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Sensitive detection of carcinoembryonic antigen using surface plasmon resonance biosensor with gold nanoparticles signal amplification

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ABSTRACT

A new method for real-time detection of carcinoembryonic antigen (CEA) in human serum with high sensitivity and selectivity using surface plasmon resonance (SPR) biosensor was developed. Two kinds of antibodies were used to recognize CEA at different epitopes with high affinity and specificity. Gold nanoparticles (GNPs) modified with streptavidin (SA) were used to further enhance signal specifically via biotin–streptavidin interaction. The binding capacity of the streptavidin-modified gold nanoparticles (SA–GNPs) for ligand biotin was quantified by titration with biotin (5-fluorescein) conjugate to be 10.54 biotin binding sites per 100 nm^2. The developed GNPs enhanced sandwich SPR biosensor successfully fulfilled the sensitive detection of CEA in the range of 1–60 ng/mL with a detection limit of 1.0 ng/mL. Compared to the direct assay format, sandwich format without GNPs and SA–GNPs enhanced sandwich format led to 4.2-fold and 13.8-fold in the sensitivity, respectively. This sensor also showed good selectivity for CEA in the interference study. The results demonstrated that the proposed method could provide a high sensitivity and selectivity in the detection of CEA and offer a promising alternative for cancer biomarker than traditional clinical examinations.

1. Introduction

In recent years, biomarkers, chemical and biological substances that increase in concentration during the formation of cancer, have given important information for early diagnosis, effective treatment and prognosis of cancer [1]. Carcinoembryonic antigen, a cell adhesion glycol–protein that has 70 kD molecular weight normally and becomes 180 kD when glycosylated, is a widely used tumor marker for diagnostic and therapeutic purposes in gastrointestinal, breast and lung cancer [2]. The increase in CEA level in serum above the normal value (2.5 ng/mL, 5 ng/mL for smokers) is an indication of possible disease [3]. Therefore, detection of CEA with high sensitivity and specificity in clinical level is crucial to cancer patients.

Many different analytical methods for determination of CEA such as electrochemical [4–6], fluorometric analysis [7–9], enzyme-linked immunoassay [10] and chemiluminescence immunoassay [11–13] provide sensitive and specific techniques, but generally require multiple steps and time-consuming procedures such as labeling and sample treatment prior to detection. However, these labeling methods are not suitable in some cases, because labeling materials may occupy the important binding sites or cause steric hindrance, resulting in false information. Surface plasmon resonance (SPR), an optical phenomenon occurred in total internal reflection of light at a metal film–liquid interface [14], is one of the powerful analytical techniques for direct monitoring of molecular interactions, without the need for intrinsic or extrinsic labeling to the target molecules. Biosensors based on SPR have been extensively used to monitor molecular interactions for its outstanding sensitivity, reliability, reproducibility as well as its capability to monitor multiple interactions successively [15,16]. SPR biosensors have been applied for the detection of CEA in previous studies [17,18]. SPR based biosensors have also been used for the detection of other biomarkers such as gastric carcinoma-associated antigen MG7-Ag [19], vascular endothelial growth factor receptor (sVEGFR-1) [20] and prostate specific antigen (PSA) [21].

To obtain clinically relevant results, it is essential to improve the sensitivity and enhance the signal. A series of strategies was employed to achieve this goal such as the use of secondary antibodies [17,18,21], functionalized nanoparticles [22–24], quantum dots [25,26] and atom transfer radical polymerization [27]. Due to their huge mass, high dielectric constant, and electromagnetic

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coupling between GNPs and Au film, GNPs have been used widely in a variety of works [21,23,28–33].

In this paper, we developed a novel method to detect CEA in buffer and human serum spiking samples using a SPR biosensor with GNPs to enhance signal. Different methods were used to enhance and amplify the signal including sandwich immunoassay and the second signal amplification by SA–GNPs. The results show that it can be used to detect CEA in buffer or in spiking serum samples with sensitivity and selectivity and realize early diagnosis of cancer in an invasive surgical procedure.

2. Experimental

2.1. Reagents

CEA, alpha fetal protein (AFP), and PSA were obtained from Zhengzhou Biocell antibody center (Zhengzhou, China). Mouse anti-CEA antibodies (clone number: C3) (mAbCEA-C3), mouse anti-CEA antibodies (clone number: B5) (mAbCEA-B5), biotin conjugated mouse anti-CEA antibodies (bio-mAbCEA-B5) and streptavidin were purchased from Bioss (Beijing, China). Bovine serum albumin (BSA) was purchased from Aladdin (Shanghai, China). N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), 10 nm gold nanoparticles (5.38×10^{12}–6.5×10^{12} particles/mL) and biotin (5-fluorescein) conjugate were purchased from Sigma-Aldrich. The human serum was from healthy patient in affiliated Hospital of Datong university. All reagents were of analytical grade and used without further purification. Deionized water was used for the preparation of aqueous solution.

2.2. Instrumentations

A two-channel Biacore X™ (Uppsala, Sweden) and CM5 sensor chips were used for the assays. Running buffer was phosphate buffer solution (PBS, 0.01 M phosphate solution, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4). The operating temperature of the assays was 25 °C and the flow rate of the buffer was 5 μL/min. The SPR response signals were calculated according to the difference subtracted the signal of the control channel (flow cell 1) (Fc1) from that of channel (flow cell 2) (Fc2). So that non-specific binding and buffer induced bulk refractive index changes can be reduced to minimum. All buffers for experiments were filtered (0.22 μm) and degassed before use.

UV–visible spectra were carried out on an UV/VIS spectrophotometer (Lambda 35, PerkinElmer, USA). A fluorescence spectrophotometer (Hitachi, F-2500, Japan) was used to record excitation and emission spectra.

2.3. Modification of GNPs with SA

SA–GNPs conjugate was used for further amplification. SA–GNPs were prepared according to the literature [34] with some modification. Briefly, 1.0 mL colloidal gold solution was initially adjusted to pH 7.4 using K_2CO_3, and then 1.0 mL of SA (20 μg/mL) was added, after incubated for 12 h at 4 °C in a shaker, the mixture was centrifuged (12,000 rpm) at 4 °C for 1 h, then the obtained SA–GNPs conjugates were resuspended into 0.2 mL pH 7.4 PBS containing 1.0% BSA. Conjugates were stable by storing in refrigerator between 2 and 8 °C for several days.

2.4. Immobilization of antibodies

A CM5 dextran chip was first docked into the Biacore instrument and primed with running buffer (PBS, 10 mM, pH 7.4) at a flow rate of 10 μL/min. N-hydroxysuccinimide (NHS) (0.1 M) was mixed 1:1 with 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (0.4 M) and injected (70 μL at 10 μL/min) to activate the surface on the Fc2 of the CM5. This was immediately followed by coating antibodies (mAbCEA-C3) (70 μL at 10 μL/min) prepared in 10 mM sodium acetate buffer (pH 4.3). The surface was then deactivated with ethanolamine (1 M, pH 8.5). Each step, including activation, immobilization, and blocking was carried out for 420 s. Fc1 was activated and then deactivated as a reference flow cell channel and used as the background control. The resulting chip was used as a sensing surface for detecting CEA.

2.5. Quantitative detection of SA on the GNPs surface

The coverage factor of SA on GNPs was detected by the fluorescence quenching value of biotin (5-fluorescein) conjugate [35]. 60 μL 1.0 μg/mL biotin (5-fluorescein) conjugate in 10 mM PBS buffer (pH 7.4) and various volumes of 1.0 μg/mL SA in 10 mM PBS buffer were mixed in a centrifuge tube and the total volume was adjusted to 2.0 mL with PBS and incubated for 30 min in the dark. The fluorescence of the solution was measured at $\lambda_{ex}/\lambda_{em}$=492 nm/522 nm with a spectrophotometer, which excitation and emission slits were set at 5 nm and 5 nm, respectively. The changes of fluorescence intensity were plotted as a function of the SA concentrations. The fluorescence of one-tenth volume of total supernatant was determined to quantify the amount of SA in the supernatant.

2.6. Quantitative detection of the binding sites of SA on the GNPs surface

Various volumes of 1.0 μg/mL biotin (5-fluorescein) conjugate in 10 mM PBS buffer and supernatant containing the SA–GNPs were added into a centrifuge tube and the total volume was adjusted to 2.0 mL with PBS and incubated for 30 min in the dark. Control titrations without SA and with solution containing 2.0 μg SA were performed with same procedure. The fluorescence was measured at $\lambda_{ex}/\lambda_{em}$=492 nm/522 nm with slits set at 5 nm and 5 nm. Fluorescence intensity was plotted as a function of the concentrations of free biotin (5-fluorescein) conjugate. The increasing part of this function was linearly fitted and the *x*-axis intercept was used to quantify the binding sites of the immobilized SA on the GNPs surface.

2.7. CEA detection assay

CEA solutions were prepared by diluting into appropriate concentrations (1–700 ng/mL) with PBS. These solutions were then injected over mAbCEA-C3 modified surfaces for 6 min to allow binding assays. Following this, the sensor surface was regenerated by injection of 100 mM HCl (1 min) or the assay was continued to perform a sandwich assay. For the sandwich assay, after the binding of CEA to the sensor surface, 4 μg/mL mAbCEA-B5 was injected on the sensor surface for 6 min. To obtain much more sensitive results, SA–GNPs were used for further amplification. After the binding of CEA to the sensor surface, 4 μg/mL bio-mAbCEA-B5 and then 4.25 μg/mL SA–GNPs were injected to the sensor surface. After a 3 min dissociation period under running buffer flow, the surface was regenerated by injection of 100 mM HCl (1 min) and 20 mM NaOH (0.5 min).

In order to demonstrate whether the binding of CEA to mAbCEA-C3 was solely through the antigen-specific targeting pattern, other cancer biomarkers AFP and PSA were treated with the same strategy to test the cross-reaction. All the data points presented are the averages of triplet measurements unless otherwise stated.