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Biocompatible electrochemiluminescent biosensor for choline based on enzyme/titanate nanotubes/chitosan composite modified electrode

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ABSTRACT

A new biocompatible ECL biosensor based on enzyme/titanate nanotubes/chitosan composite film was developed for the determination of analytes in biological samples. In the fabrication of the new ECL biosensor, biocompatible titanate nanotubes (TNTs) and a model enzyme, i.e., choline oxidase (ChOX), were immobilized on a chitosan modified glassy carbon electrode (GCE) via electrostatic adsorption and covalent interaction, respectively. By this ECL biosensor, choline was enzymatically oxidized to hydrogen peroxide and detected by a sensitive luminol ECL system. The use of TNTs not only provided a biocompatible microenvironment for the immobilized enzyme, which resulted in an excellent stability and long lifetime of the ECL biosensor, but also exhibited great enhancement towards luminol ECL and thus led to a significant improvement in sensitivity of ECL biosensor. Satisfactory results were obtained when employing this biosensor in assaying the total choline in milk samples. The work would provide a common platform to develop various sensitive, selective and biocompatible ECL biosensors based on using enzyme/TNTs/CHIT composite films.

1. Introduction

In the past years, ECL biosensor, the combination of very sensitive ECL detections with extremely selective biological recognition patterns, for example enzymatic reactions, immunoassays or DNA-probe assays, has been a hot topic in the field of biosensing (Karsten et al., 2001; Miao, 2008; Richter, 2004). More recently some reviews involving the development of ECL sensors have been reported (Bertoncello and Fobert, 2009; Forster et al., 2009). In particular, Bertoncello's review has in detail described the application of carbon nanotubes (CNT) and quantum dots (QDs) in ECL application (Bertoncello and Fobert, 2009).

In the development of ECL biosensor, $Ru(bpy)_3^{2+}$ and its derivatives have been used widely as ECL labels in immunoassays (Miao and Bard, 2003, 2004a,b), and DNA assays (Miao and Bard, 2003, 2004a,b; Xu and Bard, 1995; Xu et al., 1994), while luminol ECL system has been often combined with enzymatic reactions that produce hydrogen peroxide (Karsten et al., 2001; Leca and Blum, 2000; Miao, 2008; Richter, 2004; Wilson et al., 1997). Recently, many publications have been focused on improving the performance of ECL-based sensing platform, such as sensitivity and stability, by utilizing various nano-sized functional materials (Cui et al., 2003; Guo and Dong, 2004; Jie et al., 2007; Jie et al., 2008; Liu and Ju, 2008; Wang et al., 2007; Xiong and Cui, 2008; Zhang and Dong, 2006). For examples, Au or Ag nanomaterials were reported to enhance significantly the ECL emission of luminol (Cui et al., 2003; Wang et al., 2007; Xiong and Cui, 2008). Carbon nanotubes were also utilized for the architecture of improved sensitivity ECL biosensor based on luminol ECL system (Chen et al., 2007; Lin et al., 2008). Most recently, QDs as new and attractive ECL luminophores were used for constructing ECL immunosensors (Jie et al., 2008), ligand-receptor binding ECL biosensor (Jie et al., 2007), and enzyme-sensitized ECL biosensor (Liu and Ju, 2008).

In general, the issue of biocompatibility should be considered in the construction of biosensors, since the biological recognition molecules used in the biosensors, such as immobilized enzymes, antibodies and DNAs may lose their biological activities at biologically incompatible environments. Unfortunately, not much attention has been paid to the biocompatibility of ECL biosensors especially that in luminol based ECL system. CNT, one of the most widely used nanomaterials, play important role in improving the conductivity of composite films. However, recent researches have demonstrated cytotoxicity of carbon-based nanomaterials, which is size-dependent and maybe even enhanced when the surfaces of these nano-carbon materials are functionalized by treating with acid (Jia et al., 2005; Magrez et al., 2006). The most used QDs in

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biosensors are Cd-based semiconductor crystals, however, a latest report shows that they have very strong cytotoxicity, even higher than that of the heavy metal ion Cd^{2+} (Priester et al., 2009). Therefore, it encourages us to explore other nanomaterials to construct a new type of biosensor, which is not only able to enhance the response of the biosensor, but also exhibits well biocompatibility towards biological recognition elements.

A new kind of TiO_2-derived material known as titanate ($H_2Ti_3O_7$) nanotube (TNT) has received great interest for its unique characteristics of nanostructure, such as uniform nanometer-sized channels, large specific surface area (Liu et al., 2005a,b), excellent physical and chemical stability (Hodos et al., 2004; Thorne et al., 2005), and satisfied optical and catalytic properties (Murciano et al., 2007; Umek et al., 2005; Xu et al., 2006). Interestingly, TNTs have been recently reported to have very attractive biocompatibilities (Kasuga, 2006). TNTs have high porous structures, contain large amount of water molecules and hydroxyls functional group on the nanotubes' surface, which provide super hydrophilic and aqueous-like microenvironments for immobilized enzymes and thus may maintain their stability in biosensing film (Kasuga, 2006; Liu et al., 2005a,b; Miyauchi et al., 2007; Wenzel, 1949). Hence, such special properties of TNTs would be beneficial to the construction of nanotubes based bio-devices. However, the application of TNTs in ECL research is still limited. The unique properties of TNTs, especially its excellent biocompatibility, encourage us to investigate its possible applications in ECL biosensing.

Choline is distributed in central and peripheral nervous systems of mammals. It has very similar fat-modifying effects on the membranes of our cells which allow our cell membranes to operate with greater flexibility in handling both water- and fat-soluble molecules. So that, without choline, many fat-based nutrients and waste products could not pass in and out of our cells. Fortunately, sufficient choline could be achieved from daily diet including milk, oranges, oats, corn, etc. Some departments of countries thus have established the daily intake of choline in infants and adults, respectively. During the past few years, choline oxidase (ChOX) based biosensors have emerged as the most promising alternative for direct monitoring of choline (Marquette and Blum, 2003; Tsafack et al., 2000).

Herein, a new biocompatible ECL biosensor based on using enzyme/TNTs/chitosan (CHIT) composite film as the recognition unit was fabricated and used for the determination of analytes in biological samples. In this new ECL biosensor, TNTs and a model enzyme, i.e., ChOX, were immobilized on chitosan modified glassy carbon electrode (GCE) via electrostatic adsorption and covalent interaction, respectively, while the enzymatic oxidation product of choline, hydrogen peroxide (H_2O_2) was detected by the sensitive luminol ECL system. To the best of our knowledge, this is the first report on developing ECL biosensor by using a nano-sized material (i.e., TNTs) that exhibits both excellent biocompatibility and strong enhancement in ECL response. Satisfied results were obtained by employing this biosensor in assaying the total choline in the milk sample. The work would be very interesting in developing various sensitive, selective and biocompatible ECL biosensors based on using easily prepared enzyme/TNTs/CHIT composite films.

2. Experimental

2.1. Instruments and chemicals

ECL intensity versus potential was detected by a homemade ECL system, consisting of a BPCL Ultra-Weak Chemiluminescence Analyzer (Institute of Biophysics, Chinese Academy of Sciences) for detecting light emission and a CHI 620 electrochemical analyzer (Shanghai Chenghua Instrument Co., China) for controlling waveforms and potentials. The detailed description of this ECL system has been given in previous report (Dai et al., 2008). Transmission electron microscopy (TEM) and atomic force microscope (AFM) images were respectively obtained with Tecnai G2 F20 S-TWIN microscope (200 kV) and NS3A-02Nanoscope IIIa Scanning Probe Microscopy.

Choline oxidase, luminol, choline solution (25%), tetraethylammonium hydroxide (TEAOH) and glutaraldehyde were obtained from Sigma and used without further purification. Chitosan (MW 1.68×10^5, 91.7% deacetylation) was purchased from Nantong Shuanglin Co (China). H_2O_2 solutions were freshly prepared by diluting 30% (v/v) H_2O_2 (Fuchen Chemical Reagent Factory, Tianjin, China) with water. Other chemicals were of analytical grade or better.

A 0.5 wt.% chitosan solution was prepared by dissolving certain amount of chitosan powder into 10 mL of hot 0.05 M acetic acid and stirred for 3 h at room temperature until complete dissolution. The pH was adjusted to 5.0 using a concentrated NaOH solution. Water used for preparing aqueous solutions was purified by Millipore Milli-Q purification system.

Titanate nanotubes (TNTs) were synthesized by a previously reported hydrothermal method (Kasuga et al., 1998). A colloidal suspension of TNTs was prepared as follows: a suitable amount of TNT powder was dispersed in a 0.1 M HNO_3 solution under stirring and centrifugalization. The obtained deposit was further dispersed in 0.05 M TEAOH under stirring, resulting in a translucent suspension solution. The prepared suspension was diluted with water and sonicated for 5 min shortly before use.

2.2. Preparation of TNTs/CHIT-modified electrodes

First, a disk glassy carbon working electrode (0.126 cm^2 in area) was polished to a mirror-like finish with define silica–alumina powder, then it was sonicated successively in 1 M HNO_3/1 M HCl, 1 M NaOH solution, ethanol and doubly distilled water for 5 min, respectively.

CHIT film modified electrode was prepared by coating 5 μL of 0.1 wt.% CHIT solution on the surface of GCE. The evaporation of water resulted in a thin CHIT film. The TNTs/CHIT multilayer films at the electrode surface were assembled by immersing the CHIT-modified GC electrode into negatively charged TNTs (5 mg/mL)–TEAOH solution. Then such modified electrode was well washed with doubly distilled water after each assembly.

2.3. Immobilization of choline oxidase on the modified electrodes

The choline oxidase was immobilized onto the modified electrode surface by cross-linking the enzyme with chitosan using glutaraldehyde. The TNTs/CHIT-modified GCE was rinsed with water and immersed into 0.25% glutaraldehyde for 30 min. After rinsing with water, 5 μL of ChOX solution (4 U) was pipetted onto the electrode and the surface was dried for approximately 1 h at room temperature. The resulted biosensor was stored in a refrigerator at 4 °C for use. The schematic principle for fabrication of this ECL biosensor was shown in Fig. 1.

2.4. Sample preparation

The preparation of milk hydrolysate samples for the determination of the total choline content was carried out by the procedure described by Sandra et al. (2007), and Woollard and Indyk (1990, 2000) used in a collaborative study for the AOAC official method.

Briefly, a 20 mL milk sample was mixed with 10 mL of 3 M HCl in a flat-bottomed flask. The flask was connected to a condenser, heated for 3 h in a covered water bath (70 ± 2 °C) and agitated periodically. The acid digest was cooled and centrifuged for 15–20 min.

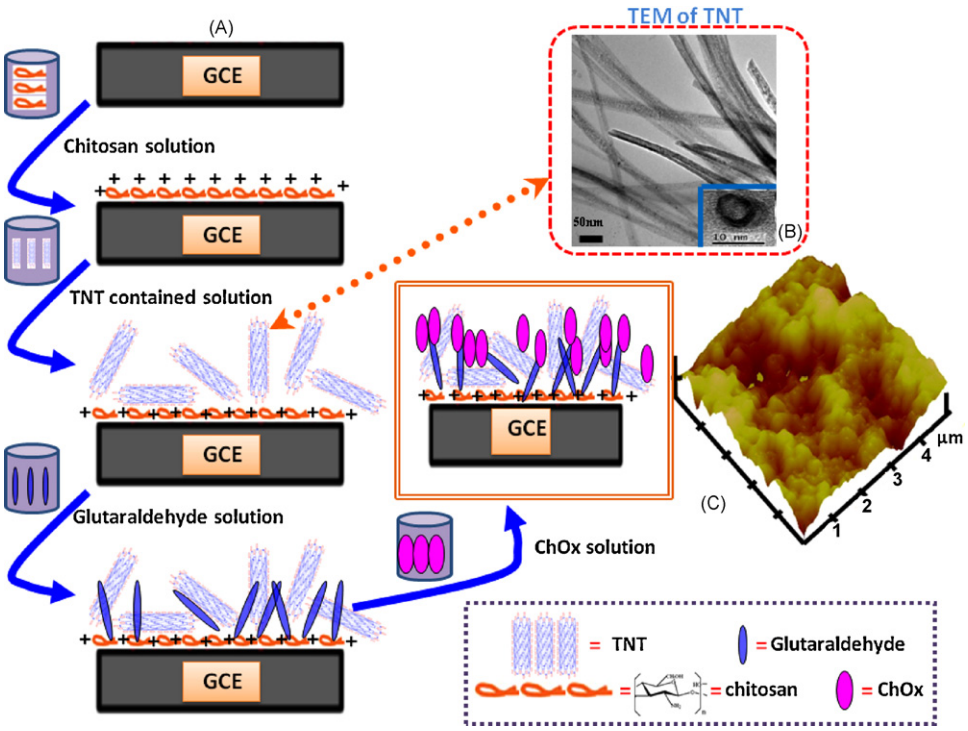

Fig. 1. A schematic steps for fabrication of the ECL biosensor (A). Insets are TEM (B) and AFM (C) images of TNTs and ChOX/TNTs/CHIT film.

The aliquot of the supernatant was then transferred to a beaker, the pH was adjusted to 7.0–7.4 with 50% (w/w) NaOH solution and then samples were filtered through a 0.45 μm cellulose acetate membrane. The samples were stored frozen at −18 °C in dark before use. When necessary, a further dilution with carrier solution was performed in order to bring choline concentration in the linear range.

3. Results and discussion

3.1. Characterization of TNTs/CHIT-modified electrode

3.1.1. TEM image of TNTs

The morphologies of TNTs were characterized by TEM. Fig. 1B shows the typical TEM images of the product, consisting of nanotubular structures. It can be seen from Fig. 1B that one-dimensional (1D) nanostructure materials TNTs were successfully synthesized, which have inner diameters of 3–5 nm, outer diameters of 8–15 nm, wall thickness of 3–6 layers with an interlayer spacing of ~0.4 nm, and the length up to micron. The inset in Fig. 1 indicates the nanotubes have open ends.

3.1.2. Formation of TNTs/CHIT film

Chitosan, as an amine-rich polysaccharide derived by deacetylation of chitin, has many primary amino groups with pK_a values of about 6.3, which results in its unique water solubility. When the solution pH is less than 6, the amino group of CHIT will combine with H^+ to form $-NH_3^+$, making chitosan to be a water-soluble cationic polyelectrolyte (Sheng et al., 2008). Contrarily, at pH above the pKa, chitosan's amino groups are deprotonated, leading to insolubility of this polymer in water. Therefore, when the cationic polyelectrolyte CHIT-modified electrode is immersed into the TNTs solutions, TNTs can be assembled on the surface of CHIT due to

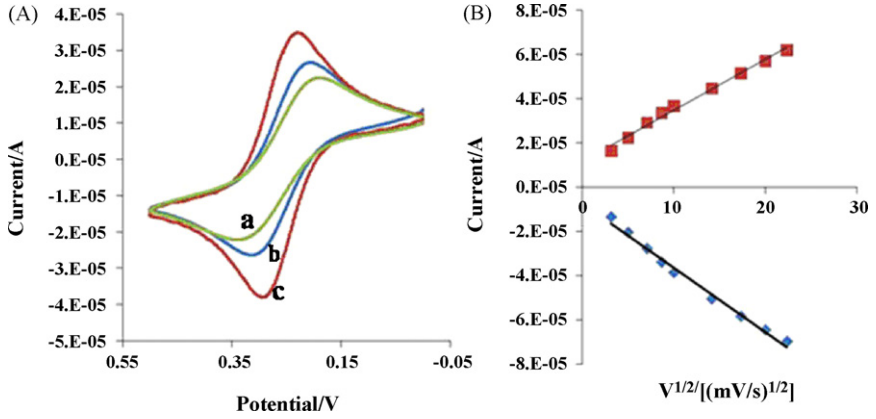

Fig. 2. Cyclic voltammograms at different modified glassy carbon electrodes (A): (a) CHIT-modified GCE; (b) TNT/CHIT-modified GCE; (c) bare GCE. Supporting electrolyte: 1.0 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ + 0.5 M KCl; scan rate, 50 mV s^{-1}. Relationship of scan rate and current intensity for the TNT/CHIT-modified GCE (B): supporting electrolyte: 1.0 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ + 0.5 M KCl; scan rate, 10, 25, 50, 75, 100, 200, 300, 400, 500 mV s^{-1}.

the electrostatic effect. In addition, amino groups of CHIT would be helpful in the deposit of TNTs on the modified electrode.

3.1.3. Electrochemical behavior of the modified electrode

Fig. 2A shows the cyclic voltammograms (CVs) of ferri/ferrocyanide solution at different modified electrodes (versus Ag/AgCl reference electrode). Well-defined redox peaks of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ couple were observed at the bare GCE (see curve (c) in Fig. 2A). Obvious decreases in peak currents and reversibility of electrode processes or an increase in peak potential separation (ΔE_p) were observed when the electrode was modified with CHIT (see curves (a) in Fig. 2A), suggesting that the modified CHIT film might act as a barrier to the interfacial electron transfer, although there was electrostatic attraction between cationic CHIT and ferri/ferrocyanide. However, when the TNTs were further coated on the CHIT-modified electrode, recoveries in peak currents and the electrochemical reversibility of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ were found (see curve (b) in Fig. 2A). By the Randles–Sevcik equation (Bard and Faulkner, 2001), the apparent electroactive surface area of TNTs/CHIT/GCE was calculated to be 0.180 cm^2, which was larger than that case of the CHIT/GCE (0.1413 cm^2). These experimental results indicated that TNTs played an important role in elevating mass and electron transfer of ferri/ferrocyanide anions in CHIT-modified electrode.

The effect of scan rate on the redox peak currents of ferri/ferrocyanide was shown in Fig. 2B. It can be seen from Fig. 2B that the peak currents are linear with the square root of scan rate, which indicates that the redox processes of ferri/ferrocyanide anions at the TNTs/CHIT/GCE are diffusion-controlled and there is fast electron transfer at the surface of TNTs.

3.1.4. Effect of TNTs assembling time on ECL emission

It was found that the ECL response of luminol was affected significantly by the amount of immobilized TNTs that could be easily controlled by assembling times. When TNTs were assembled on the CHIT based matrix, the ECL intensity of luminol was enhanced greatly with assembling time within 1 h, and tended to decrease with further increasing of assembling time. It may be explained as follow: TNTs have great promotion for ECL reaction of luminol and thus ECL will be increased with the amount of TNTs in an appropriate concentration range, however, on the other hand, abundant negative charges carried by TNTs may cause strong electrostatic repulsion between TNTs and luminol, which tends to prevent the ECL reaction of luminol on the modified electrode, and thus too much immobilized TNTs will result in the decrease of luminol ECL. Therefore, 1 h was selected as the self-assembling time in subsequent work.

3.1.5. ECL sensing behaviors of TNTs/CHIT GCE for hydrogen peroxide

It is well known that many redox enzymatic reactions may produce hydrogen peroxide. Meanwhile, trace hydrogen peroxide can enhance the ECL of luminol, based on which a series of ECL enzyme biosensors maybe designed for detection of those substrates that produce hydrogen peroxide under the catalysis of their corresponding enzymes. Apparently, it is necessary to investigate ECL sensing behaviors of TNTs/CHIT GCE for hydrogen peroxide before the construction of the ECL enzyme biosensors.

At the TNTs/CHIT composited film, the linear response for hydrogen peroxide was in the range of 1.0×10^{-9}–5×10^{-5} mol/L with detection limit of 8.5×10^{-10} mol/L. Moreover, the ECL response of this sensor displayed a good reproducibility with RSD of 1.12% at the concentration of 1×10^{-6} M H_2O_2 in 2.0×10^{-5} M luminol solution ($n = 15$). After stored at 4 °C for 4 weeks, the ECL responses of the sensor was not significantly changed, such reproducibility and stability could be acceptable for most practical applications.

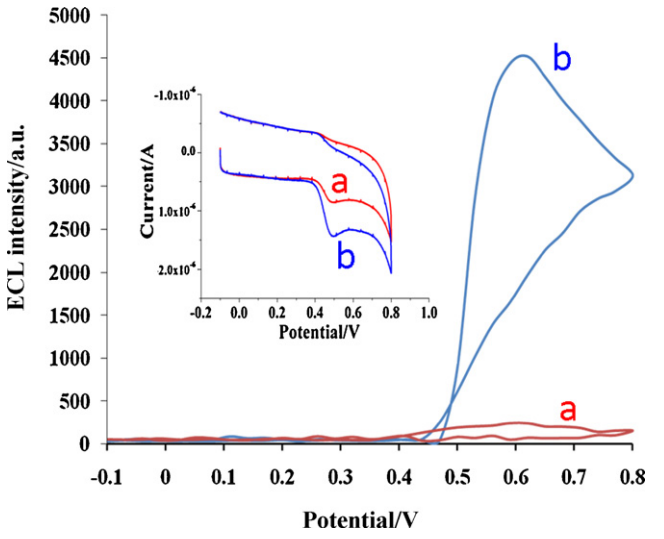

Fig. 3. Cyclic voltammograms (inset) and ECL response of luminol with (b) and without (a) 1×10^{-4} M choline on the ChOD/TNTs/CHIT-modified GCE in the 0.1 M phosphate buffer solution (pH 8.0), scan rate: 60 mV/s.

3.2. Development of an ECL enzymatic biosensor for choline

3.2.1. Electrochemical and ECL behavior of the biosensor in the presence of choline

The aim of this work is to achieve a new enzymic ECL biosensor for the detection of choline on the basis of luminol–H_2O_2 ECL system, according to the following reactions (Godoy et al., 2005):

$$\text{Choline} + H_2O + 2O_2 \xrightarrow{\text{ChoD}} \text{betaine} + 2H_2O_2 \quad (1)$$

$$\text{Luminol} + 2H_2O_2 \rightarrow \text{3-aminnophtalate} + N_2 + 3H_2O + h\nu(\lambda_{max} = 425\,\text{nm}) \quad (2)$$

The root mean square (RMS) roughnessis of the 3D images of the AFM image (Fig. 1C) is 110 nm, which suggests that ChOX has been well immobilized at the surface of the modified electrode (Gooding et al., 2003). Fig. 3 showed the CV (inset) and ECL behavior of luminol at the enzymatic biosensor in 0.1 mol/L phosphate buffer (pH 8.0) with and without choline. When certain amount of choline was added into phosphate buffer solution containing 2×10^{-5} mol/L luminol, the oxidation current (see inset of Fig. 3) and ECL intensity of luminol was obviously increased. Choline was oxidized into betaine and hydrogen peroxide by choline oxidase in the presence of oxygen; hydrogen peroxide was finally involved in the ECL reaction of luminol and emitted the light signal, which was proportional to concentration of choline in a definite range. Also, such produced hydrogen peroxide could react with luminol radicals, resulting in the larger oxidation current.

3.2.2. Optimum conditions for ECL biosensor

It is well known that luminol CL reaction is more efficient in alkaline medium. But the higher pH may lead the enzyme to lose its activity. Therefore, the ECL intensity is undoubtedly influenced by the pH of buffer solution.

The effect of pH on the performance of the biosensor was investigated in the range of 7.0–9.0, and the results showed that the enhanced ECL intensity of luminol was increased with the increasing of pH in the range of 7.0–8.0, reached the maximum at pH 8.0, and then was gradually decreased beyond pH 8.0. Therefore

pH 8.0 was chosen as the optimum pH for subsequent experiments. Moreover, considering the self-quenching effect and severe electrode contamination of high concentration luminol in solution, 2×10^{-5} M luminol was selected throughout the experimentation.

The dependence of ECL intensity on the potential scanning range was investigated. The initial potential were set at −0.1 V (versus Ag/AgCl) for all experiments, and the terminal potential was varied from 0.6 to 1.2 V (versus Ag/AgCl). The results showed that when the terminal potential was in the range of 0.6–0.8 V, the maximal ECL intensity could be obtained, beyond this range the ECL intensity would be decreased. Therefore, the scanning potential was selected in the range of −0.1 to 0.8 V.

Under the mode of CV, the effect of scanning rate on the ECL behaviors of biosensor for luminol/choline system was examined, and the result showed that the ECL intensity was increased when the scan rate was below 60 mV/s, and the ECL intensity reached the maximum at 60 mV/s, and then decreased with increasing of scanning rate. It is well known that the rate of generation/annihilation of excited state species during electron transfer process and diffusion rate co-reactant in the ECL system are the two main factors to decide the ECL efficiency (Zu and Bard, 2001; Zhang and Bard, 1988; Dai et al., 2009; Miller et al., 1991; Obeng and Bard, 1991). Usually, the ECL response increases with the increased applied scan rate due to the more easily reactions between luminophor and the co-reactant to the electrode interface. However, too high scan rate might be unfavorable for electrochemical oxidation of both luminophor and its co-reactant and the reaction between excitated luninophor and co-reactant. It ultimately results in the instability and decrease of the response of ECL system. Thus, 60 mV/s was selected as the scanning rate in subsequent experiments.

3.2.3. Linear response range and detection limit for choline

Under the optimization condition, when series concentrations of choline were added in the phosphate buffer solution containing 2×10^{-5} mol/L luminol, the ECL intensity of luminol was increased proportionally (see Fig. 4). A calibration curve shown in the inset of Fig. 4 was obtained for the determination of choline in aqueous solution. The ECL intensity is linear with the concentration of choline in the range of 1.0×10^{-7}–5×10^{-4} mol/L with a detection limit of 1×10^{-8} mol/L. The regression equation can be shown as below,

$$Lg\, I_{ECL/(a.u.)} = 5.232 + 0.394\, Lg\, C_{choline/(mol/L)}; \quad R^2 = 0.9930$$

where I_{ECL} is the ECL intensity, C is the concentration of choline.

3.2.4. Reproducibility and stability of the ECL biosensor

It was found that there was no significant change of response for the ECL intensity of luminol when the ECL biosensor was repetitively cycled for 15 times (see inset of Fig. 4). The reproducibility of ECL intensity obtained at the enzyme/TNTs/CHIT-modified electrode (RSD = 1.33% at the concentration of 1×10^{-4} M choline in 2.0×10^{-5} M luminol solution) was obviously better than that observed at the enzyme/CHIT-modified electrode (RSD = 2.18%), suggesting that the biocompatible TNTs play an important role in ensuring a good reproducibility of the enzymatic biosensor. This could be attributed to the fact that TNTs provided a super hydrophilic and aqueous-like microenvironment for the immobilized enzyme.

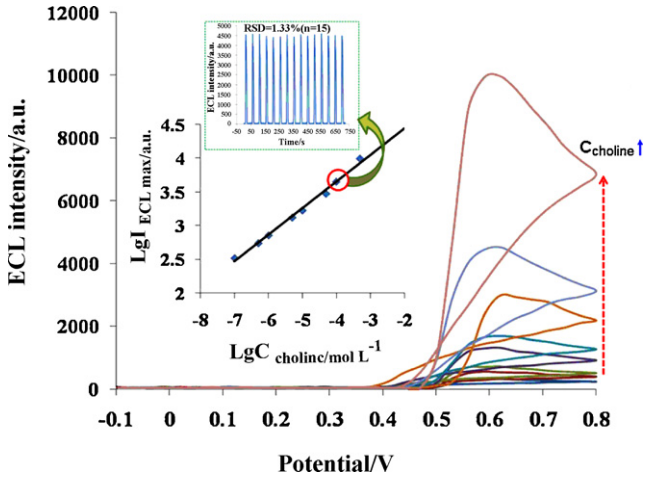

Fig. 4. ECL curves with various concentration of choline on the enzymatic biosensor: 1.0×10^{-7} M, 5.0×10^{-7} M, 1.0×10^{-6} M, 5.0×10^{-6} M, 1.0×10^{-5} M, 5.0×10^{-5} M, 1.0×10^{-4} M, 5.0×10^{-4} M (from bottom to top). Inset: linear relationship between ECL intensity and the concentration of choline. The reproducibility of the ECL biosensor [luminol] = 2×10^{-5} M; [choline] = 1×10^{-4} M; 0.1 M PBS buffer solution (pH 8.0); scan rate: 80 mV/s.

The long-term storage stability of the developed ECL biosensor was also studied for over 20 days by monitoring its ECL response to 1×10^{-4} mol/L choline in phosphate buffer solution (pH 8.0) with an intermittent usage and storage at 4 °C when it was not in use. It was found that the response of the ECL biosensor gradually decreased to almost 85% of its initial value within 15 days. However, the response of such choline biosensor in the absence of TNTs obviously decreased to only 63% of its initial response within the same period. Such result just again exhibited the well biocompatibility of TNTs.

In the same preparation of the choline ECL biosensor, involving five different GCE modifications, reproducibility within a mean value of about +15% was generally achieved for the ECL determination of 1.0×10^{-4} mol/L of choline.

3.2.5. Determination of choline in milk sample

The applicability of the choline ECL biosensor was assessed by the determination of choline concentration in milk hydrolysate samples utilizing standard addition method. For accurate analysis of each sample, five replicate measurements were performed under the optimum condition, and the results are shown in Table 1. The recovery assays were also performed for evaluating this method, and the recoveries were found to be in the range of 96.2–107%.

The concentration of total choline in the milk samples was 0.98 ± 0.05 mM ($n = 5$). The results obtained for each sample were not significantly different from those obtained by a Richard salt colorimetry (Richard et al., 1945), according to a proper t-test at the 95% confidence level. Such result validated the proposed ECL biosensor possessed good and reliable analytical performance for choline. The TNTs based hybrid system, which improved the

Table 1
Results of determination of choline in milk sample ($n = 5$).

Sample no.	$C_{choline}$ (mol/L)			Recovery (%)	Choline in sample	Average concentration of choline in milk sample (mol/L)	
	Choline in diluted sample	Choline added	Measured after adding			The proposed ECL biosensor	Richard salt colorimetry
1	1.00×10^{-6}	1.0×10^{-7}	$(1.176 + 0.027) \times 10^{-6}$	106.9	1.00×10^{-3}	$(9.87 + 0.13) \times 10^{-4}$	9.06×10^{-4}
2	9.73×10^{-6}	1.0×10^{-6}	$(1.028 + 0.020) \times 10^{-5}$	95.8	9.73×10^{-4}		
3	9.86×10^{-6}	1.0×10^{-5}	$(1.920 + 0.017) \times 10^{-5}$	96.7	9.86×10^{-4}		

biocompatible microenvironment of sensing interface, also played a satisfactory role in interfacing biological recognition events with electronic signal transduction. Therefore, this TNTs contained hybrid sensing interface, as one of new bioelectronic devices with synergistic properties originating from the components of the nano-size materials, met the increasing need for highly sensitive, reliable, reproducible and low-cost methods for on-site monitoring of choline.

4. Conclusions

A new biocompatible ECL biosenor based on enzyme/TNTs/CHIT composite film was first reported. TNTs used in the biosensor not only provided a biocompatible microenvironment for the immobilized enzyme but also strongly enhanced luminol ECL, leading to the development of a new ECL biosensor with excellent stability and high sensitivity. The ChOX/TNTs/CHIT film-based ECL biosensor has been developed and successfully applied for detecting choline in milk samples. The present work was envisioned to provide a common platform to develop various sensitive, selective and biocompatible ECL biosensors based on using enzyme/TNTs/CHIT composite. The flexibility and biocompatibility in the fabrication step presented here make it possible to incorporate other biological recognition elements into the composite film so as to further perfect the electrochemiluminescent sensing interface.

Acknowledgements

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