Preparation of a Sandwich-like Complex "MIPs - Target Molecule - Magnetic SERS Probe" and SERS Determination of Immunoglobulin G

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Abstract

Based on the reversible covalent binding between borates and glycoproteins, we proposed a scheme that can specifically recognized and detected immunoglobulin G (IgG). The application of magnetic materials to enrich SERS probes can provided the enhanced signal and the repeatability. The results showed that the prepared sandwich-like complex showed high sensitivity and excellent selectivity for IgG. The Raman peak intensity showed a good linear relationship in the concentration range of 1mg/mL ~ 1ng/mL. The linear equation was $y=657.93x+2963.9$, $R^2=0.996$. Parallel testing of this sandwich-like complex proved to have excellent repeatability. This method provided a new possibility for clinical non-immunity and quantitative detection of IgG.

Keywords: Surface-enhanced Raman scattering; Molecular Imprinting Technology; immunoglobulin G; Specific recognition
Introduction

Glycoproteins are ubiquitous in organisms and can be used as carriers of cellular information function. They can not only be used as markers for disease diagnosis, but also for monitoring and interventional therapy in the future. Immunoglobulin G (IgG) is an important component of human serum immunoglobulin and an important indicator of human immune function. It is also the main anti-infective immune antibody. Clinical trials have proved that, the level of IgG in human body can be used for early diagnosis of various diseases. Such as mycoplasma pneumonia, pulmonary tuberculosis, chronic urticaria, etc. Therefore, detect the content of IgG in human serum has important clinical significance. At present, the detection methods of glycoprotein include ChemiLuminescence method (CL), enzyme linked immunosorbent assay (ELISA), Fluorescence immunoassay (FIA), etc. Most of these methods have some shortcomings, such as low recovery, high cost and environmental sensitivity. Therefore, it is of great significance to develop a rapid, trace and highly specific non-immunized method.

Surface enhanced Raman scattering (SERS) is a detection technology with high sensitivity and simple operation, which can quickly detect the target, and has been widely used in the identification and detection of molecules and pathogens. At present, Magnetic probes are widely used in the field of surface enhanced Raman scattering. By introducing the magnetic field, not only the magnetic probe can be enriched and the sensitivity of the detection is greatly improved, but also the Raman peak of the magnetic probe can be made uniformly, thereby avoiding the situation that the Raman peak of the non-magnetic probe is high or low. Therefore, it can provide a basis for quantitative detection. Molecular imprinting technology (MIT) is a method that can remember the size, shape and functional groups of template molecules and form specific binding sites, and can perform specific recognition and selective adsorption of template molecules. Therefore, a method with high sensitivity and selectivity can be constructed by combining the two methods.
Based on magnetic probes can provide the enhanced signal and the reproducibility, this paper, based on Ye et al.\textsuperscript{12}, added magnetic probe for the first time to prepared a "molecular imprinted polymers (MIPs)-target molecule-magnetic SERS probes" sandwich-like complex, which was used for the detection of IgG in human serum. The polymerization of MIPs on four-hole array glass sheet can realize the simultaneous preparation of multiple sandwich complexes, which facilitates the control conditions and simplifies the test steps. Fig.1 showed the specific preparation. The test results showed that the MIPs prepared by this method had excellent selectivity, high sensitivity and wide biological applicability for target glycoproteins, and had good repeatability that can be used repeatedly. The method was simple, cost-effective and had high tolerance to different environments, so it was expected to be a new strategy for clinical non-immunized of glycoproteins.

**Experimental**

**Reagents and Chemicals**

Vinylphenylboronic acid (VPBA, AR, Jiuding chemical co. LTD, China), Ethyleneglycol dimethacrylate (EGDMA, AR, Aladdin reagent co. LTD, China), 2, 2′-Azobisisobutyronitrile (AIBN, AR, Aladdin reagent co. LTD, China), Butyl Alcohol (AR, Xilong chemical industry co. LTD, China), 1,4-Butylene glycol (AR, Xilong chemical industry co. LTD, China), 3-Hydroxytyramine hydrochloride (DA, AR, Xilong chemical industry co. LTD, China), Ammonium persulphate (APS, AR, Xilong chemical industry co. LTD, China), 4-mercaptophenyl-boronic acid (MPBA, AR, McLean co. LTD, China), glucose (AR, Xilong chemical industry co. LTD, China), immunoglobulin G (IgG, 95%), carcinoembryonic antigen (CEA, 90%), Bovine albumin (BSA, 90%), human Serum Albumin (HSA, 90%), Acetyl cholinesterase (AchE, 90%), lipoprotein lipase (LpL, 90%) are all from Nanjing Dulai biotechnology company.

**Preparation of magnetic probe**
Fe$_3$O$_4$ \(^{13}\): Added 1.35 g of FeCl$_3$ • 6H$_2$O, 1.0 g of PEG4000 and 2.7 g of sodium acetate to 40mL of ethylene glycol solution and stirred for 4h. Transferred the mixture to the 200°C autoclave for 8 hours. Then cooled to room temperature, washed successively with deionized water and ethanol for 2 to 3 times, and putted them in 100mL ethanol solution for use.

Fe$_3$O$_4$@SiO$_2$ \(^{14}\): Under constant stirring, dispersed the prepared Fe$_3$O$_4$ in the mixed solution of 60mL ethanol and 4mL deionized water. Then added 1mL of 25% ammonium hydroxide and 0.8mL of TEOS. After continuous stirring for 4h, washed them successively with deionized water and ethanol for 2 to 3 times, and putted them in 100mL ethanol solution for use.

Fe$_3$O$_4$@SiO$_2$@Ag \(^{15}\): Dispersed the prepared Fe$_3$O$_4$@SiO$_2$ and 10ml 3% SnCl$_2$ • 2H$_2$O in 10mL of deionized water. After ultrasound reaction for 10min, washed successively with deionized water and ethanol for 2 to 3 times, and dispersed in a certain amount of deionized water. Then added 10mL of Ag(NH$_3$)$_2$OH (25 mmol/L) to the solution. After ultrasound reaction for 30 min, the Fe$_3$O$_4$@SiO$_2$@Ag seeds was obtained by cleaning them with deionized water and ethanol for 2~3 times. Collected Fe$_3$O$_4$@SiO$_2$@Ag seed with magnet and dispersed in 200mL silver nitrate solution (20 mmol/L) containing 0.2 % PVP. Under ultrasonic conditions, added excess ammonium hydroxide (28 %) and formaldehyde (37 %) solutions at 25°C. After a few seconds of reaction, Fe$_3$O$_4$@SiO$_2$@Ag particles were obtained. Finally washed them with deionized water and ethanol for 2~3 times and store in ethanol for use.

Fe$_3$O$_4$@SiO$_2$@Ag- MPBA: Ultrasonically mixed 100μL of Fe$_3$O$_4$@SiO$_2$@Ag with 400μL (10$^{-3}$ mol/L) MPBA for 60 min. Then washed them with deionized water and ethanol for 2~3 times so that to remove the unconnected MPBA. Fe$_3$O$_4$@SiO$_2$@Ag-MPBA was obtained.

Preparation of MIPs
VPBA-CO-EGDMA\(^{16}\) skeleton: Mixed 2mg VPBA (functional monomer), 15μL EGDMA (cross-linking agent), 0.075 mg AIBN (initiator), 21μL Butyl Alcoho and 14μL 1,4-Butylene glycol (binary porogen) for use. Took 50μL of the above mixed solution onto each array point of
the 70 mm × 20 mm array glass slide and heated at 75 °C for 5~10 min. Washed 2 to 3 times with methanol and deionized water to remove excess pore-forming agent.

MIPs \(^{17}\): Added 40μL, 0.12 mg/mL of target glycoproteins (dissolved in PBS solution with pH=7.5) to the VPBA-CO-EGDMA skeleton. After 10 min reaction, washed the overloaded target glycoproteins with PBS solution. Then added 50μL of PBS solution (dissolved with 3 mg/mL DA and 10 mM APS) and react at 4 °C for 12 hours. Shock washing with 30% acetonitrile (containing 0.2 mol/L phosphoric acid) so that to remove the template molecule. The preparation of non-molecular imprinted polymer (NIPs) is the same as MIPs except that no template molecules added.

Preparation of standard solution and collection of Raman spectra

Prepared a 1 mg/mL IgG standard solution at room temperature, diluted to the desired concentration and stored at 4 °C until use. Took 40 μL the tested solution and dropped it on MIPs for 75 minutes. Washed 2-3 times with PBS buffer solution to removed unreacted IgG. Then added 50μL Fe\(_3\)O\(_4\)@SiO\(_2\)@Ag-MPBA and reacted 100min. After washing away excess Fe\(_3\)O\(_4\)@SiO\(_2\)@Ag-MPBA with PBS solution, we placed the glass piece above the magnet to completed the enrichment of the magnetic probe and then collected the Raman spectra.

Results and Discussion

Characterization of SERS probes and MIPs

We performed SEM and TEM characterization of the Fe\(_3\)O\(_4\) and Fe\(_3\)O\(_4\)@SiO\(_2\)@Ag particles, VPBA-CO-EGDMA framework, MIPs polymer and MIPs-Fe\(_3\)O\(_4\)@SiO\(_2\)@Ag-MPBA composites. The Fig. 2-a,b showed the SEM and TEM characterization results of Fe\(_3\)O\(_4\) particles. It can be seen that the prepared particles are spherical with smooth surfaces. The Fig. 2-c,d showed the SEM and TEM characterization results of Fe\(_3\)O\(_4\)@SiO\(_2\)@Ag particles. Compared with Fe\(_3\)O\(_4\), the surface was covered with a continuous layer of large particles, which proved that the Ag particles were successfully planted. It can be seen from Fig. 2-f that
VPBA-CO-EGDMA was a porous skeleton with good pores and can be used as a matrix material for MIPs. As shown in Fig. 2-g, the outer layer of the VPBA-CO-EGDMA skeleton was covered with a film. It indicated that the molecularly imprinted layer was successfully polymerized on the outer layer of the skeleton, and the MIPs prepared were also porous polymers, which contributed to the adsorption and elution of the target protein. Compared with Fig. 2-g, the surface of MIPs in Fig. 2-h was obviously connected with a large layer of particulate matter, indicating that the probe molecule Fe₃O₄@SiO₂@Ag-MPBA had been successfully connected to the surface of MIPs and can be used for SERS detection.

Research on SERS probe signal and performance

MPBA not only had strong SERS activity, but also can specifically recognized and bound glycoprotein\(^\text{16}\). So we chose MPBA as the signal molecule. Fig. 3-a showed the comparison diagram of SERS of MPBA reacted with Fe₃O₄@SiO₂@Ag before and after. It can be seen that MPBA was modified on nanoparticles. In order to investigated whether MIPs have good adsorption and SERS performance, we used Immunoglobulin G (IgG) as template molecule for Raman spectroscopy. Fig. 3-b showed the Raman spectrum of different concentrations of IgG standard solution. It can be seen that MIPs had a strong potentiating effect on different concentrations of IgG solution. Even at 1 ng/mL, two major Raman peaks at 1098 and 1594 cm\(^{-1}\) can be observed. Further studies found that with the change of IgG concentration gradient, the absorption peak intensity at 1098cm\(^{-1}\) showed a good linear relationship. The linear equation was \(y=657.93x + 2963.9\). \(R^2=0.996\). In addition, we examined the biological applicability of the prepared MIPs. As shown in Fig. 3-d, since CEA and BSA are cis-diol-containing macromolecule glycoproteins, so we used the MIPs preparation method mentioned above in this experiment. Added different template molecules (IgG, CEA, BSA) to prepared corresponding MIPs for Raman detection and different MIPs exhibited good Raman peaks. It was proved that the MIPs prepared by this method had wide biological applicability and can be used for the
detection of various glycoproteins.

To investigate the selectivity of MIPs, we used Acetyl cholinesterase (AchE), Carcinoembryonic antigen (CEA), Lipoprotein lipase (LpL), Bovine albumin (BSA), Human Serum Albumin (HSA) and glucose as distractors of MIPs and then performed Raman detection. As shown in Fig. 3-e, Compared with the blank sample, the Raman signal of the distractors yielded a bit higher, but the signal for the target glycoprotein was significantly higher. This result indicated that the MIPs had good selectivity and provided a good basis for selecting and identifying target samples. Fig. 3-f was a comparison of Raman signals that before and after the introduction of a magnetic field at the same concentration of IgG. The result showed that after the introduction of the magnetic field, the Raman signal can reach 1.5~2 times of that without magnetic field. The result was due to the introduction of the magnetic field can realized the enrichment of the probe and the enhancement of the “hot spot”. In addition, the test was also tested for repeatability, and the results were shown in Fig. 3-g, h. We collected the Raman signal after the MIPs adsorbed the target protein, and then eluted the target molecule. Repeated like this 15 times. The result showed that there was no significant change in the intensity of the collected Raman peaks. The RSD was 2.44%. Placed MIPs at 4°C for different times, then adsorbed the target molecule and performed SERS detection. The result showed that there was no significant change in the intensity of the collected Raman peaks. The RSD was 2.01%. They indicated that the prepared MIPs have good repeatability. After the prepared MIPs adsorbed the target glycoprotein and the molecular probe, we collected the SERS signals at different points (15 points) on the same polymer under the magnetic field. As shown in Fig. 3-i, there was no significant change in the Raman signal. The RSD was 2.09%. The result benefited from the synergistic effect of “hot spots” between particles, the intensity of Raman peaks of each “hot spot” was basically the same. So that this method can be used for the quantitative detection of IgG.
Sample analysis

In order to verify the utility of MIPs, we used MIPs for the detection of IgG in healthy humans and patients. Studies had shown that there was a relationship between the content of IgG in serum and the condition of patients with Hepatitis B virus\textsuperscript{18}. We diluted the serum samples of healthy people and patients with Hepatitis B virus by 1000 times, and used them as a blank sample for SERS detection. The experiment referred to the method mentioned in the "Preparation of standard solution and collection of Raman spectra" to performed Raman detection on a blank sample. The IgG concentration in the blank sample was calculated by bringing the peak intensity at 1098 cm\textsuperscript{-1} into the linear equation \(y=657.93x+2963.9\). Then added different concentrations of IgG to the blank sample, performed Raman detection and brought the results into the formula to get the recovery rate. Fig. 4 showed the serum test results of healthy humans and patients with Hepatitis B virus. The results showed that the concentration of IgG in healthy human serum was 6.24 mg/mL and the concentration of IgG in patients serum was 14.45 mg/ml. The data is close to the report of Tang et al\textsuperscript{18}. As shown in Table 1, the RSD distribution of the two serum samples was <1.27, and the recovery was between 88.7\% and 96.5\%. The above results were well proved that this method can be used for the detection of IgG content in actual samples, and had the advantages of low limit of detection and wide linearity and range.

Conclusions

This paper described a preparation method of a sandwich SERS sensor which can specifically recognize target glycoprotein, and was used for detection of immunoglobulin G in human serum samples. The results showed that the sensor exhibits excellent selectivity. The introduction of magnetic probes can enhanced the signal and reproducibility, thereby achieved the purpose of quantitative detection. In addition, specific recognition and SERS detection of various glycoproteins can be achieved by changing template molecules such as IgG, CEA, BSA, etc.,
which providing a new method for clinical non-immunological detection of human serum proteins.

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Table 1 Determination of glycoprotein in real samples (n=3)

| Sample                  | Measured C_1 (μg/ml) | Added C_3 (μg/ml) | Founded C_2 (μg/ml) | Recovery (%) P=(C_2-C_1)/C_3×100% | RSD (%) |
|-------------------------|----------------------|-------------------|---------------------|-----------------------------------|---------|
| IgG in samples of healthy people (diluted 1000-fold) | 6.24                 | 12.26             | 34.54               | 96.5                              | 1.27    |
|                         |                      | 31.2              |                     | 90.7                              | 1.09    |
|                         | 62.4                 | 61.75             | 89.0                |                                   | 1.07    |
| IgG in samples of patient (diluted 1000-fold) | 14.45                | 27.54             | 90.6                |                                   | 1.06    |
|                         | 72.25                | 82.80             | 94.6                |                                   | 1.08    |
|                         | 144.5                | 142.56            | 88.7                |                                   | 1.26    |

a. P——Spike-and-recovery experience
Fig. 1. Preparation and SERS detection of MIPs-IgG-Fe$_3$O$_4$@SiO$_2$@Ag-MPBA
Fig. 2. (a) Fe$_3$O$_4$ particle SEM diagram; (b) Fe$_3$O$_4$ particle TME diagram; (c) Fe$_3$O$_4$@SiO$_2$@Ag particle SEM diagram; (d) Fe$_3$O$_4$@SiO$_2$@Ag particle TME diagram; (e) Four-point array MIPs photo.

SEM diagram: (f) VPBA-CO-EDGMA skeleton; (g) MIPs polymer; (h) MIPs-IgG-Fe$_3$O$_4$@SiO$_2$@Ag-MPBA
Fig. 3. (a) comparison diagram of SERS with Fe$_3$O$_4$@SiO$_2$@Ag and Fe$_3$O$_4$@SiO$_2$@Ag-MPBA; (b) SERS diagram of different concentrations of IgG on MIPs; (c) different concentrations of IgG at 1098 cm$^{-1}$ Raman peak intensity standard curve (error bar: n=3); (d) SERS comparison of MIPs and NIPs prepared by different template molecules; (e) SERS diagrams of different template molecules adsorbed by MIPs and NIPs (error bar: n=3); (f) comparison diagram of SERS with magnetic field added and no magnetic field added. (g) SERS diagram of repeatability of MIPs; (h) SERS diagram of repeatability of MIPs placed at different times (i) SERS diagram of uniformity of MIPs;
Fig. 4. (a) Raman diagram for serum samples of healthy people spiked with different concentrations of IgG: a~d: blank serum; 6.24 μg/mL; 31.2 μg/mL; 62.4 μg/mL; (b) SERS signal intensity histogram of IgG at 1098 cm$^{-1}$ (error bar: n=3); (c) Raman diagram for serum samples of patients spiked with different concentrations of IgG: a~d: blank serum; 14.45 μg/mL; 72.25 μg/mL; 144.5 μg/mL; (d) and the SERS signal intensity histogram of IgG at 1098 cm$^{-1}$ (error bar: n=3).
Graphical Index

Fe$_2$O$_3@$SiO$_2@$Ag  MPBA  VPBA  Glycoprotein  Array MIPs  Magnet