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A simple and sensitive immunoassay for the determination of human chorionic gonadotropin by graphene-based chemiluminescence resonance energy transfer

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ABSTRACT

In this study, we report a strategy of chemiluminescence resonance energy transfer (CRET) using graphene as an efficient long-range energy acceptor. Magnetic nanoparticles were also used in CRET for simple magnetic separation and immobilization of horseradish peroxidase (HRP)-labeled anti-HCG antibody. In the design of CRET system, the sandwich-type immunocomplex was formed between human chorionic gonadotropin (HCG, antigen) and two different antibodies bridged the magnetic nanoparticles and graphene (acceptors), which led to the occurrence of CRET from chemiluminescence light source to graphene. After optimizing the experimental conditions, the quenching of chemiluminescence signal depended linearly on the concentration of HCG in the range of 0.1 mIU mL^{-1}–10 mIU mL^{-1} and the detection limit was 0.06 mIU mL^{-1}. The proposed method was successfully applied for the determination of HCG levels in saliva and serum samples, and the results were in good agreement with the plate ELISA with colorimetric detection. It could also be developed for detection of other antigen–antibody immune complexes by using the corresponding antigens and respective antibodies.

1. Introduction

Determination of tumor markers is a powerful technology to allow accurate diagnosis of cancer diseases and monitor the effectiveness of drug and surgery treatments (Qian et al., 2013). Nowadays, saliva has been considered as an alternative matrix for assessing a variety of tumor markers. Compared with blood collection, saliva collection is non-invasive and less stressful without the limitation of time and frequency (Matias et al., 2012; Lamy and Mau, 2012). However, due to the trace levels of tumor markers in saliva samples, it has become a great challenge to seek a simple, sensitive and selective method for determination of trace tumor markers.

Immunoassays have been considered as an effective technology to determine trace tumor markers (Wani and Darwish, 2012). Because of highly selectivity of antibody–antigen immunocomplex, sensitive signal response has become a crucial step in the determination of trace target molecules using immunoassay. Resonance energy transfer is known as extremely sensitive to separate distance between donor and acceptor, in which a luminescent donor transfers energy to a fluorescent or nonfluorescent acceptor via nonradiative dipole–dipole interaction, when brought in proximity by a bioaffinity reaction (Qin et al., 2012). Nowadays, fluorescence resonance energy transfer (FRET) is emerging as a useful tool in many fields, such as activation of receptors (Dereli-Korkut et al., 2013), cellular imaging (Lin and Hoppe, 2013), drug release (Lai et al., 2013) and trace detection (Liu et al., 2013). In contrast to FRET, chemiluminescence resonance energy transfer (CRET) is occurred by the oxidation of a luminescent substrate, which is beneficial for reducing the non-specific signals caused by external light excitation. Therefore, CRET has become an attractive light-measuring scheme in bioassays. More importantly, CRET could be used for homogeneous analysis of complex samples without separation (Huang and Ren, 2012).

Unfortunately, little study has been reported so far on CRET. One of the major problems is to identify an effective chemiluminescence (CL) donor or reaction. The most widely used CL reaction in CRET is the luminol-H_2O_2 system, catalyzed by horseradish peroxidase (HRP) (Chen and Li, 2013; Dong et al., 2013). Recently, a number of nanomaterials have been explored for the

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enhancement of CL efficiency. Magnetic nanoparticles (MNPs) have many superior characteristics such as small size, high surface-to-volume ratio, fast and effective binding to biomolecules and high magnetic susceptibility. With regards to the application of magnetic nanoparticles in CRET detection, there are two main aspects to consider: (1) The magnetic nanoparticles can effectively conjugate HRP-labeled antibody on their surface and then increase the amounts of HRP in CL reaction; (2) The superparamagnetic properties of magnetic nanoparticles provide a simple magnetic separation to attain interference-free measurement for real detection.

Another problem of CRET is poor energy-transfer efficiency and limited number of energy acceptors. Currently, small-molecule fluorophores, widely used as energy acceptors, have small Stokes shifts (Bi et al., 2009; Qin et al., 2012), which result in poor spectral separation of the acceptor emission from the donor emission. With the development of nanotechnology, carbon materials have attracted much attention in various fields (Morales-Narvaez and Merkoci, 2012; Chou et al., 2012; Dreyer et al., 2010). Especially, graphene had large Stokes shifts and long-range energy transfer efficiency (Lee et al., 2012), which is predicted to be an excellent acceptor in the CRET-based studies.

Human chorionic gonadotropin (HCG), secreted by the trophoblastic cells of the placenta chorionic vesicle, is a kind of peptide hormone with a molecular mass of about 37 kDa (Yang et al., 2010). HCG level in saliva and serum samples has been used as an important marker to diagnose germ cell tumors, trophoblastic cancer, choriocarcinoma and many diseases of pregnancy (Vartiainen et al., 2002; Birken et al., 2001). Therefore, determination of HCG in saliva and serum samples plays an important role in clinical diagnosis, treatment and prognosis.

Herein, we report a simple and sensitive immunoassay for the determination of HCG by using graphene-based CRET. In this protocol, HRP-labeled anti-HCG antibody–conjugated MNPs were incubated with a limited amount of HCG and anti-HCG antibody–conjugated graphene to form a sandwich-type immunocomplex. The close proximity led to the CRET phenomenon between CL light source and graphene as an efficient long-range energy acceptor. This immunoassay could be used for the determination of HCG in saliva and serum samples, which provided a promising potential in clinical diagnosis, treatment and prognosis.

2. Materials and methods

2.1. Reagents and apparatus

HRP-labeled anti-HCG antibody, anti-HCG antibody and HCG were obtained from Shanghai Linc-Bio Science Co. LTD. (Shanghai, China). Graphene sheet was obtained from JCNANO (Nanjing, China). Carboxyl-modified Fe_3O_4 nanoparticles and magnetic separation rack were purchased from BaseLine Chrom Tech Research Centre (Tianjin, China). (N-Morpholino)-ethanesulfonic acid monohydrate (MES), bovine serum albumin (BSA), 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), luminol and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO). Hydrogen peroxide (H_2O_2), dopamine (DA), ascorbic acid (AA), uric acid (UA), glucose and lysozyme (Lys) were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals used in this work were of analytical grade. Doubly distilled water was used throughout the experiments. Human serum and saliva samples were kindly provided by a team of volunteers and then stored at −20 °C until analysis. ELISA Kit (HCG) was purchased from Diagnostic Products (DPC, USA). CL spectra were measured with a multifunction microplate reader (TECAN Infinite 200, San Jose, CA).

2.2. Preparation of anti-HCG antibody–conjugated graphene

Anti-HCG antibody was immobilized on the surface of graphene sheet according to the literature (Lee et al., 2012) with a slight modification. Briefly, graphene (0.1 mg mL^{-1}) was mixed with a 200 μL of aqueous solution containing 2 mg mL^{-1} EDC and 4 mg mL^{-1} NHS. The mixture was then incubated for 20 min at 25 °C in a shaking water bath. Anti-HCG antibody (1 mg mL^{-1}) diluted with phosphate buffer (10 mmol L^{-1}, pH 7.5) was added and the resulting suspensions were shaken at room temperature for 6 h. After washing with distilled water, remaining NHS-active sites of grapheme sheet were blocked with 2.0% BSA in a phosphate buffer (10 mmol L^{-1}) for 3 h. The mixture was centrifuged at 15,000 rpm for 30 min at 4 °C to remove any unbound biomolecules.

2.3. Preparation of HRP-labeled antibody–conjugated MNPs

HRP-labeled antibody–conjugated MNPs were synthesized based on the literature (Lai et al., 2010). Carboxyl-modified magnetic nanoparticles (4 mg) were washed three times with MES buffer and then suspended to a final volume of 250 μL in the same buffer solution. The HRP-labeled anti-HCG antibody–conjugated MNPs were prepared by adding 0.3 mL HRP-labeled anti-HCG antibody (1 μg mL^{-1}), 0.5 mL EDC (2 mg mL^{-1}) into Fe_3O_4 nanoparticles suspension, followed by stirring at room temperature for 12 h. Subsequently, the resulting mixture were separated magnetically and then resuspended in 10 mmol L^{-1} PBS solution (pH = 7.5, containing 2.0% BSA).

2.4. Characterization

The surface morphology of the immune complex was evaluated by atomic force microscopy analyses (DI NanoScope IV AFM, Veeco Co. Ltd., USA).

Electrochemical measurements were performed on a CH 660C Electrochemical Workstation (Shanghai Chenhua Co. Ltd., China) with a conventional three-electrode system. The working electrode is a glassy carbon electrode, the reference electrode is a saturated calomel electrode (SCE), and the counter electrode is a platinum wire.

2.5. Determination of HCG by using graphene-based CRET

Schematic illustration of a graphene-based CRET platform is shown in Fig. 1. A 100 μL of HRP-labeled antibody–conjugated MNPs suspension (4 mg mL^{-1}) was firstly mixed with 100 μL of human HCG standard solution or sample solution in 96-well plate and then incubated for 30 min at 37 °C. The mixture was separated by magnetic separation rack and then incubated with 200 μL of anti-HCG antibody–conjugated graphene suspension (0.1 mg mL^{-1}) for 2.5 h at 37 °C to form a sandwich-type immunocomplex. After magnetic separation and gentle washing with distilled water, the resultant mixture was resuspended in 120 μL of PBS solution (10 mmol L^{-1}, pH = 9.0). Subsequently, 50 μL H_2O_2 (3.5 mmol L^{-1}) was added into the mixture and then 30 μL luminol solution (10 mmol L^{-1}) was introduced by using an automatic injection of TECAN Infinite 200 (Tecan, San Jose, CA). The decreased chemiluminescence intensity caused by CRET was represented as $\Delta I = I_1 - I_0$. Here, I_1 and I_0 were the chemiluminescence intensities of the system without and with adding the antibody–conjugated graphene, respectively. The chemiluminescence intensity was recorded after 30 s by using TECAN Infinite 200 microplate reader (Tecan, San