Supplemental information

A porous CuO nanowires-based signal amplification immunosensor for the detection of carcinoembryonic antigen

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2. Experimental

2.1. Apparatus and reagents

Gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$), multi-walled carbon nanotube (MWCNTs), (3-aminopropyl) triethoxysilane (APTES), ferrocenecarboxylic acid (Fc-COOH), cupric chloride dihydrate ($CuCl_2 \cdot 2H_2O$), N-Hydroxysuccinimide (NHS) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochlorideand (EDC) were from Aladdin Reagent Company (Shanghai, China). Bovine serum albumin (BSA) was supplied by Alfa Aesar (Tianjin, China). Horse radish peroxidase (HRP), CEA, alpha fetoprotein (AFP) and anti-CEA antibodies were purchased from Zhengzhou Biocell Biotechnology Co., Ltd (Zhengzhou, China). Hydrogen peroxide (H_2O_2, 30 wt %) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd (Shanghai, China). All other chemicals were of analytical grade. 0.2 M phosphate buffer solutions (PBS) at various pH values were prepared by mixing the stock solutions of

0.2 M NaH_2PO_4 and 0.2 M Na_2HPO_4 with different proportion. The pure water used to prepare all solutions in this study was purified with a water system provided.

Electrochemical experiments were carried out on a Potentiostat/Galvanostat Model 283 Electrochemical analyzer with three-electrode system (American Mattson Company, America). X-ray powder diffraction (XRD) measurements were performed on a Bruker D8 advanced X-ray diffractometer. Fourier-transform infrared (FT-IR) spectroscopic were determined using a Nicolet Avatar 360 FT-IR spectrometer. Transmission electron microscope (TEM) measurements were made on a Hitachi H-600 (Hitachi, Tokyo, Japan).

2.2. Preparation of the pCuOw@Fc/AuNPs-Ab_2 labels

2.2.1. Synthesis of pCuOw

Nanoparticle-assembled pCuOw was synthesized according to previously published by simple and scalable precipitation method [1]. In a typical procedure, First, a mixture solution of 0.68 g of $CuCl_2 \cdot 2H_2O$ and 0.15 g of citric acid in 60 mL of distilled water in a beaker was stirred vigorously at room temperature, and then 4.0 g of NaOH was added to the mixed solution to form a blue precipitate of $Cu(OH)_2$. After 10 min, the blue precipitate was filtered and washed several times with distilled water and ethanol, and then dried in an oven at 60 °C for 6 h. Finally, the blue powders were annealed at 400 °C for 4 h in a muffle furnace, and black pCuOw was obtained.

2.2.2. Preparation of amino-functionalized pCuOw

The pCuOw were functionalized with amine groups by common method using a silane coupling agent. The pCuOw-NH_2 was prepared in the typical synthetic process, 60 mg of pCuOw was dispersed in 60 mL of ethanol in a three-necked flask via sonication, and then 1.0 mL of APTES was added to the suspension with vigorous stirring. After stirring for 12 h at room temperature, the resulting mixture was cleaned with distilled water and ethanol three times. The pCuOw-NH_2 was obtained by drying at 60 °C.

2.2.3. Synthesis of pCuOw@Fc/AuNPs

Here, pCuOw were used as efficient carriers to co-support electronic mediator and secondary anti-CEA (Ab_2) as labels for amplifying the detection of CEA. The pCuOw@Fc/AuNPs were prepared as the following several steps. (i) AuNPs were prepared by the citrate reduction method. (ii) Fc was covalently bound on pCuOw by combining the carboxyl group of Fc and the amine group of pCuOw-NH_2 with the aid of EDC/NHS coupling agent. Briefly, 10 mL of 200 $\mu g \cdot L^{-1}$ Fc solution were added in a beaker, the carboxyl groups on which have been activated by addition 10 mL of the solution containing 0.4 M EDC and 0.1 M NHS for 2 h. Then, 0.1 g of pCuOw was added into the above mixture and stirred overnight. After that, the mixture was centrifuged and washed with water for three times. The final pCuOw@Fc precipitates were obtained and thoroughly washed with ultrapure water and centrifuged (4000 rpm for 10 min) to remove any un-reacted Fc. Then, the pCuOw@Fc was resuspended in 10 mL distilled water for further usage. (iii) The pCuOw@Fc/AuNPs was synthesized by as-prepared AuNPs could tightly anchor to the amine of pCuOw-NH_2 by the strong coordinating capability between AuNPs and amine group.

2.2.4. Preparation of the pCuOw@Fc/AuNPs-Ab_2 labels

As-prepared pCuOw@Fc/AuNPs was employed for the labeling of Ab_2 by the interactions between Ab_2 and AuNPs. 2 mg of pCuOw@Fc/AuNPs was dispersed in 1 mL PBS at pH 7.4. This dispersion was then mixed with 1 mL of 10 $ng \cdot mL^{-1}$ Ab_2. The mixture was allowed to react at 4 °C under stirring for 12 h, followed by centrifugation. The resulting pCuOw@Fc/AuNPs-Ab_2 labels were washed with buffer solution (pH 7.4) and then re-dispersed in 1 mL of buffer and stored at 4 °C before use.

2.3. Synthesis of CNTs-AuNPs

CNTs-AuNPs were synthesized according to previously published procedure [2]. Briefly, 1.0 mg of purified CNTs was dispersed in 1.0 mL $HAuCl_4$ solution (1 *wt* %) with the help of ultrasonic treatment to make CNTs dispersed equably. Then, the suspended solution was diluted to 100 mL distilled water and heated to boiling while stirring. Next, 4.0 mL of sodium citrate solution (1 *wt* %) was added to the boiling

solution, which was kept boiling for 10 min. After washing and centrifugation, the resulted CNTs-AuNPs nanocomposites were dispersed in 5 mL water.

3.1. XRD of CNTs and CNTs-AuNPs

CNTs and CNTs-AuNPs were characterized by XRD. As are given in Fig. 1E prominent diffraction peak of CNTs at 26° was to be seen (curve a). Diffraction peaks located at 36.2°, 43.1°, 66° and 78°, which should be attributed to the diffraction of (111), (200), (220) and (311) planes of AuNPs (curve b), Furthermore, the existence of sharp diffraction peaks (002) around a 2θ value at 26.2° demonstrates the crystalline nature of CNTs. Hence, CNTs can act as a good conductive substrate and influence the crystalline nature of the AuNPs particles that are dispersed. This result indicates that AuNPs was anchored on CNTs.

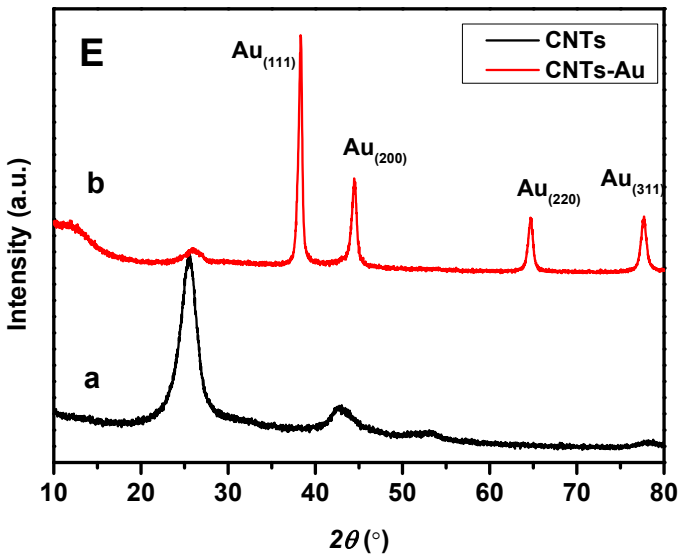


Fig. S1 XRD patterns (E) of CNTs (a) and CNTs-AuNPs (b)

3.2 Characterization of electrochemical behavior

Electrochemical impedance spectroscopy (EIS) was an important tool for monitoring the impedance changes of modified electrodes surface. To further monitor the surface conditions of the modified electrodes, EIS has been employed to characterize the surface properties of different modified electrodes. EIS consist of a semicircle at high frequencies corresponding to the electron transfer limiting process, and a line at low

frequencies resulting from the diffusion limiting step of the electrochemical process. The diameter of the semicircle corresponds to the electron-transfer resistance (R_{et}), which could be estimated from the diameter of the semicircle. EIS of the bare GCE (a), CNTs-AuNPs/GCE (b), anti-CEA/CNTs-AuNPs/GCE (c), BSA/anti-CEA/CNTs-AuNPs/GCE (d), and CEA/BSA/anti-CEA/CNTs-AuNPs/GCE (e) was recorded in 0.2 M PBS solution containing 5 mM $Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl with frequency varied from 100 kHz to 0.01 Hz. As shown in Fig. S2, Curve a shows the impedance spectra of the bare electrode, a semicircle at higher frequencies and a linear curve at lower frequencies were obtained. The semicircle diameter decreased sharply (curve b) when the bare electrode was modified with CNTs-AuNPs, which was attributed to the excellent electrode transfer ability of CNTs-AuNPs on the surface of modified GCE electrode. In contrast, when the CNTs-AuNPs/GCE modified electrode was incubated into anti-CEA and BSA solution in turn, the semicircle diameter obviously increased in turn (curve c and d) respectively, which might be attributed to the hydrophobic layer of proteins insulates hindering electronic transfer. In the following step, the modified electrode was incubated in CEA solution. The immune-reaction between CEA and anti-CEA immobilized on the electrode surface will occur. The binding leads to the semicircle diameter further increasing (curve e). The result demonstrated the successful fabricated process of the electrochemical immunosensor.

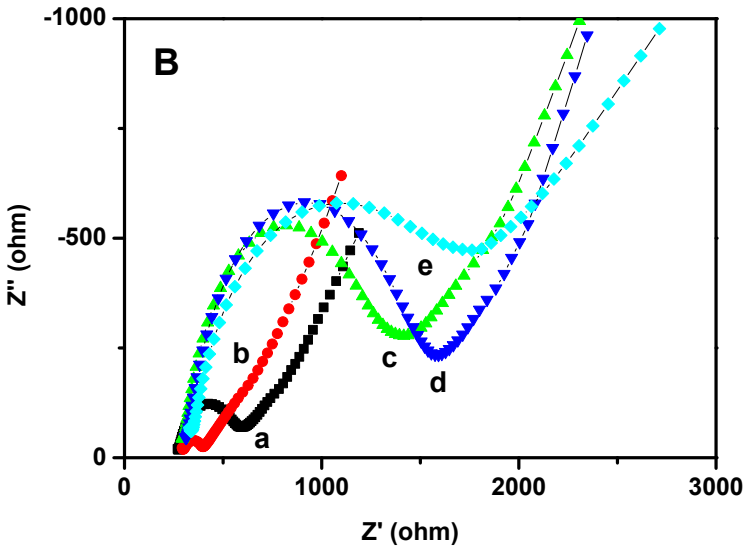
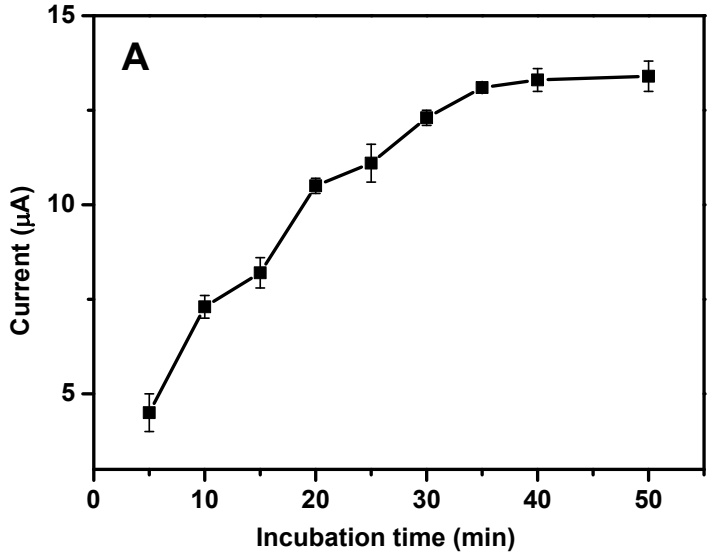
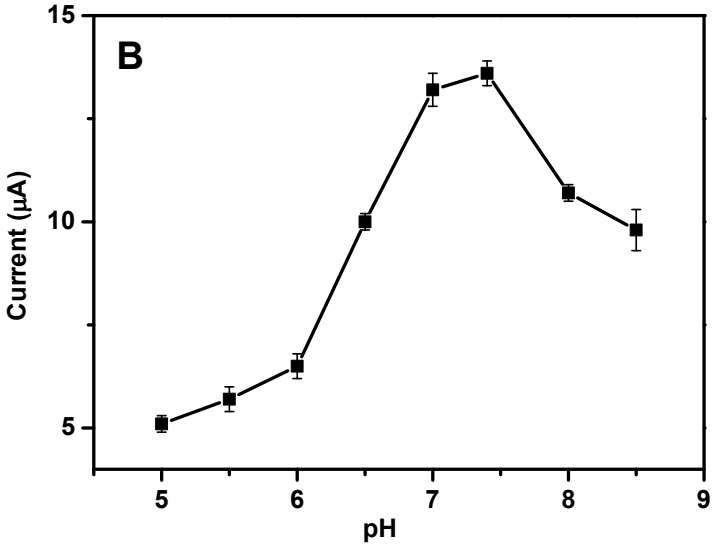


Fig. S2 EIS of the electrode at different stage in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl: (a) bare GCE electrode, (b) CNTs-AuNPs/GCE, (c) anti-CEA/CNTs-AuNPs/GCE, (d) BSA/anti-CEA/CNTs-AuNPs/GCE, (e) CEA/BSA/anti-CEA/CNTs-AuNPs/GCE, frequency range from 0.01 to 100 kHz.

3.3. Optimization of experimental conditions

In order to find the optimum the incubation time and the pH value of the detection solution, the proposed electrochemical immunosensor was incubated in 1.0 $ng \cdot mL^{-1}$ CEA, and then was tested by CV. Incubation time is an important factor in the construction of the proposed immunosensor. As shown in Fig. 4A, the current response rapidly increases from 5 min to 35 min, and then tends to level off due to the saturated formation of antigen-antibody complexes. Therefore, 35 min was chosen as the optimal incubation time. In order to optimize the pH, the proposed immunosensors are tested in a series of PBS buffer with the pH from 5.0 to 8.5. As shown in Fig. 4B, the peak current response increases from pH 5.0 to 7.4, and then decreases to pH 8.5. Therefore, pH 7.4 is chosen as the optimal pH of the detection solution. The concentration of H_2O_2 is a very important parameter in the electrochemical detection because pCuOw could act as catalysts for reduction of H_2O_2. Therefore, we investigate the dependence of the CV under a series of the concentration of H_2O_2. As shown in Fig. 4C, a significant increase of the peak current response is observed from 0.5 to 2.5 mM of H_2O_2, while insignificant differences are obtained for greater concentrations. Thus, 2.5 mM of H_2O_2 is used for detection of CEA.


A
Current (μA)
Incubation time (min)
15
10
5
0
10
20
30
40
50


B
Current (μA)
pH
15
10
5
5
6
7
8
9

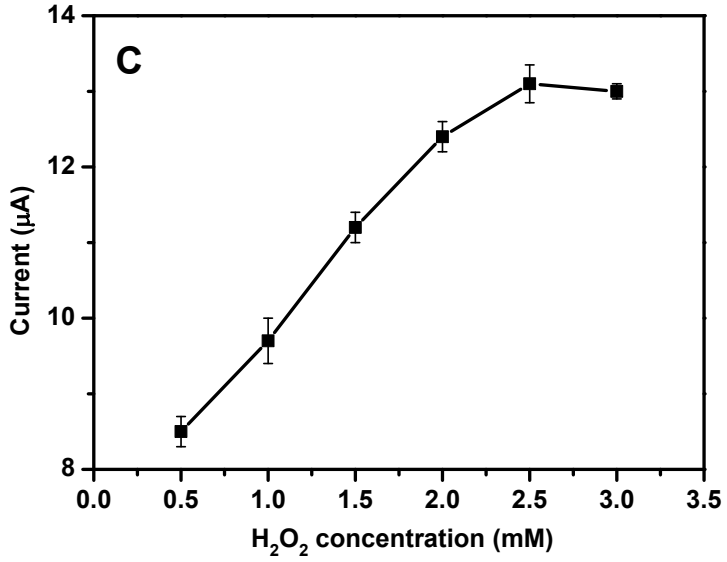


Fig.S3 Effect of incubation time (A), pH of detection solution (B) and concentration of H_2O_2 on CV responses to 1 ng·mL^{-1} CEA.

Table S1 Detection of CEA in human serum sample.

Content of CEA in the sample (ng·mL^{-1})	Added CEA (ng·mL^{-1})	Founded CEA (ng·mL^{-1})	Recovery (%)	RSD (%, n=5)
1.85	0.1	1.97	101.0	2.24
	0.5	2.32	98.7	3.72
	1	2.95	103.5	3.58
	10	11.67	98.5	3.23
	20.0	21.44	98.1	4.08

References

[1] L. Wang, K. Zhang, Z. Hu, W. Duan, F. Cheng, J. Chen, Porous CuO nanowires as the anode of rechargeable Na-ion batteries, Nano Res. 7 (2014) 199-208.

[2] R. Zhang, X. Wang, One step synthesis of multiwalled carbon nanotube/gold nanocomposites for enhancing electrochemical response, Chem. Mater. 19 (2007) 976-978.