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Shape-controlled ceria-reduced graphene oxide nanocomposites toward high-sensitive in situ detection of nitric oxide

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ABSTRACT

Nitric oxide (NO) is an important signal molecule released by most cancer cells under drug stimulation or/and disease development but it is extremely challenging to *in situ* while real-time sensitively detect NO due to its large diffusivity, low concentration and fast decay. Herein, shape-controlled reduced graphene oxide nanocomposing with ceria (rGO–CeO_2) was synthesized *via* hydrothermal reaction to construct a highly sensitive real-time sensing platform for NO detection. The crystal shape of CeO_2 nanoparticles in rGO–CeO_2 composites significantly affects the sensing performance of rGO–CeO_2, of which the regular hexagonal nanocrystal CeO_2 achieves the highest sensitivity (1676.06 mA cm^{-2} M^{-1}), a wide dynamic range (18.0 nM to 5.6 μM) and a low detection limit (9.6 nM). This attributes to a synergical effect from high catalytic activity of the specifically shaped CeO_2 nanocrystal and good conductivity/high surface area of rGO. This work demonstrates a way by rationally compose individual merit components while well control the nanostructure for a superior synergistic effect to build a smart sensing platform, while offering a great application potential to sensitively real-time detect NO released from living cells for diagnosis or/and studies of complicated biological processes.

1. Introduction

Real-time detection of bio-interesting molecules released from living cells are very critical to understand mechanisms of cellular functions and pathology while offering important applications in diseases diagnosis and drug discovery (Guo et al. 2012; Ma et al. 2014). Among these biomolecules, nitric oxide (NO) is an important bio-regulatory and signaling molecule associated with physiological and pathophysiological pathways (Lim et al. 2006; Malinski and Taha 1992), for example, regulating many biological processes including the central nervous system, cardiovascular tone, gastrointestinal tract, genitourinary system, immune process (Friedemann et al. 1996; Yang et al. 2010), antimicrobial agent, tumoricidal factor (Shin et al. 2007), Parkinson's disease (Ng et al. 2011) as well as asthma (Li et al. 2011). Nevertheless, the high diffusivity, low concentration and high reactivity of NO toward oxygen and metal-containing proteins in biological milieu make the real-time, quantitative detection very difficult (Kim et al. 2009; Woldman et al. 2009). Different strategies have been tried to ease bottlenecks of the real-time measurements of NO (Peng et al. 2008). Compared with chemiluminescence, an electrochemical sensing is able to real-time detect NO with simpleness, low-cost and miniaturization (Hu et al. 2012), and thus is very promising to real-time detect NO while avoiding damage of the cell metabolism and associated regulatory pathways of living cells.

To real time electrochemically detect low concentration of NO, a highly sensitive sensing platform is critical. Variety of materials have been employed to construct NO sensors for improvement of the sensitivity, such as graphene (Guo et al. 2012), carbon nanotubes (Chng and Pumera 2012), Pt (Park et al. 2012) and functionalized zinc oxide (Liu et al. 2009). Recently, ceria, CeO_2 containing a rare earth element has drawn research attention owing to its unique 4f shell electronic structure involved numerous transition modes for remarkable redox properties (Tang et al. 2005). Its different nanocomposites such as Au–CeO_2 (Si and Flytzani-Stephanopoulos 2008), mixed CeO_2–ZrO_2 oxides (Varez et al. 2006), Cu–CeO_2 (Bera et al. 2002), graphene–CeO_2 (Wang et al. 2011) and Pt–CeO_2 (Goguet et al. 2004) have been developed for broad applications. CeO_2 supported materials also exhibit excellent adsorption and reactivity toward NO (Dowding et al. 2012) and could be expected to be used for NO detection. However, it has not been reported yet.

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Graphene, a two-dimensional (2D) sheet of covalently bonded single-layer carbon atoms with superior physico-chemical properties for good conductivity and functionality (Lee et al. 2008; Huang et al. 2012). A rGO–CeO_2 nanocomposites could provide an ideal platform for real-time sensing of NO. However, the morphologies of rGO–CeO_2 have not been delicated tailored through the synthesis and its application for NO sensing. We synthesized different types of rGO–CeO_2 nanocomposites from GO and cerous nitrate via an alkaline hydrothermal process with controlling of the Ce^{3+}/OH^- molar ratio in two stages: initial nucleation of CeO_2 nuclei on rGO nanosheets and subsequent growth. TEM characterization shows that rGO–CeO_2 nanocomposite obtained with a Ce^{3+}/OH^- molar ratio of 1:3 has well-dispersed regular hexagonal nanocrystal CeO_2, and delivers the highest real-time sensitivity toward electrochemical detection of NO. The excellent performance could be ascribed to a synergistic effect from the composed CeO_2 and rGO, of which the former contributes to the high catalytic activity toward NO oxidation and the latter offers good conductivity and high surface area to greatly increase absorption sites for NO while enhancing electron transfer rate as well as high biocompatibility for cell attachment to *in situ* detect NO released from cells.

2. Material and methods

2.1. Reagents and chemicals

Cerium nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6H_2O$), sodium hydroxide (NaOH), polyvinylpyrrolidone (PVP, molecular weight 30,000), Acetylcholine (Ach), Hemoglobin (Hb), natural graphite, sulfuric acid (H_2SO_4), sodium nitrite ($NaNO_2$), hydrogen peroxide (30%), hydrochloric acid (HCl), potassium peroxydisulfate ($K_2S_2O_8$), phosphorus pentoxide (P_2O_5), potassium hypermanganate ($KMnO_4$) and potassium hydroxide (KOH) were obtained from Sigma-Aldrich. All other chemicals were purchased from Sigma and used as obtained. Deionized water was used throughout the experiments.

2.2. Apparatus

The electrochemical measurements were performed on a CHI660D electro-chemical work station (Shanghai, China). The conventional three-electrode system included a modified electrode as working electrode, a saturated calomel as reference electrode (SCE), and a platinum wire as counter electrode. Transmission electron microscopy was carried out on a JEM-2100 (TEM, Jeol, Japan). Scanning electron micrographs were studied with a scanning electron microscope (SEM, Hitachi, S-4800, Japan). The elemental compositions of the products were determined by energy dispersive X-ray spectroscopy (EDS, INCAX-Max250). X-ray powder diffraction (XRD) patterns were obtained using a XRD-7000 with Cu $K_{\alpha 1}$ radiation ($\lambda = 1.5406$ Å).

2.3. Synthesis of rGO–CeO_2 nanocomposite

In this strategy, graphite oxide was synthesized from natural graphite according to the Hummers' method with some modification (Guo et al. 2010). A mixed solution was prepared containing natural graphite (1 g) and H_2SO_4, $K_2S_2O_8$, P_2O_5 (weight ratio of 5:1:1), and the mixture was heated at 80 °C for 6 h. Then, the mixture was stirred overnight at room temperature and diluted with deionized water and filtered. The obtained filtered powder was dried at 80 °C under vacuum. After that it was added into H_2SO_4 (ice-cooling) solution and stirred for 15 min. Subsequently, $KMnO_4$ was slowly put into the mixture with ice-cooling under stirring. The mixture was heated at 35 °C for 6 h, and then slowly diluted with deionized water under an ice-cooling environment. After continuously stirred for 2 h at room temperature, the solution was mixed with hydrogen peroxide (30%) slowly and stirred for 30 min. Later, the mixture was diluted with deionized water and centrifuged. Graphite oxide was obtained through washing the centrifuged product with 10% aqueous HCl solution. At last, exfoliation was performed by sonicating 0.1 mg mL^{-1} graphite oxide aqueous solution under ambient condition for 60 min.

To obtain the reduced graphene oxide and ceria nanocomposites (rGO–CeO_2), a conventional alkaline hydrothermal method was performed. Briefly, 0.900 g of PVP, 0.434 g or 0.868 g of Ce $(NO_3)_3 \cdot 6H_2O$ and 500 μL GO solution (15.0 mg mL^{-1}) were dissolved in 30 mL of deionized water. Then NaOH were added into the mixture as precipitant salt with its amount ranging from 1 to 10 mmol. After stirred for 30 min under room temperature, the mixture was transferred into a 50 mL Teflon lined autoclave, which was heated at 180 °C for 24 h. The obtained precipitate was harvested by centrifuge and then washed several times with deionized water. Finally, the resultant products (rGO–CeO_2) were obtained by drying precipitates in an oven at 70 °C for 3 h. Besides, CeO_2 nanocrystals were synthesized with the same method except adding GO solution.

2.4. Fabrication of the modified electrode

NO sensing platform was prepared according to following procedure. Prior to use, glass carbon electrode (GCE, Ø = 3 mm) was carefully polished by 0.3 and 0.05 μm alumina, followed by successive ultrasonic with distilled water and ethanol for 2 min until a mirror like surface was obtained. Then, rGO–CeO_2 suspension (10.0 mg mL^{-1}) was prepared by dispersing rGO–CeO_2 power in deionized water with aid of ultrasonic. Subsequently, 5 μL rGO–CeO_2 suspensions were dropped on clean GCE surface and dried in room temperature to obtain rGO–CeO_2/GCE. The final electrode was applied to detect NO dissolved in PBS. For comparison, GO/GCE and CeO_2/GCE were also prepared with same procedure for preparation of rGO–CeO_2/GCE by replacing nanomaterials with GO or CeO_2, respectively. Scheme 1a and b illuminates the preparation process of rGO–CeO_2 composite and its electrocatalysis toward NO oxidation as well as real time monitoring of NO released from the living cells, respectively.

2.5. Cell culture and real time monitoring cell released NO molecules

Human lung carcinoma cells (A549) were cultured in a humidified incubator (95% air with 5% CO_2) at 37 °C with culture medium, which was prepared by mixing 1640 medium, 1% antibiotic and 10% fetal bovine serum in autoclaved deionized water and filtered.

A549 cells were cultured in a petri dish (Ø = 3 cm) with as-prepared culture medium for 24 h and controlled with different cell densities such as 5.0×10^4 $mL^{-1}$and 1.0×10^5 mL^{-1}. Real time monitoring NO molecules released from cells was performed by chronoamperometry. In order to ensure accuracy of measured NO concentrations, cell culture medium inside device was mildly stirred during cell released NO measurement.

3. Results and discussion

3.1. Properties of rGO–CeO_2 nanocomposite

The measured TEM images reveal that plain CeO_2 nanocrystals (Fig. 1a) have an average diameter of ~14–16 nm with