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ORIGINAL PAPER

Graphene oxide wrapped with gold nanorods as a tag in a SERS based immunoassay for the hepatitis B surface antigen

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Abstract

A composite consisting of graphene oxide and gold nanorods (GO-GNRs) was designed for the trace determination of hepatitis B surface antigen (HBsAg) using surface enhanced Raman spectroscopy (SERS). GO contains numerous carboxy and hydroxy groups on its surface and therefore can serve as the substrate for decoration with GNRs and for immobilizing antibody against HBsAg. The GNRs (carrying the SERS probe 2-mercaptopyridine) exhibit high SERS activity, and this improves the sensitivity of the biosensor. The antibody on the GO-GNRs binds HBsAg with high specificity, and it results in excellent selectivity. The SERS signal (measured at 1002 cm^{-1}) increases in the 1–1000 $pg{\cdot}mL^{-1}$ HBsAg concentrations range, and the limit of detection is 0.05 $pg{\cdot}mL^{-1}$ (at an S/N ratio of 3). The immunoassay achieves the sensitive and selective determination of HBsAg in serum and expands the potential application of GO-GNR based SERS tag in clinical research.

Keywords Carbon materials · Gold nanomaterials · Hepatitis B surface antigen · Surface-enhanced Raman scattering tag · 2-Mercaptopyridine · Immunosensor

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Introduction

Hepatitis B is caused by hepatitis B virus (HBV), which is the main reason for liver cirrhosis and liver cancer [1]. Controlling the deterioration and spread of hepatitis B is the only effective therapy strategy by now, since no other remedy can extirpate the HBV inside the body. Thus, accurate monitoring of the main serological marker-hepatitis B surface antigen (HBsAg) of HBV inside the serum has become the key strategy to achieve early diagnosis and treatments. Traditionally, enzyme-linked immunosorbent assay (ELISA) is the main approach for the diagnosis of HBsAg in the last decades [1]. However, there are still some technical disadvantages such as tedious washing steps, long testing time, the high costs as well as indirect format of detection [2, 3]. Hence, the exploitation of an economic and convenient analytical technique/method for HBsAg detection with high sensitivity, specificity, rapid response will have significant clinic application values.

Raman spectroscopy is one of important spectroscopic techniques, which can be employed to provide the information on the chemical structures and physical forms based on the processes of infrared absorption and

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Raman scattering [4]. It can be widely used to determine quantitatively or semi-quantitatively the amount of a substance by the vibrational, rotational and other low-frequency modes of the molecules [5]. Surface-enhanced Raman scattering (SERS) is a surface-sensitive technique that can enhance the Raman scattering efficiency of molecules adsorbed on rough nanostructures (normally Ag or Au nanomaterials) [6]. It can improve the sensitivity of the analytical method for the order of 10^4~10^{11} magnitudes and exhibit the great potential for the single molecules determination [7–9]. Immunoassay is the direct or indirect detection of antigen based on the specific interaction between antibody and complementary antigen, which possesses extremely high specificity. It has become a powerful analytical tool for clinical diagnoses, biochemical analyses and environmental monitoring [10–13]. Typically, SERS-based immunoassay uses a standard protocol of sandwich structure formed by the supporting substrates, SERS tags and the targeted molecules [14–16]. Among them, SERS active tags made up with Raman molecules and nanomaterials play a key role in determining the Raman response strength. Although a variety of Raman-active tags have been widely explored such as Au nanoflowers, Ag nanoparticles, Au-Ag alloy nanoparticles and aggregates etc. [17–20], looking and designing the ideal SERS tags with easy preparation, high stability, and repeatability is still highly desirable. Moreover, it will be expected to achieve the trace determination of HBsAg by incorporating both highly sensitive SERS tags and specific immunoassay.

Graphene oxide (GO) has become the subject of multi multidisciplinary studies in recent years [21]. The surface of GO contains a large number of carboxyl and hydroxyl groups that can provide the reaction sites for the modification of antibody marker and other nanomaterials [22]. GO can also be served as the substrate for the survey of SERS activity [21]. Thus, in this work, we aim to prepare the GO-gold nanorods (GO-GNRs) composites by modifying the GNRs on its surface. Based on the SPR of GNRs, the GO-GNRs composites exhibit the strong SERS activity, which is an efficient SERS substance. HBsAg antibody is labelled on the surface of GO by the esterification reaction and 2-mercaptopyridine (2-Mpy) is decorated on the surface of the GNRs via the substitution reaction [6]. The GO-GNRs composites conjugated with antibody and 2-Mpy are added into solution and reacted with HBsAg, where a sandwich structure is formed and the SERS signal of 2-Mpy is determined simultaneously by Raman instrument. The intensity of the SERS signal is proportional to the concentrations of HBsAg in the range of 1–1000 pg•mL^{-1}. The SERS-active immunosensor implements the trace determination of HBsAg in serum and expands the development of HBV analysis technique.

Experimental section

Materials and instruments

HBsAg monoclonal antibody and HBsAg were purchased from Rongsheng biotech Co., Ltd. (Shanghai, China, http://www.rsbio.com/). Gold chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium borohydride ($NaBH_4$), hexadecyl trimethyl ammonium bromide (CTAB), sodium chloroacetate ($C_2H_2ClNaO_2$), p-aminobenzenesulfonamide ($C_6H_8N_2O_2S$), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-Hydroxysuccinimide (NHS), L-ascorbic acid, disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4) and bovine serum albumin (BSA) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China, http://www.sinoreagent.com/). Tween-20 and TritonX-100 were purchased from Sangon Biotech Co., Ltd. (Shanghai, China, http://www.sangon.com/). All other reagents were analytical grade and were used without further purification. Milli-Q ultrapure water (18.2 MΩ·cm) was used throughout all experiments.

Immuno™ MaxiSorp™ was purchased from Thermo Fisher Scientific (http://www.lirunchina.com/) and Microtiter plate was purchased from Roche. High-speed freezing centrifuge (5810R, Eppendorf corporation, Germany) and BP-211D electronic analytical balance (BT214D, Sartorius Instruments Co., Ltd., http://www.gl-tp.com/) were used throughout the experiment. The acidity of all system was adjusted by the Delta 320 pH meter (Shanghai Mettler-Toledo Instruments Co., Ltd., http://www.mtchina.com). The absorbance spectra of GO-GNRs were analyzed by UV-vis spectrophotometer with a 1 cm quartz cuvette (UV-3010, Japan Hitachi Chemical Co., Ltd). The morphology of the GO-GNRs was observed by transmission electron microscopy (TEM, JEM2010, Japan electron optics laboratory Co., Ltd) at 200 kV and field emission scanning electron microscope (FESEM, Zeiss). The optical density (OD) values were measured by microplate reader (1420, PerkinElmer corporation, http://www.perkinelmer.com.cn/) to calculate the bonding efficiency of HBsAg monoclonal antibody with GO. The SERS signal of GO-GNRs was collected by Renishaw 1000 Raman microprobe with 785 nm Ar^+ ion laser source. All the Raman spectra were calibrated by using 520 cm^{-1} band of Si wafer. The accumulation time and laser power were set as 20 s and 1.0 mW for each spectrum, respectively. Rayleigh scattering light was eliminated by holographic notch filter.

Synthesis of GNRs and GO

The synthesis methods of GNRs and GO were adopted from the previous work [22–24] (See supporting information for details).

Preparation of HBsAg antibody-labelled GO

Sulfonylation of GO: Solid NaOH (5 g) and sodium chloroacetate (5 g) were added into GO solution (100 mL, 0.5 mg•mL^{-1}), and reacted in ultrasound for 2 h under room temperature. The acidity of the GO was controlled by adding the HCl solution (0.1 M) for preparation of GO-COOH. The obtained GO-COOH was washed for 3 times using deionized water and then was dialyzed in dialysis membranes with the molecular weight cut-off (MWCO) of 20 kDa for 12 h. $NaNO_2$ (80 mg) and p-aminobenzenesulfonamide (200 mg) were dissolved into NaOH solution (20 mL, 6.25 M). The mixture of p-aminobenzenesulfonamide and $NaNO_2$ were added dropwise into the HCl solution (26 mL, 0.1 M) under an ice bath to prepare diazonium salt. This diazonium salt solution was added into the cooled GO-COOH and stirred for 2 h. Finally, the reaction was terminated and dialyzed for 12 h in deionized water. The sulfonated GO (GO-SO_3H) was obtained and stored at 4 °C for further use.

Label of GO-SO_3H The GO-SO_3H was labelled by reacting with the amino group of HBsAg monoclonal antibody. EDC (2 mg) and NHS (0.1 mg) were added into the 10 mL GO-SO_3H solution under ultrasound for 2 h. HBsAg antibody (0.5 mL, 1.0 mg•mL^{-1}) was added and placed into the incubator at room temperature overnight. The reaction solution was dialyzed for 12 h and the HBsAg antibody-labelled GO was obtained.

Decoration of HBsAg antibody-labelled GO by GNRs

Different concentrations of GNRs solution were mixed with 0.5 mg•mL^{-1} HBsAg antibody-labelled GO for 24 h, and then the mixture was dialyzed extensively overnight using Spectra/Por dialysis membranes (MWCO 20 kDa) of in 0.1 M phosphate buffer (pH 7.4). Finally, the GNRs decorated and HBsAg antibody-labelled GO were collected for further use.

In order to determine the HBsAg concentration in serum by SERS signal, the Raman molecule 2-Mpy was modified on the surface GNRs. The GNRs decorated and HBsAg antibody-labelled GO was mixed with 0.1 M 2-Mpy under ultrasound for 2 h. Then the mixture was centrifuged at 8000 rpm (rcf = 7155.2 (×g)) for 5 min and the precipitation was washed 3 times using 0.1 M phosphate buffer (pH 7.4). Finally, the precipitation was re-suspended in 0.1 M phosphate buffer (pH 7.4) and stored at 4 °C for further use.

Determination of HBsAg

Immunoassay protocol The HBsAg capture antibody was fixed on the 96 well microtiter plates. Different concentrations of 100 μL HBsAg capture antibody solution (in 0.5 M Na_2CO_3-$NaHCO_3$ buffer, pH 9.5) were pipetted respectively into microtiter plates and were incubated for 12 h under 4 °C. After that, the supernatants were taken out and the HBsAg capture antibodies were rinsed using 0.01 M phosphate buffer (pH 7.4)/0.05% Tween-20 buffer for 3 times. The HBsAg capture antibody was closed using 300 μL closed buffer (0.05 M phosphate buffer, pH 7.4, 0.05% Tween-20, 1% BSA, 0.01% thimerosal) under mixing at 37 °C for oscillation 1 h. Then the buffer was removed and HBsAg capture antibodies were washed with buffer (pH 7.4, 0.01 M phosphate buffer/0.05% Tween-20) for 3 times. The HBsAg capture antibodies closed on the microtiter plates were stored at 4 °C for use after they were dried under the evaporation.

HBsAg was linked with the HBsAg antibody by ELISA assay. Different concentrations of HBsAg solutions were dispersed in the buffer (0.05 M phosphate buffer, pH 7.0, 0.1% Tween-200.5% BSA, 0.01% thimerosal) and 100 μL of them were pipetted into the closed microtiter plates. Then 50 μL HBsAg antibody labelled and GNRs-2-Mpy decorated GO probe were added into the microtiter plates for the ELISA reaction. The reaction was implemented under 37 °C for oscillation 4 h. After the consummation of the ELISA reaction, the reaction solutions were taken out and the microtiter plates were rinsed with buffer (pH 7.4, 0.01 M phosphate buffer/0.05% Tween-20) for 6 times.

Sensitive determination of HBsAg To determine the HBsAg contents more sensitive, the SERS signals of samples were collected with 785 nm Ar^+ ion laser sources. The randomly selected position within the SERS-active substrate was scanned for five times and the obtained SERS signals were averaged by origin 8.0 for quantitative analysis.

Preparation of real samples

Every 5 mL of human blood (the blood samples were obtained from Shanghai Jiao Tong University School of Medicine) from 9 infected patients was collected in a sample tube for serum preparation. The fresh serum was separated from the red cells by putting the samples in an incubator at 37 °C for 30 min, and then, they were centrifuged at 1000 rpm (157 rcf) for 10 min. The serum samples were diluted with phosphate buffer (0.1 M, pH 7.4) for the immune-Raman-active experiments as abovementioned.

Table 1 The OD values of GO before and after labelled by HBsAg monoclonal antibody

$OD_{280(pre)}$	$OD_{280(post)}$	Binding efficiency
0.3553	0.0362	89.81%

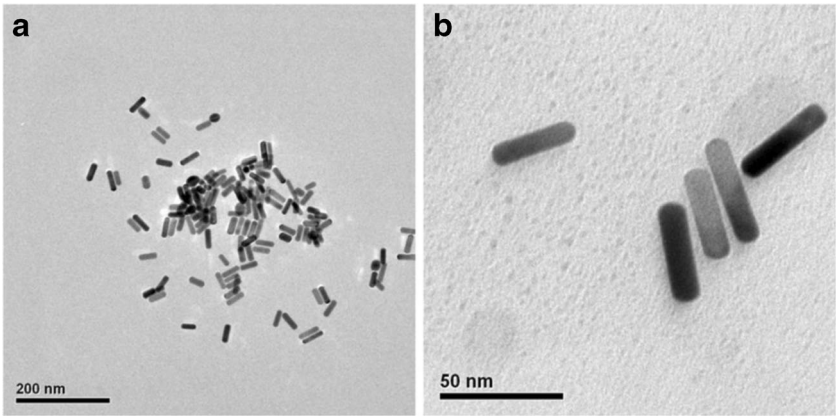

Fig. 1 TEM images of GNRs at different magnifications (**a**) the low magnification and (**b**) the high magnification

Results and discussion

The preparation of functional GO

In order to enhance the selectivity of HBsAg determination, the GO needs to be functioned by decorating HBsAg antibody on its surface. Firstly, GO was sulfonylated to obtain GO-SO_3H. Then, HBsAg monoclonal antibody was bound with the GO-SO_3H by the classic EDC-NHS reaction. The binding efficiency can be calculated according to the formula $\frac{OD(pre)-OD(post)}{OD(pre)} \times 100\%$, where.

OD(pre) and OD(post) are the optical densities of GO at 280 nm before and after binding with HBsAg monoclonal antibody, respectively. As shown in Table 1, the binding efficiency was calculated to be 89.81%, suggesting that HBsAg monoclonal antibody was successfully labelled on the surface of GO.

Preparation and characterization of GNRs and GO-GNRs

The GNRs were prepared using CTAB as coating agent by a classic seed-mediated growth method [25]. The CTAB can

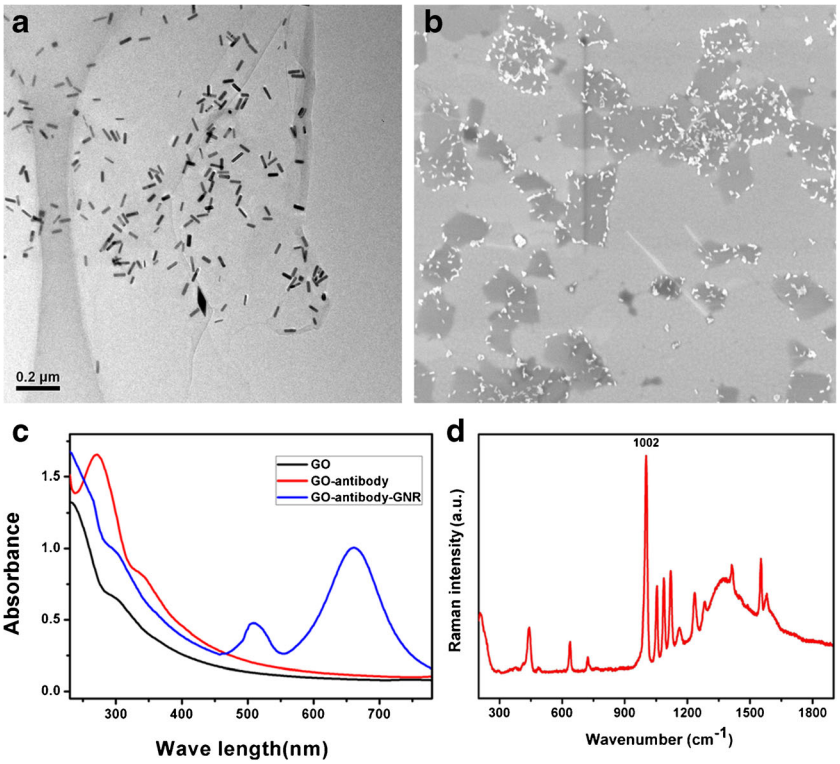

Fig. 2 (**a**) TEM and (**b**) SEM images of GO-GNRs composites, (**c**) UV-vis absorbance spectra of GO, GO-antibody and GO-antibody-GNRs, (**d**) SERS spectrum of 2-Mpy in GO-GNRs immunosensor

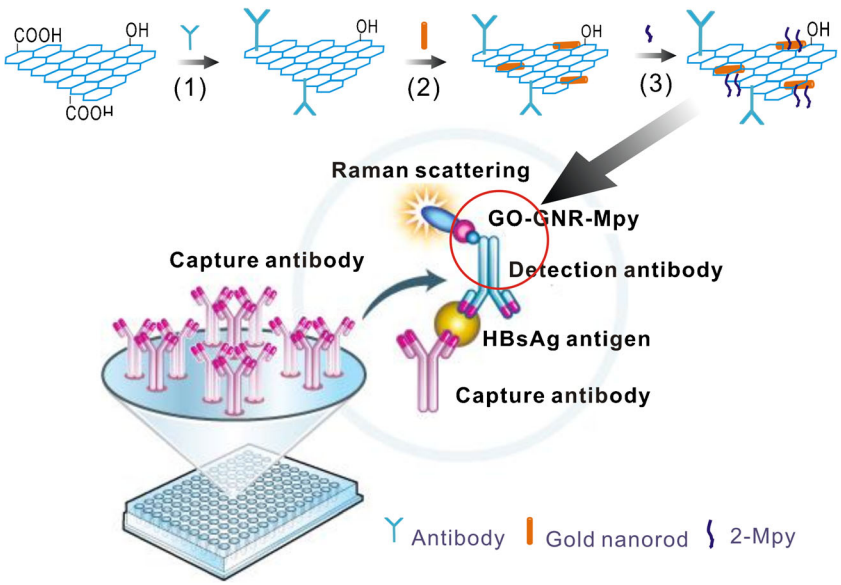

Fig. 3 Schematic illustration of Raman immunoassay based on GO-GNRs. (**1**) GO was functioned by anti-HBsAg antibody; (**2**) GNRs was decorated on the surface of GO to form GO-GNRs composites; (**3**) 2-Mpy was bound to the GNRs surface by forming the S-Au bond

form a soft template and its shape depends on the surfactant concentrations and the ionic strength of the solution. With the introduction of the seed solution into growth solution, the seed becomes a part of the soft template and the growth starts by reducing the gold atoms into the template. In the presence of Ag ions, the aspect ratio of GNRs can be controlled by slowing the growth of GNRs width. As shown in Fig. 1, the GNRs exhibit fairly uniformity and good monodispersity, the average length is about 30 nm (statistic analysis of 100 nanoparticles) and the aspect ratio of 4.0. The uniform distribution of GNRs plays a key role for the SERS signal collection of 2-Mpy.

The GNRs was positively charged due to the coating action of CTAB molecules. Thus, they can bind easily with GO via electrostatic interaction, since the surface of GO is negatively charged, which stems from the abundant carboxyl and hydroxyl groups on its surface [22].

Although a lot of carboxyl groups of GO have been reacted with HBsAg antibody, residual carboxyl and hydroxyl groups still can be served as the attaching points. As shown in Fig. 2a, with the decoration of GO surface by GNRs, the wrinkled yarn-like GO-GNRs composites are formed. According to the SEM image of GO-GNRs composites, most of GNRs are evenly distributed on the edge of the GO (Fig. 2b). The reason is that negatively charged carboxyl and hydroxyl groups is distributed mainly on the edge of the GO, which is beneficial for the electrostatic interaction between GNRs and GO. Due to the aggregation effect of GNRs, the edge of the GO is rolled up and thus the wrinkled yarn-like GO-GNRs composites are observed. As shown in Fig. 2c, GO only has a weak shoulder peak at 300 nm. With the addition of the functional HBsAg antibody, there is obvious absorbance appearance at 280 nm, suggesting the successful decoration of HBsAg antibody on the surface of GO. The UV-vis absorbance spectrum of GO-GNRs exhibit two peaks at 505 nm and 660 nm, which correspond to surface plasmon resonance peaks of the horizontally and vertically oriented GNRs, implying the successful modification of GNRs on the surface of GO.

It has been reported that GNRs exhibit excellent SERS activity due to their obvious surface plasmon resonance [24]. Based on the excitation of localized surface plasmons, the electromagnetic fields between closely packed GNRs are coupled and enhanced, which is the formation of "hot spots". When the Raman molecules are situated at the "hot spots", its Raman signal is magnified. In addition, Raman molecules can bind with GNRs to form charge- transfer complexes, which are responsible for the SERS. In order to design the SERS active probe for the determination of HBsAg, Raman molecule 2-Mpy is

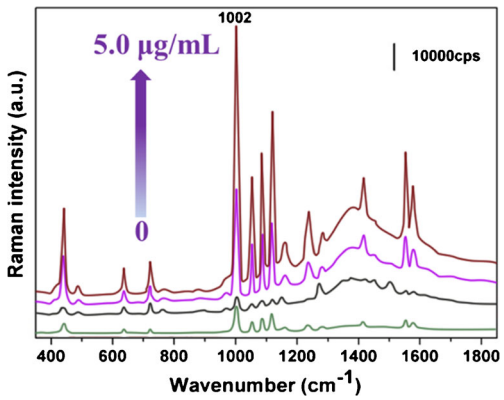

Fig. 4 SERS assays of different concentrations of HBsAg based on the GO-GNR Raman immunosensor (the concentrations of HBsAg are 0.01 pg·mL^{-1} (green), 1.0 pg·mL^{-1} (black), 10 ng·mL^{-1} (purple) and 5.0 μg·mL^{-1} (red)

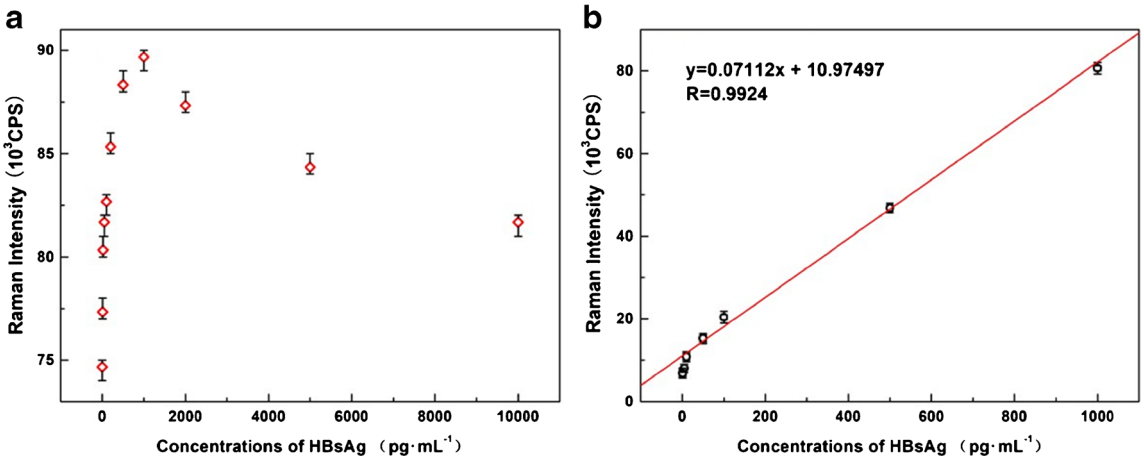

Fig. 5 **a** The relationship between SERS intensity and different concentrations of HBsAg based on the GO-GNR Raman tags (the concentrations of HBsAg were 0, 1, 10, 50, 100, 200, 500, 1000, 2000, 5000 and 10,000 pg·mL^{-1}); **b** the linear relationship of SERS intensity with HBsAg concentrations in the range of 1–1000 pg·mL^{-1}. The data were collected by averaging 5 measurements

modified on the surface of GNRs via the -SH of 2-Mpy interacting strongly with Au to form a Au-S bond. The modified GO-GNRs can greatly amplify the Raman signal and thus the highly sensitive and selective tag is constructed for the determination of HBsAg.

Before doing the ELIAS assay between HBsAg and HBsAg antibody on the surface of GO-GNRs, the effectiveness of this assay was investigated. 2-Mpy has an obvious characteristic Raman peak at ~1000 cm^{-1}. When 2-Mpy was modified on the surface of GO-GNRs, its Raman signal was largely amplified, which is also the SERS signal. As shown in Fig. 2d, at fingerprint area, SERS signal of 2-Mpy can be observed obviously and a very strong characteristic peak appeared at 1002 cm^{-1}, suggesting the effectiveness of our method. However, the characteristic SERS signals of D and G peaks from GO disappeared, which can be contributed to the masking effect of strong SERS signal from 2-Mpy. Since only the characteristic SERS signal of 2-Mpy at 1002 cm^{-1} is enough used for the determination of target substance, thus the disappearance of D and G peaks of GO will not restrict the implement of experiment. Thus, this method can be effectively used for the ELISA assay.

Determination of HBsAg

The HBsAg is the most important diagnostic marker for HBV infection. It is a secreted protein that can enter into the blood stream by viruses residing in liver cells. Thus, sensitive and selective determination of trace HBsAg in serum is a significant step for the early diagnosis of HBV [26]. SERS can amplify the Raman signal at 10^{11}–10^{12} level [27] and immunoassay can accurately determine the antigen marker [28–30], thus the establishment of SERS immunoassay will implement the high sensitive and selective determination of the trace HBsAg in serum. As shown in Fig. 3, the successful decoration of HBsAg antibody, GNRs and 2-Mpy on the surface of GO laid the foundation for the determination of HBsAg. The HBsAg in serum can be captured by the capture HBsAg antibody fixed on the 96 well microtiter plates. As the Raman active tag is introduced into the microtiter plates, immunoreaction can be induced between the HBsAg and HBsAg antibody in GO-GNRs. The unreacted antigen will be rinsed by 0.1 M phosphate buffer, thus the concentration of HBsAg depended on the SERS signal of 2-Mpy in the immunosensor. Figure 4 shows the SERS intensities of 2-Mpy at 4 different concentrations of HBsAg. With the increasing of HBsAg

Table 2 Comparison of sensing properties of the GO-GORs-based Raman-Immuno HBsAg sensor with other nanomaterials-based methods

Nanomaterials used	Methods applied	LOD	Ref.
Gold nanoflower	SERS Sensor	0.01 IU•mL^{-1}	15
Zeolietes and MWCNT Nanocomposites	Impedimetric genosensor	50 copies•mL^{-1}	29
Gold nanoparticles/DNA	SERS Sensor	0.44 fM	16
Gold nanorods	Surface Plasmon Resonance Sensor	0.01 IU•mL^{-1}	24
Gold nanoparticles	Electrochemical immunosensor	0.1 ng mL^{-1}	33
Graphen quantom dots	Electrochemical sensor	1 nM	3
Graphene Oxide and Gold nanorods Composites	Immuno-Raman Sensor	0.05 pg•mL^{-1}	This work

Table 3 Analytical results of HBsAg in human serum by GO-GNRs based Raman sensing

Serum	Found ($pg•mL^{-1}$)	RSD (%)	Added ($pg•mL^{-1}$)	Obtained ($pg•mL^{-1}$)	Recovery ($pg•mL^{-1}$)	Recovery (%)
A	33.7	0.5	50.0	82.0	49.0	98.0
B	42.3	1.6	50.0	90.9	48.6	97.2
C	55.8	3.2	50.0	107.8	52.0	104.0
D	70.9	4.3	50.0	118.3	47.4	96.0
E	72.5	2.8	50.0	124.9	52.4	104.8
F	110.5	2.7	50.0	158.8	48.3	96.6
G	128.3	3.6	50.0	179.9	51.6	103.3
H	192.7	3.4	50.0	244.9	52.2	104.4
I	205.8	0.9	50.0	253.9	48.1	96.2

concentration, the concentration of 2-Mpy will be increased, thus the SERS signal will be also increased. More information can be found in supporting information (Fig. S1).

In order to further investigate the relationship between HBsAg concentrations and SERS intensities, their scatter plots were drawn. As shown in Fig. 5a, the SERS intensity increases with the increasing of HBsAg concentrations and reaches at the maximum value at 1000 $pg•mL^{-1}$ HBsAg. Then the SERS intensity decreases gradually with the increasing of HBsAg concentrations. This is the classic "Hook Effect" or "Prozone Effect" that low response of the assay corresponds to the increasing analyte concentrations in immunometric assays, leading to a false negative error [31]. This contributes to the missed or misdiagnosis results in clinical validation. To improve the accuracy of GO-GNRs Raman tags for HBsAg determination, the optimal application range is investigated. In the range of 1–1000 $pg·mL^{-1}$, the SERS intensity increases proportionally with the increasing of HBsAg concentration (Fig. 5b). The calibration plot exhibited a good linear relationship between the SERS intensity at 1002 cm^{-1} and the HBsAg concentration. The corresponding regression equation is $y = 10.97497 + 0.07112\times$, with the correlation coefficient (r) of 0.9924 and the LOD is calculated to be 0.05 $pg•mL^{-1}$, which is much lower than the standard data (0.1 $ng•mL^{-1}$) obtained by ELISA in the hospital. In addition, the performance of our Immuno-Raman assay was also compared with other HBsAg sensors and the data were summarized in Table 2. The comparison results of LOD values show that the GO-GNRs immune-Raman method has a more satisfying performance than other HBsAg sensors (Table 2). Furthermore, the GO-GNRs based Immuno-Raman method is considered as one of potential clinical methods for the sensitive and selective determination of HBsAg in serum.

Analysis of real samples

In order to investigate the applicability of GO-GNRs Raman tags, the determination of HBsAg in serum from 9 patients were implemented. The median age was 35 (20–76) years old, 4 of female and 5 of male. 1.00 mL of serum from patient was diluted by 0.1 M phosphate buffer to the $pg·mL^{-1}$ level of HBsAg concentrations. According to the experimental method, the contents of HBsAg in patient serums were assayed and different dosages of antigen was added into the sample matrices to determine the recovery. The RSD was calculated to evaluate the precision and spike recovery test was carried out to examine the reliability of this method. In Table 3, the RSD was less than ±5% and the recovery was 96–104%, suggesting this Raman-Immunoassay possesses high accuracy for the HBsAg detection. Thus, GO-GNRs Raman-Immunoassays can be applied to the determination of HBsAg in serum and this assay affords us a potential clinical diagnosis technical for HBV infection candidate.

However, the technique still need be improved in the future research. On one hand, the amount of GNRs loaded on the surface of GO and 2-Mpy on GNRs should be accurately controlled in order to enhance the sensitivity of HBsAg detection; on the other hand, the immune-substrates can also be replaced by magnetic nanoparticles, which is benefit for the auto operation [32]. In addtion, the GO-GNRs based nanocompostes can also be used as an efficient sensing interface for electrochemical immunoassays [33].

Conclusions

In summary, we developed a GO-GNRs Raman-active tag for the determination of trace HBsAg. The HBsAg monoclonal antibody was labelled on the surface of GO to improve the accuracy and selectivity of this method via immunoreaction between HBsAg and antibody. The decoration of GNRs with surface plasmon resonance on the surface of GO endows this sensor with the strong SERS activity, amplifying the detection sensitivity of HBsAg. The SERS signal of 2-Mpy increases proportionally with the HBsAg antigen concentration in the range from 1 $pg·mL^{-1}$ to 1000 $pg·mL^{-1}$, and the LOD is 0.05 $pg·mL^{-1}$, which is lower than current ELISA method. It reveals that our GO-GNRs Raman active tag has great value and clinic potentials in determining HBsAg and other biological markers associated with diseases.

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Compliance with ethical standards The author(s) declare that they have no competing interests.

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