Electronic Supplementary Information

A label-free cytosensor for the enhanced electrochemical detection of cancer cell using polydopamine coated carbon nanotubes

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Experimental

Materials. Carboxylic group modified multi-walled carbon nanotubes (c-CNTs) with purity level above 95%, length 1.0-2.0μm, and diameter between 20 and 40 nm were obtained from Shenzhen Nanotechnology Co. Ltd. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), MES monohydrate, bovine serum albumin (BSA), dopamine (DA), folic acid (FA) and Calcein-AM were obtained from Sigma/Aldrich (St. Louis, MO). Tris was purchased from Shanghai Chemical Reagent Co. Ltd. Phosphate buffer saline (PBS, 0.01M, pH 7.4) is composed of 136.7 mM NaCl, 2.7 mM KCl, 8.7 mM Na_2HPO_4, and 1.4 mM KH_2PO_4. The pH value of PBS was adjusted by changing the ratio of Na_2HPO_4 and KH_2PO_4. All other chemicals were of analytical grade, and all aqueous solutions were prepared with doubly distilled water.

Synthesis of CNT@PDA-FA nanoprobe. For the preparation of the CNTs@PDA-FA nanoprobe, 10.0 mg of c-CNTs was first dispersed into 2.0 mL Tris buffer (pH 8.5) and sonicated at room temperature for 10 min. After the centrifugation of the mixture, the sediment was washed repeatedly with Tris buffer and dispersed into 2.0 mg mL^{-1} dopamine Tris buffer (pH 8.5) and incubated in the dark at 37 □ for 24 h. The obtained brown suspension was washed with Tris buffer and double distilled water for three times (3000rpm, 5min) to obtain the CNT@PDA nanocomposites. 5 mg of folic acid, 2.1 mg of EDC and 3.2 mg of NHS were mixed in 20 mL DMSO/PBS (v/v=1:10) solution at room temperature for 2 h to modify the terminal carboxylate group. Then, 5.0 mg of the

CNT@PDA nanocomposite was added into the solution, stirring at room temperature for 12 h. After the centrifugation (13000rpm, 10min), the collected CNT@PDA-FA nanoprobes were washed and redispersed in PBS (pH 7.4) to the concentration of 1.0 mg mL^{-1}.

Construction of the cytosensor. A glass carbon electrode (GCE) (Φ=3mm) was successively polished to a mirror by using 0.3 and 0.05 μm alumina slurry (Beuhler) followed by rinsing thoroughly with water. After successive sonication in 1:1 nitric acid/water, acetone, and doubly distilled water, the electrode was rinsed with doubly distilled water and allowed to dry at room temperature. As shown in Scheme 1, 5uL of the prepared CNTs@PDA-FA solution was dropped to the GCE and dried in a silica gel desiccator to fabricate a functional film (CNTs@PDA-FA/GCE). The modified electrode was subsequently immersed into 10.0 mg mL^{-1} BSA solution for 1 h at 37 □ to prevent the nonspecific adsorption following a carefully rinse with 0.1 M pH 7.4 PBS to remove physically absorbed protein. After that, the electrode was stored in air prior to use.

Cell culture and immobilization. Hela and HL-60 cells were cultured in a flask in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) in an incubator (5% CO_2, 37□). At the logarithmic growth phase, the cells were collected and separated from the medium by centrifugation at 1000 rpm for 10 min and then washed twice with a sterile pH 7.0 PBS. The sediment was suspended in the PBS to obtain a homogeneous cell suspension with the final concentration of 5.0×10^6 cells mL^{-1}, which was determined by using a Petroff-Hausser cell counter. The cell suspensions with various contents were prepared. Finally, 5 μL of Hela cell suspension with different concentrations was dropped to the CNTs@PDA-FA-modified GCE and was incubated at 37 □for 1 h to achieve cell immobilization. Electrochemical impedance spectroscopy (EIS) experiments were performed with an Autolab electrochemical analyzer (Eco Chemie, The Netherlands) in a 10 mM K3Fe(CN)6/K4Fe(CN)6 (1:1) mixture with 1.0 M KCl as the supporting electrolyte, using an alternating current voltage of 5.0 mV, within the frequency range of 0.01 Hz-100 kHz.

As for HL-60 cells, alternating current impedance measurements were performed on an CHI 660a workstation (Shanghai Chenhua, Shanghai, China) in 0.01M pH 7.0 PBS containing different concentration cells with a conventional three-electrode system comprised of a platinum wire as the auxiliary, a saturated calomel electrode as the reference, and the modified GCE as the working electrode. The impedance was then measured with time continuously at 10 Hz.

Apparatus. The morphologies of the CNTs@PDA nanocomposite were characterized by transmission electron microscopy (TEM, JEOLJEM 200CX) with an accelerating voltage of 200 kV. Fourier transform infrared (FT-IR) spectra were recorded on a Vector 22 FT-IR spectrometer (Bruker). Samples were thoroughly ground with exhaustively dried KBr. The static water contact angles were measured at 25□ by a contact angle meter (Rame-Hart-100) employing drops of pure deionized water. The readings were stabilized and taken within 120 s after the addition.

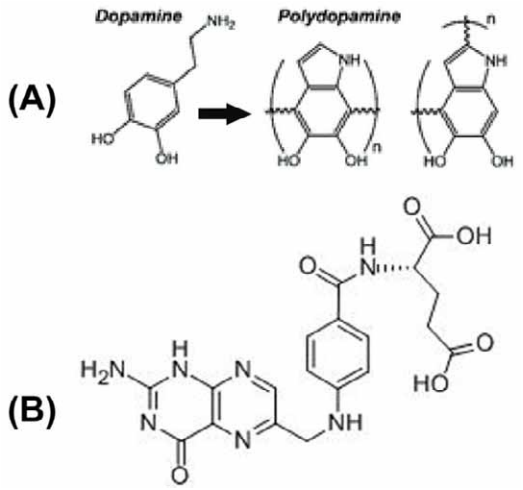

Fig. 1S (A) Schematic illustration of the spontaneous oxidative polymerization of dopamine. (B) The chemical structure of folic acid.

Characterization of the CNTs@PDA composite

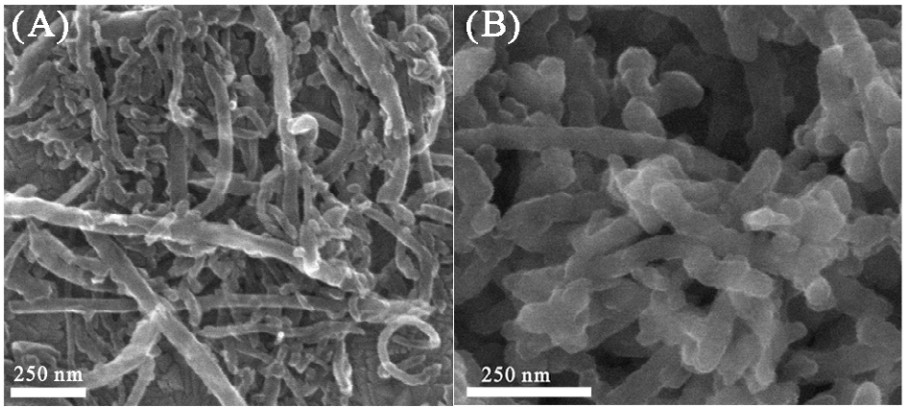

Fig. 2S Representative FESEM images of CNTs-COOH (A) and CNTs@PDA (B).

Characterization of CNTs@PDA-FA nanoprobe

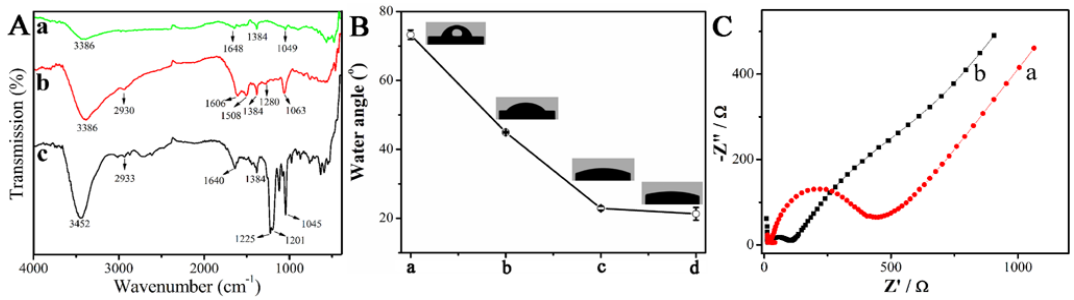

Fig. 3S FT-IR spectra of CNTs-COOH (a), CNTs@PDA (b), and CNTs@PDA-FA (c). (B) Contact angle of bare GCE (a), CNTs-COOH/GCE (b), CNTs@PDA/GCE (c), and CNTs@PDA-FA/GCE (d). (C) Nyquist diagrams of electrochemical impedance spectra recorded from 0.01 to 10^6 Hz for $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ (10 mM, 1:1) in 1.0 M KCl at the bare GCE (a) and CNTs@PDA-FA/GCE (b).