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Graphene oxide-assisted surface plasmon coupled emission for amplified fluorescence immunoassay

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ABSTRACT

We have observed the unique enhancement of fluorescence signal of surface plasmon coupled emission (SPCE) by graphene oxide (GO) on gold film. Assembling ultrathin GO by electrostatic adsorption between gold film and fluorophore layer (about 30 nm), the enhancement of fluorescence signal of SPCE reached 7 and 25 times respectively as compared to that of the regular SPCE and free space emission (FSE) without GO. The enhancement factors were studied through modifying different concentrations of GO and thicknesses of fluorophore layer, and the possible enhancement mechanisms were investigated. This GO-assisted SPCE was designed as an immunosensor to detect human IgG with a detection limit of 0.006 ng/mL, which was much lower than the detection limit of immunosensor based on regular SPCE. This strategy combing GO and SPCE to enhance the fluorescence signal provides a simple way to improve detection sensitivity in fluorescence-based sensing platforms.

1. Introduction

A strong interaction between the fluorophores in the vicinity of metal films and the surface plasmons (SPs) leading to the highly directional *p*-polarized and wavelength-resolved emission is known as surface plasmon coupled emission (SPCE) [1–5]. SPCE which can be regarded as the reverse process of surface plasmon resonance (SPR), has recently been used in biochemistry detection areas, such as bioanalysis, biomedical research, and clinical testing. Due to the enhanced fluorescence signal caused by the increased collection efficiency (exceed 50%) and the amplified electromagnetic field compared with that of isotropic fluorescence, SPCE-based methods have received wide spread attention [6–9]. Our previous work has contributed to the enhancement of the performance of SPCE by various strategies, such as combining nanomaterials [10–12], introducing external forces [13,14] and equipment upgrading [15,16], which has improved the SPCE platform for potential bioanalysis applications.

Graphene, a 2D lattice consisting of sp^2 hybridized carbon atoms, has received significant interest due to its outstanding properties, including excellent electrical conductivity, good biocompatibility and remarkable mechanical strength [9,17]. Graphene oxide (GO), known as oxidized counterparts of graphene, contains aromatic ring structures and abundant functional oxygen-based groups, such as carboxyl, epoxy and hydroxyl groups, in its basal planes and edge [18,19]. These specialties capacitate GO to be a good support for biomolecules through covalent, noncovalent, electrostatic and π-π interactions, which enable it to be served as a remarkable GO-based sensors for biometric and sensing [18,20]. In recent years, graphene materials-based SPR methods have been demonstrated their potential to amplify the optical sensitivity of SPR detections: (1) producing greater change in refractive index near the substrate, which is caused by higher loading of analytes owing to the larger surface area and rich π-conjugation structure of graphene materials [18–23]; (2) modifying the propagation constant of the SPs, which will influence the sensitivity to the change of refractive index [24]; (3) enhancing the SPR field induced by charge transfer from graphene materials to the surface of metallic film and changing the properties of SPs caused by the coupling interaction between the SPR field and graphene materials [25,26]. Normally, in SPCE, gold or silver thin films are used as experimental substrates, because their free electrons facilitate an oscillation at the electromagnetic radiation, while the resonance of other metals with large imaginary part of dielectric constants, by contrast, is generally weak. Graphene and GO spin-coated on silver film have been proved very useful for the amplification of SPCE [9,27].

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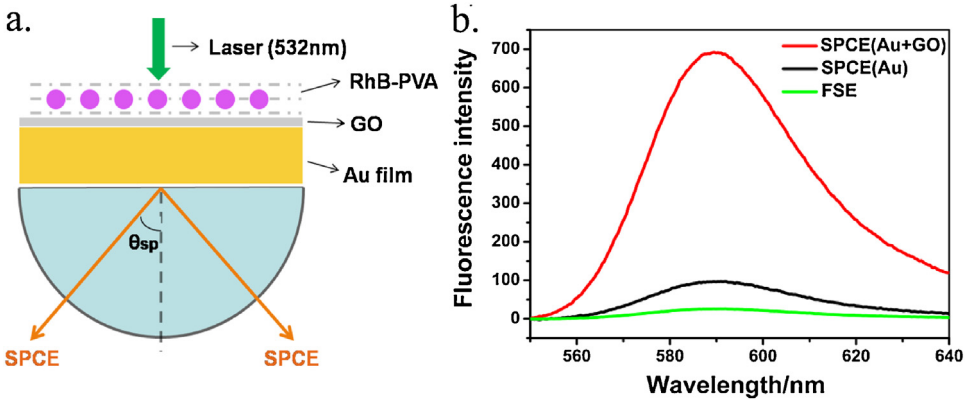

Fig. 1. (a) The experimental setup for GO-assisted SPCE of RhB. The setup is not drawn to scale. (b) The spectra of SPCE with GO assisted (red), regular SPCE without GO-assisted (black) and FSE without GO-assisted (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

However, biosensors based on GO-assisted SPCE have not been reported yet. Besides, it is hard to accurately control the thickness and homogeneity of those GO films prepared by spin-coating approach, and silver film is easy to suffer from oxidation when exposure to oxygen and less stable than gold film during biochemical modification.

Herein, we demonstrated an enhancement fluorescence signal of the assisted SPCE with GO electrostatically adsorbed on gold film, and designed an immunoassay based sensor for the sensitive detection of proteins. Electrostatic adsorption of GO on the metallic surface is homogenous and easily applicable for further surface modification. Moreover, the high stability and easy surface modification of gold film make it a better substrate than silver film in SPCE-based sensors.

2. Experimental methods

2.1. Experimental preparation for GO assistance

A 2 nm adhesive layer of chromium followed by a 50 nm gold continuous film was sequentially deposited onto a cleaned silica slide substrate via radiofrequency magnetron sputtering. The gold substrate was incubated in 1 mM cysteamine (Sigma-Aldrich) for 12 h to form amine-modified gold film and then thoroughly rinsed with ethanol and dried in pure N_2. Subsequently, the substrate was immersed into GO (Nanjing XFNANO Materials Tech Co.) solution for 5 h. The GO solutions were formed by dispersing GO in water. Next, the GO covered substrate was thoroughly rinsed with ultrapure water and dried in N_2. After that, 1 mM Rhodamine B (RhB, Sigma-Aldrich) in polyvinyl alcohol (PVA, Alfa) was spin-coated on the substrate at 4000 rpm for 40 s and dried in air to obtain dye doped film. The schematic of the experiment is shown in Fig. 1a. The fluorescence signal of SPCE was measured in the Reverse Kretschmann (RK) configuration in which the sample is excited directly by the incident light to emit the fluorescence signal through the prism.

For comparison, 1 mM RhB-1% PVA was deposited on gold substrate without GO directly by spin-coating. The fluorophore eluents were obtained by ultrasonic wash of the substrate modified with and without GO in ethanol for 5 min, and then the fluorescence spectra of eluents were investigated through a home-made fluorescence spectrometer.

For the preparation of the quartz glass substrate modified with GO, the substrate was immersed in 1% aminopropyltriethoxysilane (APTES, Acros Organics) ethanol solution for 1 h to obtain amine-modified substrate. Then, the substrate was thoroughly rinsed with ethanol and dried in pure N_2. Subsequently, the substrate was immersed into GO solution for 5 h. Next, the GO covered substrate was thoroughly rinsed with ultrapure water and dried in N_2. After that, 1 mM RhB-1% PVA was spin-coated on the substrate at 4000 rpm for 40 s and dried in air to obtain dye doped quartz glass substrate. For comparison, 1 mM RhB-1% PVA was deposited on quartz glass without GO directly by spin-coating.

2.2. Modification process for immunosensor

The schematic diagram of the sensing procedure is shown in Fig. 2. GO was modified on the gold film by electrostatic adsorption. The carboxyl groups of GO were activated with 10 mM of N-hydroxysuccinimide (NHS, Sigma-Aldrich) under the catalysis of

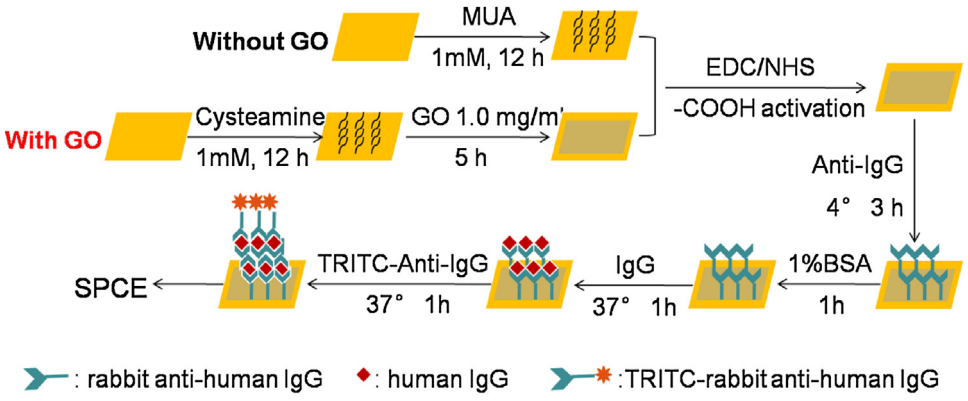

Fig. 2. Schematic illustration of the steps for the preparation of sandwich immunoassay through GO-assisted SPCE and regular SPCE without GO.