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Ultrasensitive sandwich-type immunosensor for cardiac troponin I based on enhanced electrocatalytic reduction of H_2O_2 by β-cyclodextrins functionalized 3D porous graphene supported Pd@Au nanocubes

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2.1. Chemicals and Apparatus

Prostate-specific antigen (PSA), alpha fetoprotein (AFP), Human IgG (HIgG) and bovine serum albumin (BSA, 99%) are purchased from Shanghai Linc-Bio Science Co. Ltd. (Shanghai, China). Phosphate buffered saline (PBS) containing 1/15 mol L^{-1} Na_2HPO_4 and 1/15 mol L^{-1} KH_2PO_4 is employed as an electrolyte for all electrochemical measurement. All other reagents are of analytical grade and used without further purification. Ultrapure water (18.25 MΩ cm) is used throughout all experiments.

A conventional three-compartment electrochemical cell including a platinum wire auxiliary electrode, working electrode (Φ = 4 mm) and a saturated calomel reference electrode (SCE) are used. Electrochemical measurements are operated by employing a CHI760E electrochemical workstation (Chenhua Instrument Shanghai Co, Ltd, China). The images of surface morphology are obtained with the field emission scanning electron microscopy (ZEISS, Germany). The transmission electron microscope (TEM) images are investigated with JEM-2100 microscope (JEOL, Tokyo, Japan). Energy Dispersive X-ray Spectroscopy (EDS) is recorded by JEOL JSM-6700F microscope (Japan). The specific surface area of the 3D-PG and MCS was characterized by Brunauer–Emmett–Teller (BET) analysis. The pore size distribution of MCS and 3D-PG was calculated by density functional theory (DFT) model and Barrett-Joyner-

Halenda (BJH) method, respectively.

2.2. Synthesis of MCS

Typically, resorcinol and formaldehyde solution were used as precursors for synthesize MCS. Firstly, 0.1 mL ammonia aqueous solution (NH_4OH, 25 wt%) was mixed with a solution including 8 mL absolute ethanol (EtOH) and 20 mL deionized water (H_2O), and stirred continuously for more than 10 min. Subsequently, 0.2 g phenol and 0.28 mL formaldehyde solution were added and continually stirred for 24 hours at 30 °C, and then heated for 24 hours at 100 °C under a static condition in a Teflon-lined autoclave. Finally, the solid was recovered by centrifugation and dried at 100 °C for 48 hours. The obtained solid (1.0 g) was mixed with potassium hydroxide (0.75g) and activated, the product was heated under N_2 atmosphere to 350 °C for 1 hour with a heating rate of 2 °C/min and heated to 650 °C for 4 hours with a heating rate of 5°C/min.

2.3. Synthesis of FMCS

Firstly, 0.1 g of the MCS was dissolved in 25 mL of absolute ethanol and shocked (30 min) to obtain well-dispersed solution. Then, 0.1 mL of APTES was added and heated to reflux at 70 °C for 1.5 hours.

2.4. Synthesis of AuNPs-FMCS-Th

Take the above 10 mL FMCS suspension solution in flask. Then, 10 mL Au NPs was mixed with FMCS suspension and continually ultrasoniced for 1 h. After that, the redundant Au NPs were removed out

of the solution by centrifugation. Then, 40 mg Th was dissolved in the obtained AuNPs-FMCS solution followed by stirred for 12 h. The finally synthesized AuNPs-FMCS-Th was washed three times with absolute alcohol and dried at 30 °C in vacuum.

2.5. Synthesis of 3D-PG

Coal tar pitch (3 g) was added into xylene (25 mL) under stirring for 20 min. Meanwhile, nano MgO (4 g) was mixed with KOH (2 g), then the mixture was sufficiently ground and added to xylene (15 mL) in beaker. Next, the above two mixtures were mixed and evaporated at 120°C under vigorous stirring to form the precursor. After the precursor was dried at 70 °C, the resultant brown solid was fully ground and heated at 800 °C for 2 h under Ar atmosphere. Eventually, the precipitate was washed thoroughly with 10% HCl aqueous solution and ultrapure water, and dried at 80 °C overnight.

2.6. Synthesis of CDs-3D-PG

Briefly, 3D-PG ultra-dispersed solution (10 mL, 0.5 mg mL^{-1}) was mixed with CDs aqueous solution (10 mL, 4 mg mL^{-1}) and ammonia solution (150 μL). Then, hydrazine hydrate (10 μL) was added into the mixtures. After being vigorously stirred for 10 min, the mixtures were heated at 60 °C for 3 h. Subsequently, the final product was obtained by centrifugal separation (10000 rpm, 10 min), and dried at 30 °C overnight.

2.7. Synthesis of Pd NCs

First, CTAB (228 mg) was dissolved in 30 mL doubly distilled water at 95 °C . Subsequently, the solution of Na_2PdCl_4 (1.0 mL, 22.56 mM) was added quickly under vigorous stirring and kept for 5 min. Finally, freshly prepared AA solution (1.0 mL, 0.08 M) was slowly added into the above mixture, and the reaction continued for 40 min and cooled down to room temperature.

2.8. Synthesis of Pd@Au NCs

Take the above Pd suspension solution 10 mL in flask, the solution was heated at 95 °C for 5 min under magnetic stirring. Then, 155 μL as-prepared $HAuCl_4$ solution (24.3 mM) was injected into the Pd suspension using a micro syringe. Next, the AA solution (0.5 mL, 0.08 M) was added into above mixture. The reaction mixture was continued heating at 95 °C for 1 h and cooled down to room temperature. Finally, the products were washed with ultrapure water by repeating the centrifugation and re-dispersion procedure several times to remove impurities.

2.9. Synthesis of CDs-3D-PG-Pd@Au NCs

The as-synthesized Pd@Au NCs were added to ultrapure water (5 mL) during ultrasonication for 10 min. Subsequently, CDs-3D-PG (10 mg) were added to the above solution and the mixture was stirred for 12 h so that the Pd@Au NCs absorbed onto the CDs-3D-PG sufficiently. Then, the excess Pd@Au NCs were removed out by centrifugation and the solid was obtained. The synthesized CDs-3D-PG-Pd@Au NCs was redispersed in

PBS (4 mL).

2.10. Experimental measurements

The electrochemical performance of the working electrodes were investigated by electrochemical impedance spectra (EIS), cyclic voltammetry (CV), and amperometric *i-t* measurements. EIS were recorded from 0.1 to 10^5 Hz at 0.26 V in a solution containing 0.1 mol L^{-1} KCl and 2.5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$. Amperometric *i-t* was used to monitor the analytical property of the immunosensor and the detail as followed: -0.2 V was selected as the detection potential, and after the background current stability, injected 10 μL H_2O_2 (5 mol L^{-1}) into PBS under mild stirring, and recorded the changed currents. All the measurements were performed at room temperature in PBS (pH = 7.0).

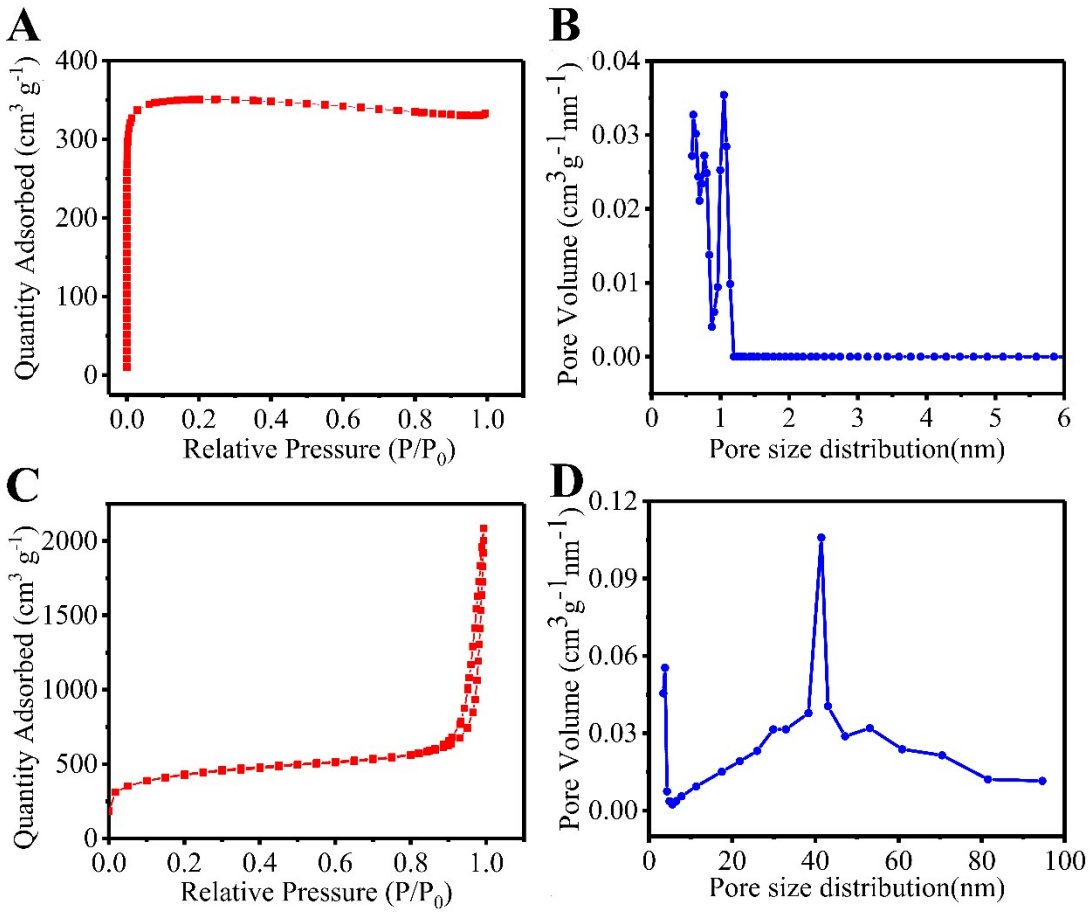

Fig. S1 (A) Nitrogen adsorption/desorption isotherm of MCS. (B) Pore size distribution of MCS calculated by DFT model. (C) Nitrogen adsorption/desorption isotherm of 3D-PG. (D) Pore size distribution of 3D-PG determined by BJH model.

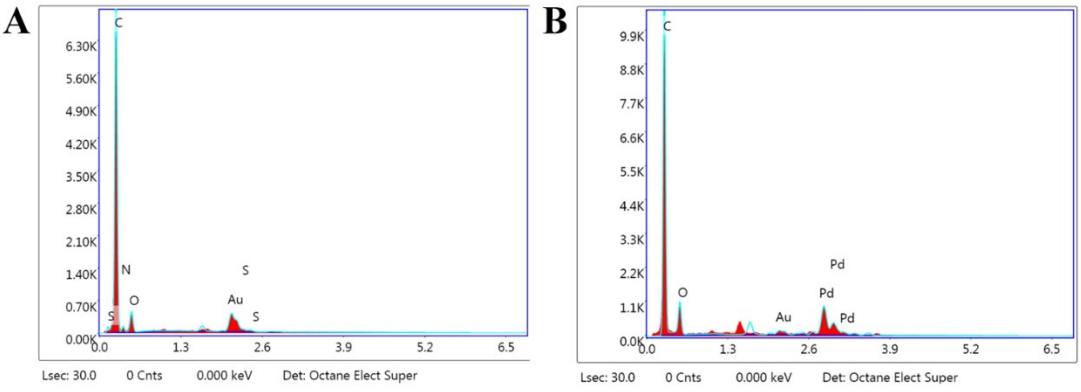

Fig. S2 (A) EDS analysis of AuNPs-FMCS-Th. (B) EDS analysis of CDs-3D-PG - Pd@Au NCs.

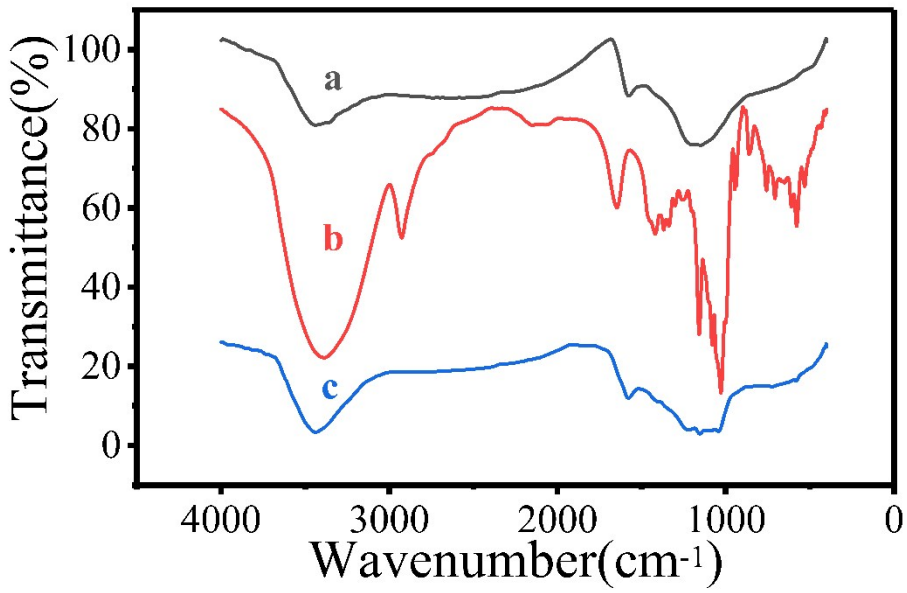

Fig. S3 FT-IR spectroscopy of 3D-PG (a), CDs (b) and CDs-3D-PG (c).

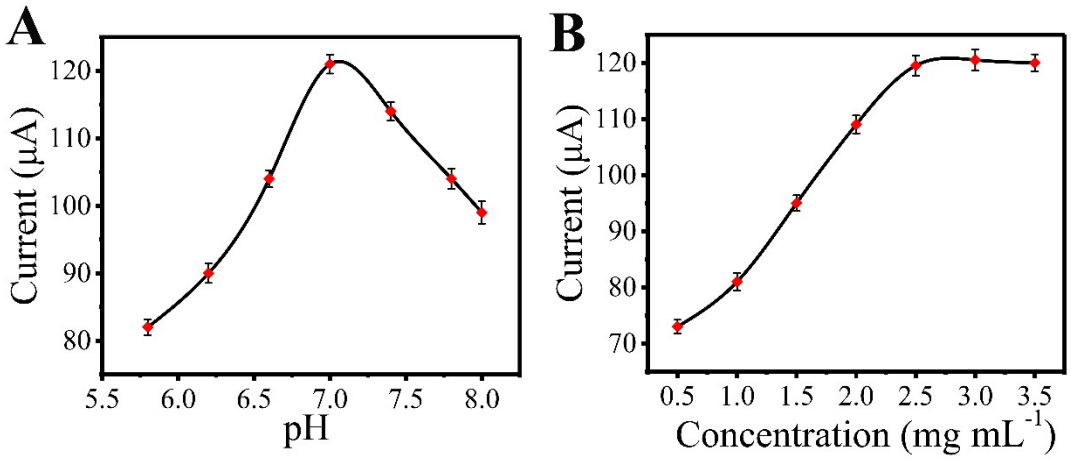

Fig. S4 Optimization of experimental parameters: (A) pH value. (B) CDs-3D-PG-Pd@Au NCs concentration. For all studies, error bar = RSD (n = 5).

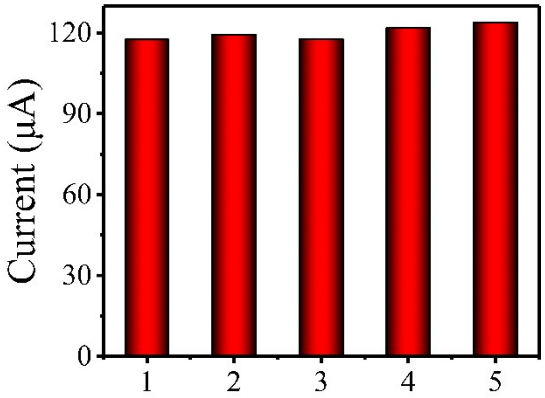

Fig. S5 Reproducibility of five developed EC immunosensors incubated with 1.0 ng mL^{-1} cTnI.

Table S1 Simulation parameters of the equivalent circuit components

Electrode	**R_s (Ω)**	**R_{et} (Ω)**	**C_{dl} (F)**	**Z_w**
GCE	58.24	146.3	4.052×10^{-6}	0.001524
AuNPs-FMCS-Th/GCE	61.87	96.4	2.303×10^{-6}	0.001468
Ab_1/AuNPs-FMCS-Th/GCE	62.44	336.5	1.540×10^{-6}	0.001216
BSA/Ab_1/AuNPs-FMCS-Th/GCE	60.59	524.6	1.608×10^{-6}	0.000887
cTnI/BSA/Ab_1/AuNPs-FMCS-Th/GCE	61.58	731.8	2.794×10^{-6}	0.000818
Ab_2-label/cTnI/BSA/Ab_1/AuNPs-FMCS-Th/GCE	63.43	782.2	1.533×10^{-6}	0.000936

Table S2 Our Proposed immunosensor for cTnI detection in real samples (n = 5).

sample	addition (ng mL^{-1})	found (ng mL^{-1})	RSD (%)	recovery (%)
1	0.5	0.51±0.03	3.7	102.0
2	1	0.98±0.06	3.3	98.0
3	5	5.07±0.22	4.5	101.4
4	10	10.12±0.42	4.0	101.2

Table S3 Human serum sample analysis using the proposed method and the ELISA method.

Sample	The detection content (ng mL^{-1})		RSD (%)		Relative error (%)
	This method	ELISA	This method	ELISA	
1	0.54±0.03	0.52±0.04	3.3	4.5	3.8
2	1.28±0.04	1.31±0.06	2.4	3.5	-2.3
3	5.25±0.23	5.34±0.33	3.6	4.9	-1.7