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Surface plasmon resonance biosensor based on Au nanoparticle in titania sol–gel membrane

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ABSTRACT

A novel surface plasmon resonance (SPR) biosensor for the determination of human IgG was introduced. The biosensor was prepared with three layers of titania sol–gel membrane by vapor deposition. The colloid Au nanoparticle (AuNP) was immobilized in the second layer of titania membrane and the AuNP coupled with rabbit anti-human IgG was encapsulated in the third layer. The AuNP in the second layer of titania membrane was proved to be effective in the sensitivity enhancement of SPR biosensor. The lowest concentration that could be detected obtained by the biosensor with AuNP is about eight times lower than that obtained without AuNP. In addition, the titania sol–gel is a porous homogeneous material that permits analytes to access the encapsulated biomolecule and can provide a controlled environment for the study of biomolecular recognition. The titania sol–gel was also confirmed to be benefit for biomolecule to keep bioactivity, which could offer a good waterish microenvironment. As a result, the modified biosensor exhibits a satisfactory response for human IgG in the concentration range of 0.30–40.00 $\mu g\,mL^{-1}$ and shows favorable bioactivity for a long time.

1. Introduction

In recent years, biomolecule immobilization has attracted considerable interest as a way to increase the sensitivity of biosensor and retain bioactivity of biomolecule. Many methods such as magnetic force [1], cross-linking [2] and chemical bond [3] have been applied to the immobilization of the biomolecule on the metal film of surface plasmon resonance (SPR) biosensor. However, some of the immobilization methods are relatively complicated and can be not widely applied. Recently, the use of sol–gel membrane to encapsulate biomolecule has been paid much attention. The sol–gel membrane has been used to encapsulate a wide variety of biomolecule in the electrochemistry, such as enzyme [4], protein [5–6] and cell [7], while there is a little application of sol–gel in the SPR biosensor [8–9]. The sol–gel membrane is a kind of three-dimensionally porous network material which can be prepared conveniently by a simple, inexpensive and practical method. In addition, the sol–gel membrane has obvious advantages in immobilizing the biomolecule. A waterish microenvironment could be offered by sol–gel membrane, which is beneficial for biomolecule to retain bioactivity. And the porous network could provide a controlled environment for the study of biomolecular recognition, as the analyte is able to diffuse toward the porous network to bind to the immobilized biomolecule.

Silica matrix is a kind of sol–gel that was often used to trap biomolecule [10]. However, the silica sol–gel matrix has some drawbacks, including fragility, hydrolysis at high acidity and complicated procedures [11], which result in the loss of protein stability and limit its applications in biosensor. Compared to silica sol–gel, titania sol–gel overcomes these drawbacks. Titania sol–gel is a suitable porous material for immobilizing biomolecule [12–13] and can be obtained easily from the vapor deposition. The vapor deposition technique provides a possibility of preparing sol–gel membrane and immobilizing different reagents at room temperature. Moreover, the silica sol–gel is prepared at high acidity, which is hostile for biomolecule to keep bioactivity. By contrast, vapor deposition technique is a more mild method to construct biocompatible neutral medium. The side effects caused by acid catalysts and organic cosolvents used in the previous sol–gel processes can be avoided. Finally, as the biomolecule can be immobilized during forming titania sol–gel membrane, the vapor deposition technique simplifies greatly the immunoassay procedure, which makes the application of titania sol–gel more convenient.

SPR has been recognized for the last two decades as a powerful optical technique for label-free detection of biomolecule at the metal layer surface [14]. This technique utilizes the evanescent wave of an incident light wave exciting a surface plasmon to detect changes occurring at the surface of a thin metal film such as Au or

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Ag. Compared with other sensor techniques, SPR spectroscopy has many advantages, such as real-time, small sample volume, label-free and non-destructive detection [8]. Owing to these features, SPR biosensor has been used to study various interaction partners, including proteins [15–16], factors [17], enzymes [18], nucleic acids [19], drugs [20] and especially antigens [21].

In this paper, a novel SPR biosensor was constructed for the determination of human IgG by trapping colloidal Au nanoparticle (AuNP) and AuNP coupled with rabbit anti-human IgG (anti-IgG) in the different layers of titania sol–gel membrane. Due to the especial virtues of colloidal Au, such as ease of preparation, high density, large dielectric constant and biocompatibility, it has been used widely to study the binding of protein to protein [22]. It has been proved that the Au nanoparticle is beneficial to improve the sensitivity of the SPR biosensor [23–24]. However, the direct deposition of AuNP on Au film could result in the enhancement of background [25]. So at first, thin titania membrane was prepared by vapor deposition technique to avoid direct deposition of AuNP to the Au film. Then the colloidal Au nanoparticle was immobilized in the second layer of titania membrane to enhance the sensitivity of SPR biosensor. Finally, the AuNP coupled with anti-IgG was encapsulated in the third layer of titania membrane. The AuNP immobilized in the third layer of titania membrane could offer a biocompatible microenvironment which is favorable for biomolecule to retain bioactivity [26]. The sensitivity of the SPR biosensor was enhanced and long-term bioactivity of biomolecule was obtained.

2. Material and methods

2.1. Reagents

Human IgG and rabbit anti-human IgG were purchased from Beijing Boisynthesis Biotechnology Company. Titanium isopropoxide (III) (98%) was purchased from JK Chemical. Hydrogen tetrachloroauratehydrate ($HAuCl_4{\cdot}3H_2O$) was obtained from Acrosl.

Human IgG and rabbit anti-human IgG were stored at −20 °C, and all other biological reagents were stored at 4 °C. All solutions were prepared with ultrapure water. PBS buffer (0.01 mol L^{-1}, pH 7.4) was used as running buffer.

2.2. Equipment

In this paper, the wavelength modulation SPR biosensor installed in our laboratory was used. It is working with Kretschmann configuration to achieve the resonant condition by attenuated total reflection (ATR) in a prism [27]. The prism was vacuum-deposited with approximately a 2 nm adhesive layer of Cr and 50 nm Au film followed. A halogen tungsten lamp is used as the excitation light source in conjunction with a constant voltage transformer. The light from this source passes through a polarizer and two lenses and becomes TM-polarized parallel light. Then the parallel polychromatic light beam passes through the optical prism and excites surface plasmon at the interface between the Au film and analyte. The output light is guided into the optical fiber and then enters the full wave spectrophotometer (Ocean Optics, Inc., USA). A 2048 element charge-coupled device (CCD) linear array detector is used as the detector. A 100 μL volume flow cell was used for the reaction. The interaction between the analyte in the solution and the biomolecule immobilized in the titania membrane produces a change in refractive index (RI) profile that is observed as a shift in the SPR resonant wavelength. The surface plasmon resonance manifests itself on a characteristic dip in a wavelength and the intensity of reflected light is minimum value at this resonant wavelength.

2.3. Assay procedure

2.3.1. Preparation of colloidal Au nanoparticle

All glassware used in the experiment were firstly washed with HNO_3–HCl (v/v, 1/3) prepared freshly for 6 h, then rinsed thoroughly with doubly distilled water and dried before use. Spherical Au nanoparticle was prepared according to the literature [28]. After 1 mL of 1% $HAuCl_4$ was added into 100 mL of H_2O, the solution was heated. When $HAuCl_4$ aqueous solution boiling, 4 mL of 1% sodium citrate solution was added. When the solution was heated for an additional 10 min, it turned dark red slowly. The colloidal solution was stored in brown glass bottle at 4 °C.

2.3.2. Preparation of Au nanoparticle coupled with anti-IgG

1 mg mL^{-1} of rabbit anti-human IgG solution was first obtained with PBS buffer. While stirring the colloid Au nanoparticle solution vigorously, a quantity of anti-IgG solution was added rapidly into colloid Au nanoparticle (v/v, 1/2). After incubation at 25 °C for 1 h, the Au nanoparticle coupled with anti-IgG was obtained. Then the solution was centrifuged at 12,500 rpm for 20 min at 4 °C and the Au nanoparticle coupled with anti-IgG was precipitated quickly. The pink supernatant in the solution was removed, and the PBS was added to dilute the precipitate.

2.3.3. Preparation of biosensors

The prism with Au film was first rinsed with doubly distilled water. Then, 10 μL of the mixture of water and ethanol (v/v, 1/2) was dropped onto the surface of Au film. The prism was immediately suspended vertically above pure titanium isopropoxide in a sealed beaker kept at a constant temperature of 25 °C for 60 min (Fig. 1). Afterwards, 10 μL of AuNP solution which diluted by ethanol was dropped onto the modified surface. In order to study the effect of the amount of immobilized AuNP on the sensitivity, the AuNP solutions at different concentrations (50% and 30%) were applied. The prism was immediately suspended vertically above pure titanium isopropoxide in a sealed beaker kept at a constant temperature of 25 °C for 75 min to form the second layer. Then 10 μL of AuNP coupled with anti-IgG solution was dropped onto the surface. The prism was immediately suspended vertically above titanium isopropoxide in a sealed beaker kept at 25 °C for 45 min. With the titanium isopropoxide hydrolysis, AuNP coupled with the antibody could be fused in the titania sol–gel membrane to form the third layer. At last, the surface of the sensor was rinsed thoroughly with doubly distilled water. The sensors modified with [TiO_2, TiO_2/AuNP (50%), TiO_2/AuNP-anti-IgG] membrane, [TiO_2, TiO_2/AuNP (30%), TiO_2/AuNP-anti-IgG] membrane were prepared. The thickness of three layers of titania membrane was detected by Dektak 150 Surface Profiler.

In addition, another biosensor without AuNP in the second layer of titania membrane was prepared. After the first layer of titania membrane formed, another 10 μL of the mixture of water and ethanol (v/v, 1/2) was dropped on the surface of prism at a constant temperature of 25 °C for 75 min. Finally, 10 μL of AuNP coupled with anti-IgG was dropped onto the surface. The prism was suspended vertically above titanium isopropoxide at 25 °C for 45 min to form the third layer of titania membrane. The surface of the sensor was also rinsed thoroughly with doubly distilled water and stored in PBS buffer at 4 °C. The biosensor based on [TiO_2, TiO_2, TiO_2/AuNP-anti-IgG] membrane was prepared.

For comparison, the anti-IgG was immobilized on the Au film by mercaptopropionic acid (MPA), which is a traditional method to immobilize antibody. The biosensor based on [MPA-anti-IgG] membrane was applied to the detection of human IgG.

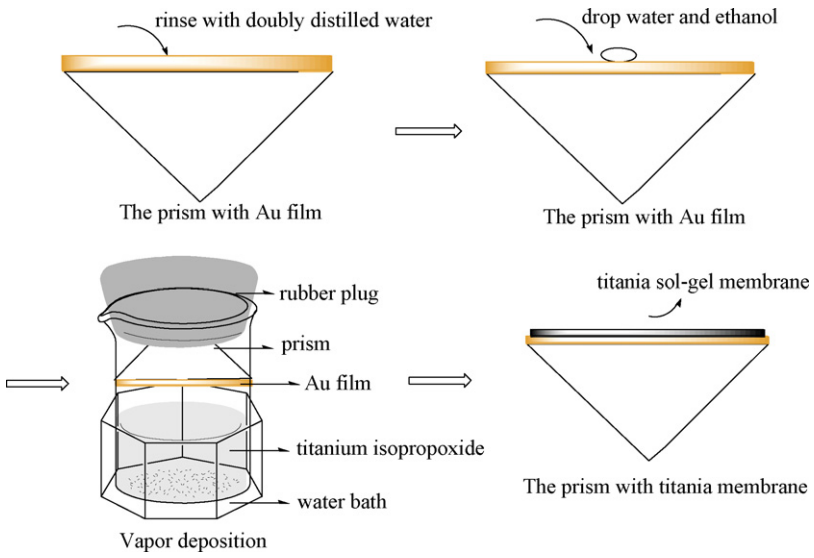

Fig. 1. Schematic diagram of construction for titania sol–gel membrane on the surface of Au film.

2.3.4. Observation of the surface morphology

The K9 glass slides with 2 nm adhesive layer of Cr and 50 nm layer of Au film were used to fabricate TiO_2 membrane and [TiO_2, TiO_2, TiO_2/AuNP-anti-IgG] membrane. The TiO_2 membrane and [TiO_2, TiO_2, TiO_2/AuNP-anti-IgG] membrane were prepared respectively on the surface of prism by the same vapor deposition as those above mentioned. Finally, the scanning electron micrographs of TiO_2 membrane and [TiO_2, TiO_2, TiO_2/AuNP-anti-IgG] membrane were obtained with JEOL FESEM 6700F electron microscope.

2.3.5. Immunoassay

Generally, the ion-concentration and pH of the sample solution are the main external factors to affect the experimental results. In order to eliminate the effect of the ion-concentration and pH of the sample solution, the sample solutions were obtained by dilution with PBS buffer. Although the sensing layer thickness may change with changing ion-concentration and pH, the ion-concentration and pH value in the analytical solutions should be almost same.

At room temperature, human IgG at different concentrations were separately injected into the flow cell of the wavelength modulation SPR biosensor system (Fig. 2). The shifts of resonant wavelength due to antibody–antigen immunoreaction were measured. After 40 min, PBS was injected into the flow cell. Due to a large amount of ions in the PBS buffer, the antigen could be washed by impulse injection of PBS buffer [29]. When the antigen was completely dissociated from the antibody on the biosensor surface, another sample solution containing human IgG at different concentration was injected into the flow cell. After the determination of human IgG was finished, the sensor was stored in PBS buffer at 4 °C. After a period of storage, the sensor was used again to detect human IgG at different concentrations and the stability of biosensor was obtained. To examine the reliability of the assay, all determinations of human IgG were conducted three times.

To evaluate the selective binding of human IgG, human serum albumin and human g-globulin were determined by the same procedure as the above mentioned.

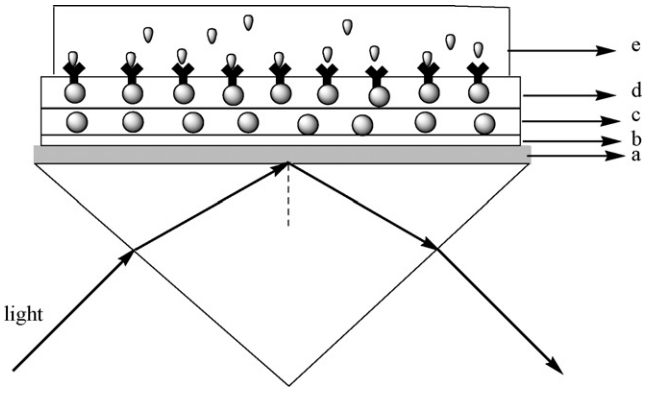

Fig. 2. Schematic diagram of SPR biosensor based on (TiO_2, TiO_2/AuNP, TiO_2/AuNP-anti-IgG) membrane. (a) The Au film, (b) the first layer of TiO_2 membrane, (c) the second layer of TiO_2 membrane, (d) the third layer of TiO_2 membrane, (e) the flow cell, ( ) antibody, ( ) antigen, ( ) Au nanoparticle.

3. Results and discussion

3.1. Titania sol–gel membrane on the Au film

The shift of the resonant wavelength ($\Delta\lambda$) is related to the thickness of the membrane, while the thickness of the titania sol–gel matrix mainly depends on temperature and vapor deposition time. Temperature affects the vapor pressure of titanium isopropoxide directly, thus controlling the hydrolysis rate. Here the suitable temperature is 25 °C [4], at which the rate of vapor deposition is equal to that of water volatilization. At too low temperature, the rate of vapor deposition is slower than that of water volatilization, which may result in poor yield of the titania sol–gel. When the preparation temperature is higher than 25 °C, too rapid vapor deposition may induce to appearance of TiO_2 powder that cannot effectively encapsulate the antibodies.

The thickness of titania membrane is also related to vapor deposition time. In order to study the effect of the vapor deposition time on the shift of the resonant wavelength, a series of assays with different vapor deposition time were performed. It can be seen clearly from Fig. 3 that the thickness of membrane increases with increasing vapor deposition time, followed with the resonant wavelength moving to longer wavelength. The shifts of resonant wavelength due to the deposition of titania sol–gel membrane on the Au film for different vapor deposition time (0, 30, 60, 90, 120, 150, 180 and 240 min) are 0, 26, 44, 69, 94, 121, 150 and 195 nm, respectively. With the increase in vapor deposition time, the thickness of titania membrane increases and the SPR curve can be expected to shift to longer wavelength, which has been proved to be benefi-

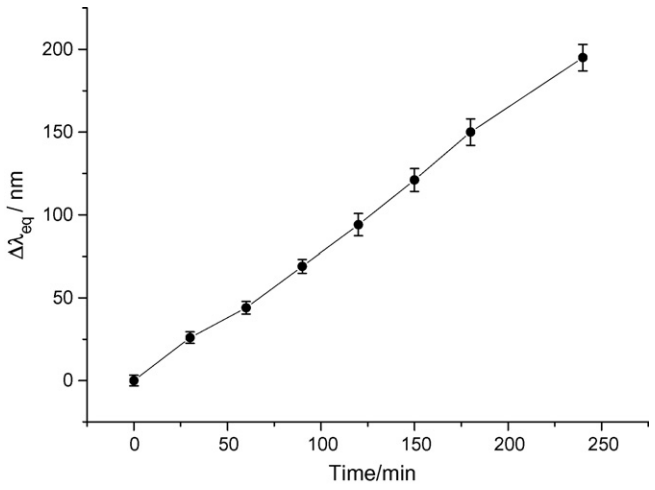

Fig. 3. The relationship between the titania sol–gel deposition time and the shift of the resonant wavelength ($\Delta\lambda_{eq}$). Error bar = ±S.D. and n = 3.

cial to the enhancement of sensitivity [30]. However, when titania membrane is too thick, the surface plasmon could not occur at the surface of Au film. As a result, distinct characteristic dip in the wavelength dependence of the reflected light intensity could not be obtained. When the shifts of resonant wavelength are within 120–150 nm, the biosensor was regarded as the best condition for detection of antigen due to the best peak shape. Therefore, the most suitable vapor deposition time for three layers of titania membrane is about 150–180 min. The error bar in Fig. 3 represents standard deviation (S.D.) obtained from three separate experiments, which were individually performed with three SPR biosensors. The relative standard deviation less than 6% illustrates that the proposed procedure is reproducible.

When Au nanoparticle was immobilized on Au film, the catalytic growth of Au nanoparticle could result in the enhancement of background. So titania sol–gel membrane was firstly constructed on the Au film surface to prevent the direct contact of the Au nanoparticle with the Au film. In this paper, the vapor deposition time of the first titania sol–gel membrane is 60 min and the corresponding red shift of the resonant wavelength is about 44 nm. In addition, the thickness of membrane was measured by Dektak 150 Surface Profiler and the result indicated that thickness was about 11 nm.

Likewise, the second titania membrane was constructed to encapsulate Au nanoparticle by vapor deposition. When the deposition time is 75 min, the corresponding red shift of the resonant wavelength is 61 nm and the thickness of the second titania sol–gel membrane is about 18 nm. In order to study the effect of Au nanoparticle, the second layer of titania sol–gel membrane without Au nanoparticle was also performed with the same vapor deposition time and the corresponding red shift of the resonant wavelength is 59 nm.

For the third layer of titania sol–gel membrane, the selection of vapor deposition time is an important parameter. For too long deposition time, the titania sol–gel membrane is too thick that may block the entrance of the antigen. As a result, the binding efficiency to the immobilized antibody decreased. If the vapor deposition time was too short, the formed titania sol–gel membrane could not cover the AuNP coupled with antibody completely. The deposition time here selected is 45 min, and the corresponding red shift of resonant wavelength is 40 nm. The thickness of the third titania sol–gel membrane is about 15 nm. When the three layers of titania membrane were formed, the resonant wavelength shifts 145 nm.

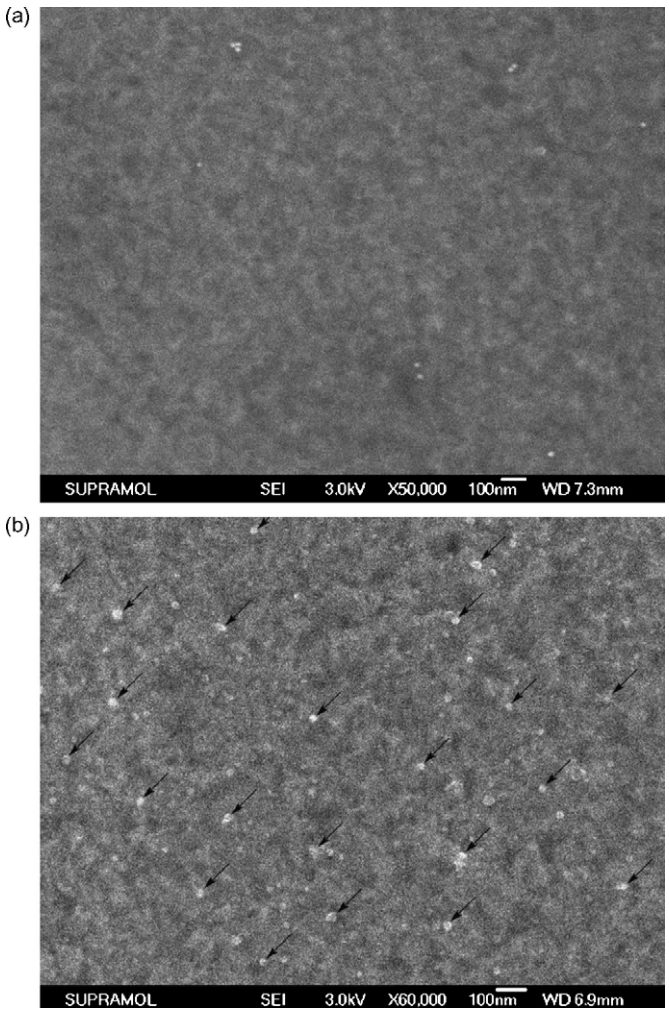

Fig. 4. Scanning electron micrographs of (a) TiO_2 membrane and (b) AuNP-anti-IgG encapsulated with TiO_2 membrane. The arrows are used to mark the AuNP-anti-IgG encapsulated.

3.2. Surface morphology of titania sol–gel membrane

The surface morphology of the titania sol–gel matrix is an important factor affecting the character of biosensor. After AuNP coupled with anti-IgG was dropped on the surface, the titania sol–gel was formed around the anti-IgG with the hydrolysis of titanium isopropoxide. In this way, the AuNP coupled with anti-IgG became a part of titania sol–gel membrane. Fig. 4 shows the morphology of titania sol–gel with and without AuNP coupled with anti-IgG encapsulated, which is obtained by scanning electron microscope. The scanning electron micrograph of the titania membrane displays a three-dimensional uniform porous structure. When the AuNP coupled with anti-IgG is immobilized in the titania sol–gel membrane, the bright particles can be observed which indicated by blank arrows (Fig. 4b). It also can be seen from Fig. 4b that the aggregates of the trapped biomolecule distribute regularly.

3.3. Determination of human IgG

To wash off non-specific binding on the sensing membrane, the biosensor surface was rinsed with PBS buffer until the resonant wavelength kept constant. Then the human IgG at different concentrations were separately injected into the flow cell for the immunoassay. Fig. 5 shows the relationship between the shift of the resonant wavelength and the concentration of

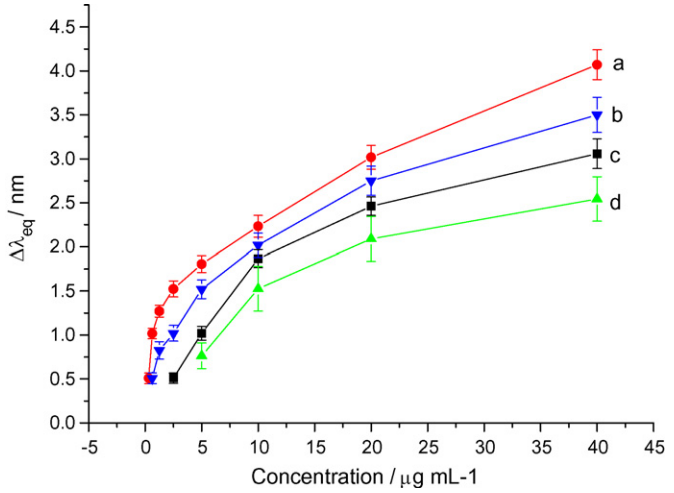

Fig. 5. The relationship between the concentration of human IgG and the shift of the resonant wavelength ($\Delta\lambda_{eq}$) based on different sensing membranes. (a) [TiO_2, TiO_2/AuNP (50%), TiO_2/AuNP-anti-IgG] membrane, (b) [TiO_2, TiO_2/AuNP (30%), TiO_2/AuNP-anti-IgG] membrane, (c) [TiO_2, TiO_2, TiO_2/AuNP-anti-IgG] membrane, (d) [MPA-anti-IgG] membrane. Error bar = ±S.D. and $n = 3$.

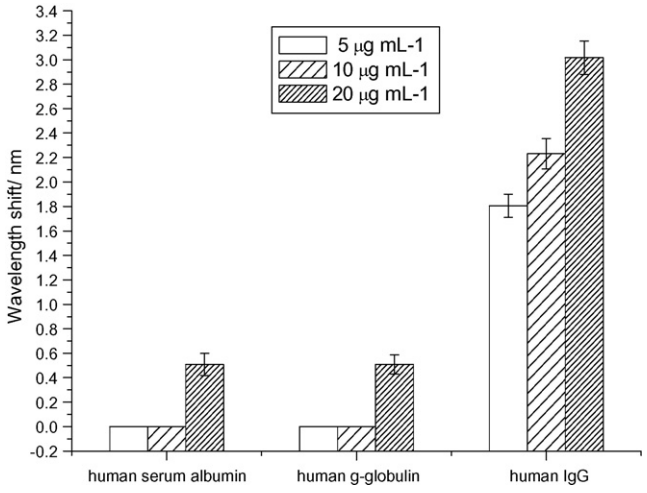

Fig. 6. The shift of resonant wavelength measured by SPR biosensor for different antigens (human serum albumin, human g-globulin and human IgG) at different concentrations. Error bar = ±S.D. and $n = 3$.

human IgG. To study the effect of the amount of immobilized AuNP on the sensitivity, the four different biosensors were applied. It can be seen from Fig. 5 that the SPR biosensors based on [TiO_2, TiO_2/AuNP (50%), TiO_2/AuNP-anti-IgG] membrane, [TiO_2, TiO_2/AuNP (30%), TiO_2/AuNP-anti-IgG] membrane, [TiO_2, TiO_2, TiO_2/AuNP-anti-IgG] membrane and [MPA-anti-IgG] membrane show a satisfactory response to human IgG in the concentration range of 0.30–40.00 μg mL^{-1}, 0.60–40.00 μg mL^{-1}, 2.50–40.00 μg mL^{-1} and 5.00–40.00 μg mL^{-1}, respectively.

These experimental results indicated that colloidal Au nanoparticle plays an important role in improving the detection capability of SPR biosensor. The lowest concentration determined by SPR biosensor with [TiO_2, TiO_2/AuNP (50%), TiO_2/AuNP-anti-IgG] membrane is about 2-fold lower than that obtained with [TiO_2, TiO_2/AuNP (30%), TiO_2/AuNP-anti-IgG] membrane, 8-fold lower than that obtained with [TiO_2, TiO_2, TiO_2/AuNP-anti-IgG] membrane and 16-fold lower than that obtained with [MPA-anti-IgG] membrane. The Au nanoparticle has its own localized surface plasmon due to the collective oscillation of its conduction electrons [24]. The coupling of local surface plasmon of AuNP with the propagating plasmon of the Au film could influence the plasmon mode. By this way, the SPR response propagation at the surface of Au film can be increased and the sensitivity of SPR biosensor is enhanced. As a result, the amount of AuNP can affect the sensitivity of SPR biosensor. The AuNP immobilized in the titania membrane is favorable for enhancing the sensitivity of SPR biosensor.

As seen from Fig. 5, when the concentration of human IgG increases, the shift of the resonant wavelength increases, but not linearly. For 1:1 reaction of antigen (Ag) and antibody (Ab): Ag + Ab = AgAb, the binding constant is K_A [31–33]:

$$K_A = \frac{[AgAb]}{[Ag][Ab]} \quad (1)$$

At equilibrium, the [AgAb] is corresponding to the SPR biosensor signal $\Delta\lambda_{eq}$, [Ag] is the concentration of antigen in the solution, [Ab] is corresponding to ($\Delta\lambda_{max} - \Delta\lambda_{eq}$). $\Delta\lambda_{eq}$ is the bound antigen measured as the biosensor response at equilibrium. $\Delta\lambda_{max}$ is a constant for a special sensing membrane, which is corresponding to the maximum capacity of binding antigen on the sensor surface.

So Eq. (1) can be expressed as:

$$\frac{\Delta\lambda_{eq}}{[Ag]} = K_A\Delta\lambda_{max} - K_A\Delta\lambda_{eq} \quad (2)$$

The increase in concentration of antigen ([Ag]) in the solution could lead to increase of AgAb in the membrane and increase of $\Delta\lambda_{eq}$. It is seen from the Eq. (2) that the $\Delta\lambda_{eq}$/[Ag] decreases with the increase of $\Delta\lambda_{eq}$, which means the slope of the relationship curve between the shift of the resonant wavelength and the concentration of human IgG decrease. The theoretical analysis is confirmed by the experimental results. When the concentration of antigen increases, the slope decreases.

The evaluation of the selective binding of human IgG was also performed by separately injecting human serum albumin and human g-globulin into the flow cell for 20 min in the concentration range of 5.00–20.00 μg mL^{-1}. There were no observable shifts of the resonant wavelength (Fig. 6), which indicated the selective binding of human IgG.

3.4. Stability of the biosensor

The stability of biomolecule immobilized in biosensor is an important parameter to affect the practical application. After a 5-day storage period at 4 °C, human IgG at different concentrations were injected again and no obvious decrease in the response to IgG was observed. After 10 days, the biosensor retains 82% of its initial response at the concentration of 10.00 μg mL^{-1} (Fig. 7a and b). After 20 days, the biosensor could retain 69% of its initial response at the concentration of 10.00 μg mL^{-1} (Fig. 7c). And after a 30-day storage period at 4 °C, the biosensor could retain 57% of its initial response at the concentration of 10.00 μg mL^{-1} (Fig. 7d).

For comparison, a biosensor based on [MPA-anti-IgG] membrane was constructed and the stability of the biosensor was also studied. After the prism stored in PBS buffer at 4 °C for 5 days, ethanolamine hydrochloride was used to block the non-specific binding sites. And then human IgG at 10.00 μg mL^{-1} was injected, no obvious shift of the resonant wavelength was observed. It can be confirmed from the above assays that there was few human IgG binding to anti-IgG. The reason could be that the anti-IgG immobilized on Au film loses bioactivity, which could not bind to human IgG. In addition, due to a large amount of ions in the PBS buffer, the anti-IgG was probable to dissociate from the MPA after 5-day storage in PBS.

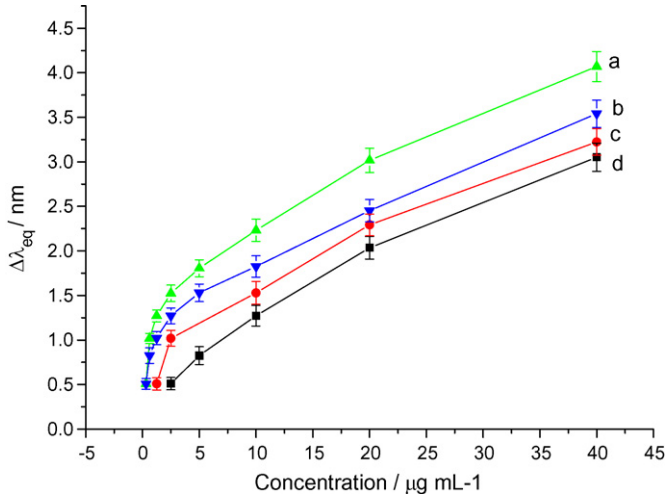

Fig. 7. The relationship between the concentration of human IgG and the shift of the resonant wavelength ($\Delta\lambda_{eq}$) after (a) 0 days, (b) 10 days, (c) 20 days, (d) 30 days storage. Error bar = ±S.D. and $n = 3$.

The fact that the biomolecule encapsulated in the titania membrane shows long-term stability is not surprising. The abundant water contained in the titania sol–gel membrane could offer a good waterish microenvironment for biomolecule. The vapor deposition of titania sol–gel membrane provides a mild immobilization process, which does not involve any additives that result in chemical modification and fouling molecules. Therefore, the bioactivity of the biomolecule encapsulated cannot be affected during the vapor deposition process. And when the biomolecule encapsulated in the pore of sol–gel membrane, the large quantities of hydroxyl groups in the sol–gel membrane can form hydrogen bonds, which could prevent dissociation of biomolecule from the membrane. In addition, the Au nanoparticle has large specific surface area and good biocompatibility, which could offer a suitable microenvironment around biomolecule. As a result, the activity of biomolecule can be kept in the membrane in the best condition.

4. Conclusions

In this work, the determination of IgG by SPR biosensor with entrapping AuNP coupled with anti-IgG in titania sol–gel membrane was developed, which is constructed by the vapor deposition. This method could provide a mild immobilization process and construct a waterish environment for the biomolecule. The biomolecule could retain its biological activity for a long term as being physically immobilized in the pores of the titania sol–gel matrix. In order to enhance the sensitivity of biosensor, the colloidal Au nanoparticle was encapsulated in the second layer of titania sol–gel membrane. This biosensor can show a satisfactory response to human IgG and retain long-term bioactivity.

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