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PtW/MoS_2 hybrid nanocomposite for electrochemical sensing of H_2O_2 released from living cells

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ABSTRACT

Hydrogen peroxide (H_2O_2) as an important reactive oxygen species (ROS) is reactive and potentially harmful to cells, causing oxidation of lipids, proteins and DNA. Herein, we report a PtW/MoS_2 hybrid nanocomposite with ultrasensitive and highly specific response for the detection of H_2O_2 released from breast cancer 4T1 cells. Upon exposure to 5 nM of H_2O_2, the electrochemical response is still visible. This PtW/MoS_2 hybrid nanocomposite could be facilely synthesized through in-situ growth of PtW nanocrystals on the surface of MoS_2 nanosheets. The incorporation of PtW nanocrystals and MoS_2 nanosheets in conjunction with each other to form hybrid nanocomposite improves the selective interaction of H_2O_2 with sensing material surface, and further increases the sensitivity and selectivity of sensor.

1. Introduction

There is now little doubt that hydrogen peroxide (H_2O_2) functions as a signal in many biological processes from relatively simple bacteria to complex multicellular plants and animals, particularly in higher organisms (Stone and Yang, 2006). For example, an increase in cellular levels of H_2O_2 has been linked to cancer development (Chen et al., 2014). A key aspect of studying H_2O_2 in living systems involves ensuring that the concentration of H_2O_2 in experimental system should be in physiologic range. For mammals, the calculated physiologic H_2O_2 concentration may reach as low as 1 nM, and the maximal intracellular H_2O_2 concentration during peak generation would be 0.5–0.7 μM (Kulagina and Michael, 2003; Weinstain et al., 2014). In accordance with these calculated levels, the specific and precise detection of H_2O_2 at the cellular level would offer an opportunity to fully understand its roles in cellular physiology and further provide reliable diagnosis of pathological conditions. Until now, various H_2O_2 physicochemical sensing materials have been developed and extensively studied (Zhang et al., 2013; Zhang et al., 2014; Qu et al., 2016). Zhang et al. reported that covalently assembled graphene quantum dots/gold electrode exhibited good current responses to H_2O_2 with a limit of detection (LOD) down to 700 nM, and could be used for living cell H_2O_2 detection (Zhang et al., 2013). A sensitive graphene-Pt nanocomposite displayed good electrocatalytic reduction performance towards H_2O_2 in the range from 0.5 μM to 3.475 mM, and used to measure the release of H_2O_2 from living cells (Zhang et al., 2014). However, new materials with tuneable sensing capabilities leading to improved performance are still very desirable.

Being an ultrathin direct bandgap semiconductor, molybdenum disulfide (MoS_2) has been considered as a promising material with potential applications due to the presence of a bandgap and versatile chemistry, which makes it attractive in various applications including catalysis, sensing, energy storage and electronic devices (Jaramillo et al., 2007; Li et al., 2015; Yu et al., 2015). Furthermore, the intercalation of other electrode materials into this layered material to form hybrid nanomaterials system would be anticipated to lower the charge transfer resistance and induce more available reaction sites (Bonaccorso et al., 2015; Yin et al., 2013). Nevertheless, the hybrid nanostructures we built must possess many functions to be effective. Conductive nanomaterials, like metal nanoparticles make them suitable for acting as “electronic wires” to enhance the electron transfer on electrode surfaces, and as catalysts to increase electrochemical reaction rate (Zhang et al., 2012). Also, introduction of second metal into monometallic catalysts, may tune their geometric structure and electronic structure, and then generates synergistic effect (Kozlov et al., 2015). In comparison with other bimetallic electrocatalyst candidates, PtW offers several advantages: (i) the cost of electrocatalysts can be

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reduced dramatically due to the cheap price and relatively abundant reserves of W; (ii) W has high tolerances towards catalyst poisons (He et al., 2007; Xiong and He, 2006); and (iii) recent studies showed that W atoms can be the active sites for adsorption and activation of H_2O_2 (Xiong et al., 2010). Therefore, by tuning the morphology of PtW into monodisperse nanocubes, and then assembling with exfoliated few-layered 2D MoS_2 nanosheets, researchers could better govern their sensing performance. Also, this kind of hybrid nanostructure can tailor the dispersion density and uniformity of bimetallic PtW nanocrystals on the surface of MoS_2 nanosheets, and open up the interlayer space to allow for more reactants and products to penetrate efficiently into the hybrid film during sensing processes (Bai et al., 2011; Huang et al., 2014).

Our motivation of this work is to construct a non-enzymatic H_2O_2 sensor with high sensitivity, low detection limit and good specific response based on PtW/MoS_2 hybrid nanocomposite without the participation of bioactive enzyme. Initially, PtW/MoS_2 hybrid nanostructure was synthesized by a facile one step approach to *in-situ* grow PtW nanocrystals on the exfoliated MoS_2 nanosheets. Having been tuned into sensors, this kind of hybrid nanocomposite could be used for the specific detection of H_2O_2, an important signalling messenger in most living organisms.

2. Experimental

2.1. Preparation of MoS_2 nanosheets

Exfoliated MoS_2 nanosheets were prepared through liquid exfoliation of the bulk MoS_2 material under sonication, with the similar method as reported elsewhere (Thanh et al., 2014). Typically, 0.5 g MoS_2 was mixed with 100 mL KOH (50 mg/mL) solution. Then, the mixture was heated up to 80 °C, and maintained at this temperature for 24 h with stirring. After that the mixture was quickly cooled down and frozen immediately by liquid nitrogen. When the ice melted, the mixture was ultrasonicated for 4 h to form a black suspension. After the suspension was filtered and washed with deionized water and ethanol for several times, the final MoS_2 nanosheets could be obtained by drying in vacuum at 60 °C overnight.

2.2. Synthesis of PtW/MoS_2 hybrid nanocomposite

In this work, cubic PtW alloy nanocrystals were *in-situ* grown on the surface of MoS_2 nanosheets to construct PtW/MoS_2 composite nanostructures. The mass ratio of PtW bimetallic nanocrystals and MoS_2 nanosheets was experimentally optimized as 1:5. Firstly, 10 mL of oleylamine was added to a flask of 25 mL in volume. Then, the obtained MoS_2 (~ 0.30 g) was dispersed into the oleylamine solution, and the mixture was ultra-sonically treated for 30 min. The obtained suspension was heated up to 120 °C, maintaining at this temperature for 10 min. After that, 10 mM of $PtCl_2$ and 10 mM of $W(CO)_6$ were added together. When the reactants dissolved completely, the mixture was heated to 180 °C, and this reaction proceeded for 10 min at this temperature. Thereafter, the temperature was continuously increased to 200 °C, and the reaction was stopped after keeping at this temperature for 25 min. After the resulting mixture cooled to room temperature, the sample of black precipitate could be collected by centrifugation at 12,000 rpm for 5 min. The obtained sample was washed with hexane for several times and dried under vacuum at 60 °C overnight. Then, the final PtW/MoS_2 hybrid nanocomposite was obtained.

2.3. Sample characterization

The phase and crystal structure of PtW nanocrystals were measured through X-ray diffractometer (Rigaku-D/MAX-2550, Japan) with a Cu Kα radiation (λ = 1.5418 Å) as X-ray source. Diffraction data were collected at the scan rate of 5 °/min with the step width of 0.02° over 2θ ranging from 30° to 90°. Transmission electron microscopy (TEM) characterization was performed with a JEM-200CX operated at 120 kV. Element analysis mapping was obtained by scanning transmission electron microscopy (STEM) combined with energy dispersive X-ray spectrometry (EDXS), which were performed in a JEOL 2010 F microscope. Atomic force microscopy (AFM) characterization was performed on a Bruker instrument (MultiMode 8 system). Scanning electron microscopy (SEM) observation was carried out by Magellan XHR 400 L instrument (FEI, USA).

2.4. Preparation of PtW/MoS_2 nanocomposite modified electrode

The prepared PtW/MoS_2 nanocomposite (4 mg) was dispersed into the mixture of ethanol (200 μL) and Nafion solution (50 μL) by 30 min sonication to form a homogeneous ink. Then, 8 μL of the obtained ink was loaded onto a glassy carbon electrode (GCE) and dried at room temperature forming a uniform thin film.

2.5. Cell culture

4T1 cells (a mouse breast cancer cell line, provided by Institute of Nanochemistry and Nanobiology, Shanghai University) were grown to confluence in 25 cm^2 flasks supplemented with high-glucose RPMI 1640 and 10% fetal bovine serum, and incubated in a humidified incubator with 5% CO_2 and 95% air at 37 °C (Wang et al., 2015). Eighty percent of confluent cells were used in all the assays.

2.6. Electrochemical measurements

The electrochemical measurements were conducted in a lab-made electrochemical cell, using saturated calomel electrode (SCE) as the reference electrode, platinum mesh (1 cm^2) as the counter electrode and PtW/MoS_2 modified GCE as the working electrode. All the measurements were conducted in N_2 saturated 0.1 M phosphate buffer solution (PBS, pH = 7.4) at room temperature and ambient pressure.

For the detection of H_2O_2 released from 4T1 cells, a pellet that contained about 2×10^6 cells was resuspended into 20 mL of PBS (0.1 M, pH = 7.4) and the mixture was saturated with N_2. A potential of −0.25 V (*vs.* SCE) was applied to the PtW/MoS_2 nanocomposite modified electrode. N-formylmethionyl- leucyl-phenylalanine (fMLP, 97%, Aldrich) was added into the solution to stimulate cells generation of H_2O_2.

Electrochemical impedance spectroscopy (EIS) was performed in a solution containing 0.1 M KCl and 2 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ and plotted in the form of complex plane diagrams (Nyquist plots) with a frequency range of 0.001 to 10^6 Hz.

3. Results and discussion

In the preparation of PtW/MoS_2 nanocomposite, exfoliated MoS_2 nanosheets were firstly dispersed in oleylamine solution. After introduction of metal precursors and elevating the temperature, PtW nanocrystals will emerge from the solution and eventually attach onto the surface of MoS_2 nanosheets. Herein, PtW/MoS_2 sample prepared from the R_{wt} value of 1/5 was chosen for *in situ* decoration of MoS_2 nanosheets as illustrated