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Dual-mode SERS-Fluorescence Immunoassay using Graphene Quantum Dot Labeling on 1D-aligned Magnetoplasmonic Nanoparticles

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ABSTRACT: A novel dual-mode immunoassay based on surface-enhanced Raman scattering (SERS) and fluorescence was designed using graphene quantum dot (GQD) labels to detect a tuberculosis (TB) antigen, CFP-10, via a newly developed sensing platform of linearly aligned magnetoplasmonic (MagPlas) nanoparticles (NPs). The GQDs were excellent bi-labeling materials for simultaneous Raman scattering and photoluminescence (PL). The one-dimensional (1D) alignment of MagPlas NPs simplified the immunoassay process and enabled fast, enhanced signal transduction. With a sandwich-type immunoassay using dual-mode nanoprobe, both SERS signals and fluorescence images were recognized in a highly sensitive and selective manner with a detection limit of 0.0511 pg mL⁻¹.

Keywords: Graphene quantum dots; Dual-mode immunoassay; Surface-enhanced Raman scattering; Fluorescence; Biosensor; Tuberculosis

1. Introduction

Tuberculosis (TB) has a long history being a deadly disease, while now it is still one of the world's most fatal infectious disease in spite of the live attenuated vaccine and several antibiotics.¹ Early infection diagnosis is vital to prevent the spread of disease, and its improvement in detection is urgently required.² Immunoassay is an analytical method of the specific interaction between an antigen and a complementary antibody, and is widely used in biochemical analyses, clinical diagnoses, and environmental monitoring.³ The sandwich-type of immunoassay is popular since it is based on nanomaterials substrates.⁴⁻⁷ Recently, multiplex immunoassay using nanomaterials have attracted significant interest.⁸⁻¹² Especially, SERS-fluorescence dual mode nanoprobe were expected to improve the imaging quality and detection sensitivity of the fluorescence signal, quick indicator; meanwhile SERS could help to distinguish specific targets in multiplex interactions.^{13,14} Utilizing SERS-fluorescence dual mode nanoprobe can be an extremely sensitive analytic tool in a variety of biomedical applications.

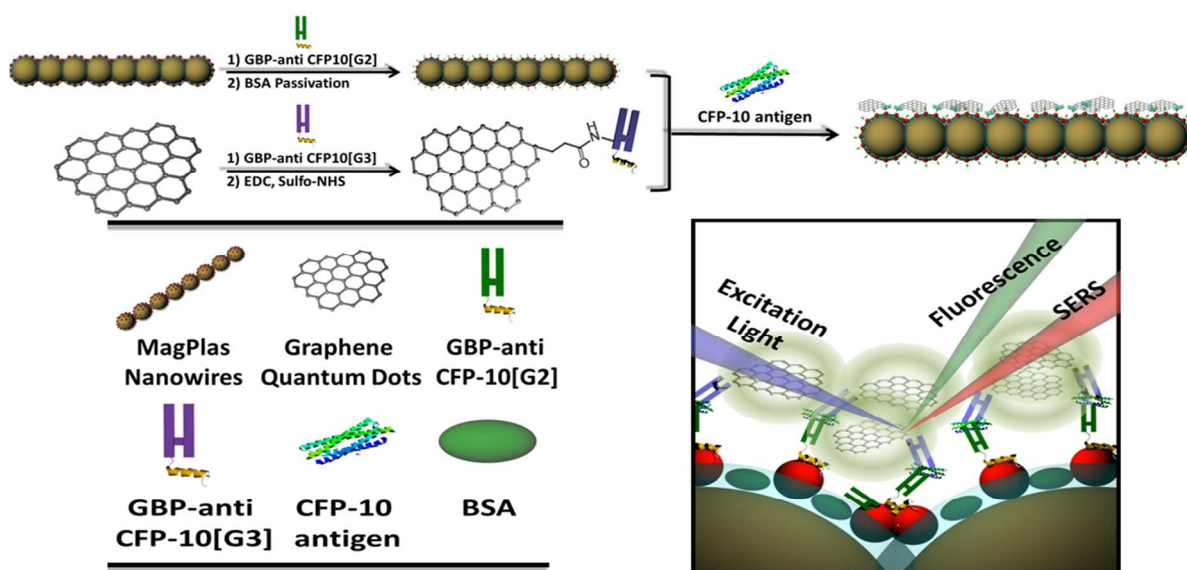
Graphene quantum dots (GQDs) are a new type of quantum dot converted from 2-dimensional graphene sheets; they are increasingly drawing the world's attention thanks to their photoluminescence (PL) and electronic properties.¹⁵⁻¹⁹ Moreover, their strong quantum confinement and edge effects are advantages for electronic and optoelectronic applications.^{20,21} In particular, Raman spectra of graphene have been well studied in visible wavelengths. For example, the peak near $1,580\text{ cm}^{-1}$, i. e. G band, is involved into an in-plane asymmetric translational motion of two adjacent carbon atoms (E_{2g} mode). The peak near $1,300\text{--}1,400\text{ cm}^{-1}$, or the D band, is associated to the in-plane carbon ring breathing

mode (A_{1g} mode). The excitation wavelength decides the peak position of the D band which is the consequence of a double resonance process at the K point of the reciprocal lattice space.²²⁻²⁸ The number of peak waves could be easily affected by the circumstances or the number of layers, while the peak intensity is in proportion to the GQD concentration. In addition to the stable PL and Raman scattering, GQDs possess low cytotoxicity and high biocompatibility, both of which have been bottlenecks for biomedical applications of semiconductor QDs.²⁹ They can also overcome the issue of extreme aggregation that occurs in graphene sheets.²⁷ Therefore, GQDs can benefit the future well-functional biosensor devices.

One dimensional (1D) alignments of nanoparticles (NPs) have been of interest for potential applications in electronic devices and sensing platforms. For example, in 1D-nanostructured Fe_3O_4 systems, the interplay between magnetic anisotropy and interparticle dipole–dipole interactions is the main factor determining the magnetic properties of the systems. Recently, magnetoplasmonic ($Fe_3O_4@Au$) core–shell NPs have been synthesized as novel hybrid nanocomposite materials, which integrate Au nanostructures with superparamagnetic Fe_3O_4 NPs to combine the plasmonic and magnetic properties in a confined cluster.³⁰ The Au coating on a magnetic core is expected to retain the chemical stability and biocompatibility of Au as well as the magnetic properties of the Fe_3O_4 core. Under the external static magnetic field, the $Fe_3O_4@Au$ NPs could be self-assembled to 1D magnetoplasmonic nanochains. The successful 1D assembled nanochain is very crucial since irregular aggregated nanoparticles could hinder specific properties of assembled nanostructures, including plasmonic coupling effects, optical bandgaps, and metamaterials effects. The novel metallic surface has long been considered as one of critical parameters for optimizing the metal-enhanced fluorescence (MEFs) and surface-enhanced Raman

scattering (SERS). The sensitivity of MEFs and SERS are strongly dependent on the gold nanomaterials of 1D magnetoplasmonic nanochains. Therefore, 1D aligned magnetoplasmonic (MagPlas) nanowires (NWs) can be used as a new sensing material for the development of sensor platforms through MEFs and SERS technologies. Sensors made of NWs exploit some fundamental nanoscopic effects and avoid signal cross-talking for high sensitivity and selectivity.³¹

In this study, we demonstrated a proof-of-concept experiment using the $\text{Fe}_3\text{O}_4\text{-Au}$ -GQDs nanocomplex to generate a novel surface-enhanced Raman scattering (SERS)-sensor and fluorescence imaging platform for protein detection. It involves dual enhancement of Raman and fluorescence signals from GQDs by localized surface plasmon resonance (LSPR) of MagPlas NWs to achieve high sensitivity. The CFP-10 antigen was monitored using two different antibodies, referred to as G2 and G3, as follows: gold binding protein (GBP)-G3 functionalized GQDs were bound to a complementary target (CFP-10) with G2-functionalized MagPlas NWs.



Scheme 1. Schematic representation of the synthesis of CFP-10 antibodies (G2 or G3)-functionalized $\text{Fe}_3\text{O}_4\text{@PEI@Au}$ NW (aka, MagPlas NW) and GQD nanocomposites; (right

box) the detection mechanism of the SERS-induced immunoassay to monitor the target CFP-10 antigen.

2. Materials and Methods

2.1 Materials.

Polyethyleneimine (PEI, branched, $MW \approx 10,000 \text{ g mol}^{-1}$), iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), hydrogen tetrachloroaurate (III) trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 99.9%), *N*-ethyl-*N*-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), bovine serum albumin (BSA), phosphate-buffered saline (0.01 M PBS buffer solution, pH 7.4), sodium acetate anhydrous (NaAc), sodium borohydride (NaBH_4), trisodium citrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 3\text{H}_2\text{O}$), sodium chloride (NaCl), potassium chloride (KCl), potassium phosphate monobasic (KH_2PO_4), sodium phosphate dibasic dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$), polyoxyethylene (20) sorbitan monolaurate (Tween-20), tris(hydroxymethyl) aminomethane (Tris), hydrogen chloride (HCl), sulfuric acid (H_2SO_4), nitric acid (HNO_3), sodium hydroxide (NaOH), and sodium carbonate (Na_2CO_3) were all obtained from Sigma-Aldrich (St. Louis, MO, USA). Pitch carbon fiber (5–10 μm in diameter) was purchased from Fiber Glast Development Corporation (Brookville, OH, USA). All chemicals were of analytical grade and were used as received with no further purification. Deionized water (Milli-Q grade, Millipore, Billerica, MA, USA) with a resistivity of $18.2 \text{ M}\Omega \text{ cm}^{-1}$ was used in all experiments. All experiments were performed under ambient temperature.

2.2 CFP-10 antigen and antibodies

The recombinant CFP-10 protein and two different monoclonal antibodies against CFP-10 (G2 and G3) were prepared following previously reported methods.⁷ The recombinant

CFP-10 protein was expressed in *Escherichia coli* (*E. coli*) and purified according to previous methods.³² As discussed before, Anti-CFP-10 monoclonal antibodies were selected from a pre-established scFv (single-chain variable fragment)-phage library^{33,34} First, the selected scFv was expressed in Chinese hamster ovary cells. Secondly, monoclonal immunoglobulin G was purified by a protein column.^{33,34} The preparation of GBP fusion CFP-10 (G2 and G3) has been described in detail elsewhere.^{35,36} CFP-10 (G2 and G3) were used for the sandwich-type immunoassay.

2.3 Synthesis of GQD and MagPlas NP

The procedure for **MagPlas** NP synthesis was as follows: $\text{Fe}_3\text{O}_4@\text{PEI}$ was prepared via the one-pot solvothermal method.³⁷ First, 0.68 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was dissolved in 20 mL of ethylene glycol by ultrasonic treatment. Then add 1.8 g of NaAc and 0.5 g of PEI into this transparent solution. After 20 min of strong stirring under 60 °C, the mixture was transferred into a 220 °C autoclave for 2 h. Rinse the resultant black products with water and ethanol for several times under exterior magnet and dry them in vacuum. The final products were ground to break aggregates by agate mortar. Then, Au coating was applied to the $\text{Fe}_3\text{O}_4@\text{PEI}$ surface to produce MagPlas NPs following the strategy developed by Wang et al.³⁷ $\text{Fe}_3\text{O}_4@\text{PEI}$ NPs were used as templates for Au coating by electrostatic forces. This strategy produces $\text{Fe}_3\text{O}_4@\text{PEI}@\text{Au}$ NPs (MagPlas NPs) with a uniform size distribution. Au NPs with a diameter of 4 nm were synthesized according to the method reported by Murphy et al.³⁸ Then, 20 mg of the well-dried $\text{Fe}_3\text{O}_4@\text{PEI}$ NPs was sonicated in the Au NP solution for 10 min and then mechanically stirred at room temperature for 2 h. The $\text{Fe}_3\text{O}_4@\text{PEI}-\text{Au}$ NPs were then magnetically separated from the mixture and washed for several times with water and ethanol, in turn.

GQDs were synthesized through chemical oxidation of micrometer-sized pitch-based carbon fibers (Fiber Glast Development Corporation) according to the method of Ajayan et al.³⁹ Further details regarding the procedure can be found in the Supporting Information.

2.4 Preparation of the sensing platform

The protocol for the aligned MagPlas NW immuno-assay is illustrated in Scheme 1. First, 7 μL of the MagPlas NPs were dropped onto micro slide glasses and an external magnetic field was engaged (1.84×10^4 Gauss, 3/4 in. $\times$ 1/10 in., disc-shaped NbFeB magnet, K&J Magnetics; Jamison, PA, USA). After 10 min in the 55 $^{\circ}\text{C}$ oven, the glass was dry. The aligned platform was investigated using optical and electron microscopy.

2.5 Preparation of the dual-mode nanoprobe

5 mL of GQDs (1 mg mL^{-1}) was first immobilized with 100 μL of G3 antibody ($32 \mu\text{g mL}^{-1}$) via the EDC/NHS bioconjugation method. Meanwhile, 10 μL of gold binding protein (GBP)-G2 antibody (0.255 mg mL^{-1}) was to bind to the aligned platform of $\text{Fe}_3\text{O}_4@\text{PEI-Au}$ NWs in 1 h under room temperature. Then, the free G2 antibody was washed twice using PBS buffer. To avoid the attachment of negatively charged GQDs to the surface of positively charged $\text{PEI}@\text{Fe}_3\text{O}_4$ NPs, 1% citrate was used to neutralize the $\text{PEI}@\text{Fe}_3\text{O}_4$ NP surface charge. Then, 20 μL of PBS buffer containing 1% BSA was used to block the nonspecific binding sites for 1 h at room temperature. After that, 10 μL of CFP-10 antigen at various concentrations ($1 \mu\text{g mL}^{-1}$ to 1 pg mL^{-1}) was added to bind with G2-MagPlas NWs for 1 h, eventually providing anchor spots for G3-GQDs. Since many immuno-conjugated structures of GQDs-G3-CFP10-G2-NWs are closely packed in the sandwich structure, many hot spots can be generated and a strong SERS-fluorescence can be

obtained.

2.6 Raman immunosensing and fluorescence imaging

Using 532 nm excitation (2.36 mW at the sample position), SERS spectra were recorded by Raman spectroscopy (Ramboss 500i, Dongwoo, Korea). The Rayleigh scattering light was screened by a holographic notch filter while the Raman scattering light was directly added to a charge-coupled Andor shamrock spectrograph. The laser spot diameter was 1 μm . The acquisition time was 10 s with 25 repetitions per spectrum; therefore, for each measurement, five spectra were observed for each area, on average. Fluorescence images were recorded using a fluorescence optical microscope (DM 2000, Leica, Heerbrugg, Switzerland) with a 10 $\times$ microscope objective and under the excitation wavelength of 488 nm.

3. Results and Discussion

3.1 Characterization of GQDs and MagPlas NPs

Graphene quantum dots are characterized with optical and electronic properties which shaped as edge-bound nanometer-scaled graphene debris. In the previous reports, many researchers have studied the excitation-dependent emission behavior of GQDs.^{40,41} Excitation-dependent PL behaviors can be widely observed in fluorescent carbon materials. And the excitation wavelength decides the PL color of GQDs. The initial GQDs exhibit a strong peak at 436 nm (blue emission) and a shoulder peak at 516 nm (green emission) while excited by 320 nm wavelength. According to previous reports, the blue emission is attributed to electron-hole recombination or quantum size effect/zig-zig effect (intrinsic state emission), while the green one is surface defects (defect state emission). Figure 1A shows the UV-vis absorption and PL spectra of GQDs. For the UV-vis spectrum of the GQDs in

aqueous solution, the typical absorption peak at 290 nm could be the result of the π - π^* transition of graphite sp^2 domains. The PL spectra of water-soluble GQDs were further studied under a 290 nm excitation wavelength. As shown in Figure 1A, the PL spectrum of GQDs showed the strongest peak at 520 nm. We can observe clear yellow light when illuminated the GQD solution with 365 nm UV light (shown in the inset of Fig. 1A). The luminescence decay profile of the GQDs was obtained by time-correlated single photon counting and record the GQD transition at 520 nm emissions and excited at 380 nm (Fig. 1B). The observed luminescence lifetime (τ_1) was 0.65 ns with a correction of 0.956. As shown in the FT-IR spectrum of the GQDs (Fig. 1C), a strong absorption peak is centered at $1,612\text{ cm}^{-1}$ because of the C=O stretching. The O-H bonding could be inferred from the broad peak centered at $3,405\text{ cm}^{-1}$, the C-O bonding from the absorption peaks at $1,184\text{ cm}^{-1}$ and C=C stretching from absorption peaks at $1,422\text{ cm}^{-1}$. The Raman spectrum (Fig. 1D) can strongly prove the fragmented graphene structure of GQDs. The peaks centered at $\sim 1,579\text{ cm}^{-1}$ (G band), caused by the vibration of the sp^2 -bonded C atoms in a 2D hexagonal lattice; while the peak at $1,358\text{ cm}^{-1}$ (D band), related with scattering of disorder structure at the edges. The relative intensity of the G/D bands was ~ 0.91 at high concentration of GQDs, which is consistent with a previous report.³⁹

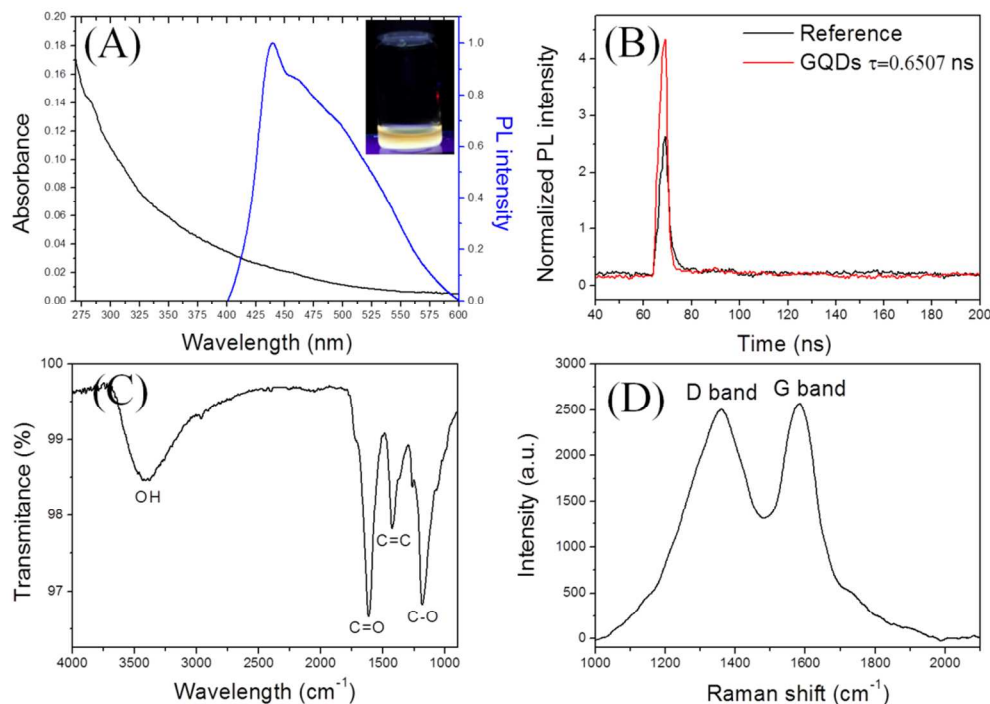


Figure 1. (A) UV & PL spectra, (B) PL lifetime, (C) FT-IR, (D) Raman spectrum of GQDs

Figure 2A and B show the transmittance electron microscopy (TEM) and scanning TEM (STEM) images of the prepared $\text{Fe}_3\text{O}_4@\text{PEI}$ NPs. The images show that $\text{Fe}_3\text{O}_4@\text{PEI}$ NPs were monodispersed with spherical shapes and an average diameter of 125 ± 11 nm. Au NPs for deposition were 4.17 ± 0.26 nm, as shown in Fig. S1. The zeta potentials of $\text{Fe}_3\text{O}_4@\text{PEI}$ NPs and Au NPs were +33 mV and -22.4 mV, respectively (Fig. S2). Therefore, the surface of $\text{Fe}_3\text{O}_4@\text{PEI}$ NPs can be coated with Au NPs via electrostatic interactions to produce MagPlas NPs. The TEM and STEM images of the MagPlas NPs are shown in Figure 2 C and D, and Au NPs were well dispersed on the $\text{Fe}_3\text{O}_4@\text{PEI}$ surface. The density of the coating layer was controlled to induce optimum magnetic interactions between magnetic cores and the external magnetic field, as well as to avoid uncontrollable agglomeration during electrostatic interactions. The assembly mechanism of MPNCs was described in our previous report.⁴²

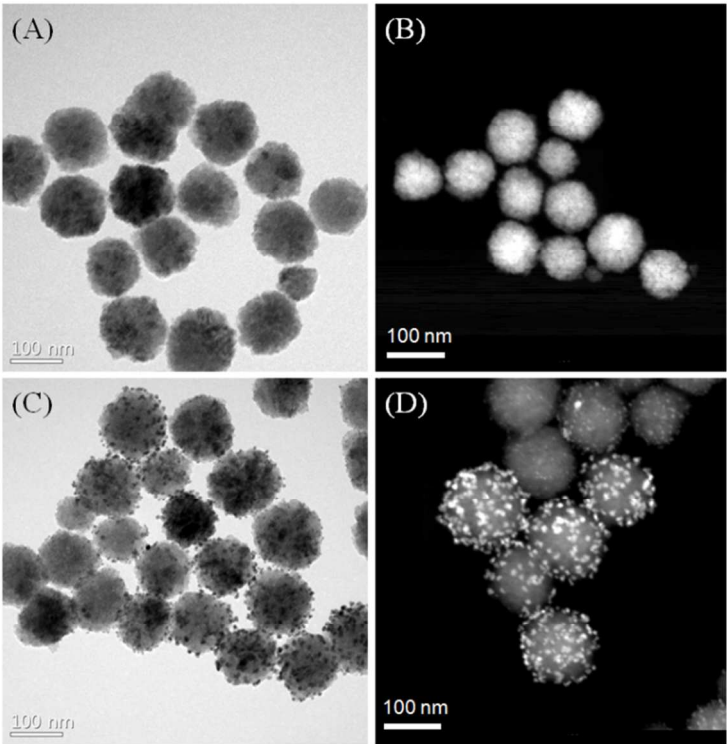


Figure 2. TEM images of Fe₃O₄@PEI NPs (A) and MagPlas NPs (C); STEM images of Fe₃O₄@PEI NPs (B) and MagPlas NPs (D)

The MagPlas NPs were aligned on the glass substrate under the external magnetic field to generate a long, single chain of NWs, as shown in Figure 3. The SEM images of the aligned NWs demonstrated that these NWs were self-assembled by single NPs to a length of a few hundred micrometers and were parallel to each other in the direction of the external magnetic field flux. The rough surface of the MagPlas NWs resulted from the deposition of Au NPs on Fe₃O₄@PEI NPs, as shown in Fig. 3D. In addition, we also measured the EDX spectrum of MagPlas NWs, as shown in Fig. S8. The spectrum confirms the presence of O, Fe and Au atoms in the MagPlas NWs.

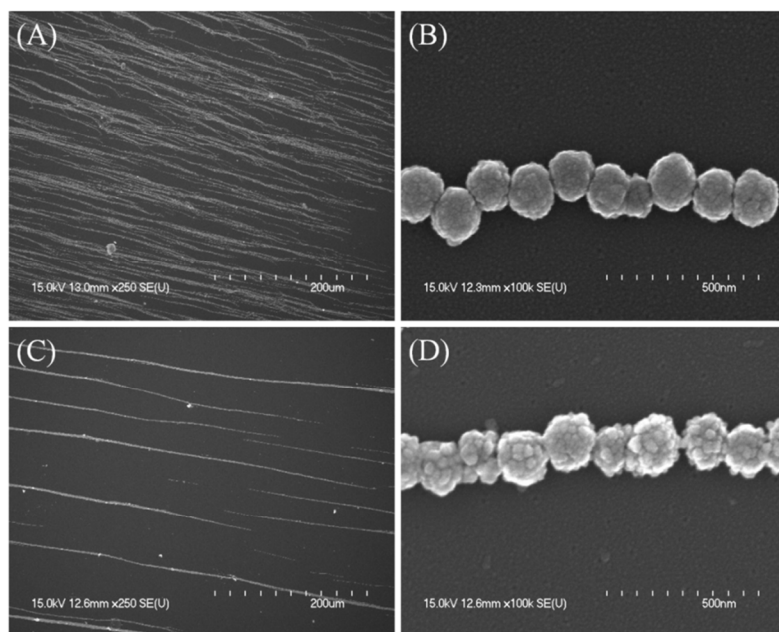


Figure 3. The SEM images of Fe_3O_4 NWs (A), magnified images of Fe_3O_4 NWs (B); the SEM images of $\text{Fe}_3\text{O}_4@\text{PEI-Au}$ NWs (C) and magnified images of $\text{Fe}_3\text{O}_4@\text{PEI-Au}$ NWs

Figure 4A and B show the TEM images of the GQDs and G3 antibody-immobilized GQDs. Figure 4C and D show high-resolution TEM images of $\text{Fe}_3\text{O}_4@\text{PEI}$ NPs and the sandwich nanocomposite of the GQDs-G3-CFP10-G2-MagPlas NPs. There were many salient points on the surface of the MagPlas NPs, as shown in the comparison between Figure 4D and Figure 4C, which were due to the immunological reaction of the GQDs-G3 antibody. Since the GBP region provides a strong and selective immobilization onto the Au (111) planes,⁴³ the Au NP coating is decisive in nanoprobe to ensure strong bioconjugation with a designated GBP-bound antibody as well as to provide a rough surface for further SERS and PL enhancement. Energy dispersive spectrometric elementary mapping was also conducted to examine the structure of the proposed nanoprobe (Fig. S2). Significant signals for representative elements in the nanoprobe were detected for Au, Fe, O, and C. We can conclude that the signals of the interior nanoprobe are mainly originated from the

Fe₃O₄@PEI-Au NPs, while the exterior ones from the GQDs. Thus, the fabricated nanoprobe actually possess expected structure. Furthermore, in our experiment, 1D alignment of MagPlas NPs was carried out because the 1D assembly of MagPlas NPs induced a more closely packed geometry at a nanoscale than did the NP structure, and this property is very beneficial for further SERS and PL enhancement for sensing and imaging applications.

3.2 SERS-fluorescence dual mode nanoprobe

The nanoprobe was able to tell the SERS and fluorescence imaging disparity by different excitation wavelengths. With a series of properties, such as narrow emission bands, high biochemical and photostability, as shown in Figure 1, GQDs can be used as contrast agents for fluorescence. In our experiment, 5 mL of GQDs (1 mg mL⁻¹) was first immobilized with 100 μ L of G3 antibody (32 μ g mL⁻¹) via the EDC/NHS bioconjugation method. As well known, the EDC/NHS crosslinkers are chemical modification reagents for activating carboxyl groups for spontaneous reaction with primary amines, enabling peptide immobilization and hapten-carrier protein conjugation. The EDC/NHS crosslinkers can bond carboxyl groups of GQDs and amine groups of G3 antibody. We measured the PL spectra of GQDs and G3 modified GQDs, as shown in Fig. S9. From our research, the unmodified GQDs can maximally absorb at 444 nm. While the slightly red shift occurred at 446 nm in the PL spectra of the G3 modified GQDs. The PL spectra and intensities of G3 modified GQDs were neither broadened nor weakened obviously. Therefore, it could be asserted to be a successful bioconjugation process on the surface of GQDs with no undue aggregation. G3 conjugates with GQDs and prevents the direct region of antibody binding each other without specific antigen. Since no such nanocomplex can be formed, no antigen

will be captured by the double specific antibodies. Thus, no SERS or fluorescence signal can be detected in the platform, as shown in Figure S3. We are expected to get detailed antigen-related information after the immunoreactions. In our research, we have measured the SERS signals and fluorescence of the nanocomplex.

GQDs and MagPlas NP avoid the non-radiative energy transfer from the GQDs to the MagPlas NPs. However, GQDs that originate from graphene oxide sheets have unique and very strong Raman bands that were ultimately utilized for our immunosensing experiment with a low concentration of antigens. The SERS performance of the nanoprobe was investigated. To achieve a strong enhancement effect of SERS, the MagPlas NPs were employed as SERS substrates after alignment under the external magnetic field. The GQDs-G3-CFP10-G2 antibody sandwich structure was adsorbed to the $\text{Fe}_3\text{O}_4@\text{PEI-Au}$ NPs to generate SERS signals, enabling SERS enhancement to be monitored. The SERS spectrum for the GQDs nanoprobe excited at 532 nm is shown in Figure 1D. The GQDs nanoprobe shows a D band and a G band, located at $1,358\text{ cm}^{-1}$ and $1,579\text{ cm}^{-1}$, respectively. From the above experiments, we can conclude that the GQD nanoprobe is capable of distinguishing SERS and fluorescence signals under different excitation wavelengths without mixing up each other.

We also measured the SERS spectra of GQDs bound on the irregular MagPlas NPs and assembled MagPlas NWs by G3-CFP10-G2 antibody sandwich, as shown in Fig. S7. We can observe that the assembled MagPlas NWs exhibit the higher enhancement efficiency which is beneficial to trap and capture probing GQDs for improving the SERS performances, compare with irregular MagPlas NPs. It attributed that the well-ordered magnetoplasmonic nanoparticles, assembled under magnetic force, exhibit a surface plasmon resonance (SPR) property which could build a strong local electric field which can

promote Raman activity in vibrational modes. Therefore, the 1D assemble MagPlas NWs is chosen as substrate in the current experiment.

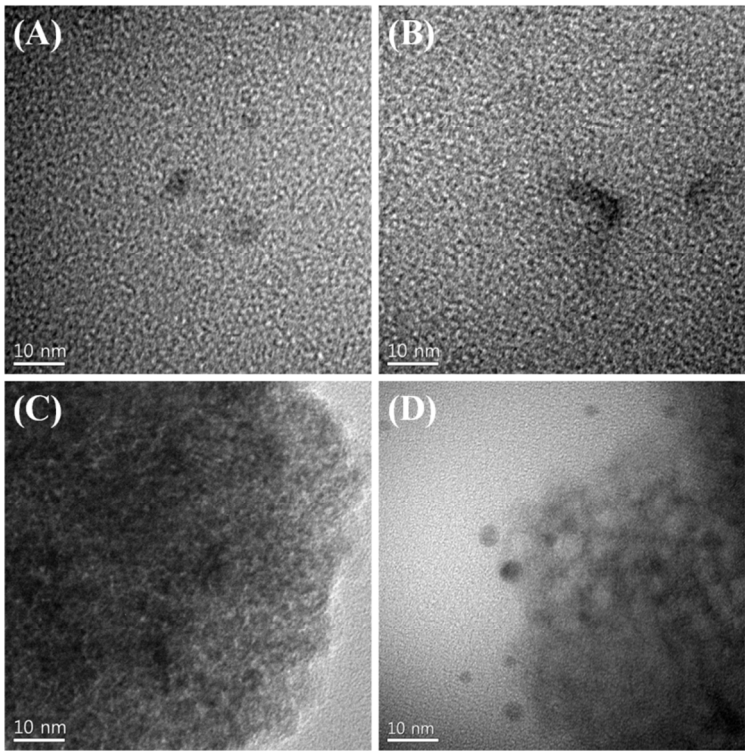


Figure 4. The HRTEM images of GQDs (A), G3-modified GQDs (B), Fe₃O₄ NPs (C), and GQDs bound on the Fe₃O₄@Au NPs via G3-CFP10-G2 antibody sandwich structure (D)

3.3 Immunoassay

The sandwich type assays were performed to validate the immunoassay protocol of multiplex bio-detection. The immune protocol was carried out following Scheme 1. The MagPlas NPs were aligned on the surfaces of silicon wafers under the external magnetic field. Then, antibodies were fixed on the surfaces of the MagPlas NPs and GQDs. So the sandwich-type nanocomplex with the target antigen (CFP10) was created. The nanocomplex was shown in Scheme 1. Briefly, the CFP-10 antigen is captured by the sandwich structure via the antibody–antigen interaction, and the MagPlas NWs shows SERS and fluorescence signals that match.

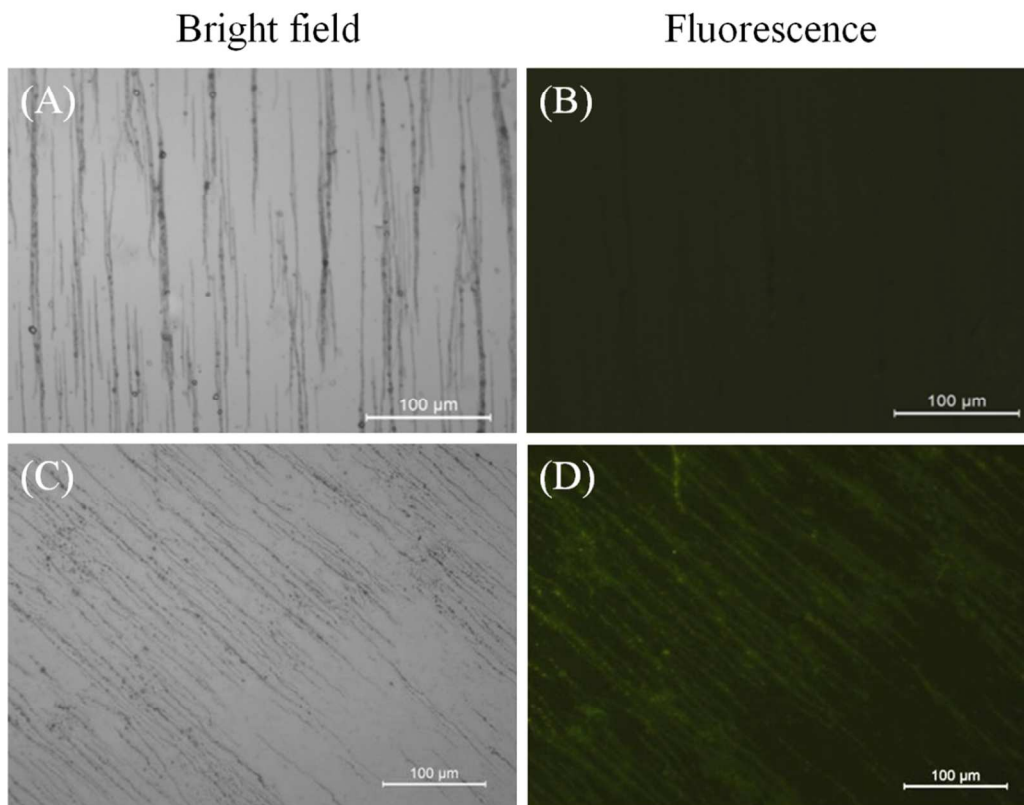


Figure 5. The fluorescence optical images of Fe₃O₄@PEI-Au NWs (A, B) and GQDs-G3-CFP10-G2 antibody sandwich structure on the surface of Fe₃O₄@PEI-Au NWs (C, D). Bright field (left) and fluorescence (right) images.

In our experiments, CFP-10 antigen was used as the target molecule for the proof-of-concept experiment. The MagPlas NPs were immobilized with GBP-G2 antibody, while the GQDs were immobilized with G3 antibody. The nonspecific binding sites on the surfaces of MagPlas NWs were blocked by BSA. First, the specificity of the immunoassay protocol was examined. Ten microliters of 10 pg mL⁻¹ CFP-10 antigen and pure PBS solution were added to the mixtures divided into two groups but both containing G3-modified GQD antibody and GBP-G2-modified MagPlas NWs. The immunoreaction proceeded for 2 h. Then we detected SERS and fluorescence signals of the nanocomplex. As expected, there is no trace of strong SERS signals until CFP-10 antigen molecules were added. Because the specific antigens help form the sandwich-type nanocomplexes which means there are more particles

in the corresponding platform than otherwise. The SERS spectra from two sets of solutions were respectively measured as shown in Figure S3. It was not hard to find that, the nanocomplex with specific antigen send out stronger SERS signals than those with no specific antigen. Fluorescence images of the nanocomplex are shown in Figure 5. In the bright field, both Figure 5A and C show the MagPlas NWs without and with antigen bounding. In the fluorescence images, however, only Figure 5D shows the green fluorescence indicating NWs owing to GQDs immobilized on the surface of $\text{Fe}_3\text{O}_4@\text{PEI-Au}$ NWs. This series of researches show that target antigens could be easily detected in such an immunoassay system. Thus, the immunoassay protocol can be used to specifically detect target biomolecules in a solid platform.

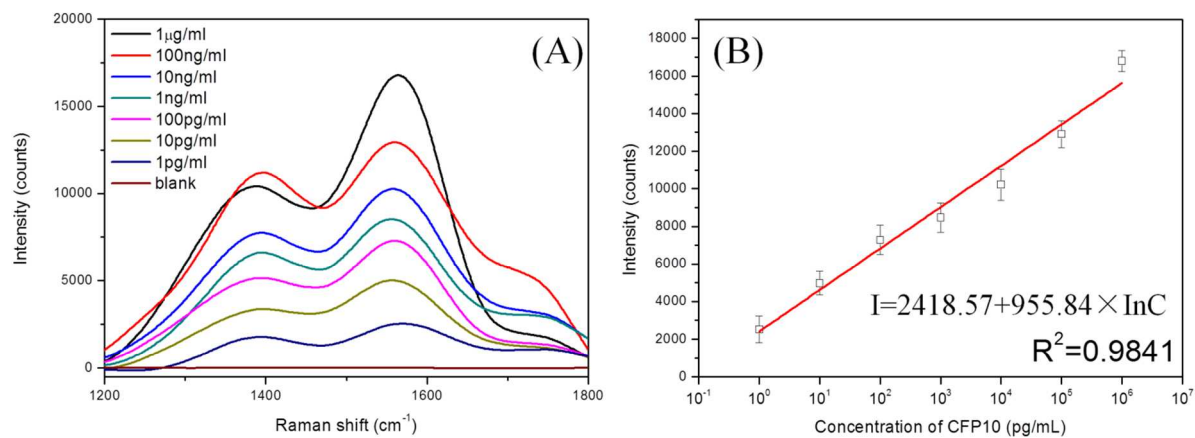


Figure 6. (A) Concentration-dependent SERS spectra of the $1,579\text{ cm}^{-1}$ peak obtained from the precipitates corresponding to different concentrations of CFP-10 antigen. (B) SERS intensity for the $1,579\text{ cm}^{-1}$ peak plotted against the logarithm of CFP-10 antigen concentrations ranging from 1 pg mL^{-1} to $1\text{ }\mu\text{g mL}^{-1}$. The error bars represent the standard deviation of 5 measurements.

Next, CFP-10 antigen was responsible for detecting the sensitivity of the exhibited immunoassay protocol as the model target biomolecules. Various concentrations of CFP-10 antigen ($1\text{ }\mu\text{g mL}^{-1}$ to 1 pg mL^{-1}) were added to 8 sets of GBP-G2-supplemented

Fe₃O₄@PEI-Au NPs and G3-modified GQDs solutions. One additional set of solution was added pure PBS solution as blank control. After 2 h of immunoreaction, each nanocomplex set was collected and measured by Raman spectroscopy. The precipitates of each solution enabled us to catch the SERS spectrum of the 1,579 cm⁻¹ peak (as demonstrated in Figure 6A). This result indicated that the concentration of CFP-10 antigen in proportion to the SERS spectra. Figure 6B shows a plot of the intensity of the 1,579 cm⁻¹ peak against the logarithm of the CFP-10 antigen concentration, clearly revealing the decrease in SERS intensity. For the SERS signals, a well linear response was achieved within the range of 1 pg mL⁻¹ to 1 µg mL⁻¹ CFP-10 antigen. The definition of detection limitation in this research is that the signal of the analyte concentration was three times stronger than that in the blank control and its value was 0.0511 pg mL⁻¹ for the immunoassay.⁴⁴ The abovementioned results proved that the immunoassay protocol can be used to realize highly sensitive and selective multiplex detection of target molecules. In addition, one peak at 1750 cm⁻¹ was clearly observed in Figure 6A. According to the previous report, we attribute the peak of 1750 cm⁻¹ to the M band due to the intravalley double resonance scattering process of bilayer graphene and few layer graphene. Similar to the G' mode, the evolution of this M band coupled with the increasing number of graphene layers could be inflicted by that of electronic band with graphene films structure.⁴⁵

Note that the ratio of intensity of D/G bands is a measure of the defects present on graphene structure.⁴⁶ In-plane vibrations of SP² bonded carbon atoms create G band whereas the D band is the result of out of plane vibrations attributed to the presence of structural defects.⁴⁷ If the D band is higher, it indicates that the SP² bonds are broken which in turn means that there are more SP² bonds. Fig. 6 shows G bands of GQDs was more enhanced than that of D band. The GQDs bound on the surface of MagPlas NWs G3-CFP10-G2

antibody sandwich in the immunoassay. It results in increasing of SP2 bonds (G band) but did not break the SP2 bonds (D band). Moreover, some GQDs overlapped each other in the immunoassay, inevitably. Thus, the M band shows weak enhancement in the peak of 1750 cm^{-1} .

This new immunoassay protocol has several advantages relative to previous methods. The $\text{Fe}_3\text{O}_4@\text{PEI-Au}$ NWs allow the detection to be performed on a solid platform, thus greatly simplifying the immunoassay process. It also has great multiplexing ability owing to the dual-mode nanoprobe because it eliminates the need to employ multiple SERS reporters or fluorescence semiconductor QDs with different emission wavelengths. GQDs can generate both SERS and fluorescence signals simultaneously. Moreover, the fluorescence signal is a fast qualitative indicator of the immunoreaction, and the SERS signal can be used for the quantitative identification of the specific target molecules.

4. Conclusions

The study of SERS and fluorescence signals helps us build a brand new immunoassay protocol. Specifically, in the dual-mode nanoprobe, the 1D aligned MagPlas NWs serve as the SERS substrate, and water-soluble GQDs was the fluorescent indicator and Raman signal. With such a structure, dual-mode nanoprobe can be used to detect SERS and fluorescence signals to monitor immunoreaction results. The biomolecules characterized with high sensitivity and selective recognition on solid platform with a detection limit of 0.0511 pg mL^{-1} was formed. Thanks to the easy operation and superior multiplexing ability (attributed to the dual-mode nanoprobe) of immunoassay protocol, it is expected to be further applied in high throughput screening of target molecules.

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■ ASSOCIATED CONTENT

Supporting Information

Additional synthesis process of GQDs, size distribution, Zeta potential, STEM images and EDX elementary mapping, SERS spectra, fluorescence optical images, EDX spectrum, PL spectra, and magnetization moments estimated for Fe₃O₄@PEI NPs. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

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Notes

The authors declare no competing financial interest.

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Table of Contents (TOC) Graphics

