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A novel sandwiched electrochemiluminescence immunosensor for the detection of carcinoembryonic antigen based on carbon quantum dots and signal amplification

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ABSTRACT

In this study, a novel sandwiched electrochemiluminescence (ECL) immunosensor for the detection of carcinoembryonic antigen (CEA) was developed. The nanocomposite of polydopamine and Ag nanoparticles (PDA-AgNPs) was prepared by the redox reaction between Ag^+ and dopamine. This nanocomposite not only provided an effective matrix for the immobilization of primary antibody (Ab_1) but also enhanced the conductivity of the electrode. Carbon quantum dots (CQDs) were immobilized on the poly(ethylenimine) functionalized graphene oxide (PEI-GO) through amido-bond. Then Au nanoparticles were decorated on the CQDs modified PEI-GO matrix, and the resulted complex AuNPs/CQDs-PEI-GO was introduced to link secondary antibody (Ab_2). The CQDs can be connected to the electrode surface through the combination of CEA with Ab_1 and Ab_2, and then the amplified electrochemiluminescence signal of CQDs was obtained with the synergistic effect of AgNPs, polydopamine, AuNPs and PEI-GO. Under the optimal conditions, the ECL intensity was proportional to the logarithm value of CEA concentration in the linear range from 5 pg mL^{-1} to 500 ng mL^{-1} with a detection limit of 1.67 pg mL^{-1} for CEA detection. The immunosensor was applied for the CEA detection in real samples with satisfactory results. The proposed ECL immunosensor showed good performance with high sensitivity, specificity, reproducibility, stability and will be potential in clinical detection.

1. Introduction

As well known to all, cancer is a kind of complex disease which greatly threatens the human health in recent years. Tumor markers are biomacromolecules which are associated with the cancer whose measurement or identification is important in diagnosis and prognosis (Kiang et al., 1990; Schneider et al., 2000; Ilantzis et al., 2002; Lin et al., 2014). Carcinoembryonic antigen (CEA) is one kind of glycoprotein most often associated with colorectal carcinomas and is widely used as a tumor marker for clinical diagnosis of liver, colon, breast and colorectal cancer (Duffy, 2001; Huang et al., 2010; Iwazawa et al., 2000; Naghibalhossaini and Ebadi, 2006). So the sensitive detection of CEA can provide essential information in early monitoring and screening disease recurrence (Kulasingam and Diamandis, 2008; Sun et al., 2013).

Up to now, a number of immunoassay techniques for the detection of CEA have been developed including fluoroimmunoassay, enzyme immunoassay, electrochemical immunoassay, radioimmunoassay, and chemiluminescence assay (Yuan et al., 2007; Wang, 2006; Tothill, 2009; Suni, 2008; Jacobs et al., 2010; Yuan et al., 2001; Lai et al., 2011). Among these methods, electrochemiluminescence (ECL) immunoassay has gained increasing attention due to the advantages such as high sensitivity, good selectivity, low background signal and rapid response (Mao et al., 2011; Zhuo et al., 2014; Blackburn et al., 1991). For the development of sandwich-type ECL immunosensor, the immobilization of labeled secondary antibodies (Ab_2) is a vital step for generating signals to improve the sensitivity of the immunosensor (Lai et al., 2011). So introducing abundant signal amplification elements in favor of the sensitive detection of CEA is the main goal.

Luminol, semiconductor quantum dots (QDs), $Ru(bpy)_3^{2+}$ and their analogues have been widely investigated as ECL-luminophores (Wang et al., 2012a; Yang et al., 2014; Xiao et al., 2013). However, these luminescence species have some disadvantages. For example, $Ru(bpy)_3^{2+}$ is difficult to be immobilized on the electrode surface modified with biomolecules due to the lack of functional groups on its structure. Luminol can generate strong ECL signal in alkaline solution. But its ECL signal is very weak in

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neutral solution, which limits its application in the biological system (Qi, 2004). Compared with $Ru(bpy)_3^{2+}$ and luminol, QDs have been widely used in immunosensors attributed to their unique properties such as controllability, biocompatibility, low toxicity and large modifiable specific surface area (Fang et al., 2012; Jie et al., 2013). Among those substances, carbon quantum dots (CQDs) is a kind of environmentally friendly material compared with the metal semiconductor QDs such as CdSe and CdTe. Recently CQDs have become a hot topic in carbon nanomaterial research (Sun et al., 2006) due to its unique optical properties of high photostability, high surface-to-volume reaction, high electrical conductivity and tunable excitation, as well as low cytotoxicity and good biocompatibility. Furthermore, there are amounts of functionalized carboxyl groups on its surface so that it can be easily labeled for ECL detection (Qiao et al., 2010). Based on the quenching effect of the analytes on the ECL of CQDs, the CQDs were used to detect many analytes such as Cd^{2+}(Li et al., 2012), Cu^{2+}(Xu et al., 2013), and S^{2-} (Wang et al., 2014). Moreover, the CQDs were employed as labeling agents for immunoassays of cancer markers (Wang et al., 2012b; Zhang et al., 2015; Zhou et al., 2015), cells (Wu et al., 2013) and adenosine triphosphate (ATP) (Lu et al., 2013).

Graphene is a single layer of aromatic carbon atoms arranged in a honeycomb two-dimensional (2D) lattice (Novoselov et al., 2004; Geim et al., 2007). Owing to its unique structure, graphene has shown some novel properties such as good mechanical strength, large specific surface area, and outstanding electric conductivity (Xia et al., 2010; Liu et al., 2011). However, its availability in electrochemistry is encumbered by the high cohesive van der Waals energy adhering graphitic sheets to one another. So the surface modification of graphene is an essential step for obtaining a molecular level dispersion of individual graphene. In this assay, we prepared poly(ethylenimine) functionalized graphene oxide (PEI-GO). The existence of PEI on graphene oxide not only provided water solubility but also endowed the nanocomposite with $-NH_2$ group, which is favorable to immobilize other CQDs and AuNPs.

In the present work, a sensitive sandwich-type ECL immunosensor was successfully designed for the detection of CEA. The ECL immunosensor was prepared using the nanocomposite of polydopamine and Ag nanoparticles (PDA-AgNPs) as platform to immobilize primary antibody (Ab_1). And the complex of poly (ethylenimine) functionalized graphene oxide (PEI-GO) modified with CQDs and AuNPs was used to immobilize secondary antibody (Ab_2). The ECL of CQDs can be obtained with the combination of CEA with Ab_1 and Ab_2. The ECL signals of CQDs were greatly amplified under the synergistic effect of AgNPs, polydopamine, AuNPs and PEI-GO, which served as favorable conductive materials to promote electron transfer rate and further improved electrochemical sensing ability.

2. Experimental

2.1. Reagents

Human colon cancer carcinoembryonic antigen (CEA) and carcinoembryonic antigen antibody (anti-CEA) were purchased from Linc-Bio Science Co. Ltd. (Shanghai, China). $HAuCl_4 \cdot 4H_2O$, bovine serum albumin (BSA), tris(hydroxymethyl)aminomethane (Tris), N-hydroxysuccinimide (NHS), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were obtained from Sigma–Aldrich. L-lysine, Poly (ethylenimine) (PEI) and dopamine hydrochloride (DA · HCl) were purchased from Aladdin Industry Corporation. Phosphate buffered solutions (PBS, pH 7.4) were prepared using 0.1 mol L^{-1} Na_2HPO_4 and 0.1 mol L^{-1} KH_2PO_4. 0.1 mol L^{-1} $K_2S_2O_8$ containing 0.1 mol L^{-1} KCl was used as the ECL electrolyte. All chemical reagents were analytical grade. Double distilled water was used through the whole study. Human serum samples were provided by Liaocheng People's Hospital.

2.2. Apparatus

Electrochemiluminescence measurements were carried out on a Model RFL-1 ECL analyzer (Xi'an Remex Instrument Co., Ltd., China) with the voltage of the photomultiplier tube (PMT) set at 800 V and with the auxiliary equipment of CHI 760C (Shanghai CH Instruments Co., China) electrochemical workstation. Electrochemical impedance spectroscopy (EIS) was performed using a CHI 760C electrochemical workstation. Transmission electron microscope (TEM) images were obtained on a JEM-2100 electron microscope operating at 200 kV (JEOL Ltd., Japan). Fluorescence spectra were obtained with a LS 55 fluorescence spectrophotometer (Perkin Elmer, USA). UV–Vis absorption spectra were obtained using Lambda 750 spectrophotometer (Perkin Elmer, USA).

2.3. Preparation of CQDs

CQDs were prepared as follows: L-lysine (0.2 g) was dissolved in the mixed solution of ethylene glycol (5 mL) and ultrapure water (10 mL) under ultrasonication. The solution was then transferred into a Teflon-lined autoclave and heated at 180 °C for 12 h. The obtained brown-yellow product was placed in a dialysis bag (MW = 3500) and then the solution out of the dialysis bag was vacuum evaporated in a rotary evaporator at 45 °C to obtain the target CQDs. The resulting solution was stored at 4 °C for further characterization and use.

2.4. Preparation of PEI functionalized graphene oxide

Graphene oxide (GO) was prepared according to Hummer's method (Hummers and Offeman, 1958). Then 0.3 g as-synthesized GO was dispersed in 200 mL of double distilled water under ultrasonication for 30 min, subsequently 0.1 g PEI was added. The mixed solution was stirred at 60 °C for 12 h. After being cooled to room temperature, the resulting dispersion was centrifuged, washed and dried. The resultant product of PEI-GO was redispersed in water for further use (Kim et al., 2012).

2.5. Preparation of Au nanoparticles

AuNPs were prepared according to the previously reported literature (Handley, 1989). Briefly, 2 mL of 50 mmol L^{-1} $HAuCl_4$ was added into 98 mL of water and then refluxed with stirring. When the solution was heated to boiling, 10 mL of 38.8 mmol L^{-1} sodium citrate was quickly added. When the color changed from pale yellow to deep red, the solution was stirred for another 20 min. Then, the solution was translated into brown volumetric flask after being cooled to room temperature and then stored at 4 °C for further use.

2.6. Preparation of AuNPs /CQDs-PEI-GO nanocomposite

25 mL CQDs solution was added into the PEI-GO solution and stirred for 3 h at 80 °C. The obtained product of CQDs-PEI-GO was centrifuged and dispersed in water again. 0.1 mL EDC and NHS (4:1) mixed solution was added into 10 mL CQDs-PEI-GO solution with stirring for 30 min, subsequently 5 mL AuNPs was added and the mixture was kept stirring for 6 h. The resulting product AuNPs /CQDs-PEI-GO was centrifuged and dispersed in PBS (pH 7.4).