Label-Free Electrochemical Immunosensor Based on a Functionalized Ionic Liquid and Helical Carbon Nanotubes for the Determination of Cardiac Troponin I

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ABSTRACT: A label-free electrochemical immunosensor for cardiac troponin I was prepared by using a helical carbon nanotube-supported aldehyde-functionalized ionic liquid. Because of the good conductivity of ionic liquid and helical carbon nanotubes, high sensitivity of the immunosensor was obtained. Functionalized ionic liquid provided binding sites for antibody, which simplified the process of sensor construction. Cardiac troponin I was detected by this immunosensor with a linear range of 0.05–30 ng/mL and a detection limit of 0.03 ng/mL. The electrochemical immunosensor had satisfactory reproducibility, high sensitivity, and acceptable specificity.

1. INTRODUCTION

Cardiac troponin I (cTnI), as a marker protein of myocardial damage, was recognized as the “gold standard” biomarker test for acute myocardial infarction.1 Accordingly, sensitivity and accuracy were very important for cTnI detection. Many detection strategies had been put forward for the quantitative detection of cTnI, such as enzyme-linked immunosorbent assay2 and photoelectrochemical immunoassay.3 Recently, fluorescent immunosensor and electrochemiluminescent immunoassay for cTnI was also reported.4,5 However, these methods had some drawbacks including the need for a tedious labeling process, bulky and expensive equipment, and highly skilled operators. The electrochemical immunosensor had aroused researchers’ interest because of its high sensitivity, handling, portability, miniaturization, and low operating cost.6,7 In most previous studies, linear carbon nanotubes (LCNTs) had widely been applied in the field of electrochemical immunosensors.8–11 However, only few electrochemical immunosensors based on helical carbon nanotubes (HCNTs) had been reported to date.12,13 The surface of HCNTs was more susceptible to chemical modification than that of LCNTs because of the high energy state as a result of inherent tensile and compressive stresses. In addition, the difference of charge density caused by the lattice defect of carbon nanotubes (CNTs) improved the electronical properties.14,15 This motivated us to fabricate an electrochemical immunosensor using HCNTs for the detection of cTnI.

Because ionic liquids (ILs) had the advantages such as high ionic conductivity, chemical stability, and good biocompatibility, they were widely used in the fabrication of electrochemical biosensors by being incorporated into other matrixes including graphene, metal nanoparticles, fullerene, and cellulose.16–20 Because of π–cation interaction between CNTs and IL, they could closely combine together to form nanocomposites which provided a platform for the fabrication of electrochemical biosensors.21,22 However, most reported papers focus on LCNTs with ILs without functionalization and a few based on HCNTs with dialdehyde-functionalized IL (DIL).

Antibody immobilization using a simple method, such as DIL,23 attracted many researchers. In this article, the DIL–HCNTs (the composite of DIL and HCNTs) was prepared by the ultrasound method, where DIL noncovalently bonded together with HCNTs through hydrogen bonding interactions.13 Subsequently, the DIL–HCNTs was used to fabricate a label-free electrochemical immunosensor for cTnI detection. As the conductivity of the sensing interface was enhanced by DIL and HCNTs, this immunosensor had a high sensitivity. Furthermore, the fabrication process was simplified because the antibody was immobilized directly by DIL.

2. RESULTS AND DISCUSSION

2.1. SEM Characterization of DIL–HCNTs. The surface characteristics of the HCNT before and after being functionalized with DIL were investigated by scanning electron microscopy (SEM, Figure 1). DIL–HCNTs (Figure 1B)
maintained the helical structure of HCNTs (Figure 1A) and looked thicker because DIL covers on the surface of HCNTs.24

2.2. CV Characterization of the Modified Electrode. In order to investigate the electrochemical characteristics of the electrochemical immunosensor, cyclic voltammetry (CV) results were measured in 5 mmol/L potassium ferricyanide/potassium ferrocyanide at a scan rate of 100 mV/s from −0.2 to 0.6 V. As shown in Figure 2, the CV curve of the bare Au electrode (Figure 2a) was a typical redox wave. Because of the obstruction electron and mass transfer of Naflon film, no peak could be observed on CV curve (Figure 2b) when the electrode was covered with Naflon. Contrarily, obvious redox peaks could be observed when the electrode was modified with Naflon film (Figure 2c), which was attributed to the high conductivity of DIL–HCNTs. After the modified electrode adsorbed antibodies and antigens, the current of redox peak decreased (Figure 2d–f) because the electron and mass transfer had been blocked.

2.3. Optimization of the Experimental Conditions. Experimental conditions, such as concentration and immobilization time of antibody and immunoreaction time between antibody and antigen, were optimized in order to improve the sensitivity and accuracy of cTnI detection. The peak current of differential pulse voltammetry (DPV) was used to evaluate the influence of the experimental conditions on detection of cTnI, and the concentration of cTnI remained at 10 ng/mL in these optimization experiments. According to the results, the peak current of DPV decreased significantly with the increase of the anti-cTnI antibody concentration from 20 to 60 μg/mL but continued to increase in the concentration of anti-cTnI antibody, resulting in a minor change of the peak current of DPV (Figure 3A). Therefore, 60 μg/mL was the optimal concentration of anti-cTnI antibody. Similarly, DPV decreased with the prolongation of the fixed time of the antibody at room temperature and remained stable after 60 min. Similarly, the peak current of DPV decreased with the immobilization time of the antibody at room temperature and remained stable after 60 min (Figure 3B). Thus, 60 min was the select immobilization time of the antibody. Finally, 30 min was the optimal time for the immunoreaction time between antibody and antigen at 37 °C (Figure 3C).

2.4. Detection of cTnI. After antibody reacted with antigen, the immunocomplex formed on the electrode surface would hinder electron transfer, resulting in the decreasing of the peak current of DPV. Under the optimal experimental conditions, the signals corresponding to different concentrations of cTnI were tested. Figure 4 shows that the peak current of DPV decreased significantly with the increase of cTnI concentration from 0.005 to 30 ng/mL. Error bars represent standard deviation, n = 3.) Experiment conditions: potential range: −0.4 to 0.6 V, pulse amplitude: 0.05 V, pulse width: 0.05 s, sample width: 0.02 s.

Figure 1. SEM images of HCNTs (A) and DIL–HCNTs (B).

Figure 2. Cyclic voltammograms of bare Au (a), Naflon/Au (b), DIL–HCNT–Naflon/Au (c), anti-cTnI/DIL–HCNT–Naflon/Au (d), BSA/anti-cTnI/DIL–HCNT–Naflon/Au (e), and cTnI/BSA/anti-cTnI/DIL–HCNT–Naflon/Au (f) in 5 mmol/L Fe(CN)₆³⁻/Fe(CN)₆⁴⁻. Scan rate was 100 mV/s.

Figure 3. Effect of the concentration of antibody (A), immobilization time (B), and immunoreaction time (C) on the peak current of immunosensor. The concentration of cTnI was 10 ng/mL.

Figure 4. DPV curves at different concentrations of cTnI. (Inset: calibration curve of the peak currents of DPV to different concentrations of cTnI. Error bars represent standard deviation, n = 3.) Experiment conditions: potential range: −0.4 to 0.6 V, pulse amplitude: 0.05 V, pulse width: 0.05 s, sample width: 0.02 s.
current decreased with the increasing concentration of cTnI. The inset indicates a linear calibration that was obtained in the range of 0.05–30 ng/mL. The limit of detection is 0.03 ng/mL determined by 3σ rule (where σ is the standard deviation of a blank solution).

Comparing the analytical characteristics including the detection limit and linear range of the proposed immunosensor with those of other cTnI immunosensors, Table 1 demonstrated that the fabricated immunosensor had a satisfied linear range and a lower detection limit.

Table 1. Performance of Different cTnI Immunosensors

| modifying material | linear range (ng/mL) | detection limit (ng/mL) | refs |
|--------------------|----------------------|-------------------------|------|
| carbon nanofiber    | 0.25–1               | 0.2                     | 25   |
| Au nanoparticle     | 0.2–12.5             | 0.2                     | 26   |
| Au/Ag nanoparticle  | 0.1–32               | 0.1                     | 27   |
| porous graphene     | 0.1–10               | 0.07                    | 28   |
| nanostructured      | 0.1–100              | 0.1                     | 29   |
| DIL–HCNT            | 0.05–30              | 0.02                    | this work |

2.5. Specificity and Reproducibility of the Immunosensor. In order to investigate specificity of the immunosensor, the peak current of DPV toward blank solution was measured (48 μA). Carcinoembryonic antigen, BSA, and α-fetoprotein were used to replace cTnI as interfering species for immunoreaction. As shown in Figure 5, the peak currents of DPV corresponding to these interfering species of 20 ng/mL were close to the peak currents of DPV obtained from blank solution, while the peak current of DPV corresponding to cTnI (20 ng/mL) was 18 μA. These results indicated that the DIL–HCNT-modified immunosensor had good specificity.

The reproducibility of the prepared immunosensor was also studied. The inter-and intra-assay coefficients of variation of detected cTnI (10 ng/mL) were 7.3 and 6.7%, respectively. These results showed that the prepared immunosensor had good reproducibility.

The immunosensor retained more than about 95% of the initial values after storing at 4 °C for 15 days. The results demonstrated that the designed immunosensor has satisfied stability.

3. CONCLUSIONS

Here, an electrochemical immunosensor based on DIL and HCNTs as a platform for the determination of cTnI was developed. The nanocomposite of DIL–HCNTs improved the conductivity of the electrode surface, resulting in high sensitivity. In addition, the DIL provided two −CHO groups for antibody immobilization, which simplified the process of sensor construction. The immunosensor for cTnI exhibited some good analytical performance such as satisfactory reproducibility and good specificity. Furthermore, the DIL–HCNT nanocomposite is an attractive modifying material in the fabrication of other electrochemical biosensors.

4. EXPERIMENTAL SECTION

4.1. Materials. cTnI and anti-cTnI (anti-cTnI monoclonal antibodies) were purchased from Shanghai Linc-Bio Science Co. Ltd. IgG (human immunoglobulin G) and BSA (bovine serum albumin) were purchased from Beijing Dingguo Biotechnology Company. PBS (0.1 mol/L, phosphate buffer solution, pH 7.0) was prepared using Na2HPO4 and KH2PO4, which were purchased from Sinopharm Chemical Reagent Co. Ltd. HCNTs were purchased from Nanjing Xianfeng Nanomaterial Technology Ltd. Nafion (Nf, 5%, v/v) was obtained from Sigma Chemical. 4-(Bromomethyl)benzaldehyde, 4,4′-bipyridine, acetonitrile, and N,N′-dimethylformamide (DMF) were purchased from Sinopharm Chemical Reagent Co. Ltd.

CV and DPV were performed on a CHI 660E electrochemistry workstation (Shanghai CH Instruments, China). The three-electrode system included a Pt electrode (counter electrode), a saturated calomel electrode (reference electrode), and a gold electrode (Au)-modified composite film (working electrode). SEM images were obtained by using a TESCAN MIRA3 (LMU) microscope.

4.2. Preparation of the DIL–HCNT Composite. 4-(Bromomethyl)benzaldehyde (0.498 g, 2.5 mmol) and 4,4′-bipyridine (0.156 g, 1 mmol) were added into 20 mL acetonitrile, and the mixture was refluxed for overnight and cooled to room temperature. Subsequently, DIL, a yellow solid (0.42 g, yield about 76%), was obtained by filtration. The DIL was directly used for the preparation of DIL–HCNTs without further purification.

The DIL–HCNTs was prepared according to ref 13 with minor modification. Typically, 1 mL of HCNTs (dissolved in DMF, 2 mg/mL) was mixed with 4 mL DIL (dissolved in ethanol, 10 mg/mL), and black DIL–HCNT suspensions were obtained after 1 h with ultrasonication. Subsequently, this mixture was centrifuged (12 000 rpm, 10 min) and washed three times with ultrapure water. Then, the DIL–HCNT product was redispersed into 1 mL ethanol solution of Nf (0.25%, v/v) under ultrasonication for 10 min. The preparation of the DIL–HCNT composite is shown in Figure 6A.

4.3. Fabrication of the Immunosensor. At first, a gold electrode (Au, 3 mm in diameter) was successively polished with 0.3 and 0.05 μm alumina slurries and ultrasonication cleaned in ultrapure water. Subsequently, 10 μL of Nf/DIL–HCNT solution was dropped on the cleaned electrode and dried in the air. Then, 10 μL of antibody (60 μg/mL) was dropped on the Nf/DIL–HCNT membrane-modified electrode and incubated at room temperature for 60 min. The unreacted antibodies were removed by washing with ultrapure water, and then, the electrode was incubated for 30 min at 37 °C with 10 μL BSA (2.0 wt %) in order to eliminate nonspecific binding. Ultimately, 10 μL of antigen solution with various concentrations was dropped on the surface of electrode and incubated for 40 min at 37 °C, followed by washing with...
ultrapure water and then measuring the electrochemical signals. The whole process of the immunosensor fabrication is shown in Figure 6B.

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Notes
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■ REFERENCES

(1) Periyakaruppan, A.; Gandhiraman, R. P.; Meyyappan, M.; Koehe, J. E. Label-free detection of cardiac troponin-I using carbon nanotube based nanoelectrode arrays. Anal. Chem. 2013, 85, 3858−3863.
(2) Cho, I.-H.; Park, E.-H.; Kim, Y.-K.; Kim, J.-H.; Paek, S.-H. Chemiluminoimetric enzyme linked immunosorbent assays (ELISA)-on-a-chip biosensor based on cross-flow chromatography. Anal. Chim. Acta 2009, 632, 247−255.
(3) Tan, Y.; Wang, Y.; Li, M.; Ye, X.; Wu, T.; Li, C. Enhanced photoelectrochemical immunosensing of cardiac troponin I based on energy transfer between N-acyetyl-L-cysteine capped CdAgTe quantum dots and dodecahedral Au nanoparticles. Biosens. Bioelectron. 2017, 91, 741−746.
(4) Seo, S.-M.; Kim, S.-W.; Park, J.-N.; Cho, J.-H.; Kim, H.-S.; Paek, S.-H. A fluorescent immunosensor for high-sensitivity cardiac troponin I using a spatially-controlled polymeric, nano-scale tracer to prevent quenching. Biosens. Bioelectron. 2016, 83, 19−26.

(5) Tang, M.; Zhou, Z.; Shangguan, L.; Zhao, F.; Liu, S. Electrochemiluminescent detection of cardiac troponin I by using soybean peroxidase labeled-antibody as signal amplifier. Talanta 2018, 180, 47−53.
(6) Campuzano, S.; Pedrero, M.; Nikoleli, G.-P.; Pingarrón, J. M.; Nikolelis, D. P. Hybrid 2D nanomaterials-based electrochemical immunosensing strategies for clinical biomarkers determination. Biosens. Bioelectron. 2017, 89, 269−279.
(7) Ji, R. Y.; Chen, S.; Xu, W.; Qin, Z.; Qiu, J. F.; Li, C. R. A voltammetric immunosensor for clenbuterol based on the use of a MoS2-AuPt nanocomposite. Microchim. Acta 2018, 185, 209−210.
(8) Pakchin, P. S.; Ghanbari, H.; Saber, R.; Omidi, Y. Electrochemical immunosensor based on chitosan-gold nanoparticle/carbon nanotube as a platform and lactate oxidase as a label for detection of CA125 oncomarker. Biosens. Bioelectron. 2018, 122, 68−74.
(9) Gulati, P.; Kaur, P.; Rajam, M. V.; Srivastava, T.; Islam, S. S. Single-wall carbon nanotube based electrochemical immunosassay for leukemia detection. Anal. Biochem. 2018, 557, 111−119.
(10) Cabral, D. G. A.; Lima, E. C. S.; Moura, P.; Dutra, R. F. A label-free electrochemical immunosensor for hepatitis B based on hyaluronic acid−carbon nanotube hybrid film. Talanta 2016, 148, 209−215.
(11) Eissa, S.; Alshehri, N.; Rahman, A. M. A.; Dasouki, M.; Abu-Salah, K. M.; Zohrob, M. Electrochemical immunosensors for the detection of survival motor neuron (SMN) protein using different carbon nanomaterials-modified electrodes. Biosens. Bioelectron. 2018, 101, 282−289.
(12) Yang, H. T.; Li, B. Y.; Cui, R. J.; Xing, R. M.; Liu, S. H. Electrochemical sensor for rutin detection based on Au nanoparticle-loaded helical carbon nanotubes. J. Nanopart. Res. 2017, 19, 354.
(13) Yan, H.; Tang, X.; Zhu, X.; Zeng, Y.; Lu, X.; Yin, Z.; Lu, Y.; Yang, Y.; Li, L. Sandwich-type electrochemical immunosensor for highly sensitive determination of cardiac troponin I using carbonyl-terminated liquid and helical carbon nanotube composite as platform and ferrocenecarboxylic acid as signal label. Sens. Actuators, B 2018, 277, 234−240.
(14) Robinson, J. A.; Snow, E. S.; Bádescu, S. C.; Reincke, T. L.; Perkins, F. K. Role of defects in single-walled carbon nanotube chemical sensors. Nano Lett. 2006, 6, 1747−1751.
(15) Childress, A.; Ferri, K.; Rao, A. M. Enhanced supercapacitor performance with binder-free helically coiled carbon nanotube electrodes. Carbon 2018, 140, 377−384.
(16) Wei, Y.; Li, X.; Sun, X.; Ma, H.; Zhang, Y.; Wei, Q. Dual-responsive electrochemical immunosensor for prostate specific antigen detection based on Au-CoS2/graphene and CeO2/ionic liquids doped with carboxymethyl chitosan complex. Biosens. Bioelectron. 2017, 94, 141−147.
(17) Dong, S.; Tong, M.; Zhang, D.; Huang, T. The strategy of nitrite and immunoassay human IgG biosensors based ZnO@ZIF-8 and ionic liquid composite film. Sens. Actuators, B 2017, 251, 650−657.
(18) Guo, S.; Wen, D.; Zhai, Y.; Dong, S.; Wang, E. Ionic liquid−graphene hybrid nanosheets as an enhanced material for electrochemical determination of trinitrotoluene. Biosens. Bioelectron. 2011, 26, 3475−3481.
(19) Shen, G.; Zhang, X.; Shen, Y.; Zhang, S.; Fang, L. One-step immobilization of antibodies for a-1-fetoprotein immunosensor based on dalddehyde cellulose/ionic liquid composite. Anal. Biochem. 2015, 471, 38−43.
(20) Sun, X.; Li, Z.; Cai, Y.; Wei, Z.; Fang, Y.; Ren, G.; Huang, Y. Electrochemical impedance spectroscopy for analytical determination of paraquat in meconium samples using an immunosensor modified with fullerene, ferrocene, and ionic liquid. Electrochim. Acta 2011, 56, 1117−1122.
(21) Zhang, X.; Dou, W.; Zhan, X.; Zhao, G. A novel immunosensor for Enterobacter sakazakii based on multiwalled carbon nanotube/ionic liquid/thionine modified electrode. Electrochim. Acta 2012, 61, 73−77.
(22) Salimi, A.; Kavosi, B.; Fathi, F.; Hallaj, R. Sensitive immunosensing of prostate-specific antigen based on ionic liquid–carbon nanotubes modified electrode: application as cancer biomarker for prostate biopsies. Biosens. Bioelectron. 2013, 42, 439–446.

(23) Shen, Y.; Shen, G.; Zhang, Y. Label-Free Electrochemical Immunosensor Based on Ionic Liquid Containing Dialdehyde As a Novel Linking Agent for the Antibody Immobilization. ACS Omega 2018, 3, 11227–11232.

(24) Tunckol, M.; Durand, J.; Serp, P. Carbon nanomaterial–ionic liquid hybrids. Carbon 2012, 50, 4303–4334.

(25) Periyakaruppan, A.; Gandhiraman, R. P.; Meyyappan, M.; Koehne, J. E. Label-free detection of cardiac troponin-I using carbon nanofiber based nanoelectrode arrays. Anal. Chem. 2013, 85, 3858–3863.

(26) Bhalla, V.; Carrara, S.; Sharma, P.; Nangia, Y.; Raman Suri, C. Gold nanoparticles mediated label-free capacitance detection of cardiac troponin I. Sens. Actuators, B 2012, 161, 761–768.

(27) Shumkov, A. A.; Suprun, E. V.; Shatinina, S. Z.; Lisitsa, A. V.; Shumyantseva, V. V.; Archakov, A. I. Gold and silver nanoparticles for electrochemical detection of cardiac troponin I based on stripping voltammetry. J. Bionanosci. 2013, 3, 216–222.

(28) Kazemi, S. H.; Ghodsi, E.; Abdollahi, S.; Nadri, S. Porous graphene oxide nanostructure as an excellent scaffold for label-free electrochemical biosensor: detection of cardiac troponin I. Mater. Sci. Eng., C 2016, 69, 447–452.

(29) Kumar, S.; Kumar, S.; Augustine, S.; Malhotra, B. D. Protein functionalized nanostructured zirconia based electrochemical immunoassay for cardiac troponin I detection. J. Mater. Res. 2017, 32, 2966–2972.