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A preliminary study of surface enhanced Raman scattering immunoassay based on graphene oxide substrate

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ABSTRACT

Graphene Oxide (GO), the oxidized form of graphene, has received considerable attention in the field of biology and medicine. Based on its advantages of surface enhanced Raman scattering (SERS) activities and high enrichment of biomolecules, here we report a sandwich-type SERS immunoassay format, which uses GO as solid substrates to attach antibodies, and Ag nanoparticles (AgNPs) covalently immobilizing 4-mercaptobenzoic acid (4-MBA) as Raman tag nanoparticles. The research results show that the new immunoassay has excellent specificity in the capture of target molecules, and the detectable limit of model antigen concentration is 2 ng/mL, which is close to the detectable level of many biomolecules or harmful substance. The preliminary study suggests that GO-based SERS immunoassay has a great potential in the field of biomedicine, food inspection, environmental monitoring, and so on.

Raman spectroscopy is a molecular vibrational spectroscopic technique that can present a chemical fingerprint information about molecular structures and compositions [1–3]. It has been extensively used as a powerful technique for the chemical analysis and medical applications [4–8]. However, Inherently weak Raman scattering signal limit its practical application. Fortunately, with the discovery of surface enhanced Raman scattering (SERS) by Fleischmann et al. in 1974, Raman spectroscopy technique has acquired rapid development [9–11]. When the sample molecules are adsorbed on the surface of some rough metal, such as silver, gold and copper, their Raman scattering signal will be enhanced up to 10^6–10^{14} times, this phenomenon is named SERS. Compared with the fluorescence spectroscopy, the major advantages of SERS are that it has potential to be used as a multiplexed readout technique through a single excitation wavelength. The main reasons are that Raman bands are much narrower than fluorescence bands. In addition, Raman signals are resistance to photo bleaching [12]. Many groups have applied SERS in many analytical systems recent years, including bacteria checking, glucose sensing, DNA detection, chemical warfare stimulant detection, and so on [13–16]. One of the most promising applications of SERS is immunoassay, which is based on a specific interaction between antigen and a complementary antibody. Immunoassay has become a powerful analytical tool in biochemical analyses, clinical diagnosis, environmental monitoring, and so forth [17–20]. However, usually the sensitivity of conventional Raman spectroscopy is too low to be used for immunoassay. In order to overcome this difficulty, various strategies have been developed. For example, Cotton et al. applied surface-enhanced resonance Raman scattering (SERRS) effect into Raman enzyme immunoassay. In their system, resonance dye molecules, p-dimethylaminoazobenzene (DAB), were covalently attached to an antibody directed against human thyroid stimulating hormone (TSH), then the resultant conjugate was used as the reported molecule in a sandwich immunoassay [21]. Xu et al. proposed an

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immunoassay method that employed immune gold labeling and Ag staining enhancement. They obtained the detecting limit of the Hepatitis B virus surface antigen as low as 0.5 μg/ml [22].

Graphene, a monolayer two-dimensional carbon nanomaterial reported for the first time in 2004, has attracted significant attention because of its fascinating optical, electronic, thermal, and mechanical properties. It has significant potential applications in the fields of photonics, nanoelectronics, and nanomechanical systems [23]. Recently, a few research groups have found that graphene oxide (GO) is one of idea materials for biomedical applications. GO is the oxidized form of graphene, it has many excellent properties suitable for biomedical applications such as good water dispersibility, facile surface modification, large surface area, biocompatible, etc. [24–27]. Structurally, GO can be visualized as a graphene sheet with its basal plane decorated by oxygen-containing groups. Owing to these groups bear affinity to water molecules, GO is hydrophilic, so it can be dissolved in water. In the previous studies, GO were employed as nanocarriers for drug loading and delivery, indicating that it has properties of enriching drug molecules. It was reported that the loading ratio (weigh ratio) of GO is much higher than that of other loading nanostructures, suggesting it has great potential to capture more target molecules [28,29].

Recently, a few research groups have found graphene can enhance Raman scattering signals of organic molecules. Zhang et al. reported that graphene leaded to an enhancement factor of 2–17 due to the charge transfer between graphene and adsorbed molecules [30]. Yu observed the chemical enhancement factor of reduced GO can be as large as 10^3 [31]. Wang group used GO/poly diallyldimethylammonium chloride (PDDA)/AgNPs hybrids as SERS substrates, they found the SERS signals of folic acid on GO/PDDA/AgNPs were much stronger than that on Ag nanoparticles. The minimum detected concentration of folic acid in water was as low as 9 nM [32].

In order to improve sensitivities of SERS immunoassay, this study describes a novel sandwich-type SERS immunoassay, which uses GO as solid substrates to attach antibody, and silver nanoparticles covalently immobilizing 4-mercaptobenzoic acid (4-MBA) molecules as Raman tag nanoparticles. Herein, 4-MBA molecules are used as both the Raman reporter molecules and the conjugation agent, which link antibody molecules and silver nanoparticles.

Graphite flake was obtained from Shanghai Yifan Graphite Co.; Ltd. 4-MBA was purchased from Sigma–Aldrich. $AgNO_3$ and sodium citrate were obtained from Sinopharm Cheimcal Reagent Co., Ltd. (Shanghai, China). 1-Ethyl-3-(3-dimethylaminpropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Shanghai Jingchun Chemical Reagent Co., Ltd. Goat anti-human IgG, goat anti-rabbit IgG, human IgG, mouse IgG, and bovine serum albumin (BSA) were purchased from Beijing Boisynthesis Biotechnology Co., Ltd. All glassware was cleaned in a bath of freshly prepared 3:1 HCL/HNO_3 and rinsed thoroughly with deionized water (resistance, 18 MΩ cm^{-1}) for further use.

AgNPs were synthesized following the reported method [32]. Briefly, 18 mg of $AgNO_3$ was added to 100 mL water under strong stirring. When the solution was boiling, 2 mL of citrate sodium (1 wt.%) was slowly added into the solution. Then the solution was boiled for 40 min and cooled in ambient conditions.

The SERS nanoprobes were prepared by following procedure shown in Fig.1(b). First, silver nanoparticles were labeled with 4-MBA molecules. 30 μL of 0.1 mM 4-MBA in methanol was added to 1.0 m L of Ag nanoparticles and then stirred for 2 h at room temperature. Afterwards, the 4-MBA-coated AgNPs were successively separated from the solution by the centrifugation at 5000 rpm for 15 min and resuspended with 1.0 m L deionized water. Second, IgG antibody was immobilized on the 4-MBA-coated AgNPs. 5 μL of 0.5 mM EDC and 5 μL of 1.0 mM NHS were added into the 4-MBA-coated AgNPs. The carboxylic groups on the particle surfaces were activated to form reactive NHS ester intermediates. After 30 min of stirring, 5 μL of 0.5 mg/mL goat anti-human IgG antibody was added to the carboxylic group-activated AgNPs. After being incubated at room temperature for 2 h, the amine groups on the antibody molecules reacted with the active ester groups on the AgNPs surfaces to form stable amide bonds. The antibody-conjugated

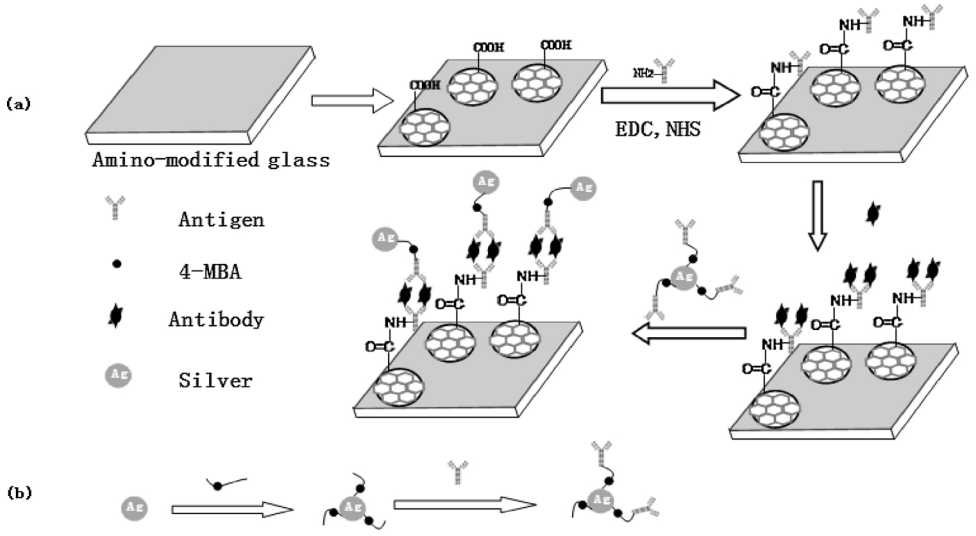

Fig. 1. (a) Scheme of preparing Silver nanoparticle-based SERS nanoprobe,(b) Schematic illustration of a GO-based SERS immunoassay.