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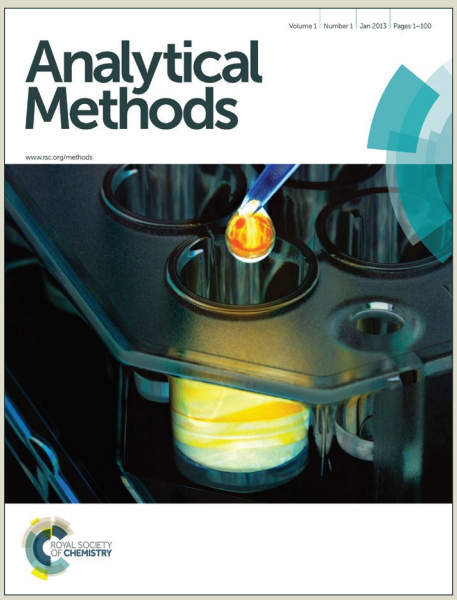

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Multi-walled Carbon Nanotubes-Chitosan with branched structure modified with ferrocenecarboxylic acid for carcinoembryonic antigen detection

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Abstract:

In this article, a novel electrochemical immunosensor was proposed based on ferrocenecarboxylic acids (Fc-COOH) attached to the branched structure of electrodes surface for the detection of carcinoembryonic antigen (CEA). The electrode firstly was modified with multi-walled carbon nanotubes-chitosan (MWCNT-CS) complexes, and the MWCNT-CS coating contains active secondary reaction functional entities that could form covalent bonds with molecules containing carboxyl or aldehyde groups. A large number of Fc-COOH were attached to the branched structure of the electrode surface, and then the electrode was further modified with polydopamine (PDA) and gold nanoparticles (AuNPs), which not only provided a favorable microenvironment and increased the loading capacity of the biomolecules to maintain the activity of the immobilized biomolecules, but also enhanced the conductivity and charge-transport properties of the modified electrode. Under optimal conditions, the immunosensor showed a low limit of detection (0.002 $ng \cdot mL^{-1}$) and a large linear range (0.01~80 $ng \cdot mL^{-1}$). With the merits of acceptable stability, high sensitivity, wide linear range and low detection limit, the new immunosensors showed great potential for the field of analytical applications.

Keywords: Multi-walled carbon nanotubes-chitosan, Branched structure, Polydopamine, Gold nanoparticles, Ferrocenecarboxylic acid, Immunoassay

1. Introduction

Carcinoembryonic antigen (CEA), an important tumor marker for colorectal and some other carcinomas, was a significant indicator to the efficacy of colorectal, breast and lung cancer [1]. The serum concentration of CEA in the range of 2.5-5.0 $ng \cdot mL^{-1}$ is considered a reference for healthy individuals. However, the level higher than 10 $ng \cdot mL^{-1}$ is a marker for colorectal carcinoma. Therefore, the sensitive detection of CEA plays a key role in clinical research and diagnosis. Many previous studies reveal a variety of analytical methods for the detection of CEA, such as Fluoroimmunoassay [2], enzyme immunoassay [3], ECL immunosensor [4] and electrochemical immunoassay [5-9]. Among them, electrochemical method has great potential for monitoring CEA duo to inherent advantages such as fast response, easy of miniaturization, low cost, high sensitivity and excellent selectivity [10-13].

In recent years, many researchers have been committed to come up with a better build strategies and methods to continuing to improve the performance of electrochemical sensor, such as selectivity, sensitivity and convenience. However, there are three key problems in fabricating such kinds of electrochemical immunosensor. The first problem is how to immobilize more mediators on the electrode surface to improve its sensitivity. The second issue is how to accelerate the electron transfer between the redox active center of mediator and the electrode surface. The third one is how to immobilize a large of antibodies on the electrode surface without denaturation. The focus of our study is around these three issues, and trying to improve the performance of immunosensor.

Chitosans (CS), a natural polysaccharide, has excellent properties such as good film-forming ability, biocompatibility, less toxic, good adhesion and a high content of

hydroxyl and amino groups [14, 15]. However, it was limited in the design of electrochemical sensor because of the non-conductive property. To improve its conductivity, many nanomaterials have been incorporated such as carbon materials (CNTs and rGO), gold nanoparticles (AuNPs). Recently, CNTs are considered a promising candidate for sensors duo to large length-to-diameter aspect ratios, high surface-to-volume ratios, intrinsic physico-chemical, excellent conductivity, thermal conductivity and mechanical properties [16-19]. CNTs-CS complexes not only have a good electron transport capability, but also have good biocompatibility and easily further modified duo to unite the interesting properties of CNTs and CS [20, 21]. Salimi *et al.* [22] report manganese oxide nanoflakes/multi-walled carbon nanocubes/chitosan nanocomposite modified electrode for chromium (III) detection, the result showed excellent catalytic activity for oxidation of Cr^{3+} at pH 3-7. Kavosi *et al.* [23] based on gold nanoparticles/PAMAM dendrimer loaded on MWCNTs/chitosan/ionic liquid nanocomposite to detected prostate-specific antigen, the immunosensor exhibited excellent stability and reproducibility and successfully used for PSA detection in serum sample. Xu *et al.* [24] report selective recognition of 5-hydroxytryptamine and dopamine on a multi-walled carbon nanotube-chitosans hybrid film-modified microelectrode array, the sensor was successfully used for selective molecular recognition and determination of DA and 5-HT. In this study, MWCNTs-CS coating not only have good biocompatibility and conductivity, but also contains active secondary reaction functional entities that could form covalent bonds with molecules containing carboxyl or aldehyde groups.

Ferrocene (Fc) and its derivatives are well known mediator due to excellent properties, such as reversibility, regeneration at low potential, and generation of stable redox states. It

was often used as a protein biomarker or redox active species for the detection of biomolecules in electrochemical systems [25-29]. However, a bottleneck of ferrocene is easily leak from electrode surface. The noncovalent monohybrid of Fc-COOH with various materials has been reported. Deng *et al.* [30] report noncovalent monohybrid of ferrocene with chemically reduced graphene oxide for detection hydrogen peroxide and choline, the sensor exhibited a marked decrease in the overvoltage and low-potential amperometric detection for electrochemical sensing when hydrogen peroxide was involved. Qu *et al.* [31] report electrochemical biosensing platform using hydrogel prepared from ferrocene modified amino acid as highly efficient immobilization matrix, the glucose biosensor exhibited good performance for the electrochemical detection of glucose. However, a drawback of such sensor is poor stability. In this study, Fc-COOH covalent branching connection on the electrode surface not only can improve the stability of the sensor, but also can immobilization a lot of Fc-COOH to improve sensitivity.

Dopamine (DA), a catecholamine neurotransmitter, has excellent biocompatibility and self-polymerizing ability. Polydopamine (PDA) can modify almost all material surfaces, such as noble metals, metal oxides, semiconductors and ceramics in weak alkaline aqueous solution [32, 33]. As well as, the PDA coating served as an extremely versatile platform for the immobilization of biological molecules. Moreover, the PDA coating possess excellent reduction ability, various metals can be readily in site grown on the PDA film surface [32]. In this study, a large of monodisperse AuNPs were in situ synthesized on the surfaces of PDA coating without any other reducing agent at room temperature using chloroauric acid as a precursor. The AuNPs of PDA coating surfaces not only can accelerate electron transfer, but

also enhanced the immobilized amount of biomolecules and maintaining biological activity [34] duo to large specific surface area, good conductivity and biocompatibility [35, 36].

In this works, a novel electrochemical immunosensor was proposed based on ferrocenecarboxylic acids attached to the branched structure of MWCNTs-CS modified electrode for the detection of CEA. The GCE/CNTs-CS electrode surface was modified by the branched structure with abundant hydroxyls groups for Fc-COOH efficient attachment. In addition, the PDA and AuNPs were modified on the above electrode surface. PDA has good biocompatibility and reductive ability, and then a large number of mono-dispersed AuNPs evenly distributed on the PDA surface and subsequently developed a new electrochemical immunosensor for detecting CEA, and the result showed have excellent properties. Thus, the proposed immunosensor showed great potential in the field of analytical applications.

2. Material and methods

2.1 Material and reagents

Glutaraldehyde (GA)，Gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$), Multi-walled carbon nanotubes (MWCNTs), Chitosan (CS) and Ferrocenecarboxylic acid (Fc-COOH) were from Aladdin Reagent Company (Shanghai, China). Dopamine (DA), p-toluenesulfonic acid (p-TSA), methylbenzene were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium ferricyanide ($K_3[Fe(CN)_6$), Sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Bovine serum albumin (BSA) and Chlorauric acid were supplied by Alfa Aesar (Tianjin, China). Horse radish peroxidase (HRP), CEA standard grade antigens, alpha fetoprotein (AFP)

standard grade antigens and anti-CEA antibodies were purchased from Guangzhou Zhonghuang Chemical Co., Ltd. (Guangzhou, China) stored at 4 ℃ before use. [2-Hydroxy-1, 1-bis (hydroxymethyl) ethyl] ammonium dihydrogen phosphate (THAMP) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Phosphate buffer solutions (PBS) with various pH values were prepared with 0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 (ShengAo Reagent Co., Ltd., Tianjin, China) solutions containing 0.1 M KCl (Guangfu Reagent Co., Ltd., Tianjin, China) as the supporting electrolyte. All reagents were of analytical-reagent grade or the highest purity available and directly used for the following experiments without further purification.

2.2 Apparatus

Cyclic voltammetry and electrochemical impedance spectroscopy were carried out on a Potentiostat/Galvanostat Model 283 Electrochemical analyzer (American Mattson Company, America).Cyclic voltammetry was carried out with the potential range from -0.2 to 0.6 V and from 0 to 0.8 V at a scan rate of 50 $mV \cdot s^{-1}$, respectively. EIS were carried out with frequencies from 0.01 to 10^5 Hz. A conventional three-electrode system was used, which consisted of a platinum wire as auxiliary electrode, an Ag/AgCl saturated KCl as reference electrode, and a bare or modified GCE as working electrode. The pH measurements were carried out on a PHS-3B exact digital pH metre (Shanghai Hongyi Instrument Co., Ltd.), which was calibrated with standard pH buffer solutions. Scanning electron microscope (SEM) measurements were made on a Leo1430VP (LEO, Germany). KQ-250B ultrasonic cleaning instrument (Kunshan Ultrasonic Instrument Co., Ltd.), HH-S digital thermostat oil bath (Jiangsu Jintan Medical Instrument Factory), RW 20 digital IKA electric mixer (Shanghai

Ruoding Instrument Co., Ltd.), GZX-9140 MBE-type electric oven blast (Shanghai Jinghong Experimental Equipment Co., Ltd.).

2.3 Preparation of MWCNTs-CS

In order to obtain MWCNTs-CS, First, the MWCNTs were purified. Briefly, 10.0 mg of MWCNTs was sonicated in 20 mL of mixture acidic solution containing sulfuric acid and nitric acid (3: 1 in volume) for 12 h. The resulting dispersion was washed with ultrapure water and centrifuged until pH 7.0. Then, 1.0 mg of purified MWCNTs were dispersed into 10 mL of acetic acid solution (1 *wt*%) containing 0.5 wt% CS by ultrasonication to obtain dispersed suspension. MWCNTs-CS was prepared successfully.

2.4 Procedures of modified electrode

The bare GCE electrode was polished carefully using 0.5 μm and 0.05 nm alumina slurries to a mirrorlike surface, and sonication in a fresh solution (HNO_3:H_2O=1:1 (v/v)), ethanol and water for 5 minute respectively. Then, the electrode was allowed to be dry with nitrogen at room temperature.

First, 10 μL of MWCNTs-CS solution was dropped on the electrode surface, and the electrode was dried. Secondly, The GCE/CNTs-CS electrode was immersed into 4% GA solution at room temperature for 16 h, and then GCE/CNTs-CS/GA electrodes were put into 4.6 mg·mL^{-1} THAMP solution for 8 h by similar procedures from previously reported method [13]. Finally, the electrodes, Fe-COOH, p-TSA, and toluene were put in a three-necked flask with water separation recycling device. The flask was placed in an oil bath and gradually heated to 140℃ for 6 h. After the reaction the electrodes were washed with ethanol and water successively. The fabrication procedure of the proposed electrochemical immunosensor was

shown in Scheme1.

2.5 Fabrication of the immunosensor

First, the GCE/CNTs-CS/GT/Fc electrode was immersed into an aqueous solution of DA (2 $mg \cdot mL^{-1}$, 10 mM Tris, pH 8.5) for about 2 h. The electrode was rinsed with deionized water and dried. Then, the electrode was immersed into $HAuCl_4$ (1*wt*%) for 6 h without any reducing agent. Next, the modified electrode was incubated at 4 ℃ for 12 h in anti-CEA solution to form the GCE/CNTs-CS/GT/Fc/PDA/AuNPs/anti-CEA. Then, the electrode was incubated in 1 wt% BSA solution for 1 h. Finally, the electrode was incubated with a series of concentration of CEA solution for 30 min. After washing, the prepared electrode was stored at 4 ℃ before electrochemical experiments.

<Scheme 1>

2.6 Immunoassay procedure

Cyclic voltammetry (CV) of pre-modified electrodes were done at room temperature, and the potential scan from 0 to 0.8 V with scan rate of 50 mv/s in 20 mL PBS buffer solution (pH 7.0) containing 0.1 M KCl. The electrochemical signals related to the different CEA concentrations could be measured.

3. Results and discussion

3.1 SEM of MWCNTs-CS

The SEM was employed to observe the as-prepared composite. As shown in Fig. 1A, the purified MWCNTs were in the form of dispersion system with about 50 nm in diameter and have good dispersibility. In Fig. 1B, the MWCNTs were fatter than the unmodified MWCNTs, indicating it was tightly wrapped by CS, and the MWCNTs-CS nanocomposite exhibited a

porous surface and many wire-like MWCNTs were distributed in the CS and form a rough surface so that increases a large surface area that connecting more ferrocenecarboxylic acid to improve the performance of electrochemical immunosensor.

<Figure 1>

3.2 Electrochemical impedance spectroscopy of the immunosensor

In order to monitor the preparation process of the electrochemical immunosensor, electrochemical impedance spectroscopy (EIS) of the different modified electrode was conducted. Electrochemical impedance spectroscopy (EIS) is an important tool for monitoring the impedance changes of modified electrodes. The impedance spectra consists of a semicircle at high frequencies corresponding to the electron transfer limiting process, and a line at low frequencies resulting from the diffusion limiting step of the electrochemical process. Since the diameter of the semicircle corresponds to the electron-transfer resistance (R_{et}), that can be estimated from the diameter of the semicircle. The equivalent circuit in inset of Fig. 3 was chosen to fit the impedance data obtained from the fabrication process and including four parameters, the ohmic resistance of the electrolyte solution (R_s), the Warburg impedance (Z_w), the double-layer capacitance (C_{dl}) and the electron-transfer resistance (R_{et}). R_s and Z_w represent bulk properties of the electrolyte solution and diffusion features of the redox probe in solution. C_{dl} and R_{et} reveal interfacial properties of the electrode, which is highly sensitive to the surface modification. Fig. 2 shows the electrochemical impedance of different layer modified electrodes in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution. It was observed that the bare GCE exhibits a small semicircle at high frequencies and a linear part at low frequencies (curve a, R_{et}=185 Ω). After the MWCNTs-CS was dropped onto the GCE, the electrode showed a much

smaller semicircle than that the bare GCE electrode (curve b, R_{et}=75 Ω), indicating that MWCNTs-CS exhibits an excellent electronic conduction ability and accelerated the electron transfer. Then, after Fc-COOH attached to the branched structure of the modified electrode and deposition of PDA film, the diameter of semicircle was in turn increased (curve c, R_{et}=350 Ω and curve d, R_{et}=425 Ω), which was ascribed to the THAMP/GA (TG) layer on the electrode retarded the electron surface and PDA prevent the electron transfer. Subsequently, monodisperse AuNPs are anchored on the surface PDA membrane, the diameter of the semicircle was decrease (curve e, R_{et}=345 Ω) due to AuNPs possesses excellent conductivity, implying that AuNPs was successfully anchored on the electrode surface. Then, the immobilization of anti-CEA on the above electrode surface, the diameter was increase (curve f, R_{et}=620 Ω) when the electrode was incubate in anti-CEA solution. The result is ascribed to the nonconductive properties of anti-CEA which insulates the conductive support and blocks the electron transfer. Finally, the modified electrode was blocked nonspecific sites with BSA, the diameter of the semicircle was increasing (curve g, R_{et}=905 Ω), because protein is insulator that can hinder the electron transfer. These results again suggest that the modification of each step is very successfully.

<Figure 2>

3.3 Optimization of detection conditions

There are many factors that affect the electrochemical performance of the CEA immunosensor. Wherein, the incubation temperature and time for the immune-reaction, and the pH value of substrate solution were the greatest impact. Thus, the purpose of this work was to control the optimal experimental value of the three factors. The pH value of detection solution has an

obvious effect because the activity of the immobilized protein may be influenced by the acidity of the solution. In order to optimize the pH value, the immunosensors were tested in a series of PBS buffer with the pH value range from 5.0 to 8.5 by CV. Fig. 3 A shows the effect of detection solution pH on the relative change in peak current. With the increasing solution pH value from 5.0 to 8.5, the peak current rises before pH=7.0, when pH value is higher than 7.0, the peak current drops because the activity of protein decline. Thus, pH=7.0 is chosen as the optimum pH of this experience.

The incubation time and incubation temperature for the immune-reaction were other two important parameters in the construction of the immunosensor. The effect of incubation time is investigated for different times from 5 to 50 min. As shown in Fig. 3B, the current response of immunosensor increased sharply with the increasing incubation time and then stable after 30 min, indicating the immune-response of between CEA and anti-CEA was reach saturation. Therefore, 30 min was chosen as the optimal incubation time. As shown in Fig. 3C, the dependence of the current response on digestion temperature was also investigated over the range of 10-45 °C. The result showed that the peak current increased with the incremental temperature until it was up to 35 ℃ which was chosen for further investigation. Thus, pH=7.0, 30 min, and 35 °C were used as the optimal acidity, incubation time and temperature, respectively.

<Figure 3>

3.4 Analytical performance

Under the optimum conditions, the performance of the immunosensor for detection of CEA was studied by CVs measurements (Fig. 4). The peak current s of CV was decreased with

increasing concentration of CEA, which due to the increased hindrance of the antigen–antibody complex to electron transfer of the mediator of Fc. The currents changed linearly with the logarithm of CEA concentration in the range from 0.01 ng·mL^{-1} to 80 ng·mL^{-1} with a detection limit of 0.002 ng·mL^{-1} (S/N=3). The calibration plot for CEA detection is illustrated in the top right inset of Fig. 4. The linear regression equations was I (μA) =70.19+23.31LogC_{CEA} (ng·mL^{-1}) (R^2=0.994), and the standard deviation (SD) of the slope and the intercept was 0.8088 and 0.9642, respectively. The results show that the proposed electrochemical immunosensor has a higher sensitivity and a wider linear detection range. The comparison of analytical performances toward CEA is summarized in Table 1. In order to verify the effect of branched connection ferrocenecarboxylic acid, the GCE/CNT-CS-Fc modified electrode was used to fabricate the electrochemical immunosensor and the immunosensor exhibited lower sensitivity and narrower linear range (Fig. 5). The reason for this might be that the way of branched connection was used to increase the amount of Fc.

<Figure 4>

< Figure 5>

<Table 1>

3.5 Stability and reproducibility of the immunosensor

Stability and reproducibility are the two most important factors in application and development of immunesensor. The reproducibility of immunesensor was investigated by a series of five electrodes for detection of 10 ng·mL^{-1} CEA. The results showed an acceptable reproducibility and accuracy with a relative standard deviation (RSD) of 2.4%. The stability of the electrochemical immunosensor was investigated by measuring periodically current

response of 10 ng·mL^{-1} CEA. The modified electrode was stored in a refrigerator at 4 °C when not in use. Every 5 days, the current responses of the electrochemical immunosensor were examined. The current response of the as-prepared immunosensor decreased 2.6% after 5-day storage. 10-day and 30-day later, the electrochemical immunosensor still retained 96.6% and 91.8% of its initial current, which indicated that the immunosensor have a good stability.

3.6 Selectivity of the immunosensor

To further investigate the specificity of the proposed electrochemical immunosensor, some contrast experiments were performed. The immunosensor were incubated in 80 ng·mL^{-1} interfering substance such as AFP, BSA, HRP and DA and in 5 ng·mL^{-1} CEA solution containing 80 ng·mL^{-1} different interfering species, respectively. The result shown in Fig.6, no remarkable difference of currents was observed in comparison with the result obtained in the blank, and the current (incubated, 5 ng·mL^{-1} CEA solution containing 80 ng·mL^{-1} different interfering species) were no significant difference with the current obtained from the 5 ng·mL^{-1} CEA. And the current variation was less than 5.2% due to the interfering substances, indicating that the selectivity of the proposed method was acceptable.

<Figure 6>

3.7 Application in detection of serum tumor marker

In order to further investigate the application potential of the proposed immunosensor for the detection of CEA, the recovery test was carried out by adding different amounts of CEA into human serum sample, and the results were summarized in Table 2. The recovery of detection CEA under CV was in the range of 99.7-104.0%, and the R.S.D was in the range

from 2.38 to 4.23%. The facts showed that the proposed immunosensor could be effectively applied to the clinical detection of the CEA in human serum.

<Table 2>

4. Conclusions

In this work, a novel electrochemical immunosensor was proposed based on ferrocenecarboxylic acid branching connection modified electrodes with MWCNTs-CS complexes for the detection of carcinoembryonic antigen (CEA) in clinical immunoassay. The modified electrode surface has a large number of mediators by branched connection ferrocenecarboxylic acid and a favorable microenvironment that improve electrochemical performance. The main advantages of the electrochemical immunosensor contribute to several aspects: (1) MWCNTs-CS has a good electron transport capability and a large of amino to providing a large of sites for the next modify. (2) The electrode surface has abundance mediator by branched connection ferrocenecarboxylic acid, which can improve the performance of the electrochemical immunosensor. (3) Via in situ reduction, a large number of monodisperse AuNPs was anchored on PDA surface without any reducing agent. The PDA-AuNPs provided a favorable microenvironment to maintain the activity of the immobilized biomolecules due to the excellent biocompatibility of PDA-AuNPs, but also increased the loading capacity of the biomolecules due to the large surface area. Therefore, the proposed immunosensor have good electrochemical performance and potential applications for CEA detection with a remarkable detection limit (0.002 $ng \cdot mL^{-1}$) and range (0.01~ 80 $ng \cdot mL^{-1}$), as well as its other advantages, such as good reproducibility, acceptable stability, high sensitivity, and simplicity, make it a promising candidate for bioanalytical

applications.

Acknowledgments

This project was partly financially supported by the National Natural Science Foundation of China (No. 21065009), and the Key Project of Chinese Ministry of Education (No. 2010250).

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Figure legends

Scheme 1. (A) The fabrication procedure of branched structure modified electrodes and the preparation procedure of electrochemical immunosensor

Fig.1 TEM of MWCNTs and MWCNTs-CS

Fig. 2 EIS of electrochemical immunosensor with different surface: bare GCE (a), GCE/CNTs-CS (b), GCE/CNTs-CS/GT/Fc (c), GCE/CNTs-CS/GT/Fc/PDA (d), GCE/CNTs-CS/GT/Fc/PDA/AuNPs (e), GCE/CNTs-CS/GT/Fc/PDA/AuNPs/anti-CEA (f), GCE/CNTs-CS/GT/Fc/PDA/AuNPs/anti-CEA/BSA (g) in 0.5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl.

Fig. 3 The optimization of detection condition with pH of detection solution (A), incubation time (B), and incubation temperature (C) on the peak current of CVs. One parameter changed while the others were under their optimal conditions and 5 $ng \cdot mL^{-1}$ CEA was used as an example. Above detections in 0.2M PBS containing 0.1 M KCl. Scan rate: 50 mV/s.

Fig.4 Calibration plots of the current at GCE/CNTs-CS/GT/Fc/PDA/AuNPs/anti-CEA/BSA versus concentration of CEA under optimal conditions. Inset：CVs curves obtained for of the oxidation current at GCE/CNTs-CS/GT/Fc/PDA/AuNPs/anti-CEA/BSA for different concentrations in 0.2 M PBS containing 0.1 M KCl. Scan rate: 50 mV/s.

Fig.5 Electrochemical response of the different connection way, (a) GCE/CNTs-CS/GT/Fc/PDA/AuNPs (b) GCE/CNTs-CS/Fc/PDA/AuNPs toward various concentrations of CEA. (a) The SD of the slope and the intercept were 0.6335 and 0.7856, respectively. (b) The SD of the slope and the intercept were 0.8088 and 0.9642, respectively.

Fig.6 The current response of the electrochemical immunosensor to blank CEA (yellow bars),

only 80 ng·mL^{-1} interferences (green bars), 5 ng·mL^{-1} CEA (red bars) and 5 ng·mL^{-1} CEA with 80 ng·mL^{-1} interferences (cyan bars). Interference: (1) AFP, (2) BSA, (3) HRP, and (4) DA. Above detections in 0.2 M PBS (pH 7.0) containing 0.1 M KCl solution. Scan rate: 50 mV/s. Error bar=SD.

Table 1. Comparison of analytical properties of different immunosensors for the detection of CEA.

Table 2. Recovery results of the proposed immunosensor in serum samples by CV in 0.2 M PBS (pH 7.0) (*n*=5).

Scheme 1.

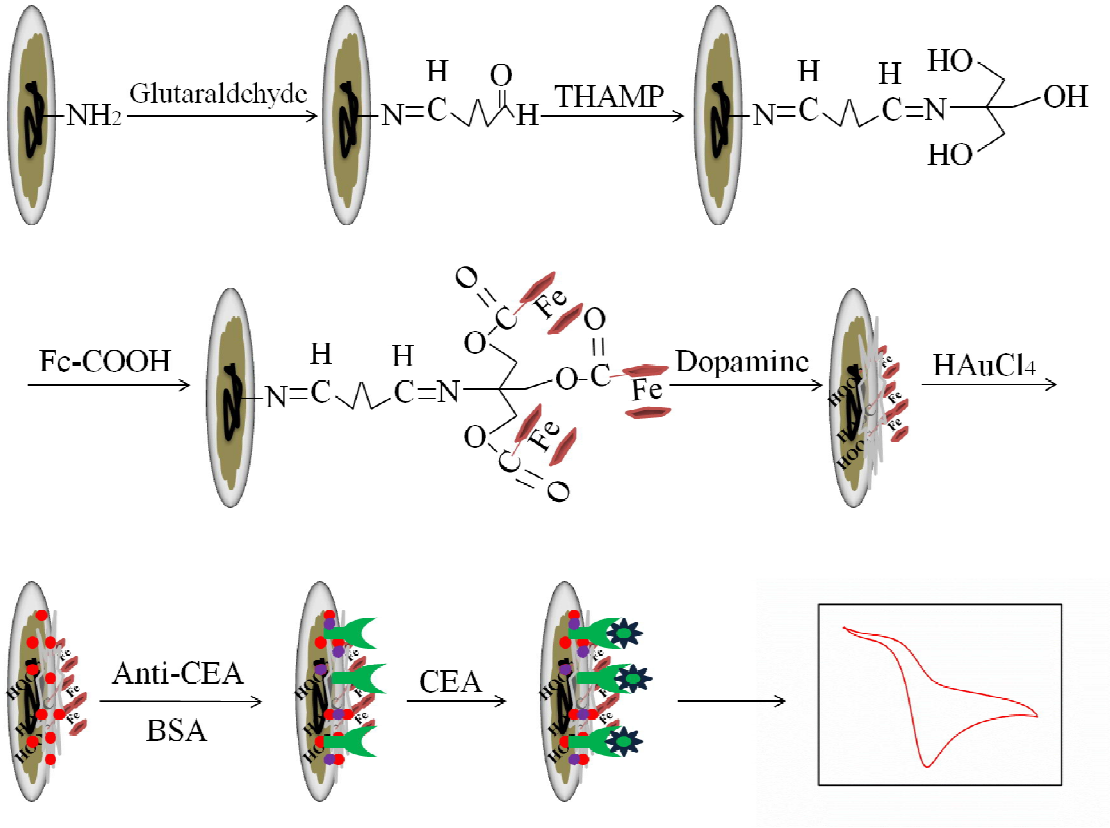

Fig.1

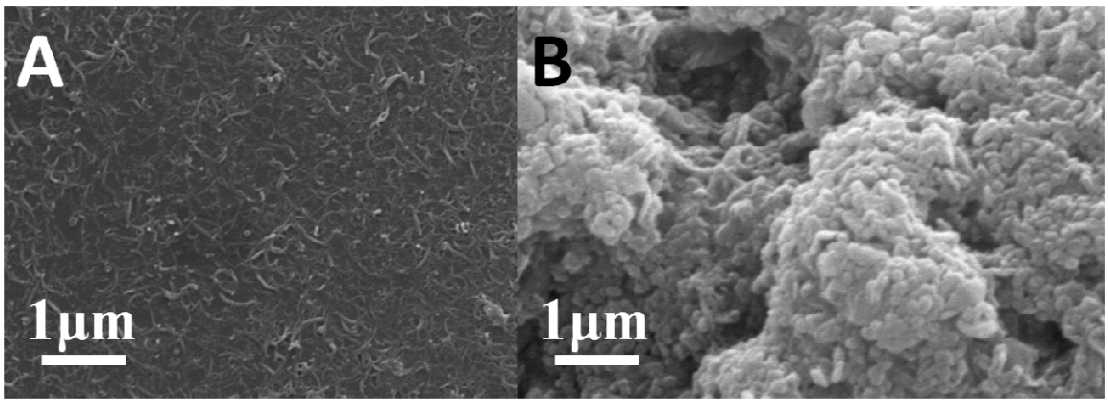

Fig.2

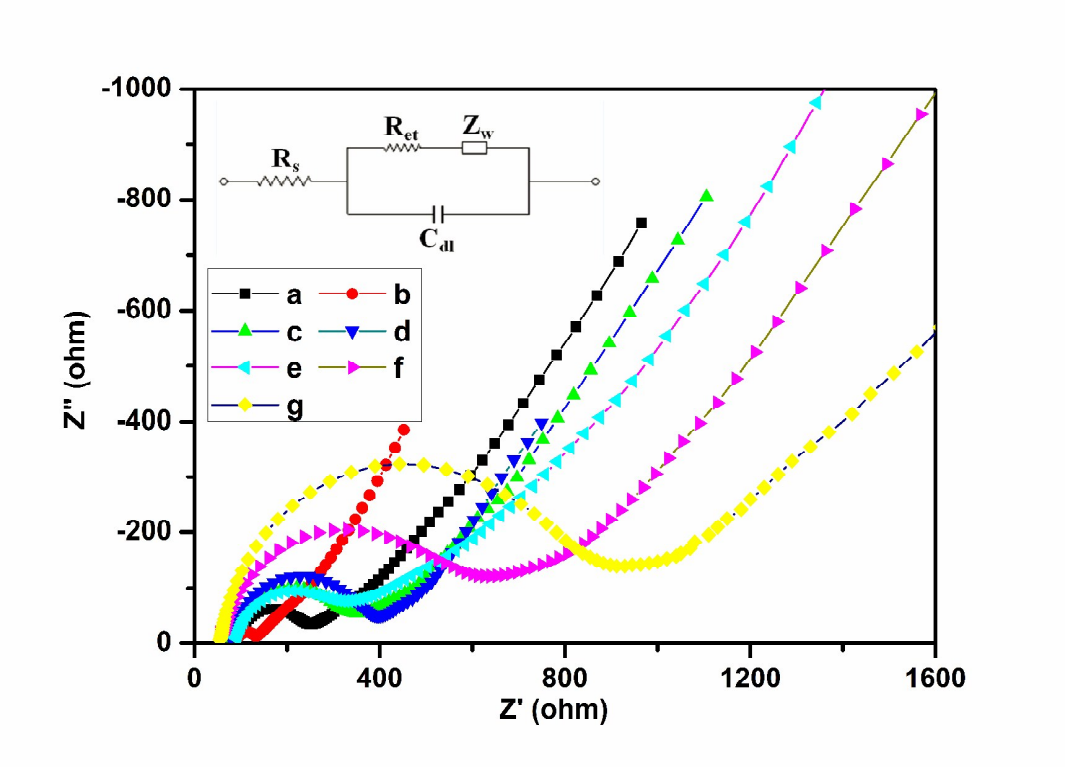

Fig.3

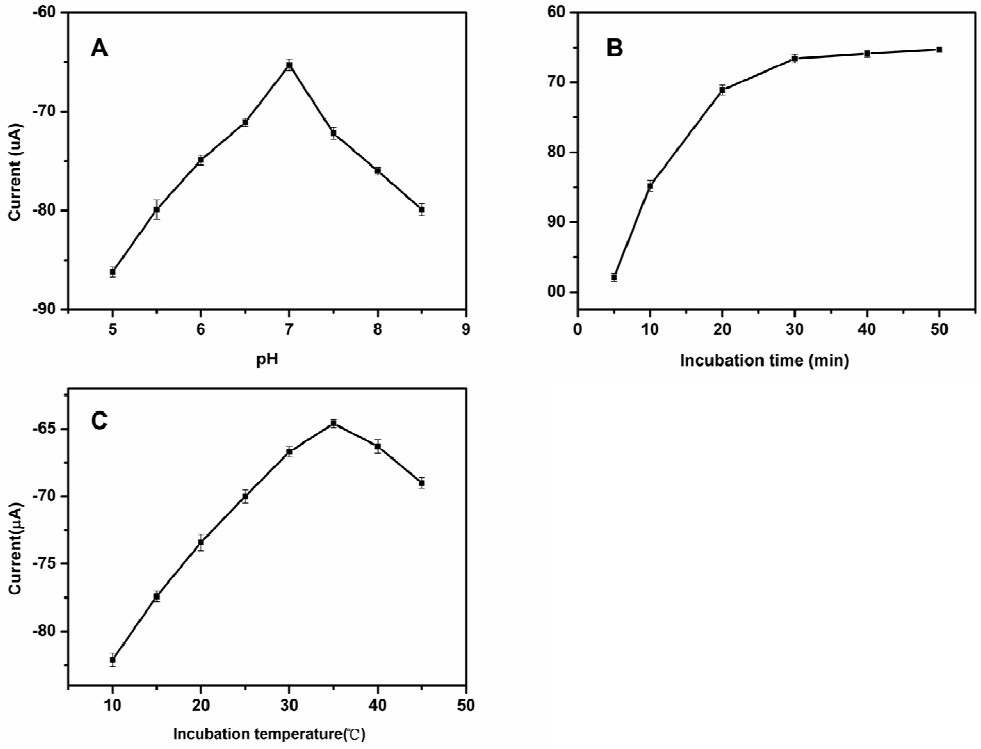

Fig.4

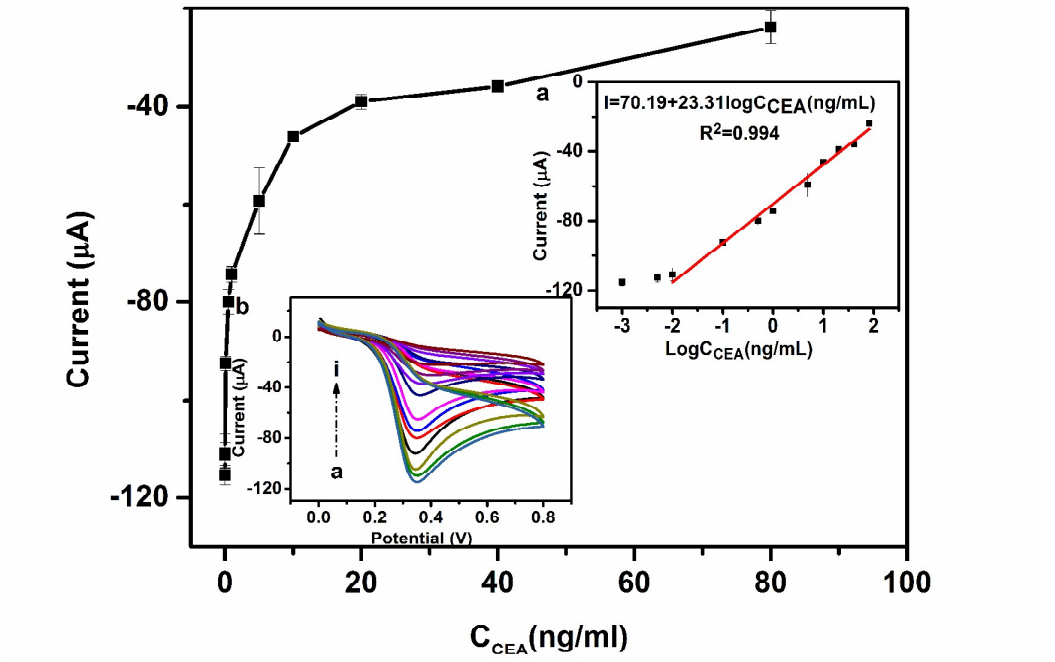

Fig.5

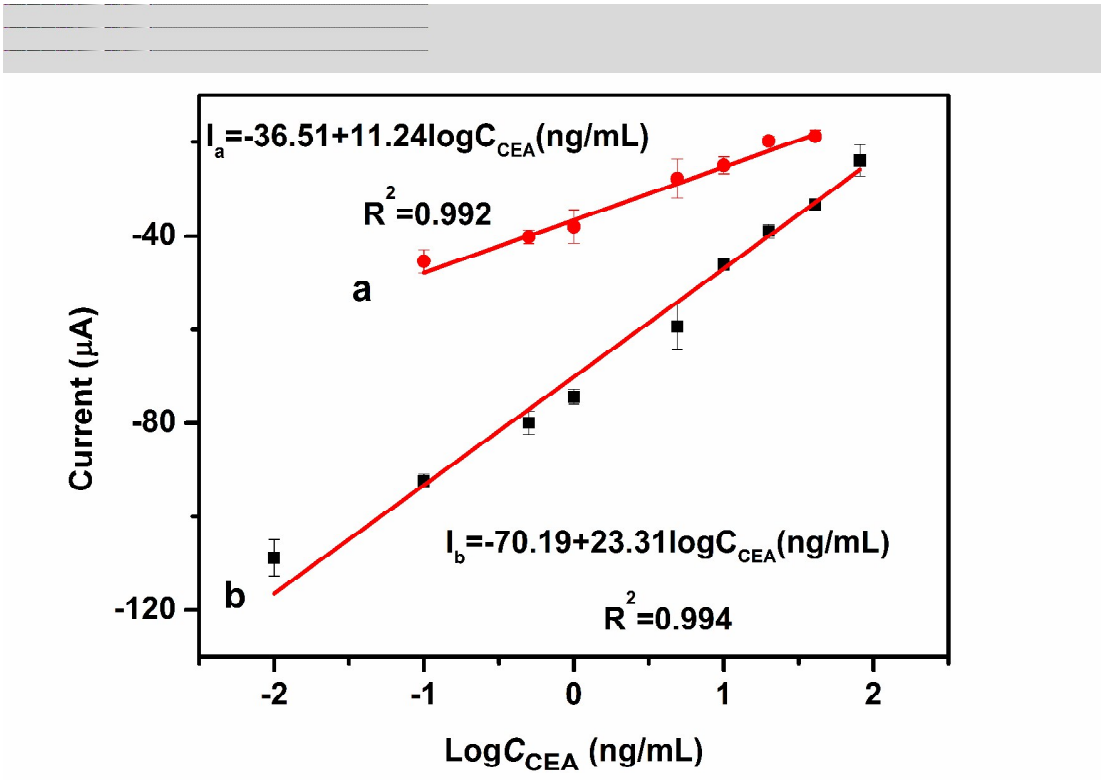

Fig.6

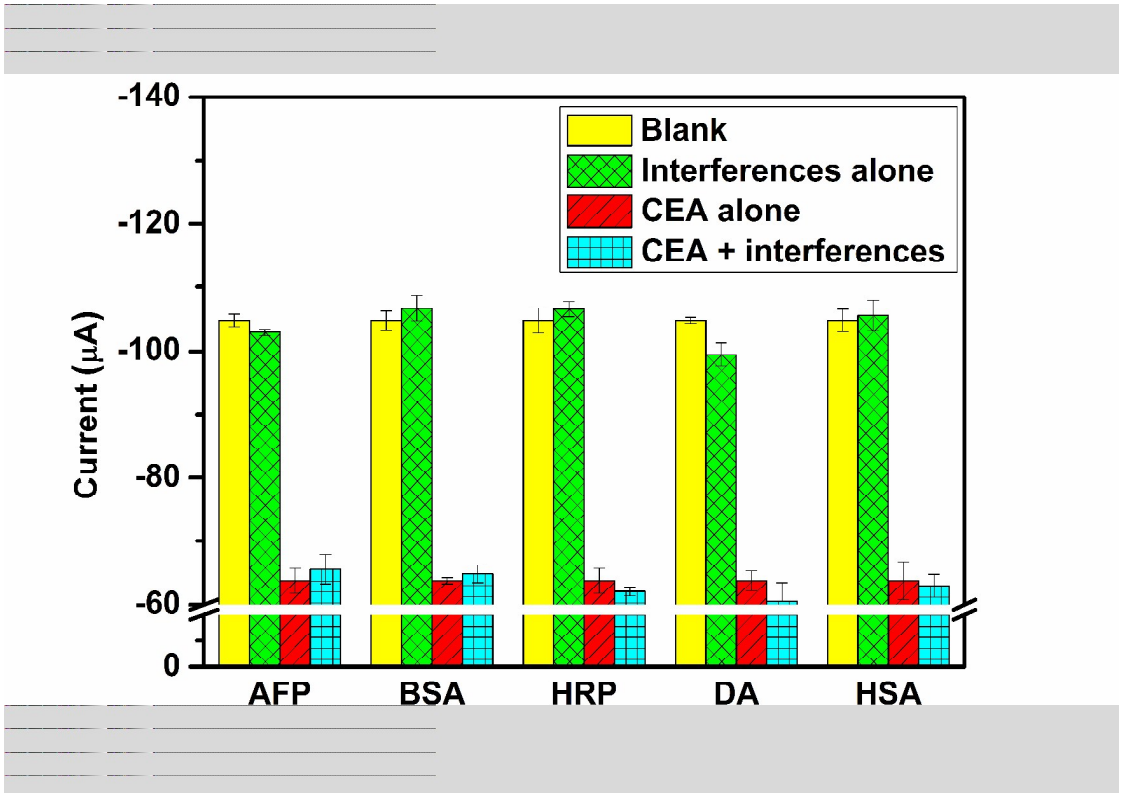

Table 1.

Comparison of analytical properties of different immunosensors for the detection of CEA.

immunosensor	Detection methods	Linear range ($ng \cdot mL^{-1}$)	Detection limit ($ng \cdot mL^{-1}$)	Sensitivity ($\mu A \cdot mL \cdot ng^{-1}$)	Reference
Anti-CEA/$(GNPs–MWNTs\text{-}THI–CHIT)_8$/MPS/GCE	CV	0.5-200.0	0.01	3.87, 0.48	[9]
Anti-CEA-HRP/Con A/PDA/3D-G	DPV	0.1-750.0	0.09	-	[12]
Anti-CEA/CHT/PB/AuNPs/GCE	DPV	0.05-100	0.02	0.5111	[37]
Anti-CEA/Nano-Au/MWCN-CHT/GCE	DPV	0.3-20	0.01	4.0685, 0.3693	[38]
Anti-CEA/CS–CNTs–GNPs/GCE	CV	0.1-200.0	0.04	1.56, 0.18	[39]
Anti-CEA/AuNPs/PB/nanoAu/GCE	CV	3.0–80	0.9	-3.5494, -0.8091	[40]
Anti-CEA/Au-CHT/$(MWNT\text{-}PEI\text{-}Au/PB)_5$/GCE	CV	0.5–160	0.08	4.85, 0.37	[41]
Anti-CEA/colloid Au/chitosan/SPCE	DPV	0.50–25	0.22	0.00095	[42]
Anti-CEA/AuNPs/SiO_2–Thi/AuNPs/Cys/AuE	CV	1.0–100	0.34	-0.7314	[43]
Anti-CEA/AuNPs/PBNPs/CS-CNTs-Au-/GCE	CV	0.30–120	0.10	0.6886	[44]
Anti-CEA/AuNPs/NiHCFNPs/AuNPs/GCE	CV	0.50–160	0.10	2.6696, 0.1704	[45]
Anti-CEA/AuNPs/CS/NG/GCE	CV	0.20–120	0.06	-1.31	[46]
Anti-CEA/AuNPs/PDA/Fc/CNTs-CS/GT/GCE	CV	0.01-80	0.002	23.31	This work

Table 2

Recovery results of the proposed immunosensor in serum samples by CV in 0.1 M PBS (pH 7.0) (*n*=5).

Content of CEA in the sample ($ng \cdot mL^{-1}$)	Added CEA ($ng \cdot mL^{-1}$)	Founded CEA ($ng \cdot mL^{-1}$)[a]	Recovery (%)	RSD(%, n=5)
1.25	0.5	1.78±0.07	102.0	3.94
	1.0	2.28±0.076	104.0	3.32
	3.0	4.24±0.10	99.7	2.38
	5.0	6.27±0.265	100.4	4.23
	10.0	11.41±0.448	101.6	3.93

[a] Mean value ± SD of five measurements.

A novel electrochemical immunosensor for the detection of CEA was proposed based on CNTs@CS complexes branching connection Fc-COOH for the detection of CEA.