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Highly sensitive fluorogenic sensing of L-Cysteine in live cells using gelatin-stabilized gold nanoparticles decorated graphene nanosheets

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ABSTRACT

A highly sensitive and selective fluorogenic sensing of L-Cysteine (L-Cys) was implemented based on gelatin stabilized gold nanoparticles decorated reduced graphene oxide (rGO/Au) nanohybrid. The rGO/Au nanohybrid was prepared by the one-pot hydrothermal method and well characterized by different physiochemical techniques. The nanohybrid exhibits a weak fluorescence of rGO due to the energy transfer from the rGO to Au NPs. The rGO/Au nanohybrid shows enhanced fluorescence activity due to the restoration of quenched fluorescence of rGO/Au nanohybrid in presence of L-Cys. The rGO/Au nanohybrid exhibits much lower detection limit of 0.51 nM for L-Cys with higher selectivity. The fluorescence sensing mechanism arose from the fluorescence recovery due to the stronger interaction between Au NPs and L-Cys, and consequently, the energy transfer was prevented between rGO and Au NPs. The practicability of rGO/Au sensor was implemented by *invitro* bioimaging measurements in Colo-205 (colorectal adenocarcinoma) and MKN-45 (gastric carcinoma) cancer live cells with excellent biocompatibility.

1. Introduction

The lower molecular weight of thiol-containing molecules such as L-Cysteine (L-Cys), homocysteine (Hcy) and glutathione (GSH) are frequently involved in various biological actions due to its wide distribution in living organisms. In addition, thiols are playing important role in reversible redox homeostasis and cellular functions in the biological system [1–4]. Especially, L-Cys as a semi-essential thiol-containing amino acid, which is most important in peptide and protein synthesis, detoxification, metabolism, and neuronal tissues [5–7]. In addition, L-Cys can be easily cross-linked with bio-macromolecules *via* disulfide bonds and coupled with enlargement and hindrance of senility of the cells and tissues in the living system [8,9]. However, a series of disorders could be associated with the problem of L-Cys such as slow growth, muscle and fat loss, hair depigmentation, weakness, edema, skin lesions, liver damage, lethargy, Alzheimer's disease, Parkinson's disease and acquired immune deficiency syndromes [10–12]. Hence, there is an urgent demand for accurate and low-level detection of L-Cys with simple and highly sensitive analytical techniques. Thus far, L-Cys was quantified with various classical analytical techniques such as UV–vis and fluorescence spectroscopy, Fourier-transform infrared spectroscopy (FT-IR) spectroscopy, mass spectrometry, gas chromatography and immunoassay, high-performance liquid chromatography (HPLC), fluorescence-coupled HPLC and electrochemical methods [13–22]. Among these traditional methods, herein the fluorescence method was used for detection of L-Cys by taking advantage of the merits of simplicity, high sensitivity, low cost, selectivity and versatility [23,24].

During the past decades, the carbon-based nanomaterials such as carbon quantum dots, graphene, and other carbon nanomaterials have been adopted widely for the fluorescence sensing of biomolecules [25–30]. Particularly, reduced graphene oxide (rGO), a two-dimensional (2D) thin layer nanosheet with hexagonal hon-

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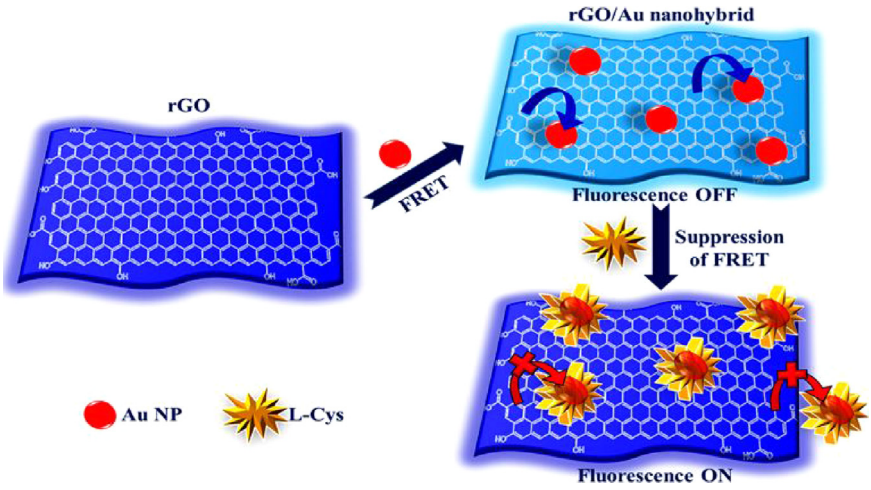

Scheme 1. Schematic illustration of fluorescence turn-on mechanism over rGO/Au nanohybrid.

eycomb lattices of carbon sheet, has been widely used in various fields such as nanotechnology, materials science and biotechnology [31]. On the other hand, the noble-metal nanoparticles, especially gold nanoparticles (Au NPs), have attracted tremendous interest in biological applications due to its specific large surface area, superior biocompatibility, and excellent conductivity. In addition, they also act as an efficient catalyst for electron transfer process [32–34]. Accordingly, the Au NPs have been widely applied for ion detection, molecular identification, and chemical catalysis, while showing the prospects in bio-labeling and bio-sensing [22]. For instance, the biocompatible polymer and peptides conjugated with Au NPs could be used for biomedical applications during the decades. Specifically, Gelatin (GEL) is a helically structured biopolymer matrix, is most abundant in animal skin and bone. Moreover, GEL is a denatured product of collagen and utilized for the entrapment of biomolecules for the preparation of biosensors [35–37]. Zhang et al. adopted gelatin as reducing and stabilizing agent to immobilize Au NPs with carboxylic single-walled carbon nanotubes for use of cytosensing and drug uptake [38].

In this work, GEL was used as a stabilizing and reducing agent for the preparation of rGO/Au nanohybrid *via* the one-pot hydrothermal synthesis. The obtained rGO/Au nanohybrid shows excellent sensitivity and selectivity towards the detection of L-Cys. The fluorescence sensing of L-Cys was investigated based on the fluorescence turn-on process. When coordinated with Au NPs, the fluorescence of rGO was diminished due to the energy transfer from rGO to Au NPs. Then, the fluorescence recovery of rGO was obtained upon the addition of L-Cys owing to the strong interaction between Au NPs and L-Cys prevents energy transfer from rGO to Au NPs. For practical application, this nanohybrid based sensor was successfully demonstrated to detection of L-Cys in Colo-205 (colorectal adenocarcinoma) and MKN-45 (gastric carcinoma) cancer live cells by using confocal fluorescence microscopy.

2. Experimental section

2.1. Materials

Pristine graphite powder (size <20 mm), gelatin from bovine skin and $HAuCl_4{\cdot}3H_2O$ were purchased from Sigma-Aldrich. L-Cys and other chemicals were obtained from Acros and Sigma-Aldrich, and used as purchased without further purification. Phosphate buffered saline (PBS, 1X, pH 7.4) was purchased from Life technology.

2.2. Instruments

Transmission electron microscope (TEM) observations were conducted with a JEOL JEM-1200 EX-II. Atomic force microscopy (Veeco, Bio-Scope SZ) was used to determine the thickness of rGO. Raman spectra were recorded by using a Raman spectrometer (Dong Woo 500i, Korea) equipped with a 50 x objective and a charge-coupled detector. The FTIR spectra were carried out using the Thermo Scientific Nicolet iS10 instrument. The UV–vis absorption spectra were recorded with a Thermo Scientific evolution 220 UV–vis spectrophotometer. The fluorescence spectra were recorded with a Perkin-Elmer LS45 spectrometer. Confocal fluorescence images of cells were performed on a Leica SP5 confocal system under 405 nm excitation light source.

2.3. Synthesis of rGO/Au nanohybrid

The graphite oxide (GO) was synthesized *via* modified Hummers' method [39]. The GEL solution was prepared as per our previous report [40]. The GEL stabilized rGO/Au nanohybrid was prepared by the one-pot hydrothermal method. Briefly, the GO was redispersed in Millipore water and stirred for 1 h to obtain a fully exfoliated GO suspension. Then, the as-prepared aqueous solution of GEL (10 mL, 3%, wt%) was added to the GO suspension. After that, 1.0 mL of the $HAuCl_4$ solution (1%, wt%) was added to the above GO/GEL suspension under magnetic stirring. Then, appropriate amount of 0.1 M NaOH solution was added to the suspension and stirred vigorously for 6 h at 80 °C. The composite was cooled at room temperature and washed with Millipore water through centrifugation to remove the unreacted Au NPs and GEL suspension. The composite was then dried for 2 h at 60 °C. The obtained GEL stabilized rGO/Au nanohybrid was further used for characterizations and fluorescence sensing studies. The plausible mechanism for fluorogenic detection of L-Cys using rGO/Au nanohybrid was illustrated in Scheme 1.

2.4. Procedure for fluorescence sensing experiments

All diverse biomolecules solutions were buffered in pH 7.4 PBS (0.1X). The stock solution of rGO/Au nanohybrid (15 μg/mL) was prepared by the dilution of concentrated rGO/Au nanohybrid with pH 7.4 PBS. The fluorescence titration measurements were carried out by adding L-Cys (up to 20 μL, with the maximum concentration ~0.4 μM) to the rGO/Au nanohybrid solution in a quartz cuvette.