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Electrochemiluminescence immunosensor using poly(L-histidine) protected glucose dehydrogenase on Pt/Au bimetallic nanoparticles to in situ generate co-reactant

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In this work, Pt/Au bimetallic nanoparticles (Pt/Au NPs) were used as nanocarriers to develop an electrochemiluminescence (ECL) immunosensor for sensitive cardiac troponin I (cTnI) detection coupling with enzyme-based signal amplification. Firstly, gold nanoparticles modified $Ru(phen)_3^{2+}$-doped silica nanoparticles ($Au@RuSiO_2$ NPs) with numerous luminophor inside were used as platform, potentially increasing the signal intensity. Secondly, Pt/Au NPs with large surface area and rich surface atoms were a superior matrix for the immobilization of numerous antibody (Ab_2), poly(L-histidine) (PLH) and glucose dehydrogenase (GDH). More importantly, the PLH protected GDH exhibited excellent enzymatic activity for the oxidation of glucose accompanying with the reduction of NAD^+ into NADH. The in situ generated NADH acted as co-reactant of $Ru(phen)_3^{2+}$ significantly enhance the ECL signal. In this way, the designed immunosensor displayed high sensitivity for the detection of cTnI in the range from 0.010 ng mL^{-1} to 10 ng mL^{-1} with a detection limit of 3.3 pg mL^{-1} (S/N=3). The proposed strategy held a new promise for high sensitive bioassays applied in clinical analyses.

Introduction

Cardiac troponin I (cTnI), a major troponin complex presented in cardiac muscle tissues, has been widely recognized as a principle diagnostic biomarker for the monitoring of acute myocardial infarction (AMI) due to its excellent specificity and great sensitivity[1-3]. The cTnI levels are normally lower than 0.4 ng mL^{-1} in healthy human and the levels greater than 2.0 ng mL^{-1} demonstrate an increased risk for future serious heart events[4-6]. To date, quantitative detection of cTnI mainly relies on immunoassay-based protocols such as enzyme-linked immunosorbent assay[7], immunochromatographic tests[8], radioimmunoassay[9] and fluorescence immunoassay[10]. However, most of these analytical methods are limited by the sensitivity, complex operating procedures, long diagnostics times and cost equipments. Hence, looking for a cost-effective and simple method for rapid detection of cTnI at lower concentration is urgent. Electrochemiluminescence (ECL), a new analytical method developed in recent years, has attracted considerable interest due to its intrinsic merits such as simple operation, low cost, wide dynamic range and high sensitivity[11-13]. Therefore, ECL immunoassay is of great interest for highly sensitive cTnI determination.

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Noble-metal nanoparticles, because of their unique optical, electronic and catalytic properties, have attained widespread use in catalysis, nanophotonics, nanomedicine, chemical and biological sensing[14]. Among them, bimetallic nanoparticles are particularly attractive because they can not only incorporate the properties of the monometallic counterparts but also exhibit superior physical and chemical properties due to their much better flexibility in local electric field and nanometer-scale structure[15,16]. It is reported that bimetallic nanoparticles were highly attractive platforms for biosensor construction by the virtue of their large specific surface area, rich surface atoms, excellent conductivity and fascinating catalytic properties[17-20]. They can also provide a favorable microenvironment to retain the activity of enzyme and make it easier for the electron transfer of enzymatic reaction[21]. In addition, it has been reported that platinum nanoparticles (Pt NPs) and gold nanoparticles (Au NPs) could enhance the electrochemical regeneration of NADH[22,23]. Therefore, it is conceivable that Pt/Au bimetallic nanoparticles (Pt/Au NPs) can present an efficient platform for protein immobilization and lead a great amplification to the ECL of ruthenium (II) complex due to its efficient electron transport capacity and excellent catalytic properties. All this merits make Pt/Au NPs a promising material for the development of favorable ECL biosensors.

Co-reactant is often introduced to ECL biosensors for signal amplification. Among them, tripropylamine (TPA) is one of the

most used co-reactant in $Ru(phen)_3^{2+}$-based ECL biosensors[24,25]. However, TPA is poisonous and highly volatile to some extent and the oxidation of TPA on the electrode surface is not as fast as desired. In consideration of reducing environmental pollution and improving ECL efficiency, NADH is an excellent alternative to TPA for the reason that NADH can be in situ generated from some biologically active substances with the catalysis of corresponding enzymes[26,27]. Glucose dehydrogenase (GDH), as a glucose oxidase, can catalyze the conversation of glucose into gluconolatone with concomitant reduction of NAD+ to NADH which can co-react with $Ru(phen)_3^{2+}$ to enhance the ECL intensity. The mechanisms to the ECL of $Ru(phen)_3^{2+}$-NADH system are as follows[28,29]:

$$\text{Glucose} + NAD^+ \xrightarrow{\text{GDH}} \text{Gluconolatone} + NADH + H^+ \quad (1)$$

$$Ru(phen)_3^{2+} - e \rightarrow Ru(phen)_3^{3+} \quad (2)$$

$$NADH + Ru(phen)_3^{3+} \rightarrow NAD+ + Ru(phen)_3^{2+*} \quad (3)$$

$$Ru(phen)_3^{2+*} \rightarrow Ru(phen)_3^{2+} + h\nu \quad (4)$$

According to the mechanisms, the ECL amplification efficiency is highly dependent on the amount of immobilized enzyme and the enzyme activity. Therefore, exploiting a method for immobilizing abundant enzyme and retaining its activity is very necessary for sensitive bioassay. Recently, He and coworkers[30] showed clearly and interestingly that enzymatic activity of poly(L-histidine) (PLH) hydrochloride-protected "sandwiched-type" glucose oxidase was significantly enhanced as compared with free glucose oxidase. Inspired by this, PLH, a kind of cationic polyelectrolytes, was used in this work to improve the enzymatic activity of the GDH. To the best of our knowledge, integration of Pt/Au NPs as nanocarrier and enzymatic reaction to in situ generate NADH as co-reactant have received little attention in ECL biosensors so far.

In our work, Pt/Au NPs were used as nanocarrier to construct a sandwich-type ECL immunosensor for high sensitive cTnI detection based on the enzymatic reaction of GDH to in situ generate NADH as co-reactant. The signal enhancement of the proposed immunosensor was accomplished by the following ways: on the one hand, gold nanoparticles modified $Ru(phen)_3^{2+}$-doped silica nanoparticles (Au@$RuSiO_2$ NPs) as platform provided numerous luminophor for the enhancement of ECL signal. On the other hand, Pt/Au NPs with large specific surface area and excellent conductivity facilitated the immobilization of Ab_2 and GDH. Moreover, the PLH protected GDH with superior enzymatic activity effectively catalyzed the oxidation of glucose with the concomitant production of NADH in the presence of NAD^+. The in situ generated NADH acted as a co-reactant of $Ru(phen)_3^{2+}$, resulting in significant ECL signal amplification and highly sensitive ECL detection. Thus, under the optimal experimental conditions, the proposed immunosensor exhibited high sensitivity for quantitative analysis of cTnI and showed great potential in clinical applications.

Experimental

Reagents

Dichlorotris(1,10-phenanthroline) ruthenium hydrate ($Ru(phen)_3Cl_2 \cdot H_2O$), β-nicotinamide adenine dinucleotide hydrate (NAD^+), glucose dehydrogenase (GDH) from pseudomonas ap., poly(L-histidine) (PLH) hydrochloride ($Mw \geqslant 5000$), bovine serum albumin (BSA), gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$) and chitosan (CS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N-ethyl carbodiimidehydrochloride (EDC) were purchased from Shanghai Medpep Co. Ltd. (Shanghai, China). Highly purified human cardiac troponin-I antigen (cTnI) and mouse anticardiac troponin-I monoclonal antibody (anti-cTnI) were obtained from Shanghai HuaYi BioTechnology Co. Ltd. (Shanghai, China). Carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), mouse IgG (IgG) and carbohydrate antigen 15-3 (CA15-3) were bought from Biocell Company (Zhengzhou, China). All other chemicals were of reagent grade and used as received. Phosphated buffered solutions (PBS, pH 7.4, 0.1 M) were prepared using 0.1 M KH_2PO_4, 0.1 M Na_2HPO_4 and 0.1 M KCl. Serum specimens were obtained from Daping Hospital of Third Military Medical University (Chongqing, China). Double distilled water was used throughout this study.

Apparatus

The ECL emission was monitored by a model MPI-A electrochemiluminescence analyzer (Xi' an Remax Electronic Science & Technology Co. Ltd., Xi' an, China). The voltage of the photomultiplier tube (PMT) was set at 800 V and the applied potential was 0.2-1.25 V (vs. Ag/AgCl) with a scan rate of 100 mV s^{-1}. All the ECL experiments were carried out with a three-electrode setup, which consisted of an Ag/AgCl (sat. KCl) as reference electrode, a platinum wire as counter electrode and a prepared glassy carbon electrode (Φ=4 mm) as working electrode. Electrochemical impedance spectroscopy (EIS) measurements were performed with a CHI 660A electrochemical workstation (Shanghai Chenhua Instrument, China). The morphologies of nanoparticles were estimated from scanning electron microscopy (SEM, S-4800, Hitachi, Japan), transmission electron microscope (TEM, TECNAI 10, Philips Fei Co., Hillsboro, OR). The Ultraviolet-visible (UV-vis) absorption spectrum was recorded with an UV-vis spectrophotometer (UV-2450, Shimadzu, Japan).

Synthesis of gold nanoparticles modified $Ru(phen)_3^{2+}$-doped silica nanoparticles (Au@$RuSiO_2$ NPs)

The uniform $Ru(phen)_3^{2+}$-doped silica nanoparticles ($RuSiO_2$ NPs) were synthesized by using the Stöber method as previously described with some minor modifications[31]. Typically, 1.0 mL of TEOS and 500 μL of 0.1 M $Ru(phen)_3^{2+}$ aqueous solutions were premixed with ethanol. After 10 min vigorously stirring, 1.0 mL ammonium hydroxide (25%) was added into the mixture. And then the solution was kept stirring for 1 h in the dark and further sonicated for 10 min before centrifugation. Subsequently, the $RuSiO_2$ NPs were isolated by acetone, and followed by centrifuging (5000 rpm, 8 min) and washing with ethanol and double distilled water several times to remove any surfactant molecule. The precipitates were redispersed into double distilled water to obtain yellow $RuSiO_2$ NPs colloid.

Then, the surface of the above $RuSiO_2$ NPs suspension (2.0 mL) was chemically functionalized by adding 1mL of BSA which could not only be absorbed on $RuSiO_2$ NPs surface through the hydrogen bonding between the -OH groups of $RuSiO_2$ NPs and the $-NH_2$ groups in BSA , but also link with the gold nanoparticles (Au NPs) via strong Au-S bonding[32]. Then, the modified $RuSiO_2$ NPs were added to 12.0 mL of Au NPs solution and the mixture was shaken for 6 h. Finally, These Au@$RuSiO_2$ NPs were collected by centrifugation and redispersed in double distilled water. Then, 2 mL of the as-prepared Au@$RuSiO_2$ NPs was mixed with 0.25*wt %* chitosan (CS) and sonicated to obtain homogeneous suspension (abbreviated as Au@$RuSiO_2$ NPs/CS). The preparation procedures of Au@$RuSiO_2$ NPs/CS were shown Fig. S1. And the prepared Au@$RuSiO_2$ NPs were characterized by UV-Vis spectra and SEM (see Electronic Supplementary Information, Fig. S2).

Synthesis of Pt/Au bimetallic nanoparticles (Pt/Au NPs)

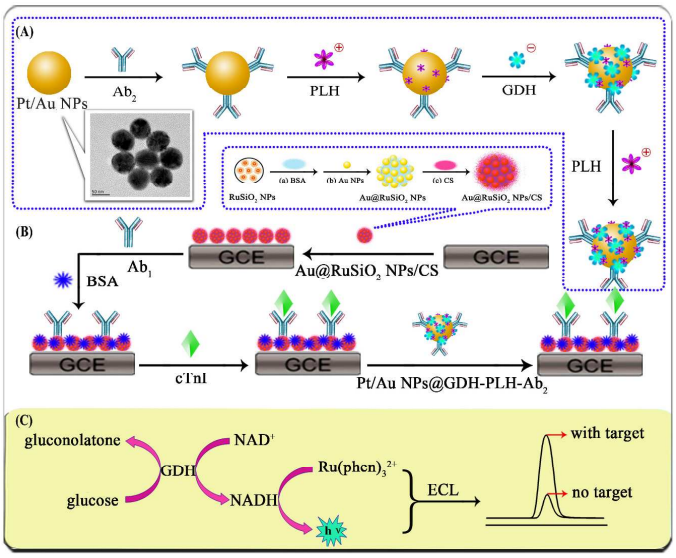
The Pt/Au NPs were synthesized according to a previous method with slight modifications[33]. Firstly, gold colloid was synthesized by sodium citrate reduction of $HAuCl_4$ according to the Frens method[31]. Subsequently, 30.0 mL of the prepared gold colloids as a seeding solution was mixed with 21.0 mL of 10.0 mM H_2PtCl_6 under magnetic stirring. And then 8.0 mL of 0.1 M freshly prepared ascorbic acid was added slowly. Within 30 min, the red color of the gold colloid changed to the dark brown color. The product was purified with centrifugation (8000 rpm, 10 min) and redispersed in 2.0 mL of 0.1 M PBS (pH 7.4). The resulted Pt/Au NPs were characterized by TEM (Scheme 1(A), inset).

Preparation of Ab_2, PLH and GDH multi-labeled Pt/Au bimetallic nanoparticles (Pt/Au NPs@GDH-PLH-Ab_2 biocon-jugates)

The Pt/Au NPs@GDH-PLH-Ab_2 bioconjugates were synthesized according to the following steps (scheme 1(A)). Firstly, the Ab_2 solution (200 μL) was slowly added into 2.0 mL of the prepared Pt/Au NPs under softly stirring and incubated for 8 h at 4 °C, followed by adding 50 μL of poly(L-histidine) (PLH) hydrochloride (0.5 mg mL^{-1}). The pH of the solution was adjusted to 6-7. Through a charge-charge interaction, the positively charged peptide layer, PLH (pI=7.59), was adsorbed onto the surface of the Au/Pt NPs. After incubation for 60 min, 200 μL of GDH (10 mg mL-1 in 0.1 M PBS, pH 7.4) was added to the obtained solution and incubated overnight. Subsequently, the mixture was purified by centrifugation and redispersed in 2 mL of PBS (0.1 M, pH 7.4). To raise the stability and enzymatic activity of GDH, another 50 μL of PLH (0.5 mg mL^{-1}) was added into the as-prepared solution at pH 6-7. At last, the mixture was purified by centrifugation and redispersed in 2.0 mL PBS (0.1 M, pH 7.4).

Fabrication of the ECL immunosensor

The fabrication of the proposed ECL immunosensor was shown in Scheme 1(B). Firstly, glassy carbon electrode (GCE, Φ = 4 mm) was carefully polished with 0.3 and 0.05 μm alumina slurries, followed by rinsing thoroughly with double distilled water and sonicating in ethanol, double distilled water separately. Subsequently, 10 μL Au@$RuSiO_2$ NPs/CS was spread on the pretreated electrode to obtain a nature hydrogel layer with abundant amino groups for further immobilization of primary antibody (Ab_1). 400 mM EDC and 100 mM NHS were used as coupling agents to immobilize the Ab_1 on the modified electrode surface with the formation of amide link between the $-NH_2$ groups of CS and the -COOH groups of the antibody molecules. Then 20 μL BSA was dropped onto the electrode and incubated for 1 h to block the remaining active groups. Afterwards, the modified electrode was incubated with 20 μL cTnI for 30 min at 37 °C. At last, as a sandwich format, the obtained electrode was immersed in the Pt/Au NPs@GDH-PLH-Ab_2 bioconjugates solution for immunereaction. The resultant electrode was rinsed thoroughly with double distilled water to remove the nonspecifically bound species after each step. Thus the proposed electrode was ready for measurement. The ECL signal amplification mechanism of the proposed immunosensor was shown in Scheme 1(C).



Scheme 1 The preparation procedure of Pt/Au NPs@GDH-PLH-Ab_2 bioconjugates (A), the illustration of the stepwise preparation of the immunosensor (B) and the ECL signal amplification mechanism (C).

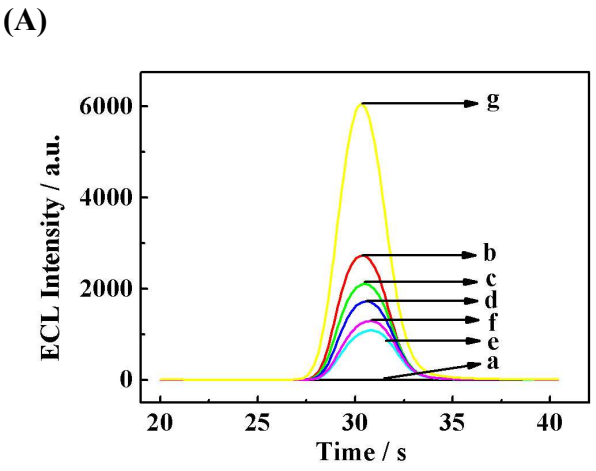
Results and discussion

Characterization of the immunosensor fabrication

The fabrication process of the ECL immunosensor was characterized by ECL in 2 mL PBS solution (0.1 M, pH 7.4). As shown in Fig. 1 (A), almost no ECL signal was observed on the bare GCE (curve a), which was attributed to the lack of luminescence reagent. When Au@$RuSiO_2$ NPs/CS complex was coated on the electrode, an ECL signal was observed (curve b). The main reason could be that great deals of luminophor were entrapped in the Au@RuSiO2 NPs/CS film and the luminophor on the electrode surface ensured improved electronic transmission and high luminous efficiency. After Ab_1 was

successfully immobilized onto the electrode via covalent bound between the active carboxylic groups of the Ab_1 and the amine groups in CS, an obvious decrease in ECL signal was obtained (curve c). The reason was that the protein on the electrode hindered the electron transfer. And the ECL signal further decreased (curve d) when BSA was used to block the remaining active sites. After incubated with cTnI, a successive decline of ECL signal was detected (curve e) due to the hindrance of antigen-antibody immunocomplex. However, when the Pt/Au NPs@GDH-PLH-Ab_2 bioconjugates were introduced to the electrode surface by ''sandwich'' immunoreactions, an increased ECL signal could be observed (curve f). Finally, when 0.01 M glucose and 200 μL NAD^+ (0.25 mM) were added into the ECL detector cell, a greatly stronger ECL signal was obtained (curve g). The reason was that Pt/Au NPs and in situ generated co-reactant NADH had a great amplification to the ECL of $Ru(phen)_3^{2+}$.

Electrochemical impedance spectroscopy (EIS) was also used to evaluate the interfacial changes of the electrode in the fabricated process. Fig. 1 (B) showed the EIS of the proposed electrode for stepwise modification in 0.1 M KCl solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$. Bare GCE showed a small semicircle domain (curve a), which demonstrated the characteristics of a diffusion-controlled electrochemical process. After the electrode was modified with a layer of Au@$RuSiO_2$ NPs/CS complex, an increased electron transfer resistance was observed due to the insulating properties of CS (curve b). Immobilization of Ab1 further increased the impedance value (curve c), which was attributed to inhibited access of the redox probe to the electrode by the insulating antibodies. After blocked with BSA, a successive increase of the electron transfer resistance was detected (curve d). Immunoreaction of cTnI in solution with the anti-cTnI immobilized on the modified electrode surface formed insulating antigen-antibody complex, resulting in a further increase of the electron transfer resistance due to the electrostatic repulsion effect to the negatively charged electrochemical probe (curve e). To gain a better understanding of the fabrication process, the cyclic voltammograms (CVs) experiments were also performed (see Electronic Supplementary Information, Fig. S3). All these results confirmed that the immunosnesor had been successfully fabricated.



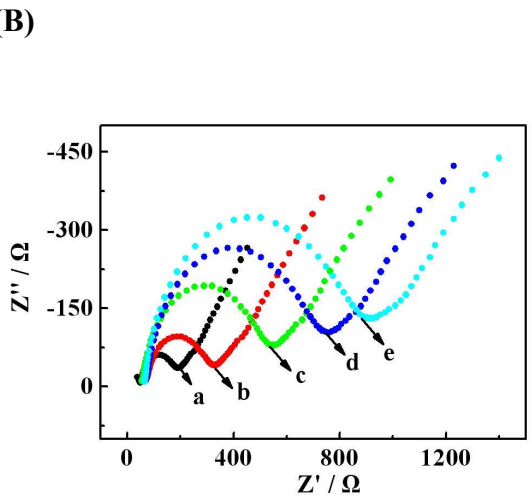


Fig. 1 (A) ECL profiles of (a)bare GCE, (b) Au@$RuSiO_2$ NPs/CS/GCE, (c) Ab_1/Au@$RuSiO_2$ NPs/CS/GCE, (d)BSA/Ab_1/ Au@$RuSiO_2$ NPs/CS/GCE, (e)cTnI/BSA/Ab_1/ Au@$RuSiO_2$ NPs/CS/GCE, (f) Pt/Au NPs@GDH-PLH-Ab_2/cTnI /BSA/Ab_1 /Au@$RuSiO_2$ NPs/ CS/GCE, in 2 mL 0.1 M PBS (pH 7.4), (g)Pt/Au NPs@GDH-PLH-Ab_2/cTnI/BSA/Ab_1/Au@$RuSiO_2$ NPs/ CS/GCE in 2 mL 0.1 M PBS (pH 7.4) containing 0.01 M glucose and 200 μL NAD^+ (0.25 mM). (B) EIS for (a) bare GCE, (b)Au@$RuSiO_2$ NPs/CS/GCE, (c)Ab_1/Au@$RuSiO_2$ NPs/CS/GCE, (d) BSA/Ab_1/ Au@$RuSiO_2$ NPs/CS/GCE, (e) cTnI/BSA/Ab_1/ Au@$RuSiO_2$ NPs /CS/GCE, (f) Pt/Au NPs@GDH-PLH-Ab_2/cTnI/BSA/Ab_1/ Au@$RuSiO_2$ NPs/CS/GCE, in 0.1 M KCl solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$. Scan rate, 100 mV s^{-1}.

Optimization of analytical conditions

The incubation time, which was closely related to the formation of immunocomplex on the electrode surface, had an important impact on performance of the immunosnesor. As shown in Fig. 2 (A), the ECL intensity of the proposed immunosnesor, which incubated with 1.0 ng mL^{-1} cTnI, increased with the increasing incubation time and then reached a constant value after 30 min. Thus, 30 min was chosen as the optimal incubation time for the immunoreaction between anti-cTnI and cTnI in this study.

The concentration of NAD^+ was another important factor for the ECL intensity of the immunosnesor. Fig. 2 (B) illustrated the ECL signal of the proposed immunosnesor at different NAD+ concentrations when incubated with 10 ng mL^{-1} cTnI. The ECL curves corresponding to different volume of NAD^+ were given in the inset. The change of NAD^+ concentration was gained by adding different volume of NAD^+ (2.5 mM) into 2 mL PBS (pH 7.4) solution containing 0.01 M glucose. As a result, the ECL signal increased with the increasing volume of NAD^+ and reached a relatively stable value when the volume of NAD^+ reached 200 μL. Hence, 200 μL was chosen as the appropriate volume of NAD^+ in this experiment.

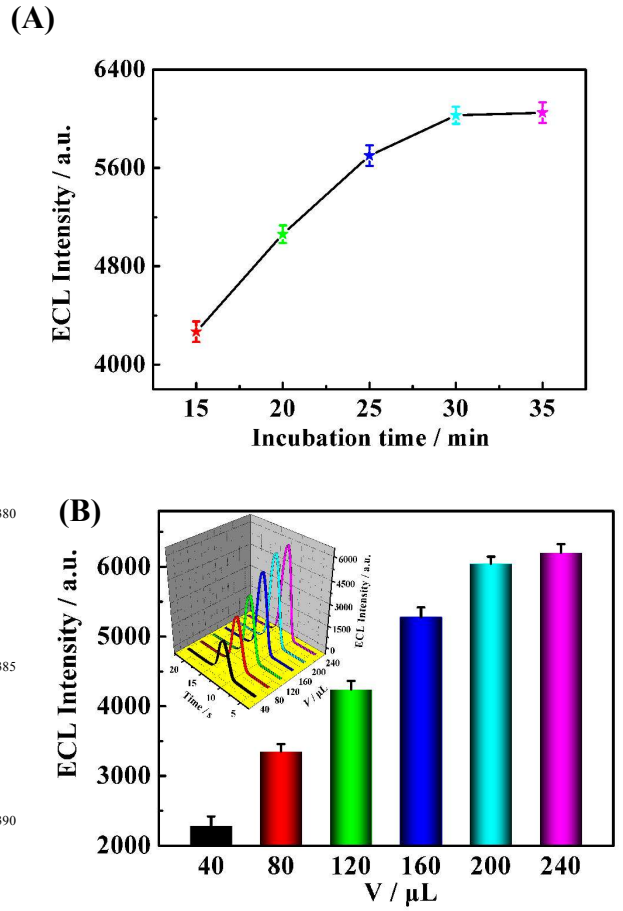


Fig. 2 (A) Effect of incubation time and (B) NAD^+ concentration on ECL signals of the immunosensor when incubated with 1.0 ng mL^{-1} cTnI. Scan rate, 100 mV s^{-1}.

ECL detection of cTnI with the immunosensor

Under the optimized experimental conditions, we explored the quantitative range of the proposed ECL immunosensor in the detection of cTnI. As shown in Fig. 3, the ECL intensity was increased with the increase of cTnI concentration (curve a-g). Fig. 3 (insert) showed that the ECL intensity was linear with the logarithm of cTnI concentrations. The linear equation was I = 6037.50 + 1870.60 log c (where I was the ECL intensity and c was the concentration of cTnI), with a correlation coefficient of 0.9980. The linear range for cTnI was from 0.010 ng mL^{-1} to 10 ng mL^{-1} with an evaluated detection limit of 3.3 pg mL^{-1} (S/N = 3). According to the linear equation, the proposed immunosensor could be applied to detect the concentration of cTnI quantitatively.

As shown in Table S1, the proposed immunosensor exhibited wider response range and much higher sensitivity compared with other cTnI detection methodologies (see Electronic Supplementary Information, Table S1).

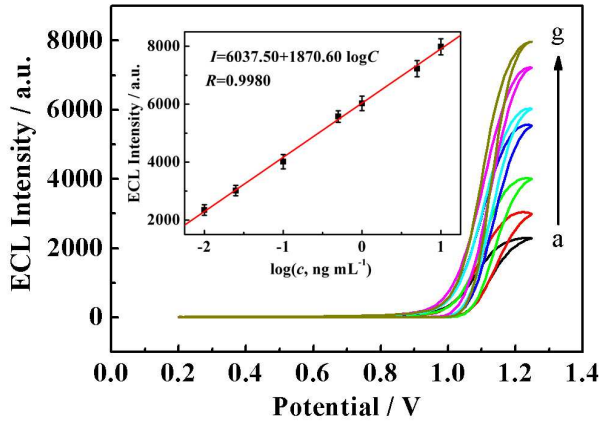


Fig. 3 ECL responses of the proposed immunosensor to different concentrations of cTnI: (a) 0.010 ng mL^{-1}, (b) 0.025 ng mL^{-1}, (c) 0.10 ng mL^{-1}, (d) 0.50 ng mL^{-1}, (e) 1.0 ng mL^{-1}, (f) 5.0 ng mL^{-1}, (g) 10 ng mL^{-1}. Inset: calibration curve for cTnI determination. All ECL signals were measured in 2 mL PBS (pH 7.4) with 0.01 M glucose and 200 μL NAD^+ (0.25 mM).

Selectivity, reproducibility and stability of the immunosensor

In order to investigate the selectivity of the proposed ECL immunosensor, several interference molecules including CEA, AFP, IgG and CA15-3 were chosen for control experiments. Fig. 4 (A) showed that in the presence of the interference molecules, no apparent change in the ECL signals was observed compared with that of the blank test. However, the presence of 1.0 ng mL^{-1} cTnI resulted in a substantial increase in ECL signal. Even when these interfering substances coexisted with cTnI, the ECL response was almost the same with that of cTnI only. This revealed the high specificity and sensitivity of the proposed immunosensor.

The reproducibility of the proposed immunosensor was evaluated by the variation coefficients (ECL signal) of intra- and inter-assays. The relative standard deviations (R.S.D.) both of the intra- and inter-assay were not more than 5.0%, which suggested the reproducibility of the proposed immunoassay was acceptable.

The stability of the ECL signals of this proposed immunosensor to various concentrations of cTnI was presented in Fig. 4 (B). It showed that the ECL intensity increased with the increasing concentration of cTnI, and a relative stable curve at every concentration could be obtained.

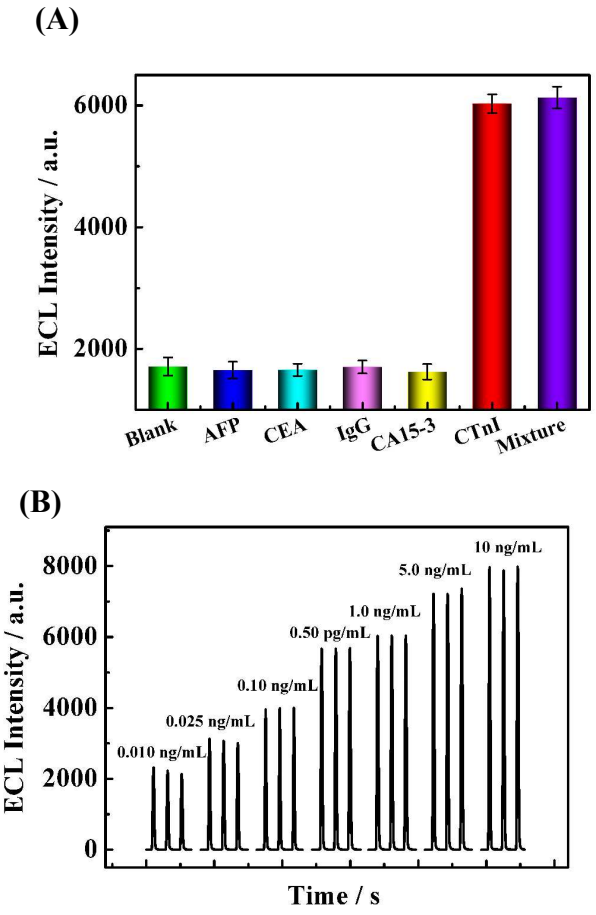


Fig. 4 (A) Selectivity evaluation of the immunosensor against the interference molecules, AFP (20 ng mL^{-1}), CEA (20 ng mL^{-1}), IgG (20 ng mL^{-1}), CA15-3 (20 U mL^{-1}) and their mixing with 1.0 ng mL^{-1} cTnI. (B) ECL stability of proposed immunosensor to various concentrations of cTnI.

Preliminary analysis of real samples

To monitor the feasibility of the developed immunosensor, recovery experiments were performed by standard addition methods in human serum. A series of samples were obtained by adding cTnI of different concentrations into human serum samples. The results were shown in Table 1 and the recovery (between 97.6% and 106%) was acceptable, which indicated that our strategy could be considered as a potential tool for the detection of cTnI in real biological samples.

Table 1 The recovery of the proposed immunosensor in normal human serum.

Sample	Add/ng mL^{-1}	Found/ng mL^{-1}	Recovery/%
1	0.010	0.0098	98.0
2	0.50	0.488	97.6
3	1.00	1.03	103
4	5.00	5.31	106

Conclusions

In summary, a sandwiched ECL immunosensor based on the Pt/Au NPs as nanocarriers and enzymatic reaction to in situ generate NADH as co-reactant has been successfully developed for high sensitive determination of cTnI. The Pt/Au NPs not only provided large surface area for the immobilization of abundant Ab_2 and GDH but also facilitated electrons transfer, thus enhancing the ECL intensity. In addition, the PLH protected GDH exhibited superior enzymatic activities in catalyzing the reduction of NAD^+ to in situ generate NADH as the co-reactant of $Ru(phen)_3^{2+}$, which dramatically amplified the ECL signal. The proposed immunosensor showed excellent performance for the detection of cTnI with high sensitivity, wide linear ranges, and good specificity. Moreover, this method might provide a versatile tool for detecting other biomolecules in bioanalysis and clinical.

Acknowledgements

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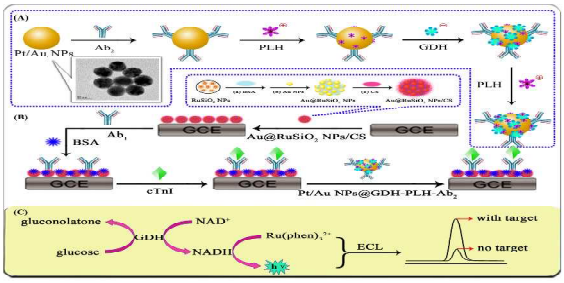

A new ECL immunosensor based on poly(L-histidine) protected glucose dehydrogenase on Pt/Au bimetallic nanoparticles to in situ generate co-reactant