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A streptavidin functionalized graphene oxide/Au nanoparticles composite for the construction of sensitive chemiluminescent immunosensor

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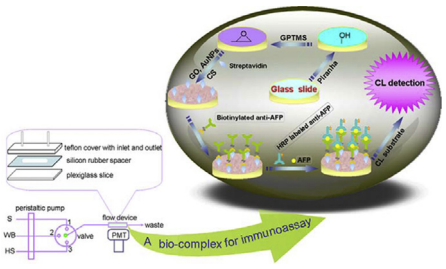
HIGHLIGHTS

- A novel streptavidin/GO/AuNPs composite is prepared for immobilizing antibody.
- A highly sensitive chemiluminescent immunosensor is constructed for tumor marker.
- The immunoassay system shows extremely low detection limit down to picogram level.
- This work provides a promising approach for ultrasensitive biosensing applications.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, a novel streptavidin functionalized graphene oxide/Au nanoparticles (streptavidin/GO/AuNPs) composite is prepared and for the first time used to construct sensitive chemiluminescent immunosensor for the detection of tumor marker. The streptavidin/GO/AuNPs composite and the immunosensor are characterized using scanning electron microscopy, static water contact angle measurement and electrochemical impedance spectroscopy. The biofunctionalized composite has large reactive surface area and excellent biocompatibility, thus the capture antibody can be efficiently immobilized on its surface based on the highly selective recognition of streptavidin to biotinylated antibody. Using α-fetoprotein (AFP) as a model, the proposed chemiluminescent immunosensor shows a wide linear range from 0.001 to 0.1 ng mL^{-1} with an extremely low detection limit down to 0.61 pg mL^{-1}. The resulting AFP immunosensor shows high detection sensitivity, fast assay speed, acceptable detection and fabrication reproducibility, good specificity and stability. The assay results of serum samples with the proposed method are in an acceptable agreement with the reference values. This work provides a promising biofunctionalized nanostructure for sensitive biosensing applications.

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1. Introduction

The development of specific and sensitive immunoassay methods relies on the ability to design advanced interface for highly efficient immobilization of proteins [1]. The physical and chemical properties of the interface play a crucial role in obtaining excellent assay performance [2,3]. The loading of proteins on the nanostructure materials surface has opened up potential avenues for fabricating excellent biosensors and biosensing devices [4–7]. Chemiluminescent (CL) immunoassay has attracted increasing attention in different fields due to its advantages such as high sensitivity, wide dynamic range, low-cost instrument and materials, suitability for miniaturization, and no radioactive pollution [8–13]. Conventionally, micro-scaled materials such as microbeads and membranes are used in CL immunoassay for the immobilization of proteins [9,10,14–18]. Due to the unique physical and chemical properties, numerous types of nanomaterials, such as metal nanoparticles, magnetic nanomaterials, carbon nanomaterials and semiconductor nanomaterials, have been extensively employed in electrochemical immunoassay [19–26]. Unfortunately, nanomaterials are seldom exploited as solid support for proteins immobilization to develop CL immunoassay system. In our previous research, biofunctionalized carbon nanotubes were prepared to develop a highly sensitive CL immunoassay method [27], which obviously improved the analytical capacity of CL immunoassay.

The continuous progress of nanotechnology in material science has led to the development of nanostructure materials with unique chemical, physical, and mechanical properties. Graphene oxide (GO), a two-dimensional nanomaterial that contains graphene-like sheets, has received tremendous attention in both experimental and theoretical scientific fields due to its unique and excellent electronic, thermal, optical, and mechanical properties as well as its high surface area [28,29]. GO is often produced by the oxidation of graphite in strong acids and oxidants, thus leading to the hydroxyl and epoxide functional groups on its basal planes, in addition to carboxyl groups located at the sheet edges [30]. The presence of these functional groups makes GO sheets strongly hydrophilic, which allows them to readily swell and disperse in aqueous media [31]. As a new class of carbon nanomaterial, GO opens a potential avenue for fabricating excellent biosensors and biosensing devices [32,33]. Biocompatible GO sheets not only provide an abundant domain for biomolecules but also play a role of signal amplification detection [34,35]. Over the last decade, Au nanoparticles (AuNPs) have attracted increasing research interest in catalysis and sensing because they can provide excellent catalytic activity and "hospitable" environment for biomolecules [36]. Recently, GO-AuNPs nanocomposite films have been prepared for the electrochemical sensing [37]. To the best of our knowledge, there has been no application of GO-AuNPs composite in CL immunoassay.

Streptavidin, a 66 kDa protein purified from the bacterium *Streptomyces avidinii*, has been widely used in immunohistochemistry [38] and immunoassay [39] due to its high specificity and strong affinity for biotin [40]. Herein, a novel streptavidin functionalized GO/AuNPs (streptavidin/GO/AuNPs) composite is, for the first time, prepared on the glass substrate for the construction of highly sensitive CL immunosensor. Based on the highly selective recognition of streptavidin to biotinylated antibody, the capture antibody can be efficiently immobilized on the functionalized composite surface. The resultant biofunctionalized composite shows large reactive surface area and excellent biocompatibility, which are beneficial to obtaining good assay performance. Using α-fetoprotein (AFP) as a model, the fabricated CL immunosensor displays a sub-picogram level detection limit, wide dynamic range, short assay time, and good reproducibility and stability. The streptavidin/GO/AuNPs composite provides a promising platform for highly efficient biosensing.

2. Materials and methods

2.1. Materials and reagents

AFP ELISA reagent kit was bought from CanAg Diagnostics, which consists of a series of AFP standard solutions from 0 to 500 ng mL^{-1}, the stock solutions of horseradish peroxidase (HRP)-labeled mouse monoclonal anti-AFP (20 μg mL^{-1}) and biotinylated mouse monoclonal anti-AFP (1.0 μg mL^{-1}). Electrochemiluminescent immunoassay reagent kit used for reference detection of AFP was provided by Roche Diagnostics GmbH (Germany). Streptavidin, 3-gycidoxypropyltrimethoxysilane (GPTMS, 98%), chitosan and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO). Graphite powder, chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$), trisodium citrate dehydrate and hydrogen peroxide (30%, H_2O_2) were bought from Sinopharm Chemical Reagent Co. Ltd. (China) and *p*-iodophenol (PIP) was from Alfa Aesar China Ltd. Luminol (Acros, Belgium) stock solution (0.01 M) was prepared in 100 mL of 0.1 M NaOH. Prior to use, luminol and PIP stock solutions were mixed and diluted using 0.1 M pH 8.5 Tris–HCl buffer. The HRP substrate solution consists of luminol (5 mM), PIP (0.6 mM) and H_2O_2 (4 mM). Coupling buffer for streptavidin immobilization was 0.01 M pH 7.4 phosphate buffer solution (PBS). Blocking buffer was PBS containing 1% BSA. To minimize unspecific adsorption, 0.05% Tween-20 was spiked into 0.01 M pH 7.4 PBS as wash buffer (PBST). The clinical serum samples were from Jiangsu Institute of Cancer Research. Distilled water was used in all runs, and all other reagents were of the best grade available and used as received.

2.2. Apparatus

The flow-through CL assay system was constructed as illustrated in Scheme 1. Teflon tubes (0.8 mm i.d.) and silicon rubber tubes (1.0 mm i.d.) were used to connect all the components in the flow system. All solutions were carried with a multichannel peristaltic pump. A multiposition valve with five inlets and one outlet was used to switch different solutions into the flow system sequentially. The flow device (Scheme 1) is composed of a Teflon cover (4.0 cm × 2.5 cm × 0.8 cm) with inlet and outlet, a silicon slice rubber spacer (2.0-mm thickness), and a transparent plexiglass slice (0.5-cm thickness), which is positioned in front of photomultiplier (PMT) of the luminescence analyzer. The CL emission was measured with the PTM operated at −700 V. Instrument control and data recording were performed using IFFM software package run under Windows 2003.

Flow-through CL measurements were performed with an IFFM-E Luminescent Analyzer (Remex Analytical Instrument Co. Ltd., Xi'an, China). The reference electrochemiluminescence immunoassay was performed with a Roche Elecsys 2010 immunoassay analyzer (Roche Diagnostics GmbH). Scanning electron microscopy (SEM) images were obtained with a Hitachi S-4800 scanning electron microscope (Japan) at an acceleration voltage of 15 kV. The static water contact angles were measured with a contact angle meter (Rame-Hart-100) using droplets of distilled water at room temperature (RT, 25 °C). Electrochemical impedance spectra (EIS) measurements were performed on an Autolab/PGSTAT30 (The Netherlands) in 0.1 M KCl solution containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The amplitude of the applied sine wave potential was 5 mV.

2.3. Synthesis of GO

GO was synthesized chemically according to Hummers and Offeman method [41]. Briefly, 5 g of graphite and 2.5 g of sodium nitrate were mixed into 15 mL of concentrated sulfuric acid. After the mixed solution was cooled to 0 °C in an ice-bath, 15 g of

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