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Label-free Electrochemiluminescence Detection of Specific-Sequence DNA Based on DNA Probes Capped ion Nanochannels

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Abstract

As one of the powerful molecular recognition elements, the functional DNA probes have been successfully utilized to construct various biosensors. However, the accurate readout of the recognition event of DNA probe binding to the specific target by the label-free means is still challenged. Here, a simple and label-free electrochemiluminescence (ECL) method for sensing the recognition event of DNA probe to sequence-specific DNA was developed. Oxalate is used as an ECL co-reactant and p53 tumor suppressor gene as a model of target analyte. In the ECL sensing platform, the nanochannel structural film, which contains silica-sol, chitosan and $Ru(bpy)_3^{2+}$, was prepared by an electrochemical deposition method; Then, DNA probes are attached on the surface of the nanochannel-based composite film electrode based on the stronger interaction between DNA probes and chitosan embedded in the ECL composite film. These nanochannels were capped by the DNA probes. As a results, the mass-transfer channel between the $Ru(bpy)_3^{2+}$ embedded in the nanochannel-based composite film and the ECL co-reactant in the bulk solution was greatly blocked and a weak ECL signal was observed. Conversely, in the presence of target sequences, the hybridizing reaction of targets with DNA probes could result in the escape of the DNA probes from the composite film due to the rigid structure of the duplex DNA. Thus, these nanochannels were uncapped and a stronger ECL signal was detected. Our results showed that this ECL method could effectively discriminate complementary from single-base mismatch DNA sequence. Under the optimal conditions, the linear range for target DNA was from 1.0×10^{-11} to 1.0×10^{-9} $mol\cdot L^{-1}$ with a detect limit of 2.7×10^{-12} $mol\cdot L^{-1}$. This work demonstrated that porous structures on the silica-chitosan composite film can provide a label-free and general platform to measure the change of DNA configuration.

Keywords: Electrochemiluminescence; $Ru(bpy)_3^{2+}$; Silica-Chitosan; Ion Nanochannels; DNA Probe

1. Introduction

In the recent years, the sensing of DNA probes binding to targets, especially the specific DNA sequence, has become more important since it have been widely used in diverse applications such as clinical diagnostics [1], bioengineering [2], pharmaceutical studies [3] and forensic applications [4]. Consequently, different analytical methods including electrochemical [5,6], fluorescence [7], quartz crystal microbalance [8], and surface plasmon resonance analytical techniques [9], have been developed for this purpose.

Among these, electrochemical technology is one of the most important methods for sensing the specific DNA sequence because it owned some obvious advantages over the other existing methods such as simple, rapid, sensitive, low-cost and portable [10-13]. To date, the electrochemical intercalator-based sensor [13, 14], the sandwich-type strategy [15-17] and the electrochemical DNA (E-DNA) sensor [18-21] were all developed to detect different target DNA sequences. Although the former presented the advantage of design simplicity and operation convenience, they often suffered from high background signals. Compared to the former, the latter two exhibited the higher sensitivity and selectivity. However, for fabricating the sandwich-type and these E-DNA sensors, procedures of labeling the electro-active tags or the thiol-group on DNA probes are always required. These complicated processes not only reduce the affinity of DNA probes to targets, but also is time-consuming, labor-intensive and high cost. In particularly, these E-DNA sensors [18-21] suffered from complicated structural design of DNA probes, the case-by-case design for different targets. Therefore, alternative protocols for developing DNA sensor with a simplified analytical procedure are important and highly desirable.

More recently, ions channel-based or nanopore-based strategies have been paid much attention for designing some label-free sensing platforms, utilizing DNA as a recognition element [22-26]. However, the complicated preparation processes were often required. On the other hand, as one of the powerful electroanalytical methods, electrogenerated chemiluminescence (ECL) or electrochemiluminescence analysis has received considerable interest in the different fields such as chemical/biochemical sensing and imaging etc. Among the reported ECL systems, solid-state $Ru(bpy)_3^{2+}$/TPA ECL system is a well-known detection method since it can provide several advantages, such as high quantum yield, simplifying experimental design, and creating a regenerable sensor [27-28]. Up to now, this detect system has been successfully applied to detect different targets [12], such as DNA [29, 30], protein [31], cell [32, 33], small molecule [34] and so on. Moreover, Yin and his co-workers have developed some label-free ECL DNA sensors that presented the advantage of preparation simplicity and the higher sensitivity [35-36]. However, in these ECL reactions, the use of TPA as an ECL

co-reactant is limited because TPA is toxic and volatile, and is always used in high concentrations level [37]. These defects always result in a nonspecific reaction between negatively charged DNA and positively charged TPA. In this case, this reaction could influence the affinity of ss-DNA, to some extent. On the contrary, although oxalate was an nontoxic $Ru(bpy)_3^{2+}$ ECL co-reactant, its ECL efficiency, especially in the silica-based matrix, was limited due to the poor mass-transfer behavior of negatively charged oxalate ion in silica matrix [38].

In the present study, we found that chitosan, silica-sol and $Ru(bpy)_3^{2+}$ could be electrochemically deposited on a graphite electrode surface and form a silica/Ru/chitosan ECL active film. In this ECL active film, the introduction of chitosan could bring about the two features. Firstly, due to the hydrogel structure and positively charged character of chitosan, the multiple anion channels were created in the composite ECL active film, which allowed oxalate to diffuse into ECL active film to do the ECL reaction. Secondly, chitosan, one of the DNA affinity polymers, could effectively adsorb ss-DNA on the composite ECL active film to cap the anion channels, leading to a weak ECL signal. Conversely, in the presence of targets, DNA probes that were adsorbed on the composite film electrode with multiple ion channels, bond to targets and its conformation changed, which resulted in the opening of the blocked ECL anion channels. As a result, many ECL co-reactant species, oxalate anion, got into the ECL active film to do the ECL reaction with $Ru(bpy)_3^{2+}$ and accompanied by the stronger ECL signal. In this case, a amplification-based ECL method for sensing the recognition event of targets with DNA probes could be achieved. Furthermore, using the novel properties of multiple ion channels film, the selectivity of this ECL sensing platform may be improved. To our best knowledge, this novel ECL DNA sensor via multiple ion channels has never been reported previously. More importantly, this new design may potentially be applied to the detection of other proteins and small molecules.

2. Experimental section

2.1 Materials

Tris(2,2′)-bipyridyl)dichlororuthenium(II) hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$) was purchased from Sigma (U.S.A.). Oxalate ($H_2C_2O_4$), potassium chloride (KCl), TEOS (Tetraethylorthosilicate), Chitosan, Tris (Trisaminomethane), and Ethanol et al. were purchased form Reagent Corporation of Xi'an (China). And the solutions and glassware were autoclaved prior to being used. All reagents were of analytical reagent grade, and Millipore

Milli-Q (18 MΩ) water was used throughout. A concentration of 10 mM phosphate buffer saline (PBS, pH 7.40, 0.10 M NaCl+10 mM KH_2PO_4/K_2HPO_4) was used as hybridization buffer and washing solution. All DNA sequences were acquired from Shanghai bioengineering Co., Ltd (China). And DNA sequences are recorded as P (probe), T1 (complementary sequence) and T2 (single-base mismatch sequence), T3 (random sequence), as shown in Table S1; Esophageal carcinoma cells, in which DNA is a complementary sequence of P, were obtained from College of Life Sciences, Shaanxi Normal University.

2.2 Apparatus

The experimental setup for ECL measurements consisted of a CHI 660C workstation (Shanghai Chenhua Instruments, Shanghai, China) and an ultraweak chemiluminescence (CL) analyzer controlled by a personal computer with the CL program (Xi'an Remax Electronic Science Tech. Co. Ltd, Xi'an, China). The applied potential for single-step potential electrolysis was achieved by CHI660C workstation. A microweighing bottle (height: 3.5 cm, diameter: 2.5 cm) was used as an ECL cell. A three-electrode system composed of a graphite electrode modified with $Ru(bpy)_3^{2+}$-Silica-chitosan composite film as working electrode, a platinum plate as counter electrode, and a Ag/AgCl (saturated KCl) as reference electrode. For detecting ECL, the cell was placed directly in front of a photomultiplier (PMT (Hamamatsu)), and the PMT window was only opened toward the working electrode to eliminate the blank CL and the ECL from the counter electrode.

The morphology and dimension of the as-prepared composited film were characterized using a Quanta-200 scanning electron microscopy (SEM, FEI Company, The Netherlands) and a JEM-2100HR transmission electron microscope (TEM, JEOL Ltd., Japan), respectively. UV-Vis absorption spectra were obtained on an UV–Vis spectrophotometer (TU-1900, Beijing Puxi, China).

2.3 Preparation of DNA ECL Probe

Firstly, a graphite electrode (Φ=0.8 cm) was carefully polished with 1.0μm of alumina slurry, rinsed with deionized water and sonicated for 3 min, respectively. After ultrasonic processing, this graphite electrode was rinsed several times with Millipore Milli-Q water and absolute ethanol in turn, and dried with highly purified nitrogen stream. Then, 2.0 mL of 0.20 $mol \cdot L^{-1}$ KCl, 2.0 mL of anhydrous ethanol and the right amount of TEOS were mixed and sonicated for 10 min. Thus, silica-sol was prepared successfully. Subsequently, the as-prepared silica-sol, 300 μL of 0.5 % chitosan and 1.0 mL of 0.05 $mol \cdot L^{-1}$ $Ru(bpy)_3^{2+}$ were immobilized on the precleaned graphite electrode surface by an electrochemically deposition

technique. The as-prepared composited film electrode was thoroughly rinsed with Millipore Milli-Q water to remove physically adsorbed silica-sol and others. Finally, 10.0 μL of 10 $\mu mol \cdot L^{-1}$ probe DNA solution containing Tris-HCl buffer was dropped on the resulting composited film electrode surface and was stored at the refrigerator for overnight. After that, it was washed with Millipore Milli-Q water to remove any nonspecific adsorption of DNA and was stored at 4℃ in the dark when not in use.

2.4 ECL detection for target ss-DNA

The composite film electrode immobilized probe DNA was immersed in PBS buffer containing different concentrations of target DNA for 25 min at 25℃. And then, this electrode was thoroughly washed three times with the same buffer to remove any physically adsorbed target DNA. Then, the resulting electrode was immersed into an ECL cell containing PBS (pH 7.4) solution and 1.0×10^{-5} $mol \cdot L^{-1}$ of oxalate solution. ECL measurement was performed with a three-electrode electrochemical system at a single step electrolytic potential of 0.1~1.2 V vs Ag/AgCl. The concentration of target DNA was quantified by the relative ECL intensity ($\Delta I= I-I_0$), where I_0 is the ECL peak height before hybridization and I is the ECL peak height after hybridization.

3. Results and Discussion

3.1 Electrodeposition and characterization of silica/$Ru(bpy)_3^{2+}$/chitosan composite film

In fact, Collinson's early investigation showed that in their hydrated state, silica-sol-gel materials could be porous, thus providing a good matrix to entrap $Ru(bpy)_3^{2+}$ [38]. However, the thin films for entrapping $Ru(bpy)_3^{2+}$ are less porous and quite dense, which go against chemical sensing. To obtain the films with greater porosity, Collinson electrodeposited silica-sol-gel films on the gold surfaces. In this method, a sufficiently negative potential is applied to the electrode surface to reduce oxygen to hydroxyl ions, which serves as the catalyst for the hydrolysis and condensation of TEOS. On the other hand, chitosan is a cationic polyelectrolyte at low pH (pH<6.0), but becomes less charged and insoluble as the pH approaches or exceeds its pKa (chitosan's apparent pKa=6.3). Consequently, in this electrodeposited process, chitosan can also be deposited at the graphite electrode because oxidation of proton or proton consumption at this electrode leads to a localized region of high pH that can exceed chitosan's solubility limit [39]. Based on these studies, analogous with Collinson and Payne's protocols [38, 39], we attempt to simultaneously electrodeposite $Ru(bpy)_3^{2+}$, sol-gel-derived silica films and chitosan on a precleaned graphite electrode

surface. When a constantly negative potential is applied to the graphite electrode, the pH may be increased at the electrode surface via the oxidation of oxygen and water, or the reduction of proton. These ions can catalyze or induce the hydrolysis and condensation of the silica-sol and chitosan-gel. Thus, a base-catalyzed sol-gel process occurs at the electrode surface. As expected, we observed that a brown colored thin layer was deposited on the surface of the graphite electrode. Further investigations show that the sol-gel mixture is effectively immobilized on the electrode surface when a constant potential of -900 mV is applied for a sufficiently time. Moreover, the amount of chitosan incorporated in the composite film significantly influenced the sensitivity of ECL analysis. As suggested in Table S2, 300 μL of 0.5% chitosan was selected for obtaining the maximum ECL signal to noise rate.

To better evaluate the electrodeposited composite films, scanning electron microscope (SEM) and transmission electron microscope (TEM), have been used in the following work. SEM is a powerful tool that can give information about the surface roughness and morphology of the films. Fig.S1 shows that the surface of $Ru(bpy)_3^{2+}$-silica film composited with chitosan (Fig.S1(b)) is coarser and more loosen than that in the absence of chitosan (Fig.S1(a)). Moreover, the porosity dimensions on the resulting electrode surface become bigger. As indicated in Fig.S1(c), there are some irregular nanopores or ion nanochannels at the surface of the composite film electrode. On the other hand, we use $[Fe(CN)_6]^{3-}$ as the electrochemical probe to indirectly evaluate whether the as-prepared composite films are compact with few direct channels to the underlying electrode surface or more porous with many channels/pores. As can be seen from Fig.1, silica/$Ru(bpy)_3^{2+}$ film composited with chitosan exhibits the higher peak current compared with that in the absence of chitosan. This indicates that there are more ion channels after the right amount of chitosan is composited on the silica/$Ru(bpy)_3^{2+}$ active film, and some anions are allowed to effectively diffuse into these ion channels for taking part in electronic transfer.

3.2 Electrochemical and ECL Behaviour of silica/$Ru(bpy)_3^{2+}$/chitosan composite film

From Fig.2, it can be seen that a pair of redox waves in the resulting electrode appeared with an oxidation potential at 1.10 V and a reduction potential at 1.03 V. This demonstrated that $Ru(bpy)_3^{2+}$, being a positively charged probe, can be immobilized at the surface of the porous composite film electrode. The effective surface concentration of $Ru(bpy)_3^{2+}$ at the electrode used in Fig.2(a) was approximately 1.2×10^{-8} mol·cm^{-2}, calculated from graphical integration of the background-corrected cyclic voltammogram[40]. Furthermore, in the presence of oxalate, the oxidation current for entrapped $Ru(bpy)_3^{2+}$ at 1.10 V (Fig.2 (b)) is

significantly higher than that in the absence of oxalate（Fig.2 (a)）. On the contrary, in the absence of oxalate, the reduction current for entrapped $Ru(bpy)_3^{2+}$ at 1.03 V is higher than that in the presence of oxalate. And the half-wave potential is shifted negatively, consistent with an electrocatalytic reaction mechanism. These results all indicates that oxalate has an obviously electrocatalytic effect for the electrooxidation state of $Ru(bpy)_3^{2+}$. On the other hand, we also explored ECL kinetic curves on two composite film electrodes, using oxalate as a co-reactant. Fig.S2 shows that the film electrode composited with chitosan exhibited the faster ECL response, compared with that in the absence of chitosan.

These above-mentioned investigations also indicated that the introduction of a suitable amount of chitosan could effectively improve the mass-transfer performance of these anion channels on the porous composited film electrode surface. Thus, transmission between electrons and oxalate at the electrode surface becomes free and fast. More importantly, the resulting electrode with anion nanochannels still maintained a certain electrochemical activity and could be suitable for the following DNA detection.

3.3 The adsorption of DNA probes on the composite film and its sensing performances

Since the surface on the prepared composite film electrode is functionalized with amines-rich chitosan, positively charged chitosan will interact with negatively charged oligonucleotides [41]. To confirm this protocol, the porous composite film electrode attached DNA probes was characterized by UV-vis spectrophotomtry, cyclic voltammetry (CV) and ECL spectrum, respectively. UV-vis spectra results showed that there was an obvious characteristic absorption peak for control probe DNA in 258 nm, corresponding to the characteristic peak of oligonucletide in 260 nm. However, no characteristic absorption peak for DNA eluant appears in 258 nm, in response to adsorption probe DNA (data not shown). This indicated that probe DNA could be effectively immobilized on the surface of porous silica/$Ru(bpy)_3^{2+}$/chitosan composite film electrode. Moreover, Fig.3 showed that the redox current at the electrode immobilized DNA probes significantly decreased (Fig. 3(b)), compared with that in the absence of DNA probes (Fig. 3(a)). Correspondingly, the ECL signals for oxalate solution were weak (Fig. 4(a)). An explanation for this phenomenon could be supposed that the existence of silica-based film and probe DNA could partly prevent electron transfer and mass transfer. However, while DNA probes hybridized with target DNA sequences, the redox current (Fig. 3(c)) and the corresponding ECL signals (Fig. 4(b)) were significantly enhanced. On the base of these above results, we speculated possible sensing performances of the porous composite film electrode as Scheme.1 showed.

Firstly, when DNA probes were capped on the surface of the $Ru(bpy)_3^{2+}$/silica /chitosan composite film electrode, the permeability of the electrodes surface became so poor that weak ECL signals were obtained. It was a possible reason that the flexibility structure of DNA probes could block some anion nanochannels on the composite film electrode surface. As a result, the effective transmission of electrons and oxalate at the prepared composite film electrode surface was weakened, to a certain extent. More importantly, the negatively charged phosphate skeleton in DNA probes could generate a certain electrostatic repulsion to oxalate, which compelled some oxalate to remove away the surface of the electrode. In this case, a "gate-closed" mode or "capping effect" was created on the surface of this as-prepared composite film electrode by the attached DNA probes. Thus, oxalate could not fully enter into the as-prepared anion nanochannels for taking part in ECL reaction, which resulted in a weak ECL signal. However, in the presence of target ss-DNA, a "gate-opened" mode or "uncapped effect" was realized on the surface of porous composite film electrode, leading to a strong ECL signal. It is a possible factor that DNA hybridization event could induce a conformational change of the matrix from flexible ss-DNA to double-helix structure and these forming double-helix DNA were displaced from the porous composite film electrodes. In this case, the mass transfer channels between $Ru(bpy)_3^{2+}$ and oxalate were opened. Thus, some active-sites on the composite film electrode were liberated and more oxalate were permitted to enter into this as-prepared anion nanochannels for doing ECL action with the electrooxidation production of $Ru(bpy)_3^{2+}$.Thus, a amplification-based stragety was realized. Therefore, in contrast to molecular beacons, the proposed method could not only be used to accurately sense binding-induced a large change of DNA conformation, but also to more selectively detect target DNA. Here is consistent with we speculated.

3.4 Optimization of the ECL behavior conditions

Electrodeposition time significantly affects roughness and ion channel dimension of this composite film electrode surface. More importantly, the dimension of ion channel on the as-prepared composite film electrode surface directly affected the selectivity of ECL analysis. To obtain the maximum signal to noise rate, we investigated the effect of different deposition time on ECL analysis. The results were showed as Fig.5 (A). Therefore, a deposition time of 25 min was chosen in following experiments to obtain an excellent sensitivity. Fig.5 (B) shows that ECL signal to noise rates changed with the increasing of hybridization time from 10 min to 60 min and reached a maximum at 20 min, and then gradually decreased. This suggests that the hybridization reaction completes within 20 min. Thus, the hybridization time of 20 min was employed in following experiments.

Moreover, we also investigated the effects of concentration of oxalate and the pH of the buffer solution on ECL signal to noise rates. The results demonstrated that maximal ECL signal to noise rate was obtained when the concentration of oxalate was 1.0×10^{-5} mol·L^{-1} (Fig.5 (C)), and the ECL signal to noise rate did not obviously change with the changing of the pH value in neutral medium. In fact, the above-mentioned studies (Fig.1 and Fig.4) showed that the introduction of chitosan could greatly improved the diffused efficiency of oxalate anion on the porous silica/$Ru(bpy)_3^{2+}$ composite film. In other words, the introduction of chitosan provided a good transfer channel for oxalate anion to take part in ECL reaction. Therefore, compared to the previous reports [40], in the present work, the concentration of oxalate as a co-reactant is very low. Herein, we emphatically studied the ECL of $Ru(bpy)_3^{2+}$ in PBS solution (pH 7.4) containing 1.0×10^{-5} mol·L^{-1} oxalate.

3.5 Linear Range and Detection Limit

Under the preliminary optimized conditions, the linear range for complementary sequence (target ss-DNA) was measured. The relative ECL intensity had a linear response with the concentration of the complementary sequence in the range of 1.0×10^{-11} to 1.0×10^{-9} mol·L^{-1} (Fig.6). The linear regression equations were $\Delta I = 151.2C + 503.0$, (unit of C is mol·L^{-1} and linear range is from 1.0×10^{-11} to 1.0×10^{-10} mol·L^{-1}) and $\Delta I = 206.6C + 460.0$ (linear range is from 1.0×10^{-10} to 1.0×10^{-9} mol·L^{-1}), respectively. The corresponding correlation coefficients were 0.9983 and 0.9959, respectively. The detection limit (3σ) for the complementary sequence was 2.7×10^{-12} mol·L^{-1}. And the relative standard derivation on the resulting composite film electrode to 1.0×10^{-10} mol·L^{-1} of complementary DNA sequences was 4.1% for eleven successive measurements. This suggested good operational stability of the ECL determination of target DNA at the porous composite film electrode. Moreover, the long-term stability of the sensor was also examined. After the developed composite film electrode was stored in PBS buffer (pH 7.4) at 4 °C over 20 days, no apparent change in ECL intensity was found, indicating a good stability of this biosensor.

3.6 Specificity of the ECL probe and ECL detection of DNA in esophageal carcinoma cells

To evaluate the specificity of this ECL biosensor, 1.0×10^{-10} mol·L^{-1} of complementary ss-DNA sequence (T1), single-base mismatch sequence (T2) and noncomplementary sequence (T3) hybridized with DNA probes on the electrodes surface at 25℃ for 20 min, respectively. Then, these resulting electrodes were washed thoroughly to remove the nonspecific adsorption DNA and then used as the working electrode for ECL measurement.

As shown in Fig.7, this complementary sequence gave a higher ECL signal (Fig. 7(d)) and the noncomplementary sequence had a lower ECL response (Fig. 7(b)). Although this single-base mismatch sequence also had a higher ECL emission (Fig. 7(c)), it exhibited an obvious difference compared to the complementary sequence DNA in ECL response. These results indicated that the proposed biosensor could be used to identify the target DNA sequence from a single-base mismatch sequence and a noncomplementary sequence DNA.

The p53 tumor suppressor gene segments related to esophageal carcinoma cells were chosen as a model of target analyte and its part sequence was the same as T1（target DNA）. Esophageal carcinoma is one of the most commonly fatal genetic diseases. As a commonly mutated gene, p53 tumor suppressor gene related to the cancer cells may lead to the loss of transcriptional activation potency and the ability to bind DNA [42]. Therefore, sequence-specific analysis of the p53 could help early diagnosis of cancer and consequently increase the success of the treatment. Herein, the proposed ECL detection of the mutagenic DNA hybridization was examined in 1000 esophageal carcinoma cells with ultrasonic treatment and 1.9×10^{-10} mol·L^{-1} of DNA was successfully quantified (Fig. 7(e)).

4. Conclusion

In this work, we reported a simple and label-free ECL method for sensing the recognition event of DNA probe to targets using ion nanochannels. The introduction of chitosan into the sensing system could effectively improve the mass-transfer performance of silica-based anion channels and lead to the attachment of probe DNA. This could result in a large change of ECL signal in the absence or presence of target DNA. On the other hand, this proposed method presents relatively sensitive DNA detection and recognition ability for a single base mismatch ss-DNA due to the small dimension of the as-prepared anion channels. Although we only showed the application of the proposed strategy for target DNA monitoring, this detection scheme can be potentially expanded for the design of different types of ECL aptasensors to quantify small molecular, protein, cancer cells and so on.

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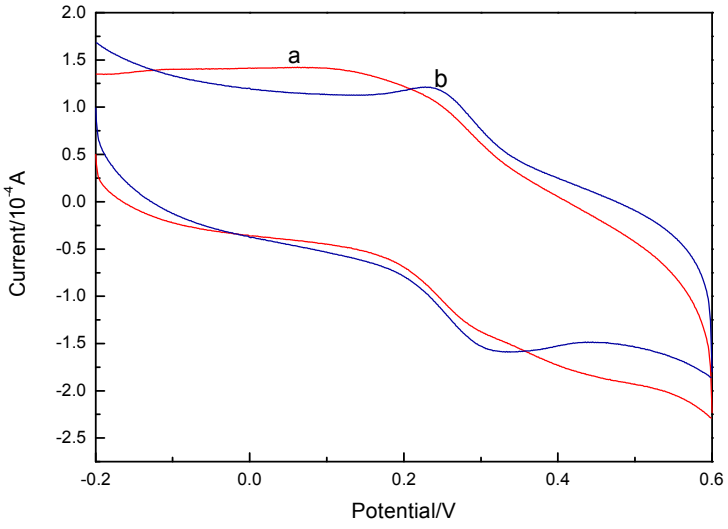


Fig. 1 Cyclic voltammograms of two different electrode (a--silica film (not composited Chitosan) electrodeposited on a graphite electrode; b--composited Chitosan) in ferricyanide potassium solution (KCl as the supporting electrolyte); Scan rate: 100 mv·s^{-1}

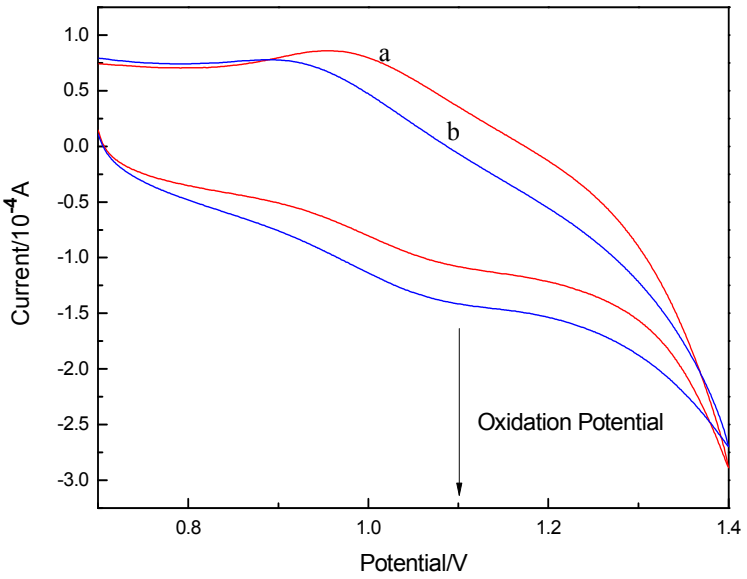


Fig. 2 Cyclic voltammograms of Silica-Chitosan-Ru(bpy)$_3^{2+}$ composite films electrode on a graphite electrode in the absence(a) and presence (b) of 1mM oxalate solution in PBS buffer solution (pH 7.4) with a scan rate of 50 mV·s^{-1}

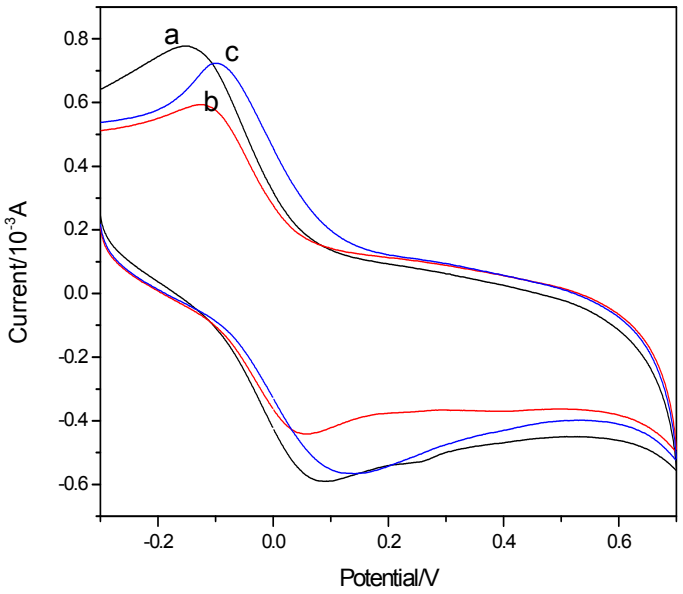


Fig. 3 Cyclic voltammograms (a) Silica-Chitosan-Ru$(bpy)_3^{2+}$ electrode

(b) ssDNA-Silica-Chitosan-Ru$(bpy)_3^{2+}$ electrode

(c) dsDNA-Silica-Chitosan-Ru$(bpy)_3^{2+}$ electrode.

Electrolyte: potassuim cyanide ferrous(1.0×10^{-2}mol·L^{-1})+potassium chloride (0.1 mol·L^{-1});

Electrolytic potentials: -0.3~0.70V; Scan rate: 100mv·s^{-1};

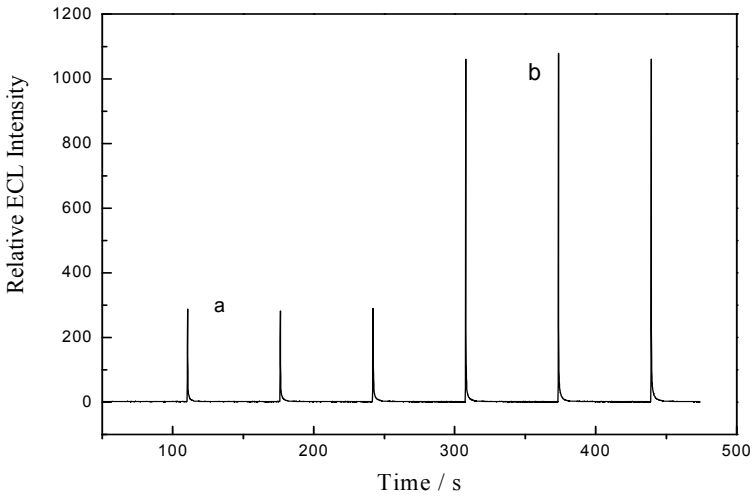
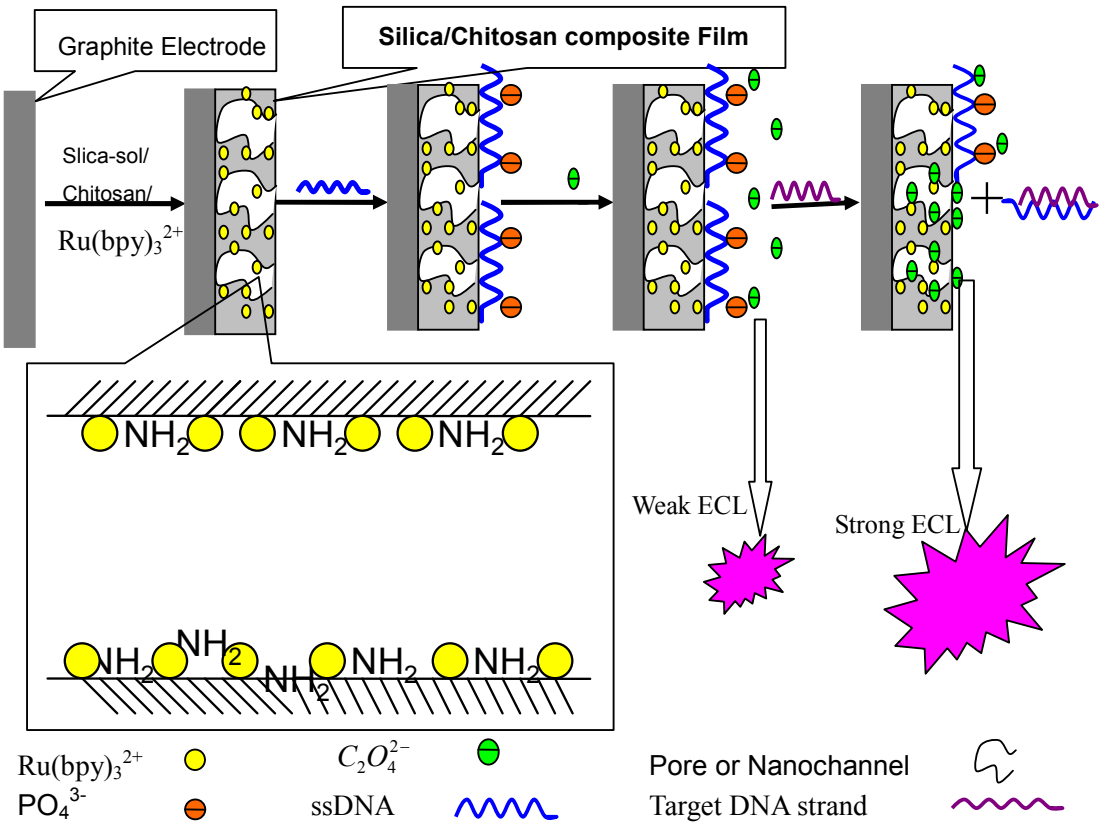


Fig. 4 The ECL signal of (a) ssDNA/ Silica-Chitosan-Ru(bpy)$_3^{2+}$ electrode;
(b) ssDNA hybrized with target DNA electrode;
Target Complementary DNA Concentration: 1.0×10^{-10} mol·L^{-1};
Oxalate concentration: 1.0×10^{-6} mol·L^{-1}; Hybridization time: 20 min;
PBS buffer solution: pH=7.4; Electrodeposition time: 30 min;



Scheme.1 Schematic diagram of the sensor detection for DNA hybridization

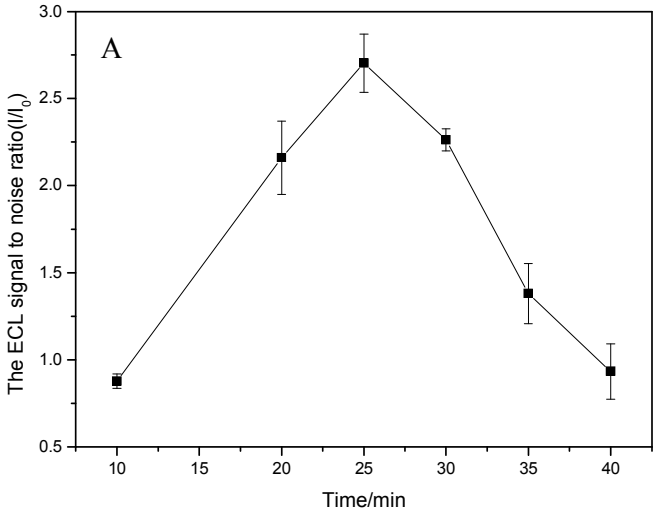


Fig. 5 (A)

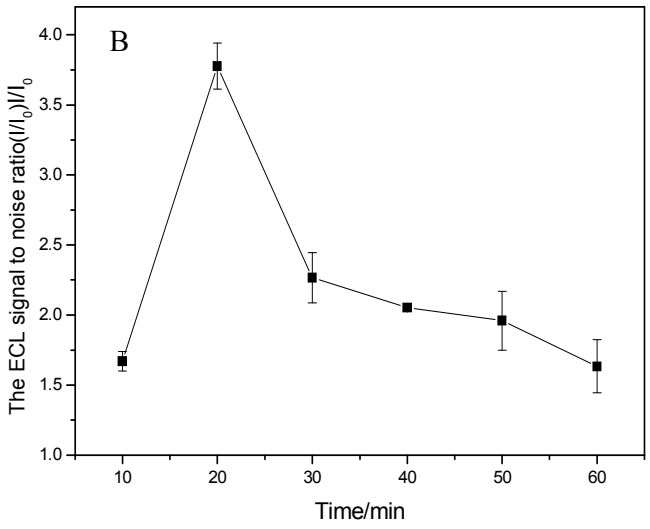


Fig. 5 (B)

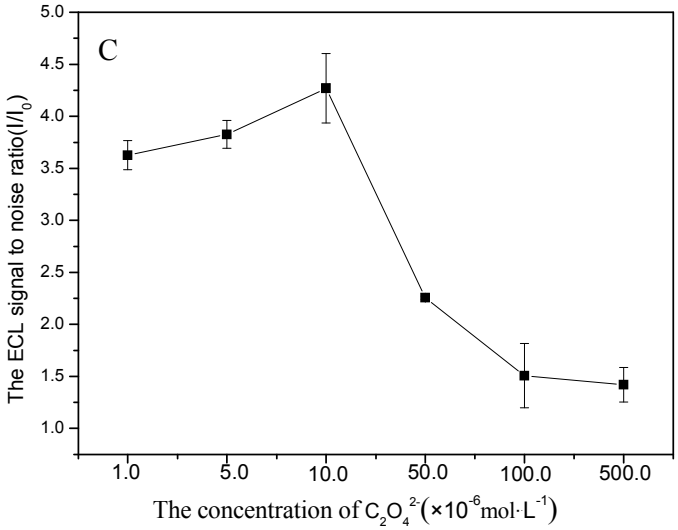
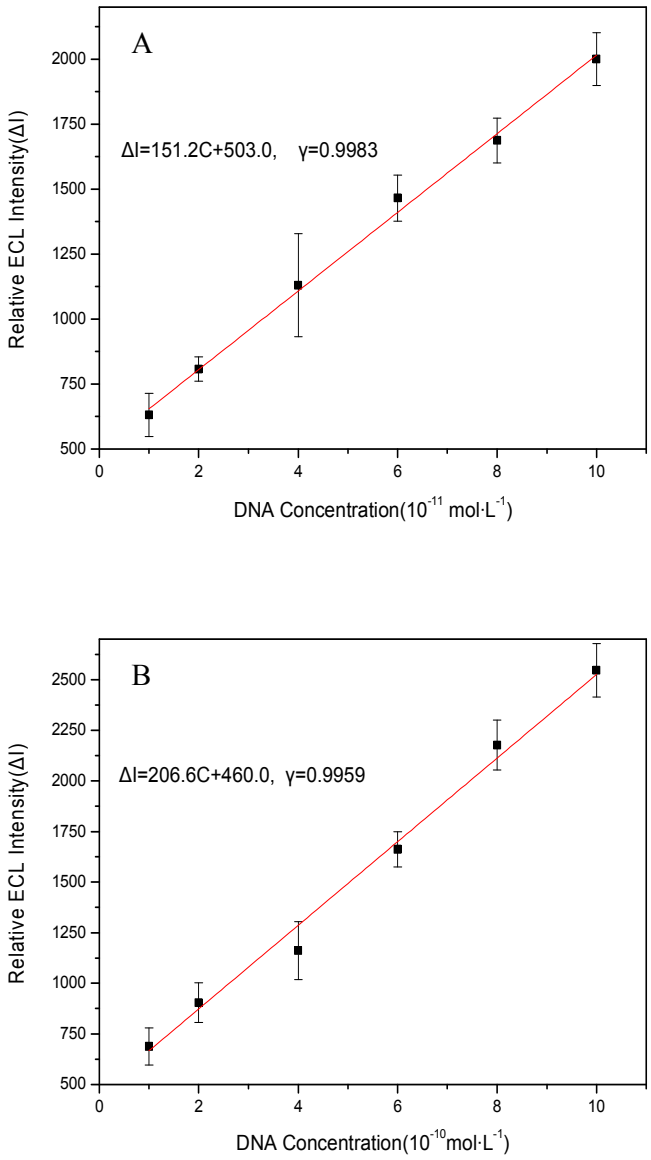


Fig. 5 (C)

Fig. 5 (A) Effect of electrodeposition time on the ECL signal to noise ratio(Oxalate Concentration: 1.0×10^{-6} mol·L^{-1}; Hybridization time: 20 min); (B)Effect of hybridization time on the ECL signal to noise ratio(Oxalate Concentration: 1.0×10^{-6} mol·L-1; Electrodeposition time: 25 min); (C) Effect of concentration of $C_2O_4^{2-}$ on ECL signal to noise ratio(Electrodeposition time: 25 min; Hybridization time: 20 min);Target Complementary DNA Concentration: 1.0×10^{-10} mol·L^{-1}; PBS buffer solution: pH=7.4



a

Fig. 6 Calibration curve of relative ECL intensity versus target DNA Concentration in different ranges

(A)The concentrations of target DNA in the range of 1.0×10^{-11}~1.0×10^{-10} mol·L^{-1} (operated at 800 V of negative high voltage), (B) the concentrations of target DNA in the range of 1.0×10^{-10}~1.0×10^{-9} mol·L^{-1} (operated at 700 V of negative high voltage)

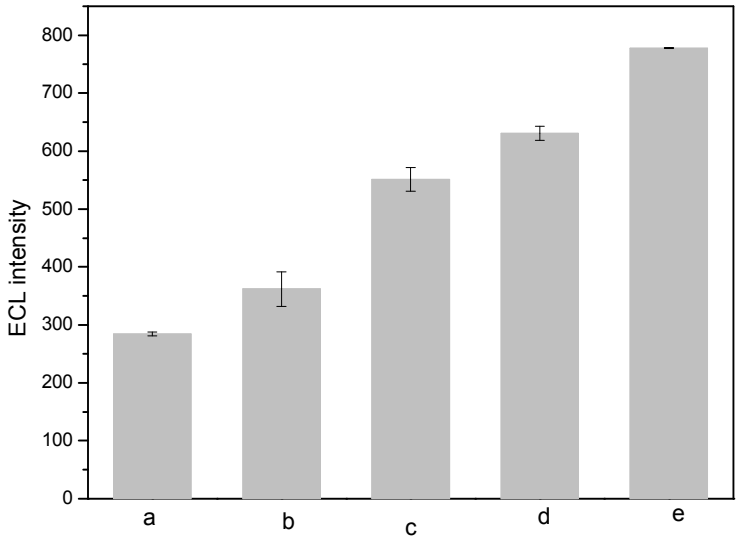


Fig. 7 ECL signals for probe DNA to recognize different DNA sequence

(a) Blank ; (b) Random DNA ; (c) Single-base mismatch DNA;

(d) Completely complementary DNA; (e) DNA in schizolytic esophageal carcinoma cells

(Operated at 700 V of negative high voltage)

A label-free electrochemiluminescence method for detecting specific-sequence DNA has been developed based on DNA probes capped ion nanochannels. This method is simple, inexpensive, and can be potentially expanded for the design of different types of ECL aptasensors.