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Streptavidin-functionalized Tin Disulfide Nanoflakes based ultrasensitive electrochemical immunosensor for detection of tumor marker

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Experimental

Materials and Reagents: A series of standard CEA solutions with a concentration of 0-75 ng·mL^{-1}, horseradish peroxidase (HRP)-labeled mouse monoclonal anti-CEA (HPR-anti-CEA) and biotinylated mouse monoclonal anti-CEA (biotin-anti-CEA) were from the CEA enzyme-linked immunosorbent reagent (ELISA) kit, which were obtained from CanAg Diagnostics. Electrochemiluminescent immunoassay (ECLIA) reagent kits for AFP used for the reference method were provided by Roche Diagnostics GmbH (Germany). Thionine was supplied by Acros Organics. Streptavidin and chitosan were bought from Sigma. Disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), potassium ferricyanide ($KFe(CN)_6$), potassium ferrocyanide ($K_2Fe(CN)_6$), hydrogen peroxide (H_2O_2, 30%), tin (II) chloride dehydrate ($SnCl.\ 2H_2O$), sublimed sulfur (S), carbon disulfide (CS_2) and ethanol (C_2H_5OH) were all obtained from Sinopharm Chemical Reagent Co. Ltd. (China). Phosphate buffer (PBS, 0.1 M) with various pH values was prepared from NaH_2PO_4 and Na_2HPO_4 solution. Moreover, clinical serum samples were provided by Jiangsu Cancer Hospital. The water used through the experiment was distilled water, and the other reagents were all analytical pure.

Apparatus: Scanning electron microscopy (SEM, S-4800, Japan) was applied to characterize the morphologies of the prepared SnS_2 NFs and immunosensor with an acceleration voltage of 15 kV. Electrochemical measurements were operated at the CHI852C electrochemistry workstation (Shanghai CH Instruments Co., China) with a conventional three-electrode system (using a saturated calomel electrode as the reference electrode, a platinum electrode as the counter electrode, and a glassy carbon electrode as the working electrode). The reference ECLIA was performed with a Roche Elecsys 2010 immunoassay analyzer (Roche Diagnostics GmbH). The adjustment of pH of the buffer solution was carried out at PHS-3C (Shanghai Leici Chuangyi Apparatus & Instrument Co., Ltd.). Electrochemical impedance spectroscopy (EIS) was measured on the Autolab/PGSTAT30 electrochemical workstation (the Netherlands), the amplitude of the sine wave potential is 5 mV and the test solution was 0.1 M KCl solution containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Besides, the static water contact angles were measured by the contact angle meter (Rame-hart-100).

Preparation of SnS_2 nanoflakes and electrochemical CEA immunosensor: SnS_2 NFs were synthesized according to our previous method [S1], and the specific steps for the synthesis are as followed. $SnCl_2{\cdot}2H_2O$ powder was mixed with excessively sublimed S powder. After being placed for 15 min, the mixture was transferred to a capped corundum crucible and then heated from room temperature to 200 °C (the average growth rate was about 4.7 °C·min^{-1}) in the electrothermal oven. Subsequently, the reactants were kept at 200 °C for 10 h before cooling naturally. Finally, the synthesized powders were rinsed with CS_2, distilled water and C_2H_5OH, respectively. After centrifugations, the product was dried in air atmosphere of 80 °C.

The glassy carbon electrode (GCE) was firstly polished with 0.3 μm and 0.05 μm alumina (Buehler), and the residual alumina was rinsed with distilled water. After being ultrasonically cleaned in a dilute nitric acid solution, the GCE was washed with C_2H_5OH and deionized water, and then dried with N_2. 2.0 mg SnS_2 NFs were dispersed in chitosan (2.0 % wt in H_2O), and then mixed with streptavidin (200 μg·mL^{-1}) at 1:1 volume ration for 2 h. Then, the pretreated GCE was modified with 5 μL of the mixture above and dried at 4 °C overnight. Subsequently, 10 μL of biotin-anti-CEA (1.0 μg·mL^{-1}) was dropped

onto streptavidin-SnS_2 NFs coated GCE and incubated for 30 min, and then rinsed with PBS to get rid of the adsorbed biotin-anti-CEA. Finally, the GCE modified with antibodies was immersed in PBS containing 1% BSA for 2 h to block nonspecific binding sites. After being washed several times with PBS, the fabricated immunosensor was placed in pH 7.0 PBS at 4 °C before use.

Immunoassay process for the detection of CEA:. The constructed CEA immunosensor was incubated in the solution which consists CEA antigen, HRP-anti-CEA and PBS, and then rinsed with PBS several times. Afterwards, the electrochemical measurements were operated in PBS (containing 0.4 mM thionine and 5.0 mM H_2O_2) through cyclic voltammetry (CV). The differential pulse voltammetry (DPV) measurement was performed from -0.4 to 0.1 V at 50 ms pulse width and 50 mV pulse amplitude. All electrochemical measurements were performed at room temperature.

References

S1 Y. C. Zhang, Z. N. Du, S. Y. Li, M. Zhang, *Appl. Catal B-Environ.*, 2010, **95**, 153-159.

Optimization of electrochemical detection conditions

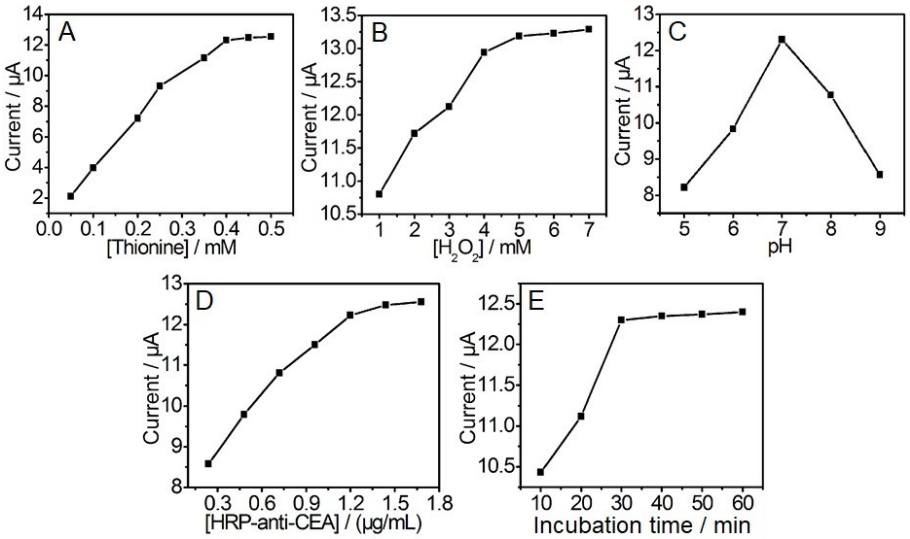

Fig. S1. Dependence of amperometric response of the immunosensor on concentrations of thionine (A), H_2O_2 (B), pH value of PBS (C), HRP-anti-CEA concentration (D), and intubation time (E).