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New Signal Probe Integrated with ABEI as ECL Luminophore and Ag Nanoparticles Decorated CoS Nanoflowers as Bis-Co-Reaction Accelerator to Develop a Ultrasensitive cTnT Immunosensor

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In this work, Ag nanoparticles (Ag NPs) decorated CoS nanoflowers (CoS NFs) were firstly used as bis-coreaction accelerator for signal amplification to establish an highly sensitive electrochemiluminescence (ECL) immunosensor for cardiac troponin T (cTnT) detection. Due to the synergistic catalysis between CoS NFs and Ag NPs, the decomposition rate of the co-reaction reagent H2O2 was largely improved, generating more ROS for signal amplification. Using the N-(4-aminobutyl)-N-ethylisoluminol (ABEI) as ECL luminophore, a new signal tag was achieved according to the assembly of ABEI functionalized Ag NPs (ABEI-Ag) on the CoS NFs via Ag-S bond, which integrated with the ECL luminophore and bis-co-reaction accelerator. In addition, to fabricate sensing interface, primary antibodies (Ab1) was immobilized on the glassy carbon electrode which decorated with Au nanoparticles (Au NPs), almost providing a zero background signal. As a result, this developed immunosensor for cTnT possessed a linear range from 0.1 fg mL\(^{-1}\) to 100 pg mL\(^{-1}\) and the limit of detection down to 0.03 fg mL\(^{-1}\) for ultrasensitive detection of cTnT, which was expected to be applied to cTnT detection in clinic acute myocardial infarction.

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Acute myocardial infarction (AMI), one of the most severe manifestation of coronary disease, leads more than 3 million each year deaths in the world, and more than a third of deaths in developed nations annually. Early diagnosis and treatment can enhance the survival rate of the patients. As available biomarker of AMI, the cardiac troponin T (cTnT) had been reported that its concentration in blood circulation above 3 pg mL\(^{-1}\) represented the apparent risk of AMI. Therefore, a sensitive and simple method for cTnT detection was a preferred strategy for early treatment of AMI. Recently, due to the advantages of simplified optical setup, low background signal, outstanding controllability, low detection limit and high sensitivity, electrochemiluminescence (ECL) has become a powerful analytical tool in the clinical analysis, food safety control, environmental monitoring, etc. Thus, to develop a ultrasensitive ECL method for cTnT detection was of great clinical significance.

During the last few years, much attention has been focused on the sandwich-type biosensors because of their low background noise, high specificity and sensitivity. It was well known the preparation of an efficient signal probe was the key step to construct sandwich-type ECL biosensors. Usually, a variety of nanomaterials have been adopted for luminophores loading and antibody labeling, such as magnetic nanoparticles, porous silica nanoparticles and noble metal nanoparticles. For instance, Cui’s group synthesized luminol functionalized gold nanoparticle (luminol-Au) as the signal probes for the sensitive detection of human immunoglobulin with a detection limit of 1.0 pg mL\(^{-1}\). They used luminol as both reducing agent and protective reagent and the excessive luminol could cover on the surface of Au nanoparticles (Au NPs), constructing an efficient ECL tag in the presence of co-reaction reagent H2O2. It was well known the co-reaction mechanism of luminol-H2O2 system was the ROS (such as O\(_2\)•\(^{-}\), \(\text{OH}\)) generated by the decomposition of H2O2 in the electrochemical process could further react with the luminol to generate more excited state (luminol\(^{+}\)) for emission. Therefore, recent research indicated that the peroxidase could catalyse the decomposition of \(\text{H}_2\text{O}_2\) to achieve a significant improvement of the \(\text{H}_2\text{O}_2\) decomposition rate and a obvious enhancement of ECL response. Although these enzyme-based immunosensors showed good selectivity and high sensitivity, the most common and serious problem with enzymatic immunosensors was insufficient long-term stability, due to the inactivation of the enzyme. To overcome these drawbacks, our groups have proposed nanozymes as co-reaction accelerator to improve the decomposition rate of \(\text{H}_2\text{O}_2\), resulting in the generation of more ROS as the intermediate to react with the luminophore for ECL signal enlargement. For instance, hemin, TiO\(_2\) nanoflowers and Ag nanoparticles (Ag NPs) were acted as co-reaction accelerator to facilitate the rate of ECL reaction between luminophore and its co-reactant. It is well documented that bimetallic catalyst broadened the density of states near the Fermi level, which could cause increasing surface molar ratios of bimetallic catalyst to yield greater catalytic activity than their single-component counterparts. In our previous work, both of Ag NPs and Fe-Fe\(_2\)O\(_3\) NPs were used as bis-co-reaction accelerator to amplify the ECL response of perylene derivatives for the highly sensitive detection of human laminin. However, Fe-Fe\(_2\)O\(_3\) nanoparticles were immobilized on electrode surface, leading to a relatively high background signal. Accordingly, in this work, a zero background signal platform and a high efficient signal probe were constructed to acquire signal amplification for enhancing the sensitivity of cTnT determination.

In this work, a new ECL signal probe was obtained by integrating with N-(4-aminobutyl)-N-ethylisoluminol (ABEI) as ECL luminophore and Ag NPs decorated CoS nanoflowers (CoS NFs) as bis-co-reaction accelerator. As shown in Scheme 1, the CoS NFs were first obtained by a traditional hydrothermal method. Simultaneously, ABEI functionalized Ag NPs (ABEI-Ag) was obtained by ABEI directly reduced AgNO\(_3\) in aqueous solution. Then, the ABEI-Ag was coated on CoS NFs via Ag-S bond linking between Ag and CoS NFs, which realized a high-efficiency signal probe construction. In this study, to fabricate sensing interface, primary antibodies (Ab1) was immobilized on the glassy carbon electrode which was decorated with Au NPs in advance. In the presence of cTnT, a significantly promoted ECL intensity could be obtained since the signal probes of ABEI-Ag/CoS NFs complex labeled cTnT second antibodies (Ab2) were trapped in immunosensor surface. With the use of the novel CoS NFs and Ag NPs as ECL bis-co-reaction accelerator for signal amplification, the ECL immunosensor achieved highly sensitive detection of cTnT in the early diagnosis of AMI. The proposed immunosensor determined cTnT down to 0.03 fg mL\(^{-1}\) within a linear range of 0.1 fg mL\(^{-1}\) to 100 pg mL\(^{-1}\). This method using novel signal probe based on bis-co-reaction accelerator to achieve signal amplification opened a new avenue for the improvement of ECL signal.

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Scheme 1. (A) Preparation of Ab2/ABEI-Ag/CoS NFs; (B) Fabrication of the immunosensor; (C) Reaction mechanism of ECL immunosensor.

Experimental

Apparatus.—The ECL emission was detected by a model MPI-A electrochemiluminescence analyzer (Xi’an Remax Electronic Science and Technology Co. Ltd., Xi’an, China). The voltage of the photomultiplier tube (PMT) was set at 800 V and the potential scan was from 0 to 0.6 V with a scan rate of 200 mV s⁻¹ in the process of ECL detection. A conventional three-electrode system contained Ag/AgCl (saturated KCl) as the reference electrode, a platinum wire as auxiliary electrode and a bare or modified GCE (⌀ = 4 mm) as the working electrode. Cyclic voltammetric (CV) measurement was conducted on a Model CHI 660C electrochemistry workstation (Shanghai CH Instruments, Shanghai, China). Different morphologies of nanocomposites were recorded by scanning electron microscope (SEM, S-4800, Hitachi, Tokyo, Japan) at an acceleration voltage of 30 kV. The ultraviolet-visible (UV–vis) absorption spectra were recorded using a model UV-2450 UV-vis spectrophotometer (Shimadzu, Tokyo, Japan).

Materials and reagents.—N-(4-aminobutyl)-N-ethylisoluminol (ABEI), gold chloride tetrahydrate (HAuCl₄, 99.9%) was obtained from Sigma (St. Louis, MO, USA). Bovine serum albumin (BSA, 96–99%) and cobaltous chloride hexahydrate (CoCl₂·6H₂O) were gotten from Chongqing Chuandong Chemical Co. (Chongqing, China). Silver nitrate (AgNO₃) was supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Human cardiac troponin T (cTnT) ELISA Kit was purchased from North Connaught Biotechnology (Shanghai, China). The phosphate buffer saline (PBS, pH 7.4) was prepared by mixing the solutions of 0.1 M Na₂HPO₄, 0.1 M KH₂PO₄, and 0.1 M KCl. Deionized water (specific resistance of 18.2 MΩ cm) was used throughout the study. All solutions were prepared with deionized water and stored in the refrigerator (4°C).

Preparation of CoS NFs.—CoS NFs were synthesized by a traditional hydrothermal method. Specifically, 15.0 mL L-cysteine (2 mM) solution and 15.0 mL CoCl₂ (2 mM) solution were added simultaneously into a Teflon-lined stainless steel autoclave (50 mL), and then the autoclave was sealed and maintained at 200°C for 8 h followed by cooling down to room temperature. The CoS NFs could be obtained by centrifuging at 10,000 rpm for 10 minutes. Finally, the CoS NFs were washed with ethanol and deionized water for three times, respectively, and then dispersed in deionized water for further use. The preparation process was shown in Scheme 1A.

Preparation of ABEI-Ag and ABEI-Au compounds.—According to some modifications of the previous literature, ABEI-Ag was synthesized using a one-step method with ABEI as reducing agent as Co (Shanghai, China). Silver nitrate (AgNO₃) was supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Human cardiac troponin T (cTnT) ELISA Kit was purchased from North Connaught Biotechnology (Shanghai, China). The phosphate buffer saline (PBS, pH 7.4) was prepared by mixing the solutions of 0.1 M Na₂HPO₄, 0.1 M KH₂PO₄, and 0.1 M KCl. Deionized water (specific resistance of 18.2 MΩ cm) was used throughout the study. All solutions were prepared with deionized water and stored in the refrigerator (4°C).
Firstly, 9 mM ethanol was added into the 7 mM AgNO₃ (3 mM) solution under stirring for 10 min to obtain the mixed solution. Then, 2 mL ABEI (20 mM) solution was added into the above mixed solution and stirred for 12 h at room temperature. The ABEI-Ag compounds could be obtained by centrifuging at 10,000 rpm for 10 min. Finally, the prepared products were washed with ethanol and deionized water for three times, respectively, and then dispersed in 3 mL deionized water for further use. To compare the performance of the different signal probes, the ABEI-Au compounds were also prepared according to the literature with some modification. Firstly, 5 mL ABEI (4 mM) solution was added into the 52 mL HAuCl₄ (0.8 mM) solution for 2 hours while stirring at room temperature. And then, 6 mL ABEI (6 mM) solution was added into the above mixture solution and stirred for 2 hours at room temperature. The ABEI-Au compounds could be obtained by centrifuging at 10,000 rpm for 10 min. Finally, the prepared products were washed with ethanol and deionized water for three times, respectively, and then dispersed in 3 mL deionized water for further use.

**Preparation of different signal probes.**—Firstly, 300 μL ABEI-Ag solution was added into 600 μL CoS NFs solution (1 mg mL⁻¹) for 12 h with shaking to obtain the ABEI-Ag decorated CoS NFs via Ag-S linking between Ag and CoS NFs (26) (the mechanism of the ABEI-Ag coated on CoS NFs was shown in the insert of Scheme 1B). Then, 200 μL Ab₂ (150 ng mL⁻¹) was dropped into the mixture under shaking for another 12 h to bind Ab₂ on the surface of ABEI-Ag/CoS NFs via Ag-S or Ag-N bond. Then, it was soaked in BSA (5%) at 37 °C for 4 h to block nonspecific binding sites. After each fabrication step, the mixture was washed and centrifuged by deionized water to remove uncombined compounds, and finally, the prepared signal probe (Ab₂/ABEI-Ag/CoS NFs) was stored at 4 °C for further use. The preparation process was shown in Scheme 1A. To compare the performance of the different signal probes, Ab₂/ABEI-Au/CoS NFs, Ab₂/ABEI/CoS NFs and Ab₂/ABEI-Ag were also prepared. Replacement of ABEI-Ag with ABEI-Au to prepared Ab₂/ABEI-Au/CoS NFs. Simultaneously, replacement of ABEI-Ag with ABEI to prepared Ab₂/ABEI/CoS NFs by electrical adsorption. Ab₂/ABEI-Ag could be prepared by omitting the step of modifying CoS NFs.

**Fabrication of the ECL immunosensor.**—Before modification, the electrodes are pretreated by conventional methods. The GCE was firstly immersed in the HAuCl₄ solution (1%) with constant potential -0.2 V for 30 s to in situ generate Au NPs on GCE. Then, 20 μL Ab₁ (0.23 ng mL⁻¹) was directly dropped on the Au NPs/GCE for 12 h at 4 °C. Then, BSA (5%) was used to block nonspecific binding sites for 40 min at 37 °C. Ultimately, the prepared Ab₁/AuNPs/GCE was stored at 4 °C for further use. The preparation process was shown in Scheme 1B.

**Measurement procedure.**—For cTnT detection, 10 μL of cTnT standard solution with different concentrations were dropped onto the Ab₁/AuNPs/GCE and incubated for 40 min at room temperature. Then, 10 μL signal probe solution was dropped onto the surface of the cTnT/Ab₁/AuNPs/GCE and incubated for 40 min at room temperature. After each step, the modified electrode was thoroughly cleaned with deionized water to remove the physically absorbed species. Finally, the obtained immunosensor was put in 2 mL PBS containing 5 mM H₂O₂ to detect ECL signal from 0.2 V to 0.8 V with a scan rate of 200 mV s⁻¹.

**Results and Discussion**

**Characterization of CoS NFs and ABEI-Ag/CoS NFs.**—SEM was used to characterize the different nanocomposites at an acceleration voltage of 15–20 kV to investigate the morphologies of CoS NFs and ABEI-Ag/CoS NFs. The Figure 1A showed the typical SEM image of CoS NFs which contained uniform particles with a diameter about 600 nm, and each particle consisted of nanosheets. Compared with CoS NFs, the ABEI-Ag/CoS NFs (Figure 1B) depicted a similar shape but could be obviously observed that large amounts of Ag nanoparticles were embedded uniformly on the surface of CoS NFs, indicating that the ABEI-Ag compounds were attached on the surface of CoS NFs as expectations.

Furthermore, the UV-vis spectra of CoS NFs (curve a), ABEI (curve b), ABEI-Ag (curve c) and ABEI-Ag/CoS NFs (curve d) were showed in Figure 1C. The UV-vis spectra of the synthesized CoS NFs (curve a) showed one peak located around 262 nm which is consistent with the literature report. The characteristic absorption peaks of pure ABEI (curve b) appeared around 290 nm and 320 nm. These are the conjugated K-band absorption peaks of the carbonyl and benzene rings, and the strong B-band absorption peaks caused by the overlap of the π-π* transition and the benzene ring. Compared with the UV-vis absorption spectra of ABEI (curve b), a new absorption around 475 nm was observed in the absorption spectra of ABEI-Ag (curve c), which was the characteristic surface plasmon resonance (SPR) peak of the Ag NFs, indicating the successful synthesis of Ag NFs. There were three peaks on the UV-vis spectra of the synthesized ABEI-Ag/CoS NFs (curve d). It could be found one peak located at around 475 nm corresponds to Ag NPs, and others around at 290 nm and 320 nm corresponds to ABEI. Meanwhile, the characteristic spectrum of CoS was not observed, which revealed that a large amount of ABEI-Ag was coated on the surface of the CoS NFs to generate a strong signal to mask the signal of the CoS NFs.

X-ray photoelectron spectroscopy (XPS) was performed for the elemental analysis of ABEI-Ag/CoS NFs. Figure 2A showed the XPS survey spectrum of the ABEI-Ag/CoS NFs. The detected elements were Co, S, C and Ag as expected for ABEI-Ag/CoS NFs. The broad peak at the center of 780.7 eV was assigned to Co 2p (Figure 2B) and the peaks at 161.61 and 167.78 eV belonged to N 1s (Figure 2C). In both of these regions, the peaks agreed well with XPS spectral characteristics of CoS NFs reported in the previous literature. Furthermore, the peaks at 399.30 and 293.08 eV belonged to N 1s (Figure 2D), and C 1s (Figure 2E), respectively. Considering that only the ABEI molecule contained the C and N, it gave the evidence that ABEI existed on the surface of CoS NFs. Additionally, the doublet at 368.18 and 374.18 eV were belonged to Ag 3d (Figure 2F), which indicated the existence of Ag NPs. Based on the above illustration, we could confirm the successful preparation of ABEI-Ag/CoS NFs.
CV and ECL characterization of the immunosensor.—To indicate the successful construction of immunosensor stepwise, CV and ECL were performed. As shown in Figure 3A, a well-defined redox peak was obtained on the bare GCE in [Fe(CN)₆]³⁻/⁴⁻ (curve a). Then the apparently enhanced peak currents (curve b) was obtained after Au NPs was modified on the bare GCE, owing to effective area increment by Au NPs generation. Then, the consecutive decreases of the redox peak currents were occurred from curve c to e when Ab1, BSA, and cTnT were incubated on the surface of AuNPs/GCE due to the electronic transmission hindranced by protein layer. Before and after the resultant electrode (cTnT/BSA/Ab1/AuNPs/GCE) was incubated with the signal probe (Ab2/ABEI-Ag/CoS NFs), the ECL behavior was monitored in 2 mL PBS containing 5 mM H₂O₂, which was displayed in Figure 3B. As shown in Figure 3B curve a, the ECL signal could hardly be measured before the incubation of signal probe, which confirmed the zero background signal could be achieved. However, after incubating signal probe (curve b), the ECL signal significantly increased owing to the luminophore ABEI and bis-co-reaction accelerator was attached to the sensor through specific binding between antigen and antibody.

Optimization the concentration of H₂O₂.—The concentration of H₂O₂ had an important influence on the signal of the immunosensor. Thus, the concentration of H₂O₂ should be controlled by optimizing the concentration of H₂O₂. As revealed in Figure 4A, the ECL intensity increased with the increasement of the concentration of H₂O₂ in the range from 0.05 mM to 7.5 mM. As revealed in Figure 4B, the ECL signal was enhanced with the concentration of H₂O₂ from 0.05 mM to 5 mM and then remained a relatively stable trend. Therefore, 5 mM was selected as the concentration of H₂O₂ for the subsequent research.

Performance comparison of different signal probes.—Contrast experiments were performed under the same condition to evaluate the performance of the immunosensors with different signal probes. The four signal probes were ABEI-functionalized silver
NPs (probe A, abbreviated as Ab2/ABEI-Ag), ABEI-functionalized CoS NFs (probe B, abbreviated as Ab2/ABEI/CoS NFs), ABEI-functionalized silver NPs decorated CoS NFs (probe C, is also the proposed signal probe, abbreviated as Ab2/ABEI-Ag/CoS NFs) and ABEI-functionalized Au NPs decorated CoS NFs (probe D, abbreviated as Ab2/ABEI-Au/CoS NFs). The detection procedures were performed toward the 10 pg mL\(^{-1}\) cTnT under the same circumstance via sandwich format immunoreaction. As shown in Figure 5, the \(\Delta I\) curves were the intensity of the proposed immunosensors (BSA/Ab1/AuNPs/GCE); the \(\Delta I\) curves were the intensity of the proposed immunosensors after the incubation with different signal probes. The changes of the intensity (\(\Delta I\)) were used to estimate the effect of signal amplification (\(\Delta I = I_b - I_a\)). It can be found the \(\Delta I\) of the immunosensor with probe A was 1208.4 a.u. (Figure 5A), which demonstrated that the luminol covered on the surface of Ag NPs was introduced on the modified electrode surface. As depicted in Figure 5B, the \(\Delta I\) of the immunosensor with probe B was 1543.7 a.u., which demonstrated CoS NFs could greatly amplify the ECL signal by providing a facile platform for loading ABEI and as the coreaction accelerator to improve the ABEI signal. Then, the proposed probe C was incubated with immunosensor towards 10 pg mL\(^{-1}\) cTnT, as shown in Figure 5C, the \(\Delta I\) significantly increased to 4955.2 a.u. The reasons for the excellent performance of probe C were as follows. CoS NFs could provided more sites for ABEI and Ag NPs to attach and it can enhance the ECL signal. Simultaneously, the CoS NFs and Ag NPs as bis-co-reaction accelerator broadened the density of states near the Fermi level, which could cause increasing catalytic activity than their single-component counterparts. Meanwhile, to verify that Ag NPs also has catalytic activity, Au NPs was substituted with Ag NPs in probe D, the \(\Delta I\) of the immunosensor with probe D was 2974.4 a.u. (Figure 5D), which demonstrated the Ag NPs could be used as an excellent co-reaction accelerator to improve ECL response. Therefore, Ab2/ABEI-Ag/CoS NFs was chosen as target signal probe.

Figure 5. ECL responses toward different Ab2 signal probes: (A) Ab2/ABEI-Ag, (B) Ab2/ABEI/CoS NFs, (C) Ab2/ABEI-Ag/CoS NFs, and (D) Ab2/ABEI-Au/CoS NFs (ECL intensity of the constructed immunosensor before (a) and after (b) the signal probe; the immunosensor was incubated with 10 pg mL\(^{-1}\) cTnT and measured in 2 mL PBS containing 5 mM H\(_2\)O\(_2\)).
Figure 6. (A) The ECL curves obtained from different concentrations of cTnT antigen, (a) 0.1 fg mL\(^{-1}\), (b) 1 fg mL\(^{-1}\), (c) 10 fg mL\(^{-1}\), (d) 10\(^2\) fg mL\(^{-1}\), (e) 10\(^3\) fg mL\(^{-1}\), (f) 10\(^4\) fg mL\(^{-1}\), (g) 10\(^5\) fg mL\(^{-1}\). (B) The corresponding relationship between the ECL intensity and the concentration of cTnT. (C) Stability of the proposed ECL immunosensor under continuous scanning for 13 cycles under 1 pg mL\(^{-1}\) cTnT and measured in 2 mL PBS containing 5 mM H\(_2\)O\(_2\). (D) Specificity investigation of the proposed immunosensor to cTnT (10 pg mL\(^{-1}\)) by comparing it to different targets: Blank; AFP (1 ng mL\(^{-1}\)); CEA (1 ng mL\(^{-1}\)); MUC1 (1 ng mL\(^{-1}\)); cTnI (1 ng mL\(^{-1}\)); A mixture containing AFP (1 ng mL\(^{-1}\)), CEA (1 ng mL\(^{-1}\)), MUC1 (1 ng mL\(^{-1}\)), cTnI (1 ng mL\(^{-1}\)), and cTnT (10 pg mL\(^{-1}\)).

Analytical performance of the immunosensor.—After different concentrations of the cTnT were incubated on the electrode for 2 h, the proposed immunosensor was detected in PBS containing 5 mM H\(_2\)O\(_2\) to evaluate the analytical performance of the method. As depicted in Figure 6A, the ECL intensity enhanced gradually with the increase of cTnT concentration from 0.1 fg mL\(^{-1}\) to 100 pg mL\(^{-1}\) (curves a-g). As depicted in Figure 6B, the ECL intensity and the logarithm of concentration presented an excellent linear relationship. The linear equation was \(I = 865.464 \lg c + 1574.357\) with the correlation coefficient square of 0.9955, where \(I\) was the ECL intensity and \(c\) was the concentration of cTnT. Additionally, the estimated limit of detection (LOD) was 0.03 fg mL\(^{-1}\). In addition, the performance of the proposed immunosensor was compared with previous reports (Table I). We could see that the proposed immunosensors exhibited better performance with a wider linear range and a lower detection limit for detecting cTnT when compared with previously researches.

The selectivity, stability, and reproducibility of the fabricated ECL immunosensor were also investigated. As shown in Figure 6C, the relative standard deviation (RSD) of the ECL signal (1 pg mL\(^{-1}\) cTnT) after 13 consecutive scans was 4.26%, indicating that the proposed immunosensor had satisfactory stability. In general, specificity and selectivity assay can be studied by artificially selecting proteins that are partially similar in structure, as well as that are present at high concentrations as interferences. In this work, AFP, CEA, MUC1 and cTnT were chosen as the interferences used in the specificity test. cTnI is also a biomarker of acute myocardial infraction and its structure was similar to cTnT, so it was chosen as the interferences used in the specificity test. Besides, although CEA, AFP and MUC1 are the most common tumor markers, their concentration in healthy human serum are usually at ng mL\(^{-1}\) levels, which is far higher than that of cTnT. Thus, they are also used to explore the selectivity of the experiment in this work. As shown in Figure 6D, the ECL signal toward different biomarkers, such as AFP, CEA, MUC1, cTnl, cTnT and their mixture solution. As expected, a negligible ECL signal was obtained from the immunosensor incubated with different interference protein (AFP, CEA, MUC1 or cTnT). While the target cTnT (10 pg mL\(^{-1}\)) and mixture solution containing cTnT depicted a significant ECL signal even its concentration was 100-fold lower than interference protein AFP, CEA, MUC1 or cTnT. This result demonstrated that the fabricated ECL immunosensor had the ability to distinguish the target from complex interference substances, depicting a superior selectivity of this immunosensor. In addition, a RSD for the reproducibility of

| Method                  | Linear range     | LOD       | Ref. |
|-------------------------|------------------|-----------|------|
| Glass fiber membrane platform | 1.0–120 pg mL\(^{-1}\) | 0.87 pg mL\(^{-1}\) | 3    |
| Immunochemistry          | 0.014–8.3 ng mL\(^{-1}\) | 3.0 pg mL\(^{-1}\) | 29   |
| Electrochemical          | 0.32–10 ng mL\(^{-1}\)  | 0.2 ng mL\(^{-1}\) | 30   |
| Electrochemical          | 10–100 pg mL\(^{-1}\)  | 6 pg mL\(^{-1}\) | 31   |
| Fluorescence             | 3.24–963.71 pg mL\(^{-1}\) | 2.21 pg mL\(^{-1}\) | 32   |
| ECL                      | 0.50–4.0 ng mL\(^{-1}\)  | 0.3 ng mL\(^{-1}\) | 33   |
| ECL                      | 10\(^{-4}\)–100 pg mL\(^{-1}\) | 0.03 fg mL\(^{-1}\) | This work |
results by the as-proposed immunosensor were consistent with those by Roche clinical results, which indicated that the lab study and the signal probe, which reduced the background signal and further increased the sensitivity of the sensor. With such design, this immunosensor has good performance, providing a promising potential tool for sensitivity enhancement in bioanalysis and diagnosis.

Table II. Recovery Study Carried out in Human Serum for cTnT Determination.

| Added concentration/fg mL⁻¹ | Mean of Founded concentration ± SD/fg mL⁻¹ | 95% confidence intervals/fg mL⁻¹ | Recovery/% |
|-----------------------------|-------------------------------------------|---------------------------------|------------|
| 1.00                        | 1.041 ± 0.026                             | 1.041 ± 0.03                    | 104.1      |
| 10.0                        | 10.14 ± 0.18                              | 10.14 ± 0.21                    | 101.4      |
| 100                         | 99.13 ± 1.5                               | 99.13 ± 1.73                    | 99.13      |
| 1.00 × 10⁵                  | 982.1 ± 8.9                               | 982.1 ± 10.10                   | 98.21      |

Roche Clinical results were obtained using cobas e601.

this ECL immunosensor was assessed to be less than 5%, indicating that the developed immunosensor has acceptable reproducibility.

Application of the ECL immunosensor in human serum.—To assess the suitability of the manufactured ECL immunosensor, the negative human serum was used to imitate the actual cTnT samples. Specifically, cTnT standard solution was added in the human serum and diluted 10-fold with PBS by standard addition method. Then, these cTnT samples were recovered using a manufactured ECL immunosensor. From Table II, we could see that the obtained recoveries of the serum samples were 104.1%, 101.4%, 99.13%, and 98.21%, respectively. Therefore, the prepared ECL immunosensor has enormous potential applications in the clinical diagnosis of cTnT.

Performance comparison between ECL immunosensors and commercial assays.—Herein, in order to more effectively investigate the feasibility of as-proposed immunosensor, six human serum samples from one normal people and five patients were used which were provided by the Xi nan Hospital (Chongqing, China) and stored at −20°C for further use. Then, 10 μL human serum samples in different concentrations were incubated on Ab1/AuNPs/GCE, respectively. After incubating with signal probe for 40 min, the ECL responses of each concentration were detected in PBS (pH 7.4, containing 5 mM H2O2). It was noted that the same concentration of serum sample was increased the sensitivity of the sensor. With such design, this immunosensor has good performance, providing a promising potential tool for sensitivity enhancement in bioanalysis and diagnosis.

Conclusions

In this work, we fabricated a highly sensitive biosensor for cTnT detection employing bis-co-reaction accelerator strategies with CoS NFs and Ag NPs. This constructed immunosensor had the following advantages: Firstly, the enhanced catalytic activity was achieved by the Ag NPs coted CoS NFs, which could realize signal amplification by improving the decomposition rate of H2O2. Secondly, ECL luminophore and bis-co-reaction accelerator were integrated onto the signal probe, which reduced the background signal and further increased the sensitivity of the sensor. With such design, this immunosensor has good performance, providing a promising potential tool for sensitivity enhancement in bioanalysis and diagnosis.

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