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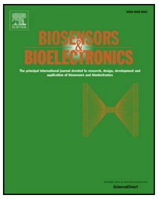

Multiple signal-amplification via Ag and TiO_2 decorated 3D nitrogen doped graphene hydrogel for fabricating sensitive label-free photoelectrochemical thrombin aptasensor

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ABSTRACT

In this study, Ag/TiO_2/3D nitrogen doped graphene hydrogel (3DNGH) was prepared for the first time and the photocurrent intensity of this material was greatly enhanced, which was attributed to the multiple enhancements accomplished in one step. The porous structure of 3DNGH could provide an exceptionally large accessible surface area, which was beneficial for the anchoring of Ag and TiO_2 nanoparticles. The introduction of nitrogen doped graphene and metal nanoparticles was capable to facilitate the charge separation efficiency and accelerate the transfer rate of the photogenerated electron-hole pairs. Then the photoelectrochemical performance was further amplified by the localized surface plasmon resonance of Ag nanoparticles. On the basis of excellent PEC properties of Ag/TiO_2/3DNGH, a sensitive label-free PEC sensor has been established for the determination of thrombin successfully. This proposed PEC biosensor exhibited good PEC performances with a wide linear in the range from 0.01 p.M. to 10 p.M. as well as a relative low detection limit of 3 fM (S/N = 3), indicating that Ag/TiO_2/3DNGH would serve as a promising photoactive material in the applications of PEC biosensors.

1. Introduction

Recently, photoelectrochemical (PEC) sensor is a novel developed and potential analytical technique, which is based on the relationship between the changes of photovoltage/photocurrent and the concentration of target analyte under light irradiation (Wang et al., 2014a; Zhang et al., 2014b). PEC sensors have attracted considerable attention owing to high sensitivity, low background current, rapid measurement speed and inexpensive platform (Freeman et al., 2013; Zhao et al., 2014; Zhou et al., 2015). It is well known that excellent photoactive materials play a crucial role in the applications of PEC sensors. As a kind of semiconductor material, TiO_2 has been attracted much attention owning to its long term stability, non-toxicity, strong oxidizing activity, and good biocompatibility (Bian et al., 2014; Liu et al., 2013). However, the low absorption efficiency in the visible light and the recombination of photogenerated electron-hole pairs seriously impede their PEC application (Fan et al., 2016a, 2016b). Consequently, significant efforts have been devoted to enhance the photoactivity of TiO_2 in the visible light region. Noble metals such as Ag, Au nanoparticles (NPs) can effectively accelerate the electron transfer and reduce the recombination rate of the photogenerated electron-hole pairs due to the localized surface plasmon resonance (LSPR) (Mubeen et al., 2011). Meanwhile, integrating graphene with TiO_2 nanoparticle was regarded as an efficient approach to improve the photoactivity performance of TiO_2 (Fan et al., 2011; Song et al., 2012).

Graphene, as a two-dimensional sp^2-hybridized carbon nanosheet, possesses a high specific surface area and high thermal conductivity (Liu et al., 2016; Wang et al., 2012). In addition, graphene has high mobility of the charge carriers, which can strongly improve the electrical conductivity of those graphene containing metal oxide nanocomposites (Liu et al., 2014; Wang et al., 2011). However, most of the composite materials with two-dimensional structure restrict the improvement of the specific surface area. Assembling graphene nanosheets into 3D architectures has been considered as one of the most promising methods to solve the above issues. Compared with 2D graphene, the three-dimensional (3D) graphene structure not only possesses the peculiar properties of individual graphene building blocks, but also has an exceptionally large accessible surface area to increase active sites for immobilizing nanomaterials, which has been widely applied in photocatalysis (Jiao et al., 2015). gas sensing (Guo et al., 2016), supercapacitors (Jiang et al., 2016b) and other electrochemical applications (Li et al., 2017). For example, Wang et al fabricated Co_3O_4 nanoflower (NF) /graphene oxide hydrogels hybrid structures, the hydrogel provided the large surface area that could disperse the Co_3O_4

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without agglomeration and facilitated the glucose redox reaction on Co_3O_4 NFs. 3D-graphene aerogels (GAs) could better adsorb organic pollutants and could provide multidimensional electron transport pathways (Le Thuy et al., 2016). Meanwhile, nitrogen doping can further facilitate charge transfer with adjacent carbon atoms and suppress the electrons and holes recombination (Liu et al., 2015; Meng et al., 2013). Thus, it is meaningful to explore the integration of Ag, TiO_2 and 3D nitrogen doping graphene hydrogel (3DNGH) and studied the performances of Ag/TiO_2/3DNGH.

Thrombin, as one of the immanent proteins in human, can convert soluble fibrinogen into insoluble fibrin. Over-expression or activity imbalance of thrombin can generate many diseases such as thrombosis, inflammation, atherosclerosis, and hemophilia (Li et al., 2014b, 2010). Therefore, it is very valuable to accurately detect thrombin concentration in the disease diagnosis and drug discovery. Several studies have reported the fabrication of PEC thrombin aptasensor with high PEC active nanocomposites, such as g-C_3N_4/TiO_2 (Fan et al., 2016a, 2016b) and graphene-CdS (Li et al., 2015a, 2015b), etc. To further improve the sensitivity, we studied the synthesis of Ag/TiO_2/3DNGH via a simple one-pot hydrothermal process for the first time and the photocurrent intensity was greatly enhanced due to the multiple signal amplification. The porous structure of 3DNGH could provide an exceptionally large accessible surface area for the anchoring of Ag and TiO_2. The introduction of nitrogen doped graphene and metal nanoparticles was capable to facilitate the charge separation efficiency and accelerate the transfer rate of the electron-hole pairs. Then the photoelectrochemical performance was further amplified by the LSPR of AgNPs. On the basis of excellent PEC properties of Ag/TiO_2/3DNGH, a sensitive label-free PEC sensor has been established for the determination of thrombin successfully with a wide linear in the range from 0.01 p.M. to 10 p.M. as well as a relative low detection limit of 3 fM (S/N = 3, $R^2 = 0.98$), indicating that Ag/TiO_2/3DNGH would be a promising material for PEC sensing.

2. Experimental section

2.1. Reagents and chemicals

Graphite was purchased from Qingdao Tianhe Graphite Co., Ltd. Graphene oxide (GO) was synthesized according to a modified Hummers method.[1] $Ti(SO_4)_2$, glycine(Gly), $AgNO_3$ and N, N-dimethylformamide (DMF) were obtained from Sinopharm Chemical Reagent Co., Ltd. Glucose oxidase (GOD), acetylcholine esterase (AchE), Lysozyme and Thrombin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Thrombin aptamer (TBA): 5´-SH-$(CH_2)_{10}$-GGT TGG TGT GGT TGG-3´was synthesized and purified by Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and Bovine serum albumin (BSA) was purchased from Aladdin Chemistry Co., Ltd. 0.1 M PBS (pH 7.4) was prepared by mixing stock standard solutions of NaH_2PO_4 and Na_2HPO_4. Double-distilled water was used throughout the study. Other reagents were of analytical grade and used as received without further purification.

2.2. Apparatus

The crystal structure identification was determined by X-ray diffraction (XRD) using a Bruker D8 diffractometer with high-intensity Cu Kα radiation (l = 1.54 Å) in the range of 2θ = 10-80º. The transmission electron microscopy (TEM) image was taken with a JEOL2100 transmission electron microscopy (JEOL, Japan) and scanning electron microscopy was conducted using (SEM, JEOL JSM-6700, Japan) equipped with an energy-dispersive spectroscopy (EDS, Oxford Inca Energy 400, UK). Raman spectra were surveyed with (RM 2000 microscopic confocal Raman spectrometer, England). X-ray photoelectron spectroscopy was performed on X-ray photoelectron spectrometer (PHI 5000 VersaProbe, Japan). All PEC measurements were performed with a CHI660B electrochemical analyzer (Chen Hua Instruments, Shanghai, China) and carried out in 0.1 M phosphate buffer (PBS) at 0 V and a 250 W Xe lamp (CHFXM 35-500W, Beijing Chang tuo) was utilized as the irradiation source (passing through a 400 nm UV-cut filter). Electrochemical impedance spectra (EIS) were conducted using ZENNIUM electrochemical workstation and the reference electrode (saturated calomel electrode), the counter electrode (platinum electrode), and the working electrode were connected respectively. Then, the working electrode, the reference electrode and the counter electrode were immersed in 0.1 M KCl solution containing 5 mM $Fe(CN)_6^{3-/4-}$ with the frequency range from 0.01 Hz to 10 kHz at 0.23 V, and the amplitude of the applied sine wave potential in each case was 5 mV.

2.3. Preparation of Ag/TiO_2/3DNGH hydrogels

Ag/TiO_2/3DNGH hydrogels were synthesized by one-step hydrothermal treatment. Typically, 5 mL (4 mg/mL) graphene oxide (GO) solution was dispersed in 5 mL water with sonication for 1 h to obtain a homogeneous suspension. Then, 100 mg Glycine (Gly), 0.24 g $Ti(SO_4)_2$ and 30 mg silver nitrate were added into the above GO solution (The silver content was optimized as presented in Fig. S1A.). After sonicated for 1 h, the above suspension was transferred into a Teflon-sealed autoclave and heated at 180 °C for 12 h. The synthetic hydrogels (Ag/TiO_2/3DNGH) were washed with water and then stored in pure water. For comparison, TiO_2/3DNGH was also prepared under the same condition without $AgNO_3$. And the Ag/TiO_2/graphene hydrogels (Ag/TiO_2/3DGH) were synthesized without Gly. Finally, the hydrogels samples were freeze dried at −37 °C for 2 days. The pure TiO_2 and the Ag/TiO_2/NG nitrogen doped graphene were synthesized by using the method as described in earlier report (Jiang et al., 2016a).

2.4. Fabrication of the modified electrodes

Primarily, the ITO electrodes were obtained by a series of pretreatment according to previous reports (Jiang et al., 2016a). 2 mg mL^{-1} Ag/TiO_2/3DNGH suspension was prepared by dispersing 2.0 mg of Ag/TiO_2/3DNGH in 1.0 mL of DMF with ultrasonic agitation. Then, 20 μL of suspension obtained was dropped onto ITO slice with a fixed area of 0.5 cm^2 and dried under an infrared lamp. After that, in order to better fix the material and prevent the material from falling off during the experiment, 10 μL of chitosan solution was covered on it, and also dried in ambient air to obtain modified ITO (Ag/TiO_2/3DNGH/ITO) electrode. For comparison, TiO_2/ITO, TiO_2/3DNGH and Ag/TiO_2/3DGH modified ITO electrodes were obtained with above similar procedure.

2.5. Fabrication of the aptasensor for thrombin

The PEC aptasensor fabrication process was illustrated in Scheme 1 as follows: 10 μL of 5 μmol L^{-1} thrombin aptamer (TBA) solution (The optimized concentration of aptamer as shown in Fig. S1B.) was dropped onto the surface of Ag/TiO_2/3DNGH modified ITO electrode and then allowed to react at 4 °C for 12 h to ensure that aptamer was successfully immobilized on Ag/TiO_2/3DNGH/ITO via the covalent bond formed between sulfydryl group of TBA and Ag. After the reaction completed, the electrodes were rinsed with PBS to remove physically absorption. Subsequently, the electrodes were covered with 10 μL of 1% bovine serum albumin (BSA) for 1 h at 4 °C to deactivate the unreacted active sites on the electrode surface and then washed thoroughly. Then, the as-prepared aptamer Ag/TiO_2/3DNGH/ITO electrodes were incubated in 10 μL of 0.1 M PBS containing different concentrations of thrombin solutions for 40 min at 37 °C (see the Supplementary material; Fig. S1C and Fig. S1D). And thrombin was immobilized on the modified electrode surface by the specific recognition affinity reaction between TBA and thrombin, followed by thoroughly rinsing with PBS. Finally, the TBA aptamer modified Ag/TiO_2/3DNGH electrodes were successfully