Highly enhanced electrochemiluminescence based on synergetic catalysis effect of enzyme and Pd nanoparticles for ultrasensitive immunoassay of protein

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1. Experimental

1.1 Preparation of luminol solution

A 0.01 M stock solution of luminol was prepared by dissolving luminol in 0.1 M NaOH solution and was kept at 4℃ when not in use.

1.2 Preparation of FCNTs-PdNPs

FCNTs adsorbed a large number of PdNPs were achieved by redox method. Firstly, 1.0 mg of FCNTs were dispersed in 2.0 mL PEI (3%) with the aid of ultrasonic agitation. Then, 2.0 mL 10 m mol L^{-1} K_2PdCl_4 aqueous solution was added in the FCNTs solution. After successively sonicated about 2 h, 2.0 mL of $NaBH_4$ (0.1 M) was slowly added under vigorously stirring. Subsequently, another 2 h of stirring was performed to complete the reaction. At last, the products were collected by

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centrifuged and washed three times with distilled water, then dispersed in 2.0 mL PBS (pH 7.4) solution. It was stored in the refrigerator at 4℃ when not in use.

1.3 Preparation of FCNTs-PdNPs@anti-AFP-GOD

Firstly, 0.5 mL anti-AFP was added into 1.0 mL prepared FCNTs-PdNPs solution, and incubated for 12 h at 4℃ to form FCNTs-PdNPs@anti-AFP bioconjugates. Then, 0.5 mL GOD (1 mg mL^{-1}) was added to the bioconjugates solution for about 1 h at 4℃ to block non-specific adsorption. Later, the solution was centrifuged at 8000 rpm for 15 min, the upper solution was removed and the lower products washed three times with PBS solution (pH 7.4). At last, the collected lower sediment was dispersed in 1.0 mL PBS solution (pH 7.4) and stored at 4℃ until use.

1.4 Fabrication of the ECL immunosensor

Au electrode (Φ=4 mm) was polished carefully with 1.0, 0.3 and 0.05 μm alumina powder, followed by washing thoroughly with distilled water. Before modification, the electrode was scanned in 0.5 M $H_2S_2O_4$ between -0.4 and 1.5 V at 50 mV s^{-1} until a reproducible cyclic voltammogram was obtained and then allowed to dry at room temperature.

The cleaned electrode was coated with 7 μL Nafion-ethanol solution (v/v, 2%) and dried in the air. Then the modified electrode was immersed into L-Cysteine (Cys) solution (pH 2.3) for 6 h. The obtained electrode was thoroughly washed with distilled water to remove the physically adsorbed Cys. Subsequently, it was immersed into the prepared Au colloid for 1.5 h to form AuNPs monolayer. Following that, the

obtained electrode was placed into prepared luminol solution at 4℃ for 1 h. Repeated the above two steps after the layer-by-layer (LBL) of (luminol-AuNPs)$_3$ were modified on the electrode. Then, the modified electrode was placed into anti-AFP solution at 4℃ for about 12 h. After that, it was incubated in BSA solution (0.5%) for 1 h at 4℃ in order to block the non-specific binding sites. Subsequently, the electrode was incubated in antigen solution for 20 min at room temperature. At last, 20 μL secondary antibody (FCNTs-PdNPs@anti-AFP-GOD) solution was dropped on the modified electrode incubated for 20 min at the room temperature, then rinsed with distilled water to remove unbound bioconjugates.

2. Results and discussion

2.1 Stability and regeneration of the proposed immunosensor

The inter-day and intra-day stability of the developed immunosensor was assessed by assaying 1 ng/mL AFP. The inter-day stability of the proposed ECL immunosensor was detected a prepared immunosensor with three different days in a week. And the relative standard deviation (RSD) of inter-day stability was 5.32%. Similarly, the intra-day stability was evaluated from the response to the same prepared immunosensor and detected three times everyday. The RSD of intra-day were 3.51%, 2.06% and 5.08%, respectively. Thus, the inter-day and intra-day stability of the proposed immunosensor were acceptable.

Furthermore, 0.05 M NaOH was chosen to break the antibody-antigen linkage. After detecting 1 ng mL^{-1} AFP, the immunosensor was dipped into 0.05 M NaOH for

5 min, removd to detect 1 ng mL^{-1} AFP, and repeated 6 times continuously. The RSD was 5.96% for six successive assays, indicated good regeneration of the proposed immunosensor.

2.2 Preliminary analysis of real samples

In order to monitor the analytical application of the developed ECL immunosensor, some human serum samples were examined by the proposed immunosensor. Firstly, human serum was appropriate dilute 50 times by PBS (pH 7.4). Then, a series of samples were prepared by adding AFP of different concentrations to human serum samples (standard addition method). As shown in Table S1, the added AFP concentrations were 1, 5, 10, 15, 20 ng/mL. Ultimatly, the six prepared immunosensors were separate to detect the diluted serum solution and the above five human serum samples. The inherent AFP in diluted human serum as the blank has been deducted, and experimental results were exhibited in Table S1. These data showed that the recovery (between 82.8% and 105.6%) were acceptable, which provided a promising alternative tool for determining AFP in real biological samples.

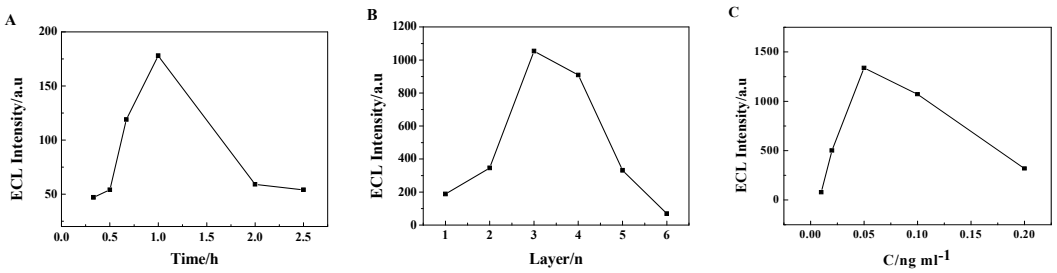

Fig. S1 ECL intensity of different modified electrodes in 0.1 M PBS (pH 7.4) solution. (A) Relationship between ECL intensity and the incubation time of luminol. The ECL intensity increased with the increase of incubation time and reached the maximum when incubation time was 1h. Thus, the incubation time of luminol was chosen as 1h in this study. (B) Relationship between ECL intensity and the layers of luminol. As the increasing layers of luminol, the ECL intensity increased and reached the maximum when assemble layers was 3. So the assemble layers of luminol was 3. (C) Relationship between ECL intensity and the concentration of glucose in the presence of detection solution. The ECL intensity increased with the increase of glucose in the detection solution, and reached the maximum as 0.05 M glucose was presence in the detection solution. Thus, 0.05 M glucose was chosen in the detection solution. The voltage of the photomultiplier tube was set at 800 V. Scan rate: 100 mV s^{-1}.

Table S1

Sample number	Add (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)
1	1	1.07	82.8
2	5	4.87	92.5
3	10	10.70	104.6
4	15	16.08	105.6
5	20	21.05	104.0

Table S 1 Determination of AFP added in normal human serum with the proposed immunosensor. All ECL signals were measured in 0.1 M PBS (pH 7.4) solution containing 0.05 M glucose. The voltage of the photomultiplier tube was set at 800 V. Scan rate: 100 mV s^{-1}.

Table S 2

Detection method	Linear range/ng·mL^{-1}	Detection limit/ng·mL^{-1}	References
electrochemical	0.5~50	8.0×10^{-2}	1
electrochemical	0.014~142	7.0×10^{-3}	2
chemiluminescent	0.008~0.3	5.0×10^{-3}	3
Flow-injection chemiluminescent	1.0~80	1.0×10^{-1}	4
Electrochemiluminescence	0.01~100	3.3×10^{-3}	5
Electrochemiluminescence	0.0001~20	3.3×10^{-5}	Present work

Table S 2 Comparation of our research with other methods for AFP detection.

Table S 2 demonstrated that the proposed immunosensor has a relative large linear range and low detection limit compared with previous reports.

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