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Design and performances of immunoassay based on SPR biosensor with Au/Ag alloy nanocomposites

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ABSTRACT

A novel method for detecting human IgG is reported, which is based on Au/Ag alloy nanocomposites for amplifying surface plasmon resonance response. Au/Ag alloy nanocomposites were characterized in detail by transmission electron microscopy (TEM), UV–vis absorption spectroscopy and X-ray photoelectron spectroscopy (XPS). Covalent immobilization of about 24 nm diameter of Au/Ag alloy nanocomposites on the Au film results in a large shift in resonance wavelength, which is due to the increase of the thickness of the sensing membrane, high dielectric constant of Au/Ag nanoparticles, and electromagnetic coupling between Au/Ag alloy nanocomposites and Au film. The SPR biosensor based on Au/Ag alloy nanocomposites exhibits a satisfactory response for human IgG in the concentration range of 0.15–40.00 $\mu g\,mL^{-1}$. While the biosensor based on Au nanoparticles shows a response in the concentration range of 0.30–20.00 $\mu g\,mL^{-1}$ and the biosensor based on Au film shows a response for human IgG in the concentration range of 1.25–20.00 $\mu g\,mL^{-1}$.

1. Introduction

Metal nanoparticles, especially Au and Ag nanoparticles, have been used to the SPR sensors because of their unique optical properties [1]. The electronic coupling between the localized surface plasmon of the nanoparticles and the propagating plasmon wave from the surface of the Au film leads to the enhanced shifts of the resonant wavelength [2]. Au nanoparticle has been used widely in immunoassays owing to its excellent properties, such as easy reductive preparation, water-solubility, high chemical stability, significant biocompatibility and affinity [3]. However, the Au nanoparticle displays a lower enhancement in the visible light region compared with Ag nanoparticle [4]. Ag nanoparticle produces a sharper peak than Au nanoparticle and shows better sensitivity, which could be used to enhance the sensitivity of the biosensor [5]. Compared with Au nanoparticles, bare Ag nanoparticles were unstable. Therefore, to address these drawbacks, Au/Ag alloy nanocomposites were introduced.

Recently, alloy nanocomposites have gained significant interest due to catalytic effect and optical properties that arise from the combination of different elements of metals on the nanoscale [6,7]. In addition, Au/Ag alloy nanocomposites possess the merit of easy preparation, good biocompatibility and tunable optical and electronic properties by changing the alloy composition. Au/Ag alloy nanocomposites combine the advantages of Au and Ag nanoparticles, which lead to their promising application in the fields of optical devices, biomedical research and electronics. It was proved for the alloy nanocomposites to be more effective than monometallic nanocrystals, although the composition of Au and Ag within the nanostructures is the same [8]. For example, one study indicated that bimetallic Au/Ag nanocomposites showed higher sensitivity than individual components in SERS analysis [9]. Au/Ag alloy nanoparticles show a single and composition-sensitive absorption band located at an intermediate position between pure Au and Ag nanoparticle surface plasmon peaks. For the preparation of Au/Ag alloy nanocomposites, a number of methods have been applied, such as laser ablation [10], hydrazine reduction [11], electrochemical synthesis [12] and photochemical method [13]. Here, the method of co-reduction of chlorauric acid and silver nitrate with sodium citrate in aqueous solution was applied.

Surface plasmon resonance (SPR) is a surface-sensitive analytical technique based on the ability to detect chemical changes occurring at the surface of a thin noble metal film [14]. In the last two decades, the development of the SPR sensor was remarkable, various SPR sensors have been developed and applied in the fields of medical diagnostic, food safety, environmental protection and biotechnology [15]. For the SPR biosensor provides a rapid, label-free and highly sensitive assay, this technique has been widely applied to the determination of affinity constants, kinetic binding parameters [16], protein–DNA interaction [17], drug–liposome interaction [18], drug–serum albumin interaction [19] and especially antibody–antigen interaction [20]. SPR is an optical–electrical phenomenon and the SPR sensor is extremely sensitive to the change in refractive index occurring within about

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a hundred nanometers from the sensor surface. Any change within this range will transform into an SPR signal. Immobilization of monolayer or multilayer onto an SPR-active film, and subsequent chemical modification on that layer can be detected at the metal surface. While, conventional SPR is unable to measure extremely small changes in refractive index, which hinders its application in ultra sensitive detection. To overcome this drawback, several approaches have been applied to enhance the sensitivity of the SPR biosensor by altering external structure, such as using of Au nanoparticles [1], the thin film of titanium dioxide [21], liposomes [22] and self-assembled PEG monolayer [23] on the biosensor surface.

In this paper, Au/Ag alloy nanocomposites were synthesized and applied in the SPR biosensor to amplify SPR response signal. The Au/Ag alloy nanocomposites could conjugate with biomolecule easily. It has been well known that thiol terminal groups are able to bind to the Au film surface stably as the formation of a monolayer which is driven by a strong, specific interaction between the thiol and the Au surface. Therefore, 1,6-hexanedithiol (HDT), a disulfide compound, was used to connect the Au film and the Au/Ag alloy nanocomposites. Then, 3-mercaptopropionicacid (MPA) was used to connect the Au/Ag alloy nanocomposites and the biological components. After the carboxyl group of MPA was activated, antibody could bind to the alloy nanocomposites steadily. The bindings of antibody to antigen lead to changes in the dielectric constant on the biosensor surface, which can be detected by the SPR sensor.

2. Materials and methods

2.1. Reagents

Human IgG and rabbit anti-human IgG were purchased from Beijing Boisynthesis Biotechnology Company. 1,6-Hexanedithiol (HDT) and 3-mercaptopropionic acid (MPA) were purchased from JK Chemical. Hydrogen tetrachloroauratehydrate ($HAuCl_4{\cdot}4H_2O$) was obtained from Acrosl. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Pierce. Silver nitrate, trisodium citrate and all other chemicals were of analytical reagent grade. All solutions were prepared with ultra pure water. Human IgG and rabbit anti-human IgG were stored at −20 °C and all other biological reagents were stored at 4 °C. Sodium phosphate buffered saline (PBS, 0.01 mol L^{-1}, pH 7.4) was used as running buffer.

2.2. Equipment

The wavelength modulation SPR analyzer installed in our laboratory was used [21]. It is working with Kretschmann configuration to achieve the resonant condition by attenuated total reflection (ATR) in a prism. A glass slide with Au film was put on base of a prism (K9 glass). A halogen tungsten lamp is used as the excitation light source. The light passes through a polarizer and two lenses and becomes TM-polarized parallel light. The parallel polychromatic light beam passes through the optical prism and excites surface plasmon at the interface between the Au film and the sample solution. The output light is guided into the optical fiber and then enters the spectrophotometer. A charge-coupled device (CCD) was used as the detector.

2.3. Procedures

2.3.1. Preparation of Au/Ag alloy nanocomposites

Au nanoparticles were prepared by the method reported previously [21]. After 100 μL of 1 g mL^{-1} $HAuCl_4$ was added into 95 mL of H_2O, the solution was heated. When the solution was boiling, 5 mL of 1% sodium citrate solution was added. The resulting solution was heated for an additional 15 min and became dark red slowly. The colloidal solution was stored in brown glass bottle at 4 °C.

Using the same method, Au/Ag alloy nanocomposites were prepared by substituting a predetermined number of moles of gold atoms by the equivalent number of moles of silver atoms in the form of silver nitrate $AgNO_3$ [6]. Au/Ag alloy nanocomposites were synthesized when the molar ratio of Au to Ag was 0.27:0.73 in this paper. After the sodium citrate solution was injected into the boiling solution, the color of the solution changed from colorless to yellowish gradually.

2.3.2. Characterization of the nanocomposites

The size and shape of Au/Ag alloy nanocomposites and Au nanoparticles were tested using a transmission electron microscope (TEM). The samples for TEM were obtained by placing drops of each sample on a copper grid. UV–vis absorption spectra of Au/Ag alloy nanocomposites and Au nanoparticles were also obtained.

2.3.3. Modification of the Au film

The glass slide was coated with a 2 nm adhesion layer of chromium followed by a 50 nm Au layer by electron-beam evaporation with 99.999% gold. The surface of the glass slide with Au film was functionalized by submerging into fresh ethanol–water solution (v/v, 2:1) containing HDT at the concentration of 10 mmol L^{-1} for 24 h, resulting in a monolayer of HDT on the Au film surface. As HDT is a disulfide compound, one of sulfhydryl groups is connected with Au film and the other can be connected with Au/Ag alloy nanocomposites. Then, the modified Au film was rinsed several times with deionized water, and dried with gaseous nitrogen. To fabricate a self-assembled monolayer of Au/Ag alloy nanocomposites or Au nanoparticles, modified Au surface was incubated in Au/Ag alloy nanocomposites solution or Au nanoparticles solution for 24 h, respectively, followed by rinsing with deionized water and dried with pure gaseous nitrogen. XPS test was carried out to show the surface characterization of the Au film after the Au/Ag alloy nanocomposites were immobilized on the Au film.

2.3.4. Immobilization of antibody

The sensor membranes fabricated as described above were mounted on the SPR instrument equipped with a flow cell. Then 10 mmol L^{-1} MPA was injected into the flow cell for 6 h. Afterwards PBS was injected into the flow cell and used as the baseline solution. A solution containing NHS (30 mg mL^{-1}) and EDC (30 mg mL^{-1}) was injected for 20 min, followed by a rinsing with PBS. After the resonant wavelength kept constant, a solution of rabbit anti-human IgG was injected into the flow cell to covalently attach to the activated carboxylic acid group. Then 1 mol L^{-1} ethanolamine hydrochloride (pH 8.0) was used to block the non-specific binding sites on the biosensor surface for 10 min, and the noncovalently bound antibody was washed off with PBS buffer, which was also used to stabilize the baseline again. A schematic diagram of the immobilization of antibody on the surface of the Au film is shown in Fig. 1.

2.3.5. Immunoassay

All SPR experiments were conducted at room temperature. After rabbit anti-human IgG was immobilized on the surface of the biosensor, different concentrations of human IgG diluted with PBS buffer were separately injected into the flow cell. Thus free antigens could interact with antibodies immobilized on the biosensor surface. The amount of antigens coupling with the antibodies immobilized on the surface was monitored as a shift of the resonant wavelength. After 40 min, PBS buffer was injected into the flow cell for 20 min. Due to large amount of ions in the PBS, the antigen can be dissociated from the antibody immobilized on the surface