GG-3') were synthesized by Shanghai Sangon Biotechnology Co. (China). Graphene oxide (GOx) was purchased from Nanjing xianfeng nano Co. (Nanjing, China). Hemin, thionine (Thi), 6-Mercapto-1-hexanol (MH, 97%), gold chloride ($HAuCl_4$) and sodium citrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other materials used were of analytical grade and purchased

from Chemical Reagent Company (Chongqing, China). Distilled water was used throughout this research. PBS was prepared with Na_2HPO_4, KH_2PO_4 and 0.1M KCl.

2.2. Apparatus

Cyclic voltammetric (CV) and DPV measurements were performed using a Zennium electrochemistry workstation (ZAHNER-Elektrik GmbH & Co. KG, Germany) with a three-electrode system containing a platinum wire as the counter electrode, a saturated calomel electrode (SCE) as the reference electrode and a bare or modified glassy carbon electrode (GCE, Φ-3 mm) as the working electrode. The characteristics of Au nanoparticles, SiO_2 nanoparticles, Au@SiO_2 nanocomposites and other nanomaterials were estimated by transmission electron microscopy (TEM) (H600, Hitachi Co., Japan) or atomic force microscopy (AFM) (SPA400, Seiko, Japan).

2.3. Preparation of GOx-Thi-Au@SiO_2 nanocomposites

The Au@SiO_2 nanocomposites were synthesized as previously described (Bikram et al., 2007; Li et al., 2013). Firstly, gold nanoparticles were produced by reported method by reducing $HAuCl_4$ with citric acid at 100 ºC for about 30 min (Yang et al., 2011). Secondly, 1 mL tetraethylorthosilicate (TEOS), 10 mL ethanol and 5 mL distilled water was mixed and stirred for 10 min. And then, 3 mL $NH_3 \cdot H_2O$ (25%) was added slowly into the mixture with keeping stirring at 30 ºC until a pale white solution formed. Immediately, 0.1 M HCl and 0.1 M NaOH solution were used to

adjust pH of the reacted solution to pH 7.0-7.5. Finally, 10 mL as-prepared gold colloidal solution was dropped into the as-prepared SiO_2 solution under ultrasonication and the reaction was kept for about 15 min. To clean the free gold nanoparticles, centrifugation-centrifugation process was used. Under 13,000 rpm centrifugation, the Au@SiO_2 nanocomposites were precipitated, while free gold nanoparticles remained in the solvent. The collected Au@SiO_2 nanocomposites were washed twice and then dispersed in PBS for later use.

In a typical procedure, the GOx (1 mg) were dispersed in 1 mL water by ultrasonication, and then mixed with 1 mL Thi (1 mM) solution at room temperature for at least 12 h. After discarding the supernatant, the GOx-Thi nanocomposites were dispersed again in 1 mL water. Subsequently, the GOx-Thi nanocomposites were added drop wisely into 20 mL of the as-prepared Au@SiO_2. The mixture was allowed to react at room temperature under stirring for 24 h, followed with centrifugation. The resulting GOx-Thi-Au@SiO_2 nanocomposites were washed with PBS, re-dispersed in 1 mL PBS (pH 7.4) and stored at 4 ºC before use.

2.4. Preparation of S_2/GOx-Thi-Au@SiO_2/DNAzyme tag

Briefly, 40 μL of 1μM thiolated G-quadruplex and 24 μL of 100 μM thiolated signal DNA (S2) were added into 1.0 mL of the GOx-Thi-Au@SiO_2 solution and incubated for 16 h with stirring at room temperature. Subsequently, the unassembled DNA was removed by centrifugation (10,000 rpm, 30 min, 4 ºC). And then, excess amount of hemin was added to the suspension and incubated for 1.5 h in dark at 4 ºC

to form hemin/G-quadruplex DNAzyme, followed with centrifugation (10,000 rpm, 30 min, 4 ºC) to remove the no-specific banded hemin. The obtained S2/GOx-Thi-Au@SiO_2/DNAzyme tag was re-dispersed in 1.0 mL of 0.10 M pH 7.4 PBS containing 0.1 M KCl and kept in dark at 4 ºC before use. The procedure for the preparation of S2/GOx-Thi-Au@SiO_2/DNAzyme tag was showed in Scheme 1.

2.5. Fabrication of proposed DNA biosensor

The procedure for the proposed DNA biosensor was showed in Scheme 1. Before the fabrication, all of the bare GCE were polished using 0.3 and 0.05 μm aluminum slurry and ultrasonicated in ethanol and distilled water respectively. Then the electrodes was taken out, cleaned with water and air-dried. Firstly, the cleaned GCE electrode was coated with 10 μL GOx and air-dried at room temperature. To attach primary S1, a nano-Au layer was formed by electrodeposition in $HAuCl_4$ (1%) solution at the potential of -0.2 V for 30 s. Secondly, these modified electrodes were immersed into S1 (40 μg/mL) for 8 h at 4 ºC, and then washed with PBS buffer (pH7.4). To eliminate nonspecific binding effects of electrode surface, the S1/nano-Au/GOx/GCE electrode was dropped on 20 mL of 1.0 mM MH solution incubated for 40 min. All of the prepared electrodes were washed with PBS and stored at 4 ºC when not in use.

Scheme 1.

The detection was based on the typical procedure of sandwich reaction. 1) The

as-prepared DNA biosensor, MH/S1/nano-Au/GOx/GCE, was soaked in 1.5 mL of detecting *E. coli* O157:H7 samples with various concentrations for 40 min at 37 ºC. 2) The obtained *E. coli* O157:H7/MH/S1/nano-Au/GOx/GCE was immersed into the prepared S2/GOx-Thi-Au@SiO_2/DNAzyme solution for 1 h at 37 ºC, followed by washing with PBS buffer to remove the nonspecific adsorption of conjugate. 3) Ultimately, the electrochemical detection was carried out in an electrolytic cell containing 1 mL 0.10 M PBS (pH 7.4) and 0.6 mM H_2O_2. The DPV scans measurement was taken with the potential range from -0.5 to 0.1 V vs. SCE.

3. Results and discussion

3.1. The electrochemical characteristics of the stepwise modified electrodes

CVs were performed step by step in 0.10 M PBS containing 5 mM $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ (pH 7.4) with the potential range from -0.3 to 0.7 V vs. SCE at a scan rate of 50 mV/s (Fig. 1A). As showed, bare GCE exhibited a pair of well-defined redox peaks of $[Fe(CN)_6]^{3-/4-}$ (curve a). The peak current increased (curve b) after GOx was coated, which implies that the GOx was with good conductivity. The peak current further increased after electrodeposition with a nano-Au layer, indicating the conductive nano-Au could accelerate the electron transfer (curve c). When S1 was immobilized on electrode surface through Au-S, the redox current decreased obviously (curve d). After the employment of MH to block nonspecific sites, a further decrease of peak current was acquired (curve e).

Fig. 1.

At the same time, AFM as a useful method to characterize the fabrication of the electrode and application of the biosensor was employed in this study also. As showed in Fig. 2 a with the AFM 3D image, the surface of the GOx was wrinkle irregularly with root mean square (RMS) roughness of 22.59 nm. After electrodeposition with a nano-Au layer (Fig. 2 b), there were some small particle-like morphology on the surface with an increased RMS roughness of 126.3 nm as the modification of nano-Au onto the GOx modified electrode. As showed in Fig. 2 c for the capture probe DNA S1 modified electrode, the RMS roughness decreased to 46.31 nm indicating the surface became relatively smooth due to the adsorption of DNA onto the nano-Au modified surface. Furthermore, the RMS roughness increased significantly to 235.2 nm in the image of proposed biosensor reacted with *E. coli* O157:H7 and signal probe linked GOx-Thi-Au@SiO_2 nanocomposites (Fig. 2 d) indicating the presence of Au-SiO_2 related nanocomposite successfully reacted onto the surface of the proposed biosensor. All of these results obtained seem to further supported the successful fabrication and potential application for the proposed biosensor.

Fig. 2.

To demonstrate the dramatically amplified electrochemical signal induced by "sandwich" reactions, a sensitive electrochemical technique, DPV, was utilized to obtain the direct electrochemical signal. After incubation with 10 nM *E. coli* O157:H7,

the proposed DNA biosensor was subjected to investigation in electrolytic cell containing 1 mL 0.10 M PBS (pH 7.4) and 0.6 mM H_2O_2. And no obviously peak current was acquired (Fig. 1B, curve a). However, when the secondary aptamer S2/GOx-Thi-Au@SiO_2/DNAzyme immobilized on electrode surface by "sandwich" (Fig. 1B, curve b), a dramatically electrochemical signal could be obtained effectively, owing to the enhancement of DNAzyme bioelectrocatalytic, which indicated the feasibility of "sandwich" strategy.

3.2. The signal amplification strategies of different conjugates

In this work, the signal amplification was derived from the GOx-Thi-Au@SiO_2 nanocomposites and the hemin/G-quadruplex simultaneously acting as HRP-mimicking DNAzyme. To evaluate the signal amplifying action of proposed DNAzyme conjugated GOx-Thi-Au@SiO_2, we compared three kinds of sandwiched DNA biosensor (incubated with 10 nM *E. coli* O157:H7) with various labeled probes by investigating DPV responses. Fig. 3a-c displayed the DPV response of hemin/G-quadruplex conjugated Thi, GOx-Thi and GOx-Thi-Au@SiO_2 probes, respectively. The electrochemical detection was carried out in an electrolytic cell containing 1 mL 0.10 M PBS (pH 7.4) and 0.6 mM H_2O_2 as enzyme substrate. As a result, the DNA biosensor with hemin/G-quadruplex conjugated GOx-Thi-Au@SiO_2 probes exhibited the maximum response current indicating the GOx and Au@SiO_2 could effectively improve the supporting amount of electron mediator Thi and hemin/G-quadruplex bioelectrocatalytic complex. On the basis of these results, we might make the conclusion that the GOx-Thi-Au@SiO_2 nanocomposites and

HRP-mimicking DNAzyme (hemin/G-quadruplex) system could be utilized for amplifying the electrochemical signal.

Fig. 3.

3.3. Analytical characteristics of the proposed DNA biosensor

In view of outstanding ability of the developed electrochemical DNA biosensor to sensitively detect target DNA, the proposed DNA biosensor was employed to detect different concentrations of *E. coli* O157:H7 standard solution. As shown in Fig. 4A, the DPV signal gradually increased with increasing *E. coli* O157:H7 concentrations and showed a good linear relationship with the logarithm of *E. coli* O157:H7 concentration in the range of 0.02-50.0 nM (Fig. 4B), indicating that the *E. coli* O157:H7 concentration could be quantitatively measured by the DPV signal with regression equation is ΔI (μA) = -11.247 log $C_{O157:H7}$ - 18.96 and detection limit of 0.01 nM estimated using 3s (where s is the relative standard deviation of a blank solution). Moreover, a comparison between the present work and previously reported studies was performed. As showed in Table 1, the performances of the proposed biosensor indicated that this method was sensitive for the detection of E. coli O157:H7 with a wide linear range and low detection limits effectively demonstrated the significant amplification of GOx-Thi-Au@SiO_2 nanocomposites and hemin/G-quadruplex biocatalytic system as expected.

Fig. 4.

Table 1

3.4. Selectivity, Repeatability and stability of the DNA biosensor

Selectivity is key factor to evaluate the performance of a DNA biosensor. In the proposed strategy, the selectivity was determined using the MH/S1/nano-Au/GOx/GCE to hybridize with different sorts of DNA sequences (S, S3 and S4). As shown in Fig. 5, high current response was obtained only when MH/S1/nano-Au/GOx/GCE hybridize with specific target sequence S (10 nM), demonstrating a sensitive detection of S. For one and two-base mismatched ssDNA (100 nM, respectively), current response was smaller, respectively, indicating that the presence of non-specific adsorption of daunomycin will not influence the response of the electrode. These results suggest that the proposed DNA biosensor exhibited good selectivity for *E. coli* O157:H7 assay.

Fig. 5.

The reproducibility of the proposed DNA biosensor was investigated by intra-assay and inter-assay coefficients of variation. The intra-assay and inter-assay precision of the analytical method was evaluated by determining ten proposed DNA biosensor, respectively. The intra-assay coefficient of variation was 7.2% (n=20), and the inter-assay coefficient of variation was 5.8% (n=15) at 10 nM *E. coli* O157:H7 solution. These results indicated that the developed strategy was robust and could be used for DNA analysis with acceptable reproducibility.

The stability of the proposed electrochemical DNA biosensor was investigated by long-term storage assay. When the DNA biosensor was stored at 4 ºC, it retained 93.6%

of its initial response after a storage period of 30 days, suggesting the satisfactory stability of the proposed biosensor.

3.5. Synthetic stool samples analysis

Recovery experiments were performed to monitor the feasibility of the prepared *E. coil* O157:H7 biosensor by standard addition methods. The cultured *E. coli* O157:H7 with different concentrations were added into stool and determined based on the studied methods. As showed in Table 2, some results of the synthetic stool samples test obtained by the proposed biosensor are acceptable with the recovery between 95.2% and 104.8%, which indicated that the proposed biosensor was feasible for the determination of *E. coil* O157:H7 and could satisfy the need for practical analyses.

Table 2

4. Conclusions

In this study, we have developed a sensitive DNA biosensor based on GOx-Thi-Au@SiO_2 nanocomposites immobilized S2 and hemin/G-quadruplex as amplified signal tag for the detection of *E. coli* O157:H7 eaeA gene. The highlights of this work can be summarized as follows: (1) GOx-Thi-Au@SiO_2 functionalized nanocomposites were used as a carrier for loading a large amounts of S2 and hemin/G-quadruplex complex; (2) the hemin/G-quadruplex simultaneously acting as a bioelectrocatalytic HRP-mimicking DNAzyme for amplifying system could greatly improve the sensitivity of the biosensors for *E. coli* O157:H7 eaeA gene. The

proposed biosensor possesses excellent performance for detection of *E. coli* O157:H7 eaeA gene with a wide linear range of 0.02-50.0 nM and low detection limit of 0.01 nM. This method could be expanded readily for detecting other pathogens and be useful for future applications in public and environmental safety.

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(1) GOx-Thi-Au@SiO_2 functionalized nanocomposites could be used as a carrier for loading large amounts of DNA and hemin/G-quadruplex complex.

(2) The hemin/G-quadruplex simultaneously acting as a bioelectrocatalytic HRP-mimicking DNAzyme could greatly improve the sensitivity.

(3) The proposed DNA biosensor possesses excellent performance for detection of *E. coli* O157:H7 eaeA gene with a wide linear range and low detection limit.

(4) The proposed method could be expanded readily for detecting other pathogens, and be useful for future applications in public and environmental safety.

Table 1 Performance compared with other references for the detection of *E. coli* O157:H7

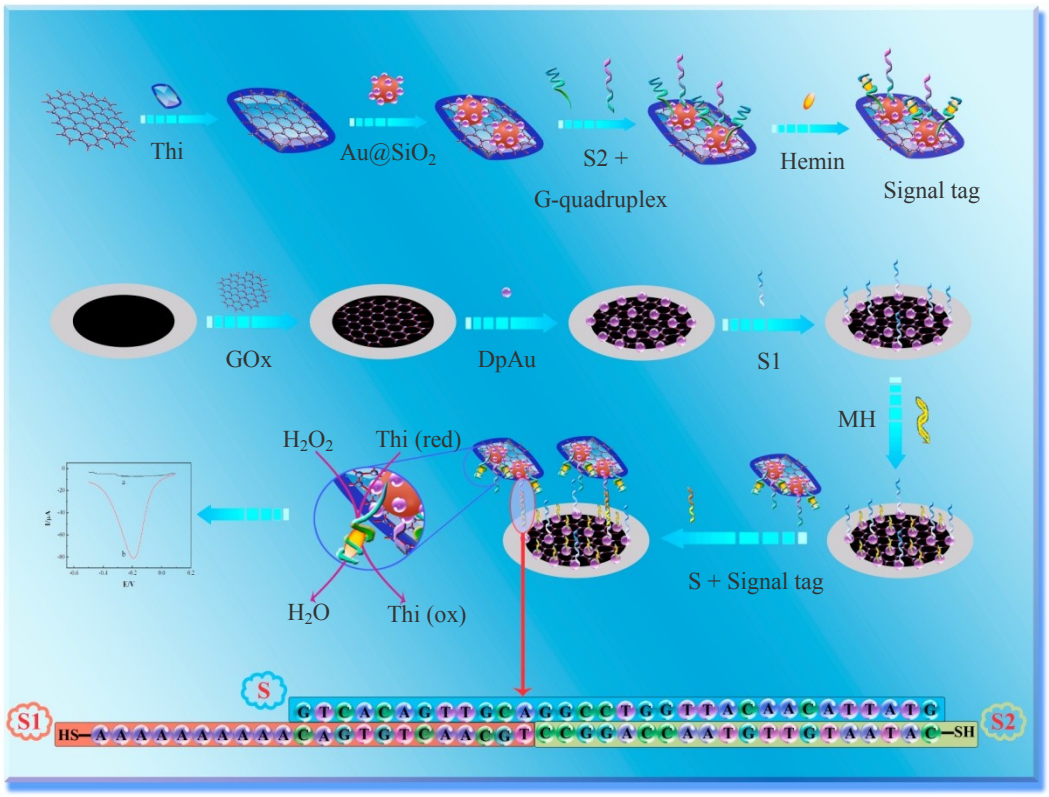
Method	Detection limits	Linear range for target	Reference
ECA[1)]	0.5 nM	n/a	Wang et. al, 2009
ECA	32 CFU/mL	3.2×10^{1} - 3.2×10^{6} CFU/mL	Li et.al, 2013 (our previous work)
ECA	412 CFU/mL	4.12×10^{2} - 4.12×10^{5} CFU/mL	Li et. al, 2.12 (our previous work)
FQDA[2)]	25 CFU/mL	4×10 - 4×10^{5} CFU/mL	Kim et. al, 2010
CLA[3)]	7.6×10^{3} CFU/mL	0 - 5×10^{5} CFU/mL	Gehring et. al, 2004
LAPS[4)]	2.5×10^{4} cells/mL	n/a	Gehring et. al, 1998
PCR[5)]	1.3×10^{4} CFU/mL	n/a	Deng et. al, 1996
ECA	0.01 nM	2×10^{-2} - 5×10 nM	Present work

Abbreviation: 1) ECA, electrochemical assay; 2) FQDA, Fluorescent QD assay; 3)CLA, Chemiluminescent assay; 4) LAPS, Light-addressable potentiometric sensor; 5) PCR, Polymerase chain reaction.

Table 2 Some determination of *E. coli* O157:H7 in synthetic stool samples.

Synthetic stool samples	Add nM	Response μA	Found nM	Recovery %
1	0.05	-4.13	0.048	96.00
2	0.1	-7.86	0.103	103.00
3	0.5	-15.80	0.524	104.80
4	1	-18.72	0.952	95.20
5	5	-26.99	5.176	103.52
6	10	-30.41	10.424	104.24
7	20	-33.53	19.745	98.73
8	30	-35.73	30.979	103.26
9	40	-36.92	39.525	98.81
10	50	-38.13	50.635	101.27

Scheme 1.



Scheme 1. Schematic diagram of preparation of GOx-Thi-Au@SiO_2 nanocomposites and fabrication and detection of the DNA biosensor

Fig. 1

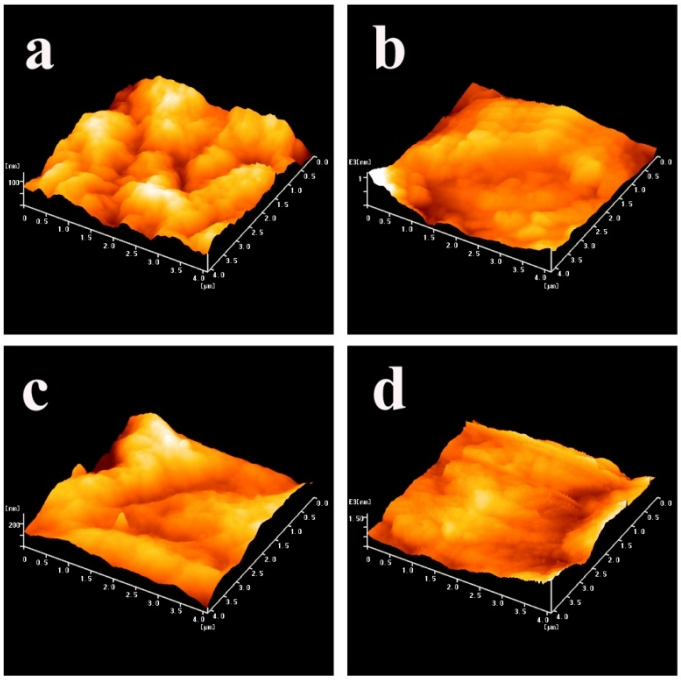


Fig. 1 AFM 3D images of GOx (a), nano-Au/GOx(b) and S1/nano-Au/GOx(c) modified electrode and the proposed biosensor reacted with *E. coli* O157:H7 and signal probe linked GOx-Thi-Au@SiO_2 nanocomposites (d).

Fig. 2

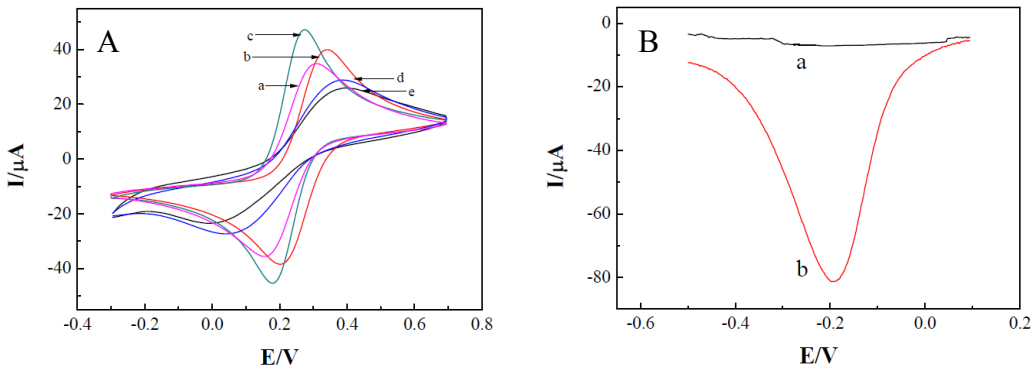


Fig. 2. (A) CVs obtained at (a) bare GCE, (b) GOx/GCE, (c) nano-Au/GOx/GCE, (d) S1/nano-Au/GOx/GCE and (e) MH/S1/nano-Au/GOx/GCE in 0.10 M PBS (pH 7.4) containing 5mM $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$, the scan rate was 50 mV/s. (B) DPVs obtained at (a) S/MT/S1/nano-Au/GOx/GCE and (b) S2/GOx-Thi-Au@SiO_2/DNAzyme/S/MH/S1/nano-Au/GOx/GCE in 0.10 M PBS (pH 7.4) containing 0.6 mM H_2O_2.

Fig. 3.

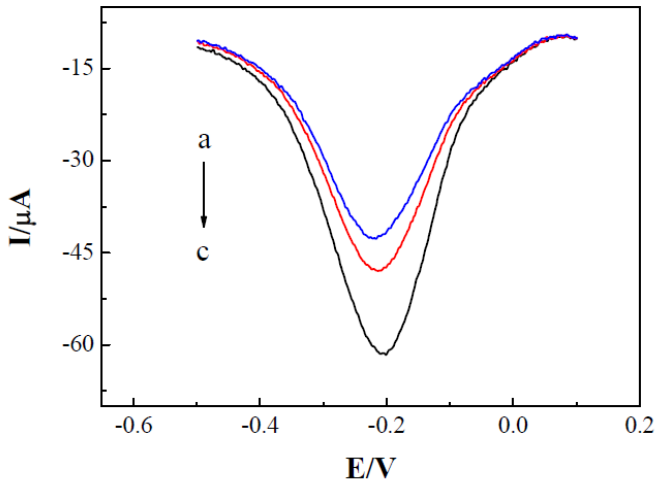


Fig. 3. DPV responses of DNA biosensor (incubated with 10 nM E.coli O157:H7) sandwiched with various labeled probes obtained in 0.10 M PBS (pH 7.4) containing 0.6 mM H_2O_2: hemin/G-quadruplex conjugated Thi (a), Thi-GOx (b) and Thi-GOx-Au@SiO_2 (c).

Fig. 4.

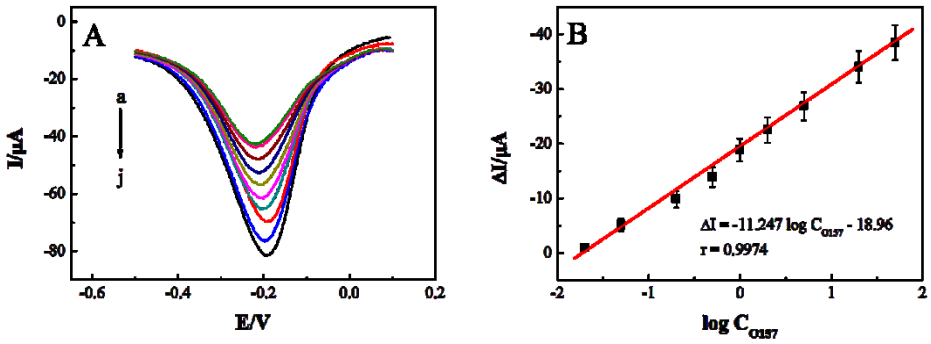


Fig. 4. (A) DPV responses and (B) the corresponding calibration curve acquired at S2/GOx-Thi-Au@SiO_2/DNAzyme/S/MH/S1/nano-Au/GCE with incubation with 0.02, 0.05, 0.2, 0.5, 1, 2, 5, 20 and 50 nM E. coli O157:H7 and detection in 0.10 M PBS (pH 7.4) and 0.6 mM H_2O_2.

Fig. 5.

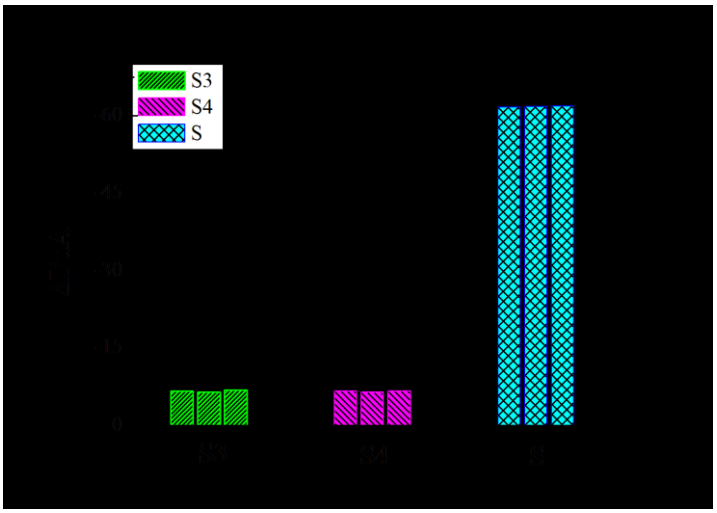


Fig. 5. Study of the possible interferences tested with the proposed DNA biosensor (S3, 100 nM; S4, 100 nM; S, 10 nM).