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Facile fabrication of 3D layer-by-layer graphene-gold nanorod hybrid architecture for hydrogen peroxide based electrochemical biosensor

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ABSTRACT

Three-dimensional (3D) layer-by-layer graphene-gold nanorod (GNR) architecture has been constructed. The resulting hybrid nanomaterials' architecture has been tested for detecting hydrogen peroxide (H_2O_2) through the electrocatalytic reaction on a three electrode disposable biosensor platform. Cyclic voltammetry and amperometry were used to characterize and assess the performance of the biosensor. The 3D layer-by-layer modified electrode exhibited the highest sensitivity compared to the active carbon, graphene-oxide, cysteine-graphene oxide and GNR coated electrodes. This research explored the feasibility of using the 3D hybrid graphene-GNR as a template for biosensor. The 3D hybrid structure exhibited higher sensitivity than GNRs alone. SEM showed the explanation that GNRs had self-aggregates reducing the contact surface area when coated on the active carbon electrode, while there were no such aggregates in the 3D structure, and TEM illustrated that GNRs dispersed well in the 3D structure. This research demonstrated a better way to prepare well-separated metal nanoparticles by using the 3D layer-by-layer structure. Consequently, other single and bi-metallic metal nanoparticles could be incorporated into such structure. As a practical example, 3D layer-by-layer nanomaterials modified active carbon electrode was used for detecting glucose showing very good sensitivity and minimum interference by ascorbic acid and uric acid in test solution, which indicated a good selectivity of the biosensor as well.

1. Introduction

Electrochemical biosensors are highly effective in detecting biomolecules due to the high sensitivity, real-time monitoring capability and low cost, compared to the relatively complex and expensive measurement techniques such as radioisotope tracing, NMR spectroscopy, and microfluorometry assay [12,25,18]. In recent years, electrochemical biosensors based on the enzymatic activity have received increasing interest, for its advantages of low cost, portability, fast response time, and ease-of-usage by non-specialist personnel [3]. Hydrogen peroxide (H_2O_2) is an electrochemical active species produced by various oxidase enzymes. Thus, the measurement of H_2O_2 in various enzymatic reactions can quantify the analyte for biomarker detections as shown in Eq. (1) [11]. The use of electrode or catalyst-modified electrode as a transducer was based on Eq. (2) of oxidation of H_2O_2:

$$\text{Analyte} + O_2 \xrightarrow{\text{oxidase}} \text{byproduct} + H_2O_2 \quad (1)$$

$$H_2O_2 \xrightarrow{\text{elecrode}} O_2 + 2H^+ + 2e^- \quad (2)$$

However, the total sensitivity of biosensors based on traditional materials is hindered due to restrictions in mass transport, enzyme loading, and electrochemical coupling, limiting the potential for miniaturization. This affects the limitation of detection of the analyte as well. Unconventional nanomaterials with good biocompatibility and electrocatalytic activities have been widely incorporated in biosensors to overcome these shortcomings [13,6,16,21].

Metal nanoparticles (NP), particularly Au and Pt, have been used in the development of electrochemical sensors and biosensors based on their catalytic activities [31,21]. They have the advantages of large surface-to-volume ratio and special binding site on the surface of nanoparticles, which lead to a fast communication between an enzymatic process and a nanoparticle response for signal transduction in biosensing or for catalytic reactions [2].

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PtNPs has superior sensitivity because of its enhanced electron transfer and reduction of overpotential for H_2O_2 oxidation [9,30]. However, electrodes modified with pure PtNPs requires a relatively high electrochemical potential (ca. +0.7 V versus Ag/AgCl) to oxidize H_2O_2 generating the oxidation current. At relatively high potential, it will oxidize ascorbic acid (AA) and uric acid (UA) in human blood resulting in an interference of the detection of the analyte (e.g. glucose) [4]. Hence, gold nanoparticles (GNPs) are considered to be another potential candidate based on its good performance in H_2O_2 related sensors with lower potential [5]. Different from spherical GNP, gold nanorods (GNR) introduces more interesting functions based on its anisotropic shape having unique localized surface plasmon resonance (LSPR), which brings applications in cancer diagnosis and monitoring local environment changes [8,1]. This can potentially expand the diversity of biosensors. However, owing to its anisotropic shape, GNR tends to self-aggregate during surface processing forming side-by-side assemblies [19,27] Therefore, it is desirable to introduce selected conducting materials to support the GNR in order to induce decent dispersity.

Carbon nanomaterials are excellent candidates as the supporting materials for metal NPs due to their unique structural, electrical, and mechanical properties [20,22]. The carbon nanomaterials can enhance the available electrochemical active surface area of electrocatalyst and provide high mass transport of reactants to the electrocatalyst. Among these carbon materials, graphene is a two dimensional monolayer of carbon atoms with high surface area, chemical stability, and thermal stability, making it as a useful substrate for electronics [24]. However, graphene sheets are difficult to exfoliate because their planar polycyclic aromatic structures favor tight packing as a result of strong π–π interactions. Also, even the graphene sheets are destacked, they can only support metal NPs on its two sides and these individual graphene-metal NP hybrid structures may lack connection to each other. Therefore, it requires a 3D structure to enhance the loading of metal NPs and interconnection between the metal NPs. In our previous work, a 3D graphene-GNR layer-by-layer nanostructure was constructed [28]. The GNRs dispersed well and linked the conductive reduced graphene layers through stable covalent Au-S bond in this 3D structure. Therefore, this architecture possesses ideal large surface area and conductive pathways for biosensing.

In this study, the prepared 3D layer-by-layer graphene-GNR hybrid nanomaterial was coated on a thick film screen-printed active carbon powder electrode as a single-use, disposable biosensor for testing H_2O_2. The performance of GNR without supporting graphene layers was tested for comparison through electrochemical analytical techniques, such as cyclic voltammetry (CV) and amperometric measurements. The interference test was executed by detecting glucose in the presence of AA and UA showing the selectivity performance of this biosensor.

2. Experimental

2.1. Materials and instruments

All chemicals and solvents were purchased from commercial suppliers and used without further purification. $HAuCl_4$ was 30 wt% in diluted HCl solution. UV–visible spectra were collected on a PerkinElmer Lambda 25 UV–Vis spectrometer at the resolution of 1 nm. For transmission electron microscopy (TEM) observation, solution samples were first dispersed on TEM Cu grids pre-coated with thin holey carbon film (Cu-400 HN) purchased from Pacific Grid Tech. After completely dried, they were digitized using a FEI Tecnai TF20 FEG TEM equipped with a Gatum 4 k UltraScan CCD camera. For scanning electron microscopy (SEM), it was obtained in a FEI Quanta 450 FEG SEM. All the experiments were conducted at room temperature. Phosphate buffer solution (PBS) (0.1 M) of pH 7.4 solution was prepared with 0.15 M KCl as the supporting electrolyte, and KH_2PO_4, K_2HPO_4, and deionized water were used in appropriate portions.

2.2. Preparation of the 3D layer-by-layer hybrid structure

The 3D layer-by-layer nanostructure was prepared as described in supporting information. The characterization of the 3D layer-by-layer structure will be discussed in Section 3.1. Fig. 1 shows steps of the synthesis of this 3D layer-by-layer hybrid structure.

2.3. Electrochemical measurements

Prior to experiments, PBS was first deoxygenated with nitrogen gas. The structure and dimensions of this biosensor prototype is shown in Fig. 2. Solutions of GO, GO-Cys, GNR, GO-Cys-GNR were drop cast on the working electrode. During preparation, the concentration of GNRs in solutions of GNR and GO-Cys-GNR was maintained same (ca. 0.02 mg/ml). After drying, PBS solution containing various amount of H_2O_2 was drop casted (6 μl) on the center part covering all the electrodes. A work station (CHI 660C, CH Instrument, Inc., Austin, TX) was used for cyclic voltammetry and amperometry investigations. Cyclic voltammetric studies were arranged over a voltage range of −0.2 V to + 1.2 V versus Ag/AgCl reference with a voltage scan rate of 0.1 V/s.

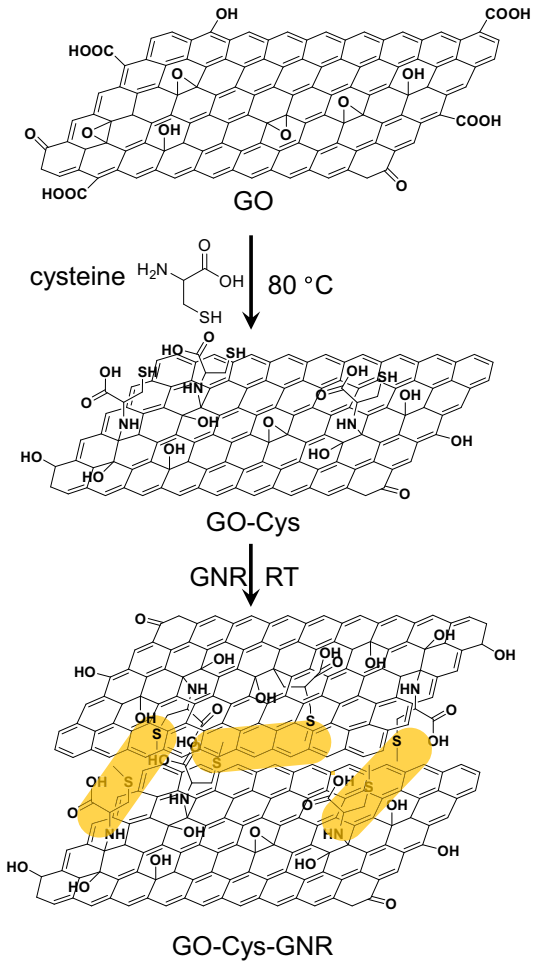

Fig. 1. Fabrication of 3D layer-by-layer hybrid structure.