A sensitive SPR biosensor based on hollow gold nanospheres and improved sandwich assay with PDA-Ag@Fe_3O_4/rGO

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A sensitive SPR biosensor based on hollow gold nanospheres and improved sandwich assay with PDA-Ag@Fe_3O_4/rGO

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Abstract

A novel surface plasmon resonance (SPR) biosensor based on hollow gold nanospheres (HGNPs) and an improved sandwich assay was developed to detect rabbit IgG. The electromagnetic coupling between the HGNPs and Au film, and the notable plasmonic fields spread over the inner and outer surfaces of HGNPs, led to the considerable amplification of the SPR signal. Polydopamine-Ag@Fe_3O_4/reduced graphene oxide (PDA-Ag@Fe_3O_4/rGO) was introduced to bind detection antibody (Ab_2) to form the improved sandwich structure. Ag nanoparticles were excited to produce SPR and their hot electrons were doped on graphene thin films, which amplified the response of biomolecules. Magnetic nanoparticles (Fe_3O_4) simplified the collection of Ab_2-PDA-Ag@Fe_3O_4/rGO. An external layer of polydopamine (PDA)

permitted the efficient immobilization of Ab_2 without activation via abundant functional groups and protected the nanoparticles from etching or agglomeration. In addition, because of its large mass, Ab_2-PDA-Ag@Fe_3O_4/rGO also played a key role in the further amplification of the SPR response signals. This novel SPR biosensor exhibited an effective response to the rabbit IgG at the different concentrations ranging from 0.019 to 40.00 μg mL^{-1}. This value is 132 times lower than that observed for a traditional SPR biosensor based on Au-3-mercaptopropionic acid and 8 times lower than that obtained from an Ab_2 sandwich assay, which indicates that the SPR sensor has high sensitivity. In addition, the designed biosensor showed satisfactory recoveries to detect the rabbit IgG spiked in serum samples. Therefore, the novel SPR biosensor with high sensitivity and acceptable recovery has potential for practical applications.

Keywords:

Hollow gold nanoparticles

Polydopamine

Ag@Fe_3O_4/reduced graphene oxide

Sandwich structure

Surface plasmon resonance biosensor

1. Introduction

Surface plasmon resonance (SPR) technology is a molecular biological detection technology, which has been widely used for ligand interaction and analysis based on SPR biosensors [1-4]. However, the sensitivity of SPR biosensors for the detection of low concentration, low molecular weight biomolecules is not sufficient; hence, it is imperative to explore new methods to compensate for this limitation.

Noble metals, particularly Au and Ag nanoparticles have been widely used as ideal signal amplification materials in the biosensor field [5]. Ag nanoparticles can produce a sharper SPR resonance peak and exhibit higher sensitivity compared to other noble metal nanoparticles under the same morphology and size, therefor, they can be used to increase the sensitivity of SPR biosensors. However, they still have some disadvantages, such as instability and facile oxidation without protection [6]. Moreover, although Au nanoparticles exhibit lower enhancement in the visible-light region than Ag [7], they are still particularly attractive in SPR biosensor applications because of their facile reductive preparation, low toxicity, and extremely high biocompatibility and affinity [8]. With the development of synthetic technologies, different morphologies of Au nanoparticles have been synthesized and applied in SPR biosensors to improve the sensitivity, including gold nanobipyramids [9], gold nanorods [10], and gold nanostars [11]. In the last few years, hollow gold nanospheres (HGNPs) have attracted considerable interest in bio-medical applications [12]. Compared with other morphologies, HGNPs exhibit advantages of a large surface-to-volume (S/V) ratio, low density, and the highest sensitivity to the changes

in the effective refractive index [13], which are extremely conducive for applications in SPR biosensors.

Meanwhile, a sandwich-type assay is an extremely effective method in immunoassay, which can increase the SPR sensitivity via the formations of a sandwiched immune complex between the immobilized capture antibodies and detection antibodies [14]. In order to further optimize the sand-type assay to improve the performance of SPR biosensor, many effective functional materials are introduced into the system, such as HGNPs, polyamidoamine, reduced graphene oxide (rGO) and polydopamine (PDA) [15, 16].

Graphene oxide (GO) exhibits high surface area with functional groups, which make it possible to interact with other functionalities of different materials [17]. Futhermore, GO sheets contain sites for the deposition of metal or metal oxide nanoparticles on its surface, which can be easily reduced to rGO via hydrothermal treatment [18]. RGO has the structure and properties similar to grahpene, which can be used as a lamellar scaffold for compounds and provide electrons by the sp^2 hybridization of carbon atoms.

Mussels are marine organisms that can be firmly adhered to the surface of various materials in a wet environment via the super adhesion property of the protein from the foot glands [19]. Inspired by this strong adhesion protein, researchers have found that polydopamine (PDA) not only has a similar structure with the mussel adhesive protein, but also exhibits a superior adhesion property [20]. In an alkaline solution, the PDA film can be expeditiously formed on the substrate surface, leading

to the improved hydrophilicity and chemical versatility of substrate because of the hydroxyl and amino groups of dopamine [21]. Meanwhile, a PDA layer can graft thiol groups, amino groups of polymers, and some biomolecules on the surface by the Michael addition or the Schiff base reaction [20]. Because of the above advantages, PDA has been widely used in sensing applications, including the detection of small organic molecules [22], biomolecules [22], and heavy metal ions [23].

In this study, a novel SPR biosensor was fabricated based on HGNPs and an improved sandwich assay to detect rabbit IgG. HGNPs were used to induce electromagnetic coupling between the HGNPs and Au film, and amplify the response of the binding of antigen to capture antibody (Ab_1). PDA-Ag@Fe_3O_4/rGO was introduced to bind the detection antibody (Ab_2) to form Ab_2-PDA-Ag@Fe_3O_4/rGO, and the SPR response signal was further amplified via electronic coupling between the Ag nanoparticles and surface plasmon wave of rGO and, due to higher mass of Ab_1/antigen/ Ab_2-PDA-Ag@Fe_3O_4/rGO with sandwich structure.

2. Material and methods

2.1 Reagents and materials

Rabbit IgG, goat anti-rabbit IgG, human IgG, bovine IgG, and bovine serum albumin (BSA) were purchased from Beijing Biosynthesis Biotechnology Company. 1,6-hexanedithiol (HDT), 3-mercaptopropionic acid (MPA), Dopamine hydrochloride, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide

(NHS) were purchased from Aladdin Industrial Corporation. Tris(hydroxymethyl)aminomethane (Tris, 10 mmol L^{-1}, pH 8.5) and sodium phosphate buffered saline (PBS, 0.01 mol L^{-1}, pH 7.4) were used as buffer. All of the other chemicals were of analytical reagent grade and ultrapure water was used in all the preparations.

2.2 Equipment

In this study, the SPR biosensor was assembled by our experimental group. The Kretschmann configuration used in the SPR biosensor represents a condition to obtain the attenuated total reflection of the interface between a prism and a thin metal layer. A K9 glass slide with 50-nm-thick Au film is fixed on the optical prism surface using the suitable cedar oil. An iodine tungsten-lamp is used as the light source, and the light beam passing through the two lens and a polarizer is converted into the transverse-magnetic parallel polarized light. The incident light produces a total internal reflection at the interface between the glass and metal, which is refracted from the other side of the prism. The refracted light is focused by the next lens, coupled to the optical fiber, and then enters the spectrophotometer (HR2000+, Ocean Optics Inc., USA).

2.3 Procedures

2.3.1 Synthesis of hollow gold nanospheres (HGNPs) and PDA-Ag@Fe_3O_4/rGO

HGNPs were prepared by Schwartzberg's method [24] with slight modifications (See the support materials S1).

Ag@Fe_3O_4/rGO was synthesized using Liu's method [18] with slight modifications (See the support materials S2). PDA-Ag@Fe_3O_4/rGO was sythesized by the oxidative polymerization of dopamine in the presence of Ag@Fe_3O_4/rGO. Typically, 50 mg of dopamine and 2 mL of the prepared Ag@Fe_3O_4/rGO were dissolved in 100 mL of Tris [20], and the solution was mechanically stirred at room temperature for 3 h. The PDA-Ag@Fe_3O_4/rGO products were magnetically collected and washed several times, then dissolved in 10 mL of ultrapure water.

2.3.2 Synthesis of antibody-conjugated PDA-Ag@Fe_3O_4/rGO

For the synthesis of Ab_2-PDA-Ag@Fe_3O_4/rGO, 12.5 μL of PDA-Ag@Fe_3O_4/rGO was added into 0.5 mL of PBS [25] containing 50 μg of goat anti-rabbit IgG (Ab_2) with vortex vibration for 24 h at room temperature. The prepared Ab_2-PDA-Ag@Fe_3O_4/rGO was collected by a magnet, followed by its addition into 0.5 mL of PBS containing 0.5 mg BSA with vortex vibration for 12 h. Finally, the products were magnetically collected and washed with PBS. Then, isolated Ab_2-PDA-Ag@Fe_3O_4/rGO was dissolved in 0.5 mL of PBS and stored at 4 °C for further use.

2.3.3 Modification of Au film

The 50 nm Au film layer was dipped into a 10 mmol L^{-1} HDT solution overnight.

Then, the Au film was washed using ethanol and ultrapure water, followed by drying under N_2. The modified film was dipped into the HGNP solution for 6 h. Then, the HDT-HGNPs modified Au film was removed from the solution and washed with ethanol and ultrapure water, followed by drying under N_2. Next, the HGNP-HDT Au film was dipped into a 10 mmol L^{-1} MPA solution overnight, then washed with ultrapure water and dried under gaseous N_2.

2.3.4 Antibody immobilization

The sensing substrate prepared was fixed in the reactor. First, NHS and EDC were injected into the reactor for 20 min to activate the carboxyl group of MPA. Second, PBS was injected to remove the unreacted NHS and EDC and stabilize the baseline. After the resonant wavelength was maintained constant, goat anti-rabbit IgG (Ab_1) was injected and covalently immobilized on the sensor substrate surface within 12 h. To prevent nonspecific interactions, 10 μL of 10 mg mL^{-1} BSA solution was injected.

By control, the MPA-modified Au film was also used to immobilize the antibodies. The 50-nm Au film layer was dipped into a 10 mmol L^{-1} MPA solution overnight, then washed with ethanol and ultrapure water and dried under N_2. The following processes were the same as described above.

2.3.5 Immunoassay

A series of different of concentrations of rabbit IgG diluted with PBS was

separately injected into the reactor. After 40 min, the specific interaction between Ab_1 and rabbit IgG was completed. PBS was injected to remove the unbound rabbit IgG and maintained a steady resonant wavelength. Then, the prepared Ab_2-PDA-Ag@Fe_3O_4/rGO was injected in the reactor for 30 min to form a sandwich structure with the antigen on the sensor substrate. Finally, PBS was used to remove unbound Ab_2-PDA-Ag@Fe_3O_4/rGO in the reactor, and the result of resonant wavelength shift was recorded. Figure 1 shows the schematic of the assay procedure. To ensure the reliability of the assay, all experiments were conducted in triplicate.

3. Results and discussion

3.1 Characterization of HGNPs and Ab_2-PDA-Ag@Fe_3O_4/rGO

The morphology and size of the prepared HGNPs, Ag@Fe_3O_4/rGO, and PDA-Ag@Fe_3O_4/rGO were investigated by transmission electron microscopy (TEM). In Figure 2a, HGNPs exhibited a hollow spherical structure with an average shell diameter of 54 nm and a wall thickness of 8 nm. According to the apparent contrast from Figure 2b, the core–shell structure of the Ag–Fe_3O_4 nanocomposites was distributed on the rGO, with a homogeneous size distribution, an average core diameter of 50 nm, and a shell thickness of (20 ± 2) nm. In the image, the Ag nanoparticles as the core were darker than Fe_3O_4 nanoparticles because of the higher electron density of Ag. The TEM image shown in Figure 2c revealed that PDA was deposited on Ag@Fe_3O_4/rGO with a thickness of 16 nm to form

PDA-Ag@Fe_3O_4/rGO. As-grown product was homogeneously suspended in an aqueous solution because of plenty of hydrophilic hydroxyl groups and amino groups on the nanocomposite surface without an applied magnetic field. In an applied magnetic field, the nanocomposites were rapidly concentrated, and the bulk solution became clear and transparent (Figure 2d). The application of magnetic separation ensured the facile separation of Ab_2-PDA-Ag@Fe_3O_4/rGO from Ab_2.

Figure 3a shows the X-ray diffraction (XRD) patterns of GO and Ag@Fe_3O_4/rGO. The XRD patterns were identified by comparison with those for standard GO, Ag nanoparticles, and Fe_3O_4 nanoparticles. The diffraction pattern of GO exhibited a strong peak centered at $2\theta = 10.4°$, corresponding to its (001) reflection. After synthesis, diffraction peaks corresponding to Ag and Fe_3O_4 were clearly observed. The diffraction peaks observed at $2\theta = 38.0°$, 44.2°, 64.4°, and 77.5° were indexed to the (111), (200), (220), and (311) planes of Ag in the cubic phase, respectively, and those observed at $2\theta = 30.0°$, 35.3°, 43.0°, 56.8°, and 62.4° were indexed to the (220), (311), (400), (511), and (400) planes of Fe_3O_4, respectively. This demonstrated that AgNPs were well covered by Fe_3O_4 nanopaticles which could protect AgNPs from being oxidized. No diffraction peaks corresponding to GO were observed, indicating that GO was completely reduced to rGO [18].

Figure 3b shows the ultraviolet–visible (UV-vis) absorption curve of 100 μg mL^{-1} of Ab_2 and the antibody in supernatant. 100 μg mL^{-1} of Ab_2 exhibited an absorption of 3.32 with a maximum absorbance peak at 205 nm. To prove the

successful synthesis of Ab_2-PDA-Ag@Fe_3O_4/rGO, the composites were collected using a magnet after subjecting PDA-Ag@Fe_3O_4/rGO and Ab_2 to vortex vibration for 24 h, followed by the ultraviolet absorption characterization of the supernatant. The Ab_2 in the supernatant exhibited an absorption of 0.89 at 205 nm; this value is far less than the absorption intensity of 100 μg mL^{-1} of Ab_2, demonstrating that Ab_2 is attached to PDA-Ag@Fe_3O_4/rGO for the successful formation of Ab_2-PDA-Ag@Fe_3O_4/rGO (Figure 3b).

The Fourier transform infra-red (FT-IR) spectra of PDA-Ag@Fe_3O_4/rGO before and after the attachment of the antibodies were also investigated to further demonstrate the conjugation of Ab_2. For PDA-Ag@Fe_3O_4/rGO, the peak at 1610 cm^{-1} represents the overlap absorption band of the stretching vibrations of C＝C in aromatic rings of both rGO and PDA [16]. The peaks at 1505 cm^{-1}, 1382 cm^{-1}, and 1285 cm^{-1} are characteristic peaks of PDA, which could be assigned to the scissoring vibration of N−H, bending vibration of O−H in catechol groups, and stretching vibration of phenolic C−O [26]. After the conjugation, two new peaks appear at 1648 cm^{-1} and 1536 cm^{-1} corresponding to the presence of amide I (C＝O stretching) and amide II (overlap of N−H bending and C−N stretching) bands [27], respectively, which suggests that the antibodies are successfully attached on the PDA-Ag@Fe_3O_4/rGO nanosheets.

3.2 HGNP-based antibody biochip

HDT permits the linkage of HGNPs with the Au film surface through its

disulfide bonds. MPA is a good linker for attaching HGNPs with the antibody via the activated carboxylic groups of MPA. To optimize the antibody concentration, different concentrations of Ab_1 were injected into the reactor. As can be observed from Figure 4a, the resonant wavelengths had different red shifts with increasing Ab_1 concentrations. Moreover, if there is no Ab_1 in PBS, the wavelength shifts are very constant at about 0 nm, which means there were no other interferences when we investigate the optimal concentrations of Ab_1. Then, the resonant wavelength maintained a constant value, suggesting that the antibody adhered on the chip surface reaching saturation. At Ab_1 concentrations of 75 μg mL^{-1}, 100 μg mL^{-1}, and 125 μg mL^{-1}, the resonant wavelength shifts were 6.8 nm, 10.8 nm, and 11.0 nm, respectively. Relatively high antibody concentration may hinder the binding of the antigen. Hence, 100 μg mL^{-1} is the optimal concentration for rabbit IgG detection.

The traditional substrate based on the MPA-modified Au film was also investigated. At the optimal concentration of Ab_1, the resonant wavelength shift of HGNP-modified Au film was longer than the resonant wavelength shift of MPA-modified Au film (Figure 4b). The better performance of the HGNP film compared to MPA is possibly related to the stronger plasmon resonance effect produced by inner and outer surfaces of the hollow structures of the HGNPs, considerably magnifying the SPR response signals. Meanwhile, the red shift of the resonant wavelength was favorable to increasing the sensitivity of the wavelength-modulated SPR biosensor [28].

3.3 Determination of rabbit IgG

The performance of the SPR biosensor designed herein was tested by detecting different concentrations of rabbit IgG. Figure 5 shows the relationship between the shifts in the resonance wavelengths and concentrations of analyte rabbit IgG in SPR analysis. The Au-MPA-based SPR biosensor exhibited a response to rabbit IgG at concentrations ranging from 2.50 to 40.00 μg mL^{-1}. The minimum shift in the resonant wavelength was 0.2 nm at 2.50 μg mL^{-1}, and the maximum resonant wavelength shift was 2.0 nm at 40.00 μg mL^{-1}. The Au-HGNP-based SPR biosensor exhibited a good response to rabbit IgG at concentrations ranging from 0.30 to 40.00 μg mL^{-1}. The minimum shift in the resonant wavelength was 0.2 nm at 0.30 μg mL^{-1}, and the maximum shift in the resonant wavelength was 7 nm at 40.00 μg mL^{-1}. The Au-HGNP-based SPR biosensor with the Ab_2 sandwich assay exhibited considerable response to rabbit IgG at concentrations ranging from 0.15 to 40.00 μg mL^{-1}. The minimum shift in the resonant wavelength was 0.2 nm at 0.15 μg mL^{-1}, and the maximum resonant wavelength shift was 11.9 nm at 40.00 μg mL^{-1}. The Au-HGNP-based SPR biosensor with the Ab_2-PDA-Ag@Fe_3O_4/rGO sandwich assay exhibited the best response to rabbit IgG at concentrations ranging from 0.019 to 20.00 μg mL^{-1}. The minimum shift in the resonant wavelength was 1.7 nm at 0.019 μg mL^{-1}, and the maximum shift in the resonant wavelength was 7.8 nm at 40.00 μg mL^{-1}. Under the same conditions, the lowest concentration determined by the present method based on the Au-HGNP substrate with the Ab_2-PDA-Ag@Fe_3O_4/rGO

sandwich assay was 132 and 16 times less than those determined by the method based on Au-MPA and Au-HGNPs, respectively, which considerably enhanced the sensitivity of the SPR biosensor.

Experimental results indicated that the Au-HGNP-based biosensor with the Ab_2-PDA-Ag@Fe_3O_4/rGO sandwich assay exhibits considerable advantages for the determination of analytes. Because of their large mass, high dielectric constant and large S/V ratios with a strong SPR effect, HGNPs considerably amplified the response signal. Moreover, the surface structure of the HGNP provides more binding sites for the antibody than flat. A conventional double antibody sandwich assay involves the use of a specific combination of the detection antibody and antigen to amplify the response signal by increasing mass of the test medium in the vicinity of the metal surface. With the Ab_2-PDA-Ag@Fe_3O_4/rGO sandwich assay, the electronic coupling between the Ag nanoparticles and the surface plasmon wave of rGO led to the amplification of the SPR response. Moreover, the large mass of Ab_2-PDA-Ag@Fe_3O_4/rGO on the sensing film directly increases the real component value of SPR, which in turn made the response signal corresponding to the minimum concentration greater than the others. Meanwhile, with the increase in the antigen concentration, the considerable space-hindrance effect of Ab_2-PDA-Ag@Fe_3O_4/rGO made the maximum concentration that can be detected considerably less than that of the other methods. The comparison of the present method with other reported methods for the analysis of antigens in terms of detection limit is given in Table 1.

The specificity of the designed biosensor was examined by testing human IgG, bovine IgG and a blank sample without rabbit IgG. After the immobilization of the goat anti-rabbit IgG on the biosensor surface, human IgG and bovine IgG at a concentration of 10 μg mL^{-1} were separately injected into the reactor cell for 40 min. The results indicated that controlling proteins exhibit no response signals compared to rabbit IgG (Figure 6). In addition, after sealing the sensing platform with BSA, Ab_2-PDA-Ag@Fe_3O_4/rGO was injected to the reactor. The resulting resonant wavelength shift was close to zero, confirming the negligible nonspecific interaction between the antibody and sealed surface of the sensing film. Therefore, the present SPR biosensor can selectively detect rabbit IgG.

3.4 Analysis of actual samples

The possibility of applying the designed biosensor to an actual sample was evaluated by recovery experiments. A series of different amounts of rabbit IgG were added to undiluted chicken serum and PBS to make actual samples and control samples, respectively. The rate of recovery of this method was defined as the ratio of the shifts of the resonance wavelength caused by the chicken serum ($\Delta\lambda_{serum}$) and PBS ($\Delta\lambda_{PBS}$) containing same level rabbit IgG. The recovery (between 89.2% and 124%) was acceptable, indicating the designed biosensor demonstrates feasibility for analyzing complex biological samples (Table 2).

4. Conclusion

In summary, a new SPR biosensor based on HGNPs and Ab_2-PDA-Ag@Fe_3O_4/rGO sandwich assay was demonstrated. The introduction of HGNPs provided strong electromagnetic field enhancement effect and the notable plasmonic fields. Meanwhile, PDA provided effective binding sites for Ab_2, making it possible to form sandwich structures, and the large mass and high refractive index of Ab_2-PDA-Ag@Fe_3O_4/rGO further increased sensitivity of the biosensor. With the design strategy, the novel SPR biosensor exhibited the sensitive response to rabbit IgG at concentrations ranging from 0.019 to 20.00 μg mL^{-1}. The lowest concentration detected by the novel designed biosensor based on the HGNPs substrate with the improved sandwich assay was 132 and 16 times lower than those detected by the biosensor based on MPA and HGNPs, respectively, which demonstrated that our sensitive improved SPR biosensor is well suited for specific protein detection in many practical applications.

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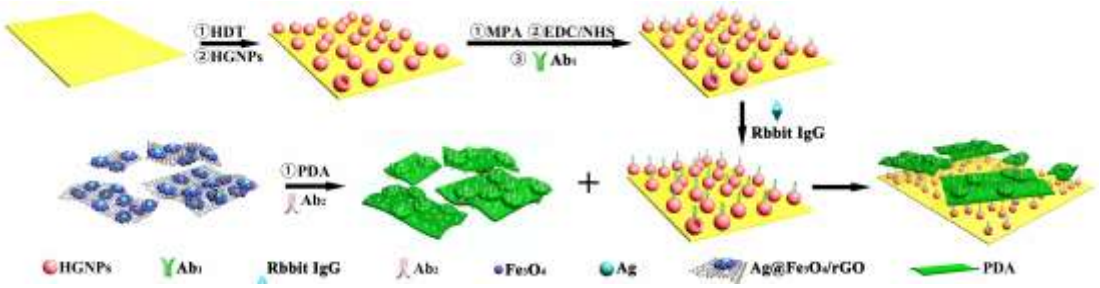


Figure 1. Schematic diagram of experimental procedure.

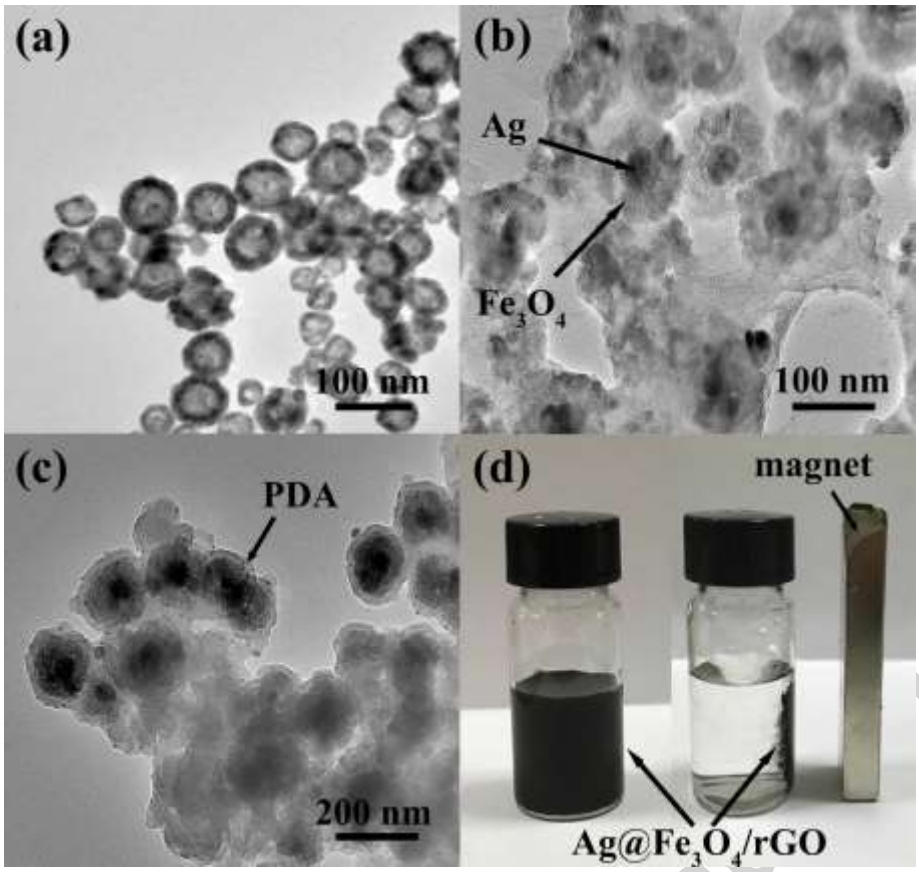


Figure 2. TEM images of (a) HGNPs, (b) Ag@Fe_3O_4/rGO, (c) PDA-Ag@Fe_3O_4/rGO, and (d) a photo of PDA-Ag@Fe_3O_4/rGO solution in an applied magnetic field.

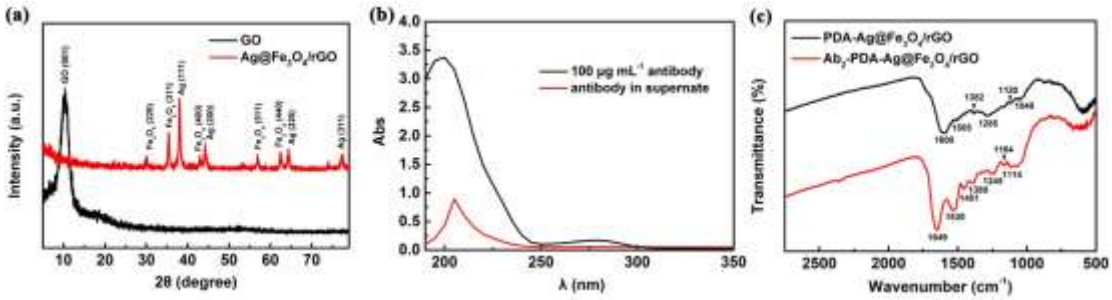


Figure 3. (a) XRD patterns of graphite oxide and $Ag@Fe_3O_4/rGO$ composite, (b) UV–vis absorption spectrum of 100 μg mL^{-1} antibody and the antibody in supernatant, (c) FTIR spectra of (a) PDA-$Ag@Fe_3O_4/rGO$, (b) Ab_2-PDA-$Ag@Fe_3O_4/rGO$.

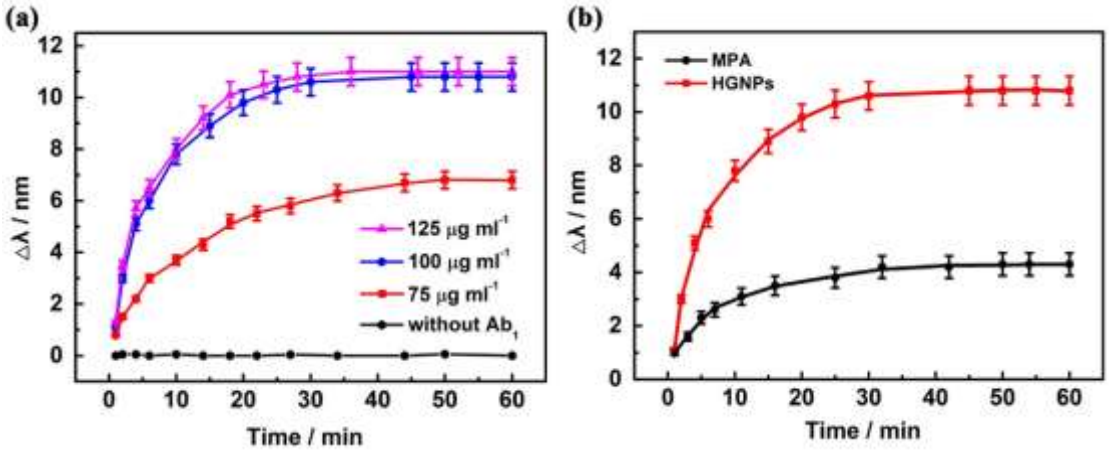


Figure 4. (a) Kinetic adsorption curves of goat anti-rabbit IgG at different concentrations on the HGNP-modified Au film, (b) Kinetic adsorption curves for the immobilization of goat anti-rabbit IgG at a concentration of 100 μg mL^{-1} based on different substrates as MPA-modified Au film and HGNP-modified Au film.

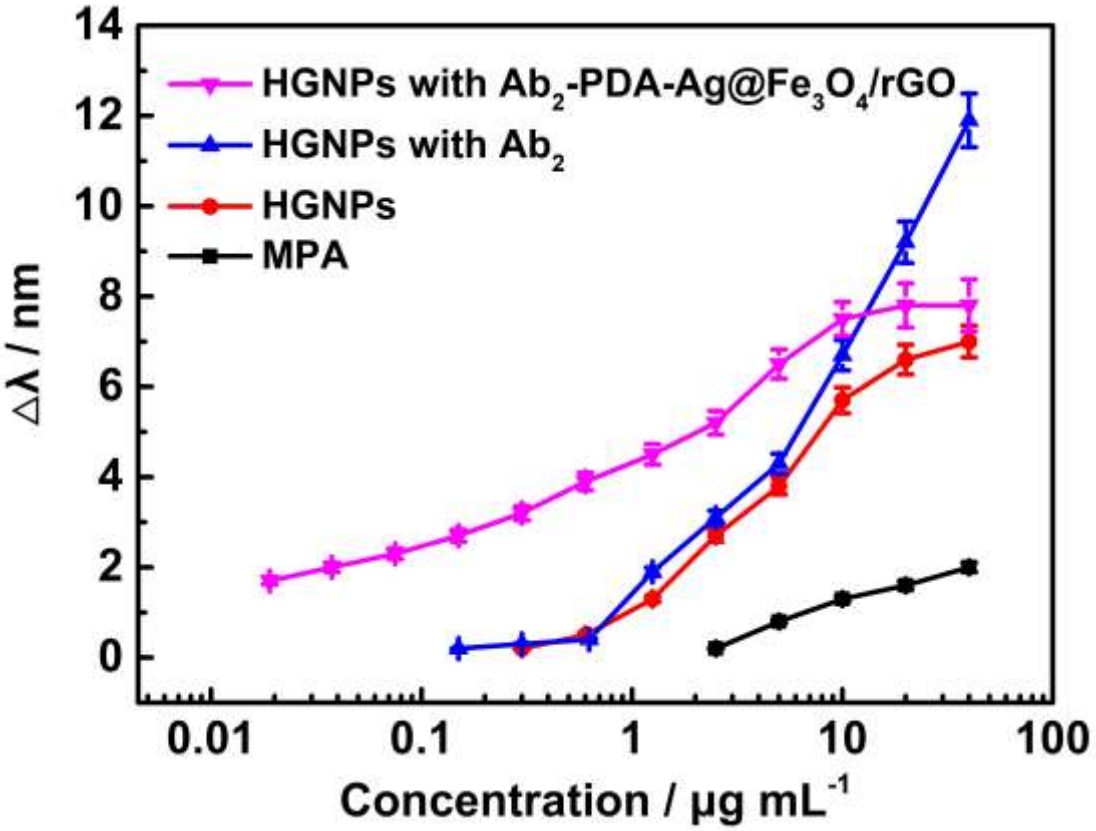


Figure 5. Relationship between the shifts of the resonant wavelength and the concentration of the rabbit IgG: biosensor based on MPA-modified Au film; HGNP-modified Au film; HGNP-modified Au film with the Ab_1 sandwich assay; HGNP-modified Au film with the Ab_2-PDA-Ag@Fe_3O_4/rGO sandwich assay.

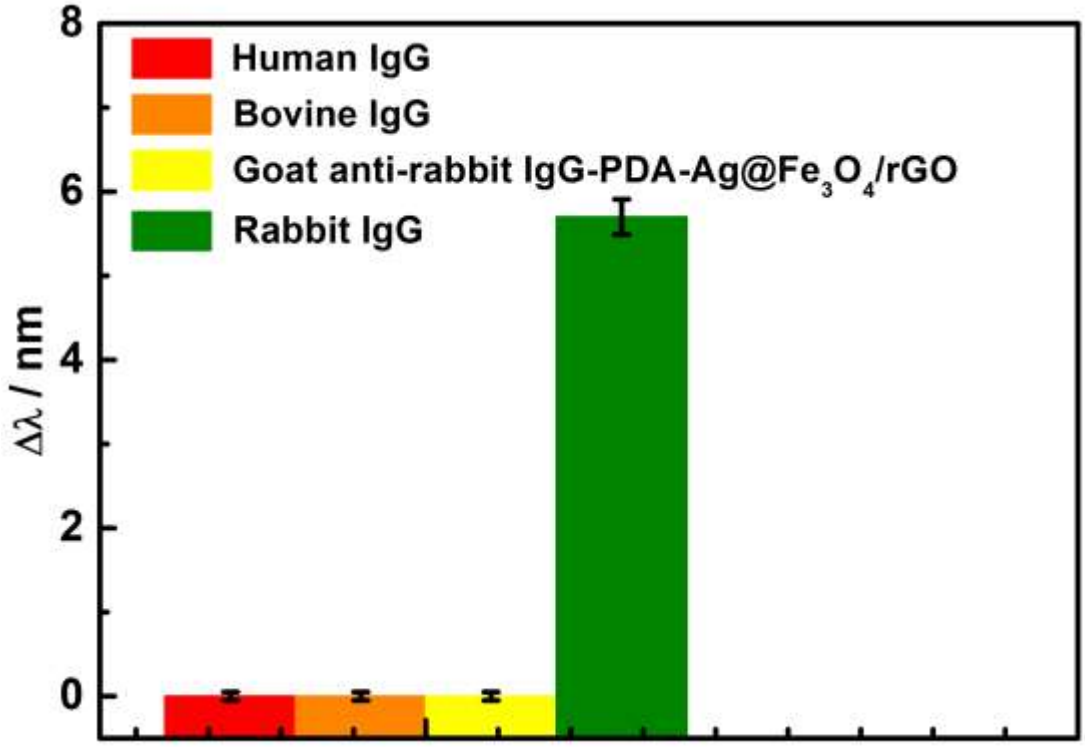
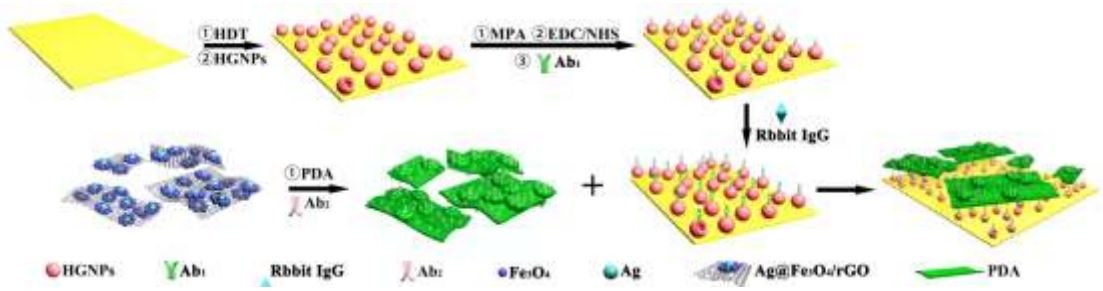


Figure 6. Shifts of the resonant wavelength measured with different antigens and goat anti-rabbit IgG-PDA-Ag@Fe_3O_4/rGO-based different assays.

Graphical abstract



Schematic of the procedure for a novel surface plasmon resonance biosensor based hollow gold nanospheres and improved sandwich assay with PDA-Ag@Fe_3O_4/rGO.

Table 1. Comparison with reported methods for detecting antigens.

Method	**Detection limit**	**Reference**
gold nanorods-Fe_3O_4 SPR assay	0.15 μg mL^{-1}	[29]
Ag nanocubes/chitosan SPR assay	0.6 μg mL^{-1}	[30]
Au bipyramid nanoparticles with graphene oxide-based SPR assay	0.03 μg mL^{-1}	[31]
graphene oxide sheet modified with gold bipyramids SPR assay	0.15 μg mL^{-1}	[32]
anti-IgG-anchored liquid crystal microdroplets assay	0.016 μg mL^{-1}	[33]
HGNPs based PDA-Ag@Fe_3O_4/rGO sandwich typed SPR assay	**0.019 μg mL^{-1}**	**This work**

Table 2. Analytical results of rabbit-IgG in chicken serum (n = 3).

Content of rabbit IgG ($\mu g\ mL^{-1}$)	Spiked ($\mu g\ mL^{-1}$)	Shifts in PBS (nm)	Shifts in serum (nm)	Recovery (%)
none	0.075	2.3	2.9	126
none	0.6	3.9	4.5	115.4
none	5	6.5	5.8	89.2

Highlights

- A sensitive and versatile SPR biosensor with an improved sandwich assay was constructed for detection of rabbit IgG.
- Hollow gold nanospheres and PDA -Ag@Fe_3O_4/reduced graphene oxide used to amplify the response signals.
- The detection limit of the proposed biosensor is 132 times less than that of traditional SPR biosensor based on Au-3-mercaptopropionic acid, and 8 times less than that of Ab_2 sandwich assay.