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An Electrochemical Immunosensor Based on AuNPs/ZIF-8/helical Carbon Nanotubes Nanocomposites for the Detection of Cardiac Troponin I

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ZIF-8, one of the metal organic frameworks, has hierarchical structure and high surface area, which can be used as a carrier material for the fabrication of biosensors. However, the poor electrical conductivity hinders its applications in electrochemical biosensors. Here, using the nanocomposites of Au nanoparticles, metal organic frameworks and helical carbon nanotubes, we developted the immunosensor for the detection of cardiac troponin I. The thin films made of Au nanoparticles, ZIF-8 and helical carbon nanotubes were bio-interfaced with antibody for the use of immuno-electrode. The conductivity of electrode surface improved greatly by adding helical carbon nanotubes into ZIF-8. Signal measuremenst were performed by differential pulse voltammetry (DPV) technique. The proposed immunosensor was used to detect cardiac troponin I in the linear range of 0.1-40 ng/mL with a detection limit of 0.04 ng/mL.

Keywords Metal organic frameworks; ZIF-8; Au nanoparticles; Helical carbon nanotube; Cardiac troponin I

1. INTRODUCTION

Acute myocardial infarction is a very terrible disease because it can result in death and disability. The concentration of cardiac troponin I (cTnI) in human serum has been considered to be a critical standard in the diagnosis of acute myocardial infarction [1]. Thus, it is very important for life-saving to detect cTnI quickly and sensitively. Recently, a method of enzyme-linked immunosorbent assay for cTnI [2] has been reported. Numerous biosensors based on surface plasma resonance [3,4],

fluorescence [5,6], chemiluminescence [7,8] have also been developed to quantitatively detect for cTnI. Although these methods possess high sensitivity and selectivity, some shortcomings such as expensive equipment, time-consuming, tedious labeling process, and highly skilled operators hinder their application in the diagnosis and laboratory research. Electrochemical immunosensors have gained significant attention due to its properties of easy-to-miniaturize, low cost, convenient operation, high sensitivity, and low sample volumes [9-11]. Recently, many electrochemical immunosensors for cTnI have been constructed. For example, using carboxylated multi-walled carbon nanotube and whiskered nanofibres, a sandwich-type electrochemical immunosensor for cTnI was developed [12]. In addition, a label-free amperometric immunosensor based on gold nanoparticles and graphene for cTnI was also reported [13]. Although great progress has been made in electrochemical immunosensor, it is still of great significan to seek new research methods for the detection of cTnI. Antibody immobilization would create influences on the performance of immunosensors. Because metal-organic frameworks **(**MOF) possess good features such as chemical stability, loading capacity, high porosity, and large surface area [14], many MOF-based electrochemical immunosensors have been developed and applied in fields of food, environment and clinical testing [15-17]. Unfortunately, inherent characteristics of low conductivity and weak electrical activity hinder their development in the field of electrochemical immunosensor. In order to overcome these shortcomings, on one hand, MOF-based electrochemical immunosensors were fabricated using sandwich strategy [18,19]. For example, using sandwich-type electrochemical immunosensor based on Fe-MOF, Tang and coworkers detected Gal-3 sensitively [20]. On the other hand, various MOF composites were mixed with conductive materials and then modified on the surface of electrode. For example, Hu reported an electrochemical immunosensor based on the use of mixtures of Zn/Ni-ZIF-8-800 and graphene for the determination of monensin in milk [21]. As we all know, linear carbon nanotubes (LCNT) have been widely applied in the field of electrochemical immunosensors [22-25]. However, few papers about helical carbon nanotubes (HCNT) for the application in electrochemical immunosensors were reported. Different from LCNT, HCNT is in a higher energy state, its surface is thus easy-to-chemical modification. In addition, HCNT has a particular 3D-helical structure which lead to high surface area, good electrocatalytic activity and excellent conductivity [26,27]. This motivated us to fabricate an electrochemical immunosensor using HCNT for the detection of cTnI.

In this work, we try to use ZIF-8 and HCNT for the development of cTnI immunosensor. First, homogeneous suspension of ZIF-8, HCNT and chitosan (Chit) was dropped on the surface of electrode. Then, Au nanoparticals (AuNPs) solution was modified on the surface of electrode surface to immobilize anti-cTnI antibodies and improve the conductivity of sensing interface. As a result, a novel label-free electrochemical immunosensor was fabricated to detect cTnI based on ZIF-8@ HCNT composites modified electrode.

2. EXPERIMENTAL

2.1. Reagents and apparatus

Cardiac troponin I and anti-cardiac troponin I monoclonal antibodies (anti-cTnI) were

purchased from Shanghai Linc-Bio Science Co. Ltd. (Shanghai, China). Human immunoglobulin G (IgG) and bovine serum albumin (BSA) were provided by Beijing Dingguo Biotechnology Company (Beijing, China). Helical carbon nanotubes and ZIF-8 were purchased from Nanjing Xianfeng Nanomaterial Technology Ltd. (Nanjing, China). $HAuCl_4$ and sodium citrate were purchased from Sigma-Aldrich.

CHI 660E electrochemistry workstation (Shanghai CH Instruments, China) was used to measure the signals of cyclic voltammetric (CV) and differential pulse voltammetry (DPV). The CV measurements were carried out under the potential range of -0.2 to 0.6 V and scan rate of 100 mV/s. The conditions of DPV measurements were the following: the potential range -0.2 to 0.6 V; pulse amplitude 0.05 V; pulse width 0.05 s; and sample width 0.02 s. Three-electrode system includes glassy carbon electrode (GCE) which is used as a working electrode, Pt electrode which is uses as a counter electrode, saturated calomel electrode which is used as a reference electrode.

2.2. Preparation of AuNPs

About 16 nm-diameter AuNPs were synthesised according to the method reported by Shen [28]. Before preparation of AuNPs, the round-bottom flask was cleaned. A 50 mL of $HAuCl_4$ aqueous solution (0.01%) was introduced into the round-bottom flask, heated to boiling and vigorously stirred. Then, a 1 mL of 1% sodium citrate was added quickly to the above solution. The change of color was observed. The resulting Au colloid solutions were stored at 4 $^{\circ}$C before use.

2.3. Preparation of AuNPs/ZIF-8/HCNT/Chit-modefied GCE

Before the GCE was modefied, it was polished with alumina powders with different size (0.3, 0.05 μm) followed by washing with distilled water repeatly. 5 mg of ZIF-8 and 5 mg of HCNT were added into chitosan solution (0.5%), a well-dispersed suspension was obtained under ultrasonic dispersion for 1 h. ZIF-8/HCNT/Chit-modefied electrode was obtained by coating 10 μL of above suspension on the electrode surface and then overnight (ZIF-8/HCNT/Chit). Then, AuNPs were introduced on ZIF-8/HCNT/Chit-modefied electrode by dropping 10 μL of AuNPs solution on the electrode surface and dried at room temperature (ZIF-8/HCNT/Chit/AuNPs).

2.4. The fabrication of immunosensor

The immunosensor was fabricated as following: (1) A 10 μL of anti-cTnI solution (60 μg/mL) was dropped onto the ZIF-8/HCNT/Chit/AuNPs-modefied electrode surface, After incubated for 40 min, the electrode was washed with PBS to eliminate unbound anti-cTnI (ZIF-8/HCNT/Chit/AuNPs/ant-cTnI). (2) The antibody-modefied electrode was incubated with 10 μL BSA (2.0 wt%) for 30 min to block nonspecific active sites(ZIF-8/HCNT/Chit/AuNPs/ant-cTnI/BSA) (3) 10 μL of cTnI with various concentrations was drop-casted onto the BSA-blocked electrode and incubated for 60 min, washed with PBS (ZIF-8/HCNT/Chit/AuNPs/ant-cTnI/BSA/cTnI). The diagram of

preparation of the electrochemical immunosensor for cTnI was shown in Fig. 1.

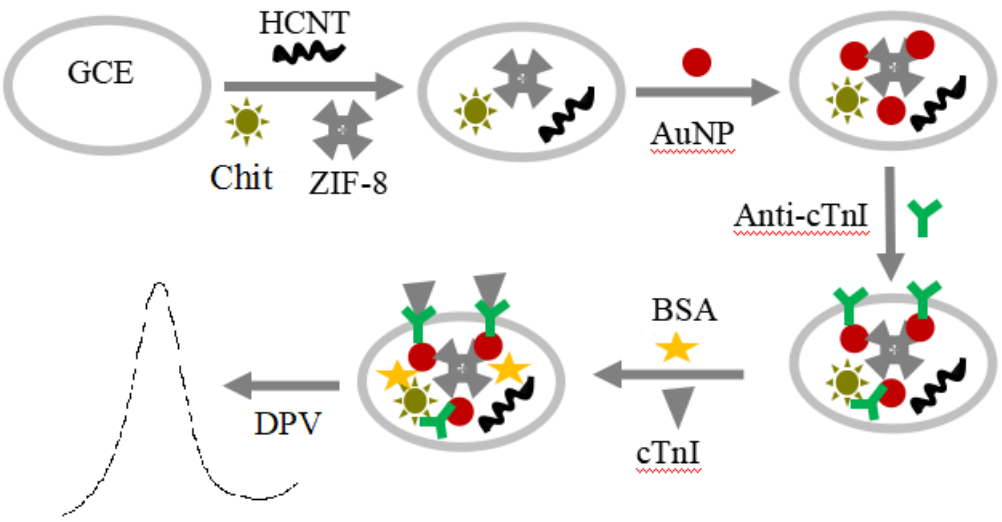

Figure 1. The preparation of the electrochemical immunosensor

3. RESULTS AND DISCUSSION

3.1. CV characterization of the modified electrode

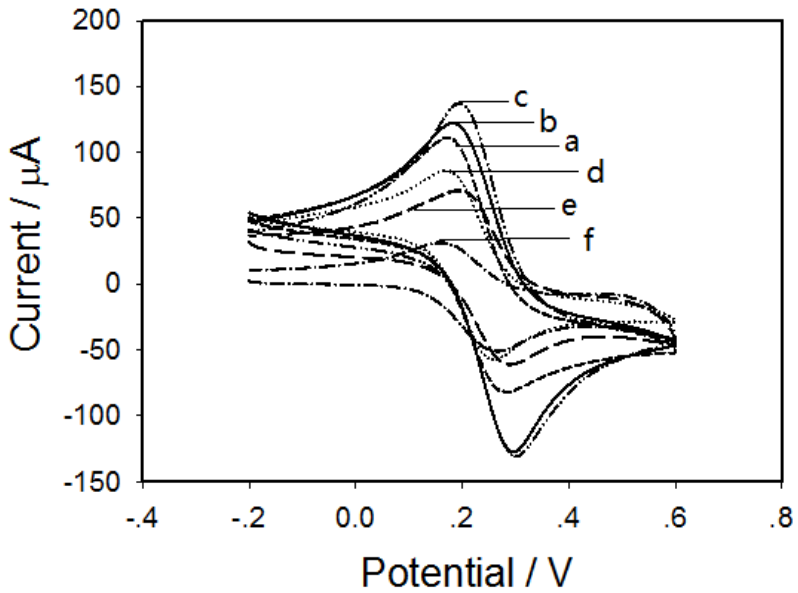

Figure 2. Cyclic voltammograms of bare GCE (a), ZIF-8/HCNT/Chit-modefied electrode (b), ZIF-8/HCNT/Chit/AuNPs-modefied electrode (c), ZIF-8/HCNT/Chit/AuNPs/anti-cTnI-modefied electrode (d), ZIF-8/HCNT/Chit/AuNPs/anti-cTnI/BSA-modefied electrode (e) and ZIF-8/HCNT/Chit/AuNPs/anti-cTnI/BSA/cTnI-modefied electrode (f) in 5 mmol/L $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$. Scan rate was 100 mV/s.

During the immunosensor preparation, every step will result in the changes of electrochemical properties. Here, we studied the changes of electrochemical properties by CV method which was adopted by many researchers [29-31]. The CV curve of the bare GCE was a couple of reversible well-shaped redox peaks (Fig. 2, a). The peaks of CV increased after ZIF-8/HCNT/Chit film was covered on the electrode surface (Fig. 2, b), which resulted from the high conductivity of HCNT. After AuNPs were modified on the electeode, the peak current response increased again (Fig. 2, c),indicating that AuNPs increased conductivity of electrode surface. This result is consistent with that reported in the literatures [32,33]. The peak current response decreased when antibody (60 μg/mL) was immobilized on the electrode surface (Fig. 2, d), which was because that antibodies hinder the interfacial electron transport [34]. The use of BSA for blocking residual active sites lead to decreasing of peak current of CV (Fig. 2, e). When antigens reacted with antibodies modefied on the electrode, peak current decreased obviously (Fig. 2, f). The results suggested that immunocomplex of antibody-antigen blocked electron transfer.

3.2. Optimization of the experimental conditions

The performance of the immunosensor was influenced by concentration of antibody solution [35], immobilization time of antibody [36], incubation time of antigen. Here, we investigate these experimental conditions using cTnI with a concentration of 15 ng/mL. ΔI represents the peak current changes before and after incubation of 15 ng/mL cTnI.

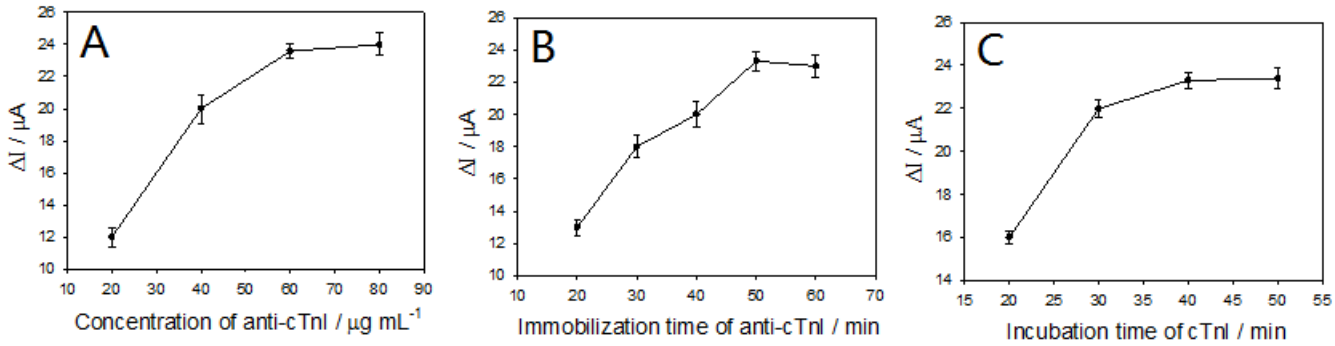

Figure 4. Effect of the concentration of antibody (A), immobilization time of antibody (B) and incubation time of cTnI (c) on ΔI of immunosensor. Error bars represent standard deviation, n=3

The impact of the anti-cTnI concentration on the performance of the immunosensor was investigated. Fig. 3A showed that when the concentration of anti-cTnI changed from 20 to 60 μg/mL, the peak current changes of DPV (ΔI) increased. when the concentration of anti-cTnI was 80 μg/mL, ΔI almost was the same as that of 60 μg/mL. Thus, 60 μg/mL was selected for further experiments. Antibody immobilization time was a crucial factor for the immunoassay. We investigated the incubation time from 20 to 60 min. As can be seen from Fig. 3 B, ΔI increased when the incubation

time enhanced from 20 to 50 min and then trended to a constant current value, indicating a saturated binding of antibody was obtained. Therefore, antibody immobilization time was selected as 50 min for this work. The incubation time of antigen was also investigated from 20 to 50 min. Fig. 3 C demonstrated that 40 min was an optimal immuno-reaction time.

3.3. The detection of cTnI

Under the optimal experimental conditions, the fabricated immunosensor was used to the immunoassay of cTnI. Here, different concentrations of cTnI standard solutions were measured in $[Fe(CN)_6]^{3-/4-}$ solution. Fig. 4A showed when the concentration of cTnI increased from 0 to 40 ng/mL, the DPV signals decreased successively. Fig. 4B exhibited a linear response toward the concentration of cTnI from 0.1 to 40 ng/mL. The limit of detection was 0.04 ng/mL (S/N = 3).

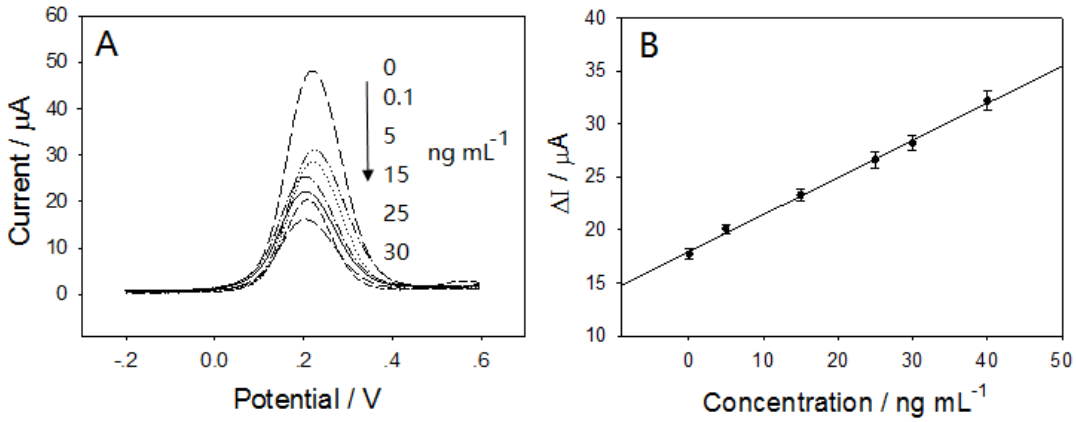

Figure 4. DPV curves corresponding to different concentrations of cTnI (A). Calibration curve of the immunosensor (B). Error bars represent standard deviation, n=3

Table 1. Comparison of the proposed immunosensor and other cTnI immunosensors.

Modification	Linear range (ng/mL)	detection limit (ng/mL)	References
AuNPs	0.2-12.5	0.2	[37]
Porous grapheme	0.1-10	0.07	[38]
Vertically aligned carbon nanofibres	5-100	0.2	[39]
Au /Ag nanoparticles	0.1-32	0.1	[40]
AuNPs and grapheme	0.05-3	0.05	[13]
Whiskered nanofibres	0.5-100	0.04	[12]
ZIF-8/HCNT/AuNPs	0.1-40	0.04	This work

The performance of the fabricated immunosensor were compared with other immunosensors for cTnI. Table 1 showed that the presented immunosensor had relatively broader linear range and an acceptable detection limit.

Although Yan's group developed a sandwich-type electrochemical immunosensor with a wider linear range (0.01-60 ng/mL) for cTnI [41], the production process is much more complicated .

3.4. Specificity, reproducibility and stability of the immunosensor

Immunosensors are analytical tools for monitoring specific reactions of antigen and antibody.Thus, the selectivity is very important for immunosensors' practical applications. Carcinoembryonic antigen (CEA), α-fetoprotein (AFP) and prostate specific antigen (PSA) were selected as potential interferents to investigate the specificity of the fabricated immunosenor. The concentration of these interfering species is 50 ng/mL. DPV curves of these interfering species were measured. We found that there are no significant differences among changes of peak current corresponding to these interfering species (Figure 5). However, the ΔI of the mixture of interferents and cTnI was almost identical to that of cTnI (15 ng/mL) alone, demonstrating the specificity of the proposed immunosensor was satisfied. In addition, inter-and intra-assay of the immunosensor for cTnI (15 ng/mL) was carried out. The coefficients of variation of inter-assay was 8.8% by measuring five independent biosensors. The coefficients of variation of intra-assay was 6.4% obtained by measuring the same immunosensor five times. Here, the stability of the immunosensor was also investigated by measuring the DPV after long-term preservation. The immunosensor stored at 4 ^{o}C in refrigerator was tested after 5, 10, 15 days, the DPV signal retained 96.8%, 92.7% and 89.4% of the initial current value. The results demonstrated the designed immunosensor had satisfied stability.

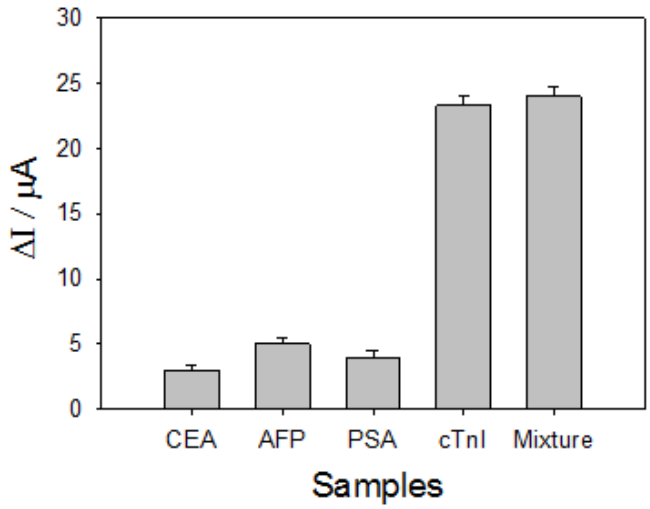

Figure 5. The ΔI corresponding to CEA (50 ng/mL), AFP (50 ng/mL), PSA (50 ng/mL), cTnI (15 ng/mL), and mixtures. Error bars represent standard deviation, n=3

3.6. Real sample analysis

We determinated the cTnI concentrations of three real human serum samples. The analysis results were compared with that of ELISA and described in Table 2. As can be seen from Table 2, a good correlation between ELISA and this proposed method was obtained, the proposed immunosensor could be reasonably applied in the clinical determination of cTnI in real samples.

Table 2. Serum sample analysis and the comparison with ELISA method (n=5).

Samples	ELISA (ng mL^{-1})	This method (ng mL^{-1})	Relative error (%)
1	1.43	1.31	-8.4
2	5.82	6.37	9.5
3	13.66	14.53	6.4

4. CONCLUSIONS

Here, a novel immunosensor based on ZIF-8/HCNT/Chit/AuNPs-modified GCE was developed for the first time to detect cTnI. The electronic signal were improved because of the synergetic electron-conducting properties of AuNPs, ZIF-8 and HCNT. AuNPs provided a support to immobilize the antibodies, which simplified the process of antibody immobilization. The electrochemical immunosensor exhibited a high sensitivity to cTnI concentrations as low as 0.04 ng/mL, a wide linear range from 0.1 to 40 ng/mL, and good selectivity. The new strategy provided an efficient method to detect the concentration of cTnI. Meanwhile, this platform supplied enormous potential to fabricate other immunosensors by replacing the capture antibody.

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