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"Sugarcoated haws on a stick"-like MWNTs–Fe_3O_4–C coaxial nanomaterial: Synthesis, characterization and application in electrochemiluminescence immunoassays

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ABSTRACT

In this paper, a carbon coated magnetic nanoparticle (Fe_3O_4–C) was first synthesized via solvothermal reaction and carbonization of glucose under hydrothermal condition. The electrochemiluminescence (ECL) property of Fe_3O_4–C was studied, and exhibited a peak at 1.21 V. In the goal to amplify the ECL intensity for sensitive detection, a novel coaxial carbon coated magnetic nanomaterial (MWNTs–Fe_3O_4–C) was synthesized. Fourier transform infrared (FT-IR) spectroscopy, transmission electron microscopy (TEM), thermal gravimetric analysis (TGA), powder X-ray diffraction (XRD) and powder X-ray photoelectron spectrometry (XPS) were applied as powerful tools to characterize and to demonstrate the named nanomaterial. MWNTs–Fe_3O_4–C showed better ECL property than Fe_3O_4–C. Furthermore, an ultrasensitive ECL immunosensor based on MWNTs–Fe_3O_4–C was developed for the determination of carcinoembryonic antigen (CEA). The prepared ECL immunosensor exhibited high sensitivity, good reproducibility, long-term stability, and acceptable precision on the detection of CEA in clinical human serum samples.

1. Introduction

Recently, there has been increasing interest in fabricating nanomaterials that have important applications in life and materials science. Moreover, the nanomaterials of interest are expected to possess unique physical or chemical properties (Shin et al., 2009; Khandare et al., 2012). Towards this direction, the possibility of bringing together several different functional components into a single nanostructure offers great potential for increased efficiency and versatility for numerous applications (Khandare et al., 2012).

Electrochemiluminescence (ECL) detection offers an inexpensive assay, low background noise, high detection sensitivity, and a wide dynamic range (Jie and Yuan, 2012; Li et al., 2011). Furthermore, nanomaterial-based ECL detection has received considerable attention during the past several decades, owing to its versatility, simplified optical setup, and good control. So far, carbon-based nanomaterials (CNMs) have been studied for the ECL property. In addition, carbon quantum dots (Dong et al., 2010) and graphene quantum dots (Li et al., 2012) have been found with great ECL property, which can be used in ECL detection. And the CNMs-based ECL sensors have been applied in detection of biomolecules (Yan et al., 2012) for their superiority in chemical inertness and biocompatibility and low toxicity.

As one of the important CNMs, amorphous carbon (AC) is an interesting and unique coating material for nanostructures (Liu et al., 2011; Sun et al., 2006), and can be obtained via inexpensive and environmentally benign hydrothermal processes using glucose as a precursor (Wu et al., 2010; Qi et al., 2010). Lately, core/shell structured nanoparticles with a carbon shell have stimulated great interest (Qi et al., 2010; Xia et al., 2011). Fe_3O_4 has been a promising core candidate for carbon coating due to several characteristics: (i) the ease of isolation of the magnetism (Chen et al., 2010); (ii) high electrical conductivity (Zhang et al., 2011); (iii) low cytotoxicity (Zhu et al., 2008); and (iv) easily available of Fe_3O_4. Carbon-encapsulated Fe_3O_4 nanoparticles (Fe_3O_4–C) have been synthesized by the hydrothermal reaction of Fe_3O_4 microspheres and glucose in water (Liu et al., 2011; Gao et al., 2011). Fe_3O_4–C can be well stabilized in biological systems and used as hybrid materials due to their large surface area, easily functionalized surfaces, perfect biocompatibility and magnetic responsiveness. Herein, we discovered the ECL property of Fe_3O_4–C and further investigated it.

To apply Fe_3O_4–C in an ECL sensor, we introduced multiwall carbon nanotubes (MWNTs), and designed a novel "sugarcoated haws on a stick"-like coaxial carbon coated magnetic nanomaterial

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(MWNTs–Fe_3O_4–C). It has been known that MWNTs have stimulated great attention due to their unique structure, high specific surface area, remarkable chemical stability, and special electronic properties (Cheng et al., 2012; Kauffman and Star, 2008). More importantly, MWNTs with incorporated metal and metal oxide nanoparticles (Fernandez-Abedul and Costa-Garcia, 2008) have also been frequently used as sensing materials thanks to their competitive performance. Nanocomposites composed of MWNTs and Fe_3O_4 have been fabricated using solvothermal method (Song et al., 2011). And, the size of the Fe_3O_4 deposited on MWNTs can be controlled by adjusting the amount of sodium acetate in the reaction system (Fernandez-Abedul and Costa-Garcia, 2008). Herein, we synthesized "sugarcoated haws on a stick"-like MWNTs–Fe_3O_4. In a further step, the surface of Fe_3O_4 was coated with carbon shell through hydrothermal reaction to obtain the MWNTs–Fe_3O_4–C. When the ECL reagents are applied in bioanalysis, to the same amount of biomolecules, the MWNTs–Fe_3O_4–C-based ECL sensor provides a better signal amplification than the Fe_3O_4–C-based ECL sensor.

To demonstrate the application of MWNTs–Fe_3O_4–C in analytical chemistry, an ultrasensitive ECL immunosensor for CEA was developed using MWNTs–Fe_3O_4–C as signal amplifier. Moreover, the flexible ECL immunosensor exhibits not only high sensitivity and specificity, but also excellent performance in real human serum assay.

2. Experimental section

2.1. Reagents

All the reagents were of analytical-reagent grade or the highest purity available, and directly used for the following experiments without further purification, except where otherwise noted. CEA, CEA primary antibody (Ab_1), CEA secondary antibody (Ab_2) and bovine serum albumin (BSA) were purchased from China Shanghai Linc-Bio Science Co., Ltd. (dispatched from U.S.A. Sigma). Polyethylene glycol-1000 (PEG-1000), graphite powder, chloroauric acid ($HAuCl_4$), ethylene glycol N-ethyl-N-(3-(dimethylamino)-propyl) carbodiimide (EDC), N-hydroxysuccini mide 4-iodomethylbenzoate (NHS) and poly(diallyldimethylammonium chloride) (PDDA) were obtained from Sinopfarm Chemical Reagent Co., Ltd (Shanghai, China). MWNTs (purity C 95%, ~30–50 nm outer diameter, ~10–30 μm length) were obtained from Beijing University of Chemical Technology. PBS (10 mmol L^{-1}, made from Na_2HPO_4, KH_2PO_4, and H_3PO_4) was employed as phosphate-buffered saline and supporting electrolyte. CEA, Ab_1, Ab_2 and BSA solutions were prepared with PBS (pH 7.4, 10 mmol L^{-1}) and stored at 4 °C. All the water used in assays and solution is Milli-Q water (Milli-Q, Millipore, 18.2-MΩ resistivity).

2.2. Characterization

The ECL measurements were carried out on an MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remex Analytical Instrument Ltd., Co.) equipped with a photomultipier tube (PMT, detection range from 300 to 650 nm) biased at 800 V. Infrared spectroscopy (IR) was achieved on a FT-IR Spectrum RX (PerkinElmer Spectoment). SEM images were recorded using a JEOL-JSM-6300 scanning electron microscope. TEM images were obtained from a JEOL JEM-1400 microscope (Japan). TGA was performed with a Simultaneous Thermal Analysis-STA 409EP. XRD patterns were recorded under a Japan Rigaku Rotaflex diffractometer (D/MAX 2200PC) equipped with a rotating anode and using Cu-Kα radiation ($\lambda = 1.5418$ Å, 40 kV, 40 mA), over the range $20° \leq 2\theta \leq 80°$. XPS spectrum were measured by using a Thermofisher ESCALAB 250 X-ray photoelectron spectrometer with monochromatized Al-Kα X-radiation in ultra high vacuum ($< 10^7$ Pa). A three-electrode system was used in the experiment with a bare and modified GCE (3 mm in diameter) as the working electrode, respectively. An Ag/AgCl electrode (saturated KCl) and a Pt wire electrode were used as reference electrode and counter-electrode, respectively.

2.3. Preparation of MWNTs–Fe_3O_4

MWNTs were firstly oxidated to carboxylic group-functionalized MWNTs (MWNTs–COOH; see Supporting Information). Then, 0.81 g $FeCl_3 \cdot 6H_2O$ (3 mmol) was dissolved in 40 mL ethylene glycol to form a clear yellow solution under magnetic stirring for 0.5 h. To the yellow solution, 0.020 g MWNTs–COOH was added and sonicated for 0.5 h till well dispersed. Then, 3.60 g natrium aceticum (NaAc, 43.9 mmol) and 1.09 g PEG-1000 were added to the solution. Afterwards, the mixture was stirred vigorously for 0.5 h and then sealed in a Teflon-lined stainless-steel autoclave (50 mL capacity). The autoclave was sealed and heated at 200 °C for 20 h and allowed to cool to room temperature. The black product was collected with the help of a magnet field, and washed six times with ethanol and dried at 60 °C for 6 h (Wang et al., 2011).

2.4. Preparation of MWNTs–Fe_3O_4–C

In the next step, 0.2 g MWNTs–Fe_3O_4 was sonicated for 10 min in 40 mL HNO_3 (0.1 mol L^{-1}), followed by washing with water. Then, the treated MWNTs–Fe_3O_4 were redispersed in aqueous glucose solution (0.5 mol L^{-1}) and sonicated for another 10 min. Next, the suspension was sealed in a Teflon-lined stainless-steel autoclave (50 mL capacity). The autoclave was heated and maintained at 180 °C for 6 h. When it cooled to room temperature, the MWNTs–Fe_3O_4–C composite material was isolated with the help of a magnet field and washed with water six times. Finally, the obtained MWNTs–Fe_3O_4–C was dried under vacuum at 50 °C for 12 h (Qi et al., 2010).

2.5. Preparation of MWNTs–Fe_3O_4–C–Ab_2 (or Fe_3O_4–C–Ab_2)

The synthesis of Fe_3O_4–C is illustrated in the Supporting Information. To 0.010 g MWNTs–Fe_3O_4–C (or Fe_3O_4–C), 1 mL of freshly prepared EDC (20 mg mL^{-1}, pH 7.4) and 1 mL of NHS (10 mg mL^{-1}, pH 7.4) were added. Then, the mixture was sonicated for 15 min and incubated at room temperature for 2 h. After the incubation, the excess EDC and NHS were removed with the help of a magnet field and washed with water three times. Then, 2 mL of Ab_2 solution (20 μg mL^{-1}, pH 7.4) was added to the resulting black product. After being shaken for 10 min, the mixture was incubated at 4 °C (as the biomolecules could retain their bioactivity for a long time at this temperature) for 2 h. The excess Ab_2 was removed with the help of a magnet field and washed with PBS three times. To block the excess amino group and nonspecific binding sites of the MWNTs–Fe_3O_4–C–Ab_2 (Fe_3O_4–C–Ab_2), 2 mL BSA (1%) was added and shaken for 10 min. Then, the mixture was incubated at 4 °C for 2 h. After being isolated with the help of magnet field and washed with PBS, the resultant MWNTs–Fe_3O_4–C–Ab_2 (Fe_3O_4–C–Ab_2) was finally diluted with PBS to a final volume of 2 mL and stored at 4 °C until use.

2.6. Fabrication of the ECL immunosensor

The immunoassay procedure was illustrated in Scheme 1. GCE with a diameter of 3 mm was successively polished using 1, 0.3, and 0.05 μm Al_2O_3 slurry and then washed ultrasonically in ethanol and water for 5 min, respectively. Firstly, 5 μL of AuNPs–EGN (see Supporting Information) solution was dropped on the