Label-Free Electrochemical Immunosensor Based on β-Cyclodextrin-Functionalized Helical Carbon Nanotube and Ionic Liquid Containing Ferrocene and Aldehyde Groups

Guangyu Shen†,‡*,†,‡,§ and Youming Shen†,‡

1Hunan Province Cooperative Innovation Center for The Construction & Development of Dongting Lake Ecological Economic Zone and 2College of Chemistry and Material Engineering, Hunan University of Arts and Science, Changde 415000, China

ABSTRACT: We fabricated an electrochemical immunosensor for the detection of cardiac troponin I using β-cyclodextrin-functionalized helical carbon nanotube and ionic liquid functionalized with ferrocene and aldehyde groups. β-Cyclodextrin-functionalized helical carbon nanotube was first modified on the electrode surface. Then, ferrocene- and aldehyde-functionalized ionic liquid was modified on the surface of the electrode through host–guest interaction, resulting in an interface with aldehyde groups. The aldehyde groups attached to the ionic liquid capture antibody directly, which simplifies the fabrication of immunosensor. Because of the use of ionic liquid and helical carbon nanotube, the conductivity of the sensing interface was improved. Thus, the sensitivity of the fabricated immunosensor was increased. The immunosensor for cardiac troponin I shows a linear range from 0.05 to 20 ng mL⁻¹ with a detection limit of 0.04 ng mL⁻¹ (S/N = 3).

INTRODUCTION

Acute myocardial infarction has seriously threatened people’s lives. An important standard in the evaluation of patients with acute myocardial infarction has been accepted as cardiac troponin I (cTnI).¹ Thus, rapid, sensitive, and accurate detection for cTnI is of great significance for saving lives. In recent years, a lot of methods for the quantitative detection of cTnI have been developed.²⁻⁵ Although these methods possess high sensitivity, they need tedious labeling process, highly skilled operators, and expensive equipment, which hinder them to be widely applied in clinical detection. Recently, electrochemical immunosensors have been attracting researchers’ attention because of its high specificity, easy miniaturization, handling, and low cost.⁶,⁷ Material modified on the electrode is a key factor affecting the working of sensors. Carbon nanomaterials including graphene,⁸⁻¹⁰ carbon fibers,¹¹ carbon dots,¹² and linear carbon nanotubes (LCNTs)¹²,¹³ were applied in the development of electrochemical immunosensors. Compared with LCNT, helical carbon nanotubes (HCNTs) have high area, excellent electrocatalytic activity, and good electronic properties.¹⁴ However, few papers about the fabrication of electrochemical immunosensors using HCNTs were reported.¹⁵

β-Cyclodextrin (CD) has a special structure with seven D-glucose units and a toroidal shape. It is composed of a hydrophobic inner cavity and a hydrophilic exterior, which result in high molecular binding ability and supramolecular recognition ability.¹⁶ Thus, CD and some molecules are easy to form stable host–guest complex. For example, Xie developed a biosensor based on supramolecular recognition ability between CD and ferrocene.¹⁷ In addition, CD can improve the dispersity of carbon nanomaterials.
used to directly immobilize antibody. On the other hand, because of the use of the HCNT and IL, good conductivity of the sensing interface was obtained, which avoided tedious labeling process for signal amplification. Thus, the novel platform based on HCNT−CD/Fc-IL-CHO was convenient and sensitive for the fabrication of electrochemical immuno-sensors.

**RESULTS AND DISCUSSION**

**SEM Characterization of HCNT−CD/Fc-IL-CHO.** The surface characteristics of the HCNT before (Figure 1A) and after being functionalized with CD/Fc-IL-CHO (Figure 1B) were investigated using scanning electron microscopy (SEM). As shown in Figure 1A, HCNT looked similar to a helical structure. Figure 1B showed that the HCNT−CD/Fc-IL-CHO was thicker than HCNT because CD and Fc-IL-CHO cover the surface of HCNT. The results are in good agreement with the reported literature.24

**Cyclic Voltammetric Characterization of the Electrode Modified.** To study the electrochemical characteristics of the electrode modified step-by-step, cyclic voltammetric (CV) measurements were carried out in 5 mM K$_3$[Fe(CN)$_6$]/K$_4$[Fe(CN)$_6$]. The scan rate is 100 mV s$^{-1}$, and the range is from −0.2 to 0.6 V. As shown in Figure 2, the CV of the bare Au electrode is a well-defined redox wave. When HCNT−CD/Fc-IL-CHO was introduced to bare Au electrode, the peaks of CV increased again (Figure 2b). It should be attributed to the PE1-IL-CHO cover the surface of HCNT and IL. After antibody (100 μg mL$^{-1}$) was immobilized on the electrode surface (Figure 2c), the peak current decreased. Bovine serum albumin (BSA) was also modified on the electrode covered by antibodies to eliminate nonspecific adsorption, and the corresponding peak current of CV decreased (Figure 2d). After antibody captured antigen (5 ng mL$^{-1}$), the peak current of CV decreased again (Figure 2e). It was probably because the antibody−antigen immunocomplex hindered the electron transfer.

**Optimization of the Experimental Conditions.** Some experimental conditions including concentration of antibody, immobilization time of antibody, concentration of BSA, and reaction time between antibody and antigen need to be optimized. In these experiments, the concentration of cTnI is 5 ng mL$^{-1}$.

The influence of concentration of anti-cTnI antibody on the peak current of DPV was investigated from 20 to 120 μg mL$^{-1}$. According to the results shown in Figure 3A, the anti-cTnI antibody concentration of 100 μg mL$^{-1}$ was an optimal selection. The influence of antibody immobilization time on the signals was studied in the range of 20−80 min. When the time changed from 20 to 60 min, the peak current of DPV decreased gradually. Then, when the time was longer than 60 min, the peak current of DPV was stable (Figure 3B). Thus, we selected antibody immobilization time of 60 min for this work. The concentrations of BSA were investigated from 1.0 to 4.0 wt %, the signals decreased with the increase in concentration until 3.0 wt % (Figure 3C). Thus, we selected 3.0 wt % as the optimal concentration of BSA.

**Detection of cTnI.** The formation of antibody−antigen immunocomplex would hinder the electron transfer and therefore decrease the peak current of DPV. Here, various concentrations of cTnI were detected by the fabricated immunosensor. Figure 4 showed the DPV response to different cTnI concentrations. As shown in Figure 4, the peak current decreased with the increasing concentration of cTnI. The inset of Figure 4 indicated a good linear relationship between the cTnI concentrations, and peak currents were obtained in the range of 0.05−20 ng mL$^{-1}$. The detection limit was 0.04 ng mL$^{-1}$ (S/N = 3).

We compared the detection limit and linear range of the fabricated immunosensor with that of other immunosensors for cTnI. These results described in Table 1 showed that the fabricated immunosensor had an acceptable linear range and detection limit.

**Specificity, Reproducibility, and Stability of the Immunosensor.** To study the specificity of the immunosensor, carcinoembryonic antigen (CEA), BSA, and α-fetotropin (AFP) were selected as interfering species. The peak currents of DPV corresponding to CEA, BSA, and AFP of carcinoembryonic antigen (CEA) were close, demonstrating that the specificity was satisfied. The reproducibility of proposed immunosensor was also studied. Inter- and intra-assay at 5 ng mL$^{-1}$ cTnI were performed. The coefficients of variation was 5.5% for interassay and 6.8% for intra-assay. These results indicated that the fabricated immunosensor had a good reproducibility. After 10 days, the current response of the immunosensor was retained at 89% of the initial response, indicating that the stability of the immunosensor was acceptable.

**Analytical Application.** To demonstrate the feasibility of the proposed immunosensor in real samples, recovery was investigated using standard addition methods. We selected three spiked human serum samples for experiments. The results are described in Table 2. The recoveries were from 97.5...
Here, a label-free electrochemical immunosensor was developed using CD-functionalized HCNT and IL-functionalized with ferrocene and aldehyde groups as a substrate. The utilization of HCNT and IL improved the conductivity of the sensing interface, resulting in a high sensitivity of the immunosensor. In addition, aldehyde groups attached to IL can be directly used to immobilize antibody, meaning the fabrication of the immunosensor was easy and convenient.

■ EXPERIMENTAL SECTION

Reagents and Apparatus. cTnI and anti-cTnI monoclonal antibodies (anti-cTnI) were provided by Shanghai LincBio Science Co. Ltd. (Shanghai, China). We purchased BSA and human immunoglobulin G from Beijing Dingguo Biotechnology Company (Beijing, China). Using Na₃HPO₄ and KH₂PO₄, we prepared 0.1 M phosphate buffer solution (pH 7.0). HCNT was obtained from Nanjing Xianfeng Nanomaterial Technology Ltd. (Nanjing, China). Fc-IL-CHO was prepared by our group according to previous reports.²¹

Electrochemical measurements including DPV and CV were carried out on an electrochemistry workstation (CHI 660E, Shanghai CH Instruments, China). All electrochemical measurements were performed in a conventional three-electrode cell made of a saturated calomel electrode as the reference electrode, a Pt electrode as the counter electrode, and a gold electrode (Au) as the working electrode (Au). The morphology of the HCNT before and after functionalization with CD/Fc-IL-CHO was investigated with a TESCAN MIRA3 (LMU) microscope.

Preparation of HCNT–CD/Fc-IL-CHO. The composite of HCNT–CD was prepared according to the reported method with minor modification.¹⁶ HCNT (25 mg) and CD (200 mg) were added to 50 mL of ultrapure water and stirred for 12 h. Then, the mixture was washed three times by centrifugation. Fc-IL-CHO (20 mg) and CD–MWCNT (10 mg) were added to 10 mL of dichloromethane solution and stirred for 6 h. HCNT–CD/Fc-IL-CHO was obtained by centrifugation. The preparation of HCNT–CD/Fc-IL-CHO is shown in Figure SA.

Fabrication of the Immunosensor. Before the preparation of the immunosensor, the gold electrode with 3 mm diameter (Au) was polished carefully using 0.3 and 0.05 μm alumina slurries, followed by sonication in doubly distilled water. Subsequently, a 10 μL homogeneous suspension of HCNT–CD/Fc-IL-CHO was added on the pretreated electrode and dried in the air. After the electrode was washed with doubly distilled water, a 10 μL of antibody solution (100 μg mL⁻¹) was dropped on the electrode modified by HCNT–CD/Fc-IL-CHO film, followed by incubation for 60 min in the air. To remove excess antibody, the electrode was washed with doubly distilled water. Then, a 10 μL of BSA solution (3.0 wt %) was added on the electrode and incubated for 30 min at 37°C.

Table 1. Comparison of the Proposed Immunosensor and Other cTnI Sensors

| materials modified on electrode | linear range (ng mL⁻¹) | detection limit (ng mL⁻¹) | RSD (%) | references |
|-------------------------------|------------------------|---------------------------|---------|------------|
| streptavidin-microsphere      | 0.1–10                 | 0.2                       |         | 25         |
| Au nanoparticle               | 0.2–12.5               | 0.2                       |         | 26         |
| whiskered nanofibers          | 0.5–100                | 0.04                      |         | 27         |
| 3-aminopropyl triethoxy silane | 1–250              | not reported              |         | 28         |
| nanostructured ZrO₂           | 0.1–100                | 0.1                       |         | 29         |
| HCNT/CD-Fc-IL-CHO             | 0.05–20                | 0.04                      |         | this work  |

Table 2. Determination of cTnI in Human Serum Samples with the Proposed Sensor (n = 3)

| concentration of cTnI in the sample (ng mL⁻¹) | added (ng mL⁻¹) | founded (ng mL⁻¹) | recovery (%) | RSD % (n = 3) |
|---------------------------------------------|----------------|-------------------|--------------|--------------|
| 0.12                                        | 0.1            | 0.24              | 109.1        | 6.3          |
| 0.5                                         | 0.65           | 104.8             | 4.7          |              |
| 1.5                                         | 1.58           | 97.5              | 7.2          |              |

Fabrication of the Immunosensor. Before the preparation of the immunosensor, the gold electrode with 3 mm diameter (Au) was polished carefully using 0.3 and 0.05 μm alumina slurries, followed by sonication in doubly distilled water. Subsequently, a 10 μL homogeneous suspension of HCNT–CD/Fc-IL-CHO was added on the pretreated electrode and dried in the air. After the electrode was washed with doubly distilled water, a 10 μL of antibody solution (100 μg mL⁻¹) was dropped on the electrode modified by HCNT–CD/Fc-IL-CHO film, followed by incubation for 60 min in the air. To remove excess antibody, the electrode was washed with doubly distilled water. Then, a 10 μL of BSA solution (3.0 wt %) was added on the electrode and incubated for 30 min at 37°C.

Figure 3. (A) Effect of the concentration of antibody on the peak current of immunosensor. (B) Effect of immobilization time of antibody on the peak current of immunosensor. (C) Effect of the concentration of BSA on the peak current of immunosensor. The concentration of cTnI is 5 ng mL⁻¹.

Figure 4. DPV responses of the immunosensor to different concentrations of cTnI. Inset: Calibration curve of the immunosensor. Error bars represent standard deviation, n = 3. The potential range was from −0.4 to 0.6 V, pulse amplitude was 0.05 V, pulse width was 0.05 s, and sample width was 0.02 s.

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| 1.5                                         | 1.58           | 97.5              | 7.2          |              |
to block active sites of nonspecific binding. Finally, a 10 μL of antigen solution of different concentrations was dropped on the surface of electrode and incubated for 40 min. After the electrode was washed with doubly distilled water, the electrochemical measurements were performed in 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆]. The preparation of the immuno-sensor is shown in Figure 5B.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: sgyrab@163.com. Phone: +86-736-7186115.

ORCID
Guanyu Shen: 0000-0003-1121-3098

Notes
The authors declare no competing financial interest. This article does not contain any studies with animals performed by any of the authors.

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