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Magneto-controlled electrochemical immunosensor for direct detection of squamous cell carcinoma antigen by using serum as supporting electrolyte

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ABSTRACT

A new magneto-controlled microfluidic device for direct electrochemical determination of squamous cell carcinoma antigen (SCC-Ag) in serum was designed by using anti-SCC antibody (SCC-Ab)-functionalized magnetic mesoporous nanogold/thionine/$NiCo_2O_4$ hybrid nanostructures as immunosensing probes (P_1-Ab) and horseradish peroxidase-SCC-Ab conjugates-labeled nanogold/graphene nanosheets as signal tags (P_2-Ab). In the presence of the analyte SCC-Ag, the sandwich immunocomplex was formed between the immunosensing probes and the signal tags. With the aid of an external magnet, the formed immunocomplex was attached to the microfluidic device. The assay was implemented in newborn calf serum (NBCS) containing 2.5 mM H_2O_2 based on the labeled peroxidase on the P_2-Ab toward the catalytic reduction of H_2O_2. Under optimal conditions, the increase in the current was proportional to the concentration of SCC-Ag from 2.5 pg/mL to 15 ng/mL. The detection limit (LOD) was 1.0 pg/mL SCC-Ag at $3s_B$. The electrochemical immunoassay displayed an acceptable precision, selectivity and stability. Clinical serum specimens were assayed with the method, and the results were in acceptable agreement with those obtained from the referenced electrochemiluminescent method. Importantly, the method can be suitable for on-line use in the mass production of miniaturized lab-on-a-chip devices and open a new opportunity for protein diagnostics and biosecurity.

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1. Introduction

The development of assays capable of determining disease-related proteins with the aim of making portable and affordable devices has become a major analytical tool in clinical diagnosis, drug delivery and gene expression profiling (Nie et al., 2009; Qian et al., 2010). In general, the assay is performed using certain affinity ligands, such as antibodies or aptamers, that specificially interact with the protein, thus mediate a target-responsive signal transduction cascade (Liu et al., 2011; He et al., 2010; Chen et al., 2009; Wu et al., 2009). Despite the high sensitivity of conventional immunoassays such as RIA and ELISA, they have some limitations such as the short shelf life of ^{125}I-labeled antibody, radiation hazards, a complicated wash procedure, and a relatively long analysis time (Tang et al., 2007). Magneto-controlled molecular electronics and bioelectronics have been new topics on monitoring the biomolecules with the aid of external magnetic field based on the electronic signal of the functional magnetic beads associated with probes (Willner and Katz, 2003). Mirkin and his colleagues reported a magneto-controlled assay device for the detection of free prostate-specific antigen (PSA) at low attomolar concentration by using an antibody-labeled magnetic bead, DNA barcodes, and an antibody/DNA-conjugated gold nanoparticle (Nam et al., 2003).

In these magneto-controlled immunoassays, batch-type magnetic separators have been fabricated on a single chip that trap magnetic particles in flowing fluids by using an external magnet, and then the particles are eluted from the system (Laschi et al., 2011; Szymanski et al., 2011). Various assay protocols based on magneto-controlled sensing platform have been utilized for the detection of biomolecules, such as the cocaine detection *via* rolling circle amplification of short DNA strand separated by magnetic beads (Ma et al., 2011), aptamer-barcode based immunoassay for the instantaneous derivatization chemiluminescence detection of IgE coupled to magnetic beads (Peng et al., 2011), DNA bio-barcode assays (Trevisan et al., 2010), and color-barcoded magnetic microparticles for multiplexed bioassays (Lee et al., 2010). Recently, our group fabricated a magneto-controlled electrochemical immunoassay of carbohydrate antigen 125 by using functional magnetic beads as immunosensing probes and antibody-coated

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nanometer-sized enzyme-doped silica beads as signal tags (Tang et al., 2010). However, we later found that the capture capacity of antibody-functionalized magnetic beads toward the analyte in the sample is limited. The reason might be random distribution of both the functionalized magnetic beads and the target analyte, which decreases the probability of an interaction between two species, especially at very low concentration. The phenomenon might be improved by enhancing the surface coverage of magnetic beads, and increasing the immobilized amount of biomolecules. Most recently, we synthesized a magnetic mesoporous $NiCo_2O_4$ nanosheet with three-dimension channels composed of a magnetic mesoporous $NiCo_2O_4$ nanosheet, an interlayer of Nafion/thionine organic molecules and a nanogold layer (Li et al., 2011). The synthesized nanostructures possess straight edges, gradually transform from semiconductors to semimetals as their width increases. We expected that the use of magnetic mesoporous $NiCo_2O_4$ nanostructures could enhance the sensitivity of the electrochemical immunoassay in this work.

Another important issue for the successful development of the electrochemical immunoassay is to accurately evaluate the level of disease-related protein in the real serum. Usually, clinical serum specimen was assayed or diluted by using certain buffer solution, such as phosphate-buffered saline solution or tris-buffer solution (Tang et al., 2011a; Triroj et al., 2011; Wu et al., 2011; Liang et al., 2011, Gu et al., 2011). In principle, use of buffer solution might change the microenvironment of the proteins, thus affecting the conformation and bioactivity of the proteins in the sample. As a result, the assayed concentration of the protein might be higher or lower than the normal level. To avoid this issue, direct detection of the samples by using serum as supporting electrolytes is preferable.

Squamous cell carcinoma antigen (SCC-Ag) was first found in the uterine cervical squamous cell carcinoma (SCC) by Kato and Torigoe (1977). The serum level of SCC-Ag is increased in parallel to the growth of the tumor size or the recurrence of the disease (Schedel et al., 2011). Therefore, measurement of the serum level of SCC-Ag has been used clinically for the diagnosis and the management of SCC other uterine cervix as well as other various organs. To the best of our knowledge, there is no report focusing on electrochemical detection of SCC-Ag based on the magneto-controlled immunosensor. Herein, we designed a nanoparticle-based magnetic immunoassay for direct electrochemical detection of SCC-Ag (as a model) in the serum without the participation of other supporting electrolytes with a flow-through system. With the flow-through system, trapped magnetic nanostructures could efficiently capture the analyte due to the unique nanostructures of the immunosensing probes. The assay is based on a sandwich immunoassay format by using biofunctionalized magnetic mesoporous nanostructures as immunosensing probes and functional gold-graphene nanosheets as signal tags. The method allowed for the detection of low levels with signal amplification by the multifunctional signal tags, and reduced sample pretreatment requirement due to the presence of magnetic particles.

2. Experimental

2.1. Materials and chemicals

SCC-Ab, SCC-Ag and horseradish peroxidase (HRP)-labeled polyclonal antibody against squamous cell carcinoma (HRP-SCC-Ab) was purchased from Shanghai Fengxiang Biotechnol. Co. Ltd. (China). Nafion (5 wt%), bovine serum albumin (BSA, lyophilized powder, ~66 kDa), and thionine acetate salt (Th, dye content ≥85%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). $HAuCl_4{\cdot}4H_2O$ was purchased from Sinopharm Chem. Re. Co. Ltd. (Shanghai, China). All other reagents were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all runs. Newborn calf serum (NBCS) was purchased from Dingguo Biosci. Co. (Beijing, China). Clinical serum samples were provided from Fujian Provincial Hospital, China.

2.2. Synthesis and bioconjugation of magnetic mesoporous nanogold/thionine/$NiCo_2O_4$ hybrid nanomaterials (i.e. immunosensing probe, P_1-Ab)

Synthesis of immunosensing probes mainly consisted of the following two steps: (i) high crystalline mesoporous $NiCo_2O_4$ were successfully prepared through the nanocasting strategy, using KIT-6 silica as a hard template, and (ii) nanogold/thionine/$NiCo_2O_4$ hybrid nanomaterials were synthesized by using the seed-mediated growth. The as-prepared magnetic mesoporous nanostructures were characterized by using XRD spectrometry, transmission electron microscopy (TEM), N_2 adsorption–desorption measurement, vibrating sample magnetometry (VSM) and X-ray photoelectron spectroscopy (XPS). The synthesized process and characteristics of the hybrid nanostructures were described in detail in our most recent paper (Li et al., 2011). Following that, the as-prepared hybrid nanostructures were used for the label of SCC-Ab. Briefly, 200 mg of magnetic nanogold/thionine/$NiCo_2O_4$ was initially dispersed into 2 mL of pH 9.0 Tris buffer, and then 100 μL of SCC-Ab (1.0 mg/mL) was added into the solution. After incubated for 12 h at 4 °C with slightly stirring, the mixture was centrifuged for 10 min at 8000 × *g*. The obtained precipitation was added into 2.5 wt% BSA solution, and incubated for 1 h at room temperature (RT) to block possible remaining active sites of nanomaterials and avoid the non-specific adsorption. The obtained bionanostructures (designed as P_1-Ab, $C[P_1\text{-Ab}] \approx 100$ mg/mL) were dispersed in 2 mL of NBCS and stored at 4 °C until use.

2.3. In situ synthesis and bioconjugation of gold nanoparticles on graphene oxide (i.e. signal tags, P_2-Ab)

Prior to experiments, graphene oxide (GO) nanosheets were prepared according to a modified Hummers method (Hummers and Offeman, 1958). 0.5 g of graphite powder was initially dispersed in 23 mL of H_2SO_4 at 0 °C, and 0.5 g of $NaNO_3$ and 3 g of $KMnO_4$ were then dropwise added. The well-mixed slurry was stirred for 1 h at a 35 °C water bath. Following that, 140 mL of H_2O was added in the mixture, and the temperature was raised to 90 °C. Afterwards, 3 mL of H_2O_2 (30 wt%) was injected, and the mixture changed to a light brown color. Consequently, graphene oxide was obtained by filtering, washing and centrifugation at 4000 rpm. The obtained GO was characterized by TEM, which has lateral dimensions between 300 nm and 12 μm with an average size of ~3 μm.

Next, the well-dispersive gold nanoparticles on graphene oxide (AuNP-GO) were *in situ* synthesized consulted to the literature with some modification (Chen et al., 2011). Prior to experiment, GO aqueous solution was prepared by dispersing 50 mg of the as-prepared GO into 100 mL of distilled water. Following that, 5.0 mL of GO aqueous solution and 0.5 mL $HAuCl_4$ aqueous solution (10 mM) were added into a 10-mL volumetric flask, and the mixture was vigorously stirred for 2 h at 90 °C. Afterwards, the mixture was washed twice with distilled water and centrifuged for 10 min at 8000 rpm. Finally, the obtained AuNP-GO nanostructures were utilized for the label of HRP-SCC-Ab by adopting the above-mentioned method of P_1-Ag. The purified HRP-SCC-Ab-AuNP-GO (designed as P_2-Ab, $C[P_2\text{-Ab}] \approx 0.5$ mg/mL) was dispersed into 2 mL of NBCS and stored at 4 °C when not in use.

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