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Fabricating carbon-nanotubes-based porous foam for superoxide electrochemical sensing through one-step hydrothermal process induced by phytic acid

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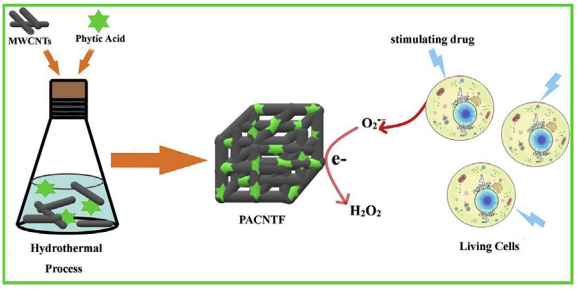
HIGHLIGHTS

- A facile way to fabricate carbon-based porous foam for $O_2^{\bullet-}$ sensing from widely-used carbon nanotubes.
- The improved activity of PACNTF was attributed to the enhanced defect and disorder degree.
- The recommended sensor successfully caught the transient signal of cell-released $O_2^{\bullet-}$.

GRAPHICAL ABSTRACT



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ABSTRACT

The detection of superoxide anions ($O_2^{\bullet-}$) is widely considered as a potential way for cancer diagnosis and the development of enzyme-mimic catalysts is the main challenge in the establishment of electrochemical sensors for $O_2^{\bullet-}$ sensing in real samples. Here we present a novel enzyme- and metal-free electrochemical catalyst for superoxide ($O_2^{\bullet-}$) sensing based on the widely-used carbon nanotubes (CNT). Through a one-step hydrothermal process induced by phytic acid (PA), CNT-based porous foam (PACNTF) was successfully obtained. Characterizations demonstrated the enhanced defect and disorder degree of PACNTF after PA treatment, which leaded to the increased active sites of PACNTF for electron transfer and the adhesion of $O_2^{\bullet-}$ during the electrochemical process. As a result, the PACNTF presented higher conductivity and larger current response toward $O_2^{\bullet-}$ sensing when compared with CNT precursor and CNTF without PA treatment. The sensitivity of PACNTF/SPCE was calculated to be 1230 $\mu A\,cm^{-2}\,mM^{-1}$ in the linear range of 0–193.6 μM ($R^2 = 0.965$) and 373 $\mu A\,cm^{-2}\,mM^{-1}$ in the linear range of 193.6 –1153.6 μM ($R^2 = 0.995$) with a limit of detection of 0.16 μM (S/N = 3). Further, the PACNTF/SPCE presented fast response toward cell-released $O_2^{\bullet-}$ stimulated by Zymosan A. The above results indicated that the fabricated sensor holds potential usage in biological samples.

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1. Introduction

ROS (reactive oxygen species), which are produced from oxygen in organisms, are demonstrated to be involved in various metabolism process such as immune clearance, the scavenging of aging and fading cells, the synthesis of thyroxine, prostaglandin and prothrombin, and so on [1–3]. Quick analysis and in situ monitoring of ROS has been regarded as an efficient method for the diagnosis of some ROS-related diseases such as cancers [4]. As reported in previous references, cancer cells usually release more ROS than normal cells do, and in turn, a high concentration of ROS could increase the risk of canceration by changing DNA expression and signal paths in living cells [5–7]. Among all kinds of ROS, superoxide anions ($O_2^{\bullet-}$) is generally regarded as the origin of some ROS such as H_2O_2. Meanwhile, the ability of $O_2^{\bullet-}$ to transfer through cell membrane has further increased its damage toward different normal cells. Therefore, in recent years, the monitoring of cellular $O_2^{\bullet-}$ has become one of the current hot spots [8–10].

Due to the short existing time of $O_2^{\bullet-}$ in aqueous solution, $O_2^{\bullet-}$ sensors used in real samples are always required with fast response and high sensitivity. Furthermore, the concentration of $O_2^{\bullet-}$ in biological system usually varies in a wide range of 10^{-8}~10^{-4} M [11,12]. Thus the established $O_2^{\bullet-}$ sensors should present low limit of detection as well as wide linear range to catch the signal of $O_2^{\bullet-}$ in biological samples. Among all kinds of methodologies developed for $O_2^{\bullet-}$ sensing, which includes fluorescent probes [13], electrochemical sensors [14], and electron paramagnetic resonance [15], the electrochemical sensors can provide real-time data in the in situ dynamic detection of $O_2^{\bullet-}$, being regarded as an ideal methodology for the monitoring of cellular $O_2^{\bullet-}$. The performance of electrochemical sensors is mainly determined by the electrochemical catalysts and functional nanomaterials modified on the working electrodes [16–20]. Most electrochemical catalysts used for $O_2^{\bullet-}$ sensing are based on enzymes such as superoxide dismutase (SOD) and cytochrome C currently, which usually present poor stability and reproducibility due to their unstable protein structure. Therefore, the exploration and application of non-enzymatic catalysts for $O_2^{\bullet-}$ dismutation is significant to enhance the performance of electrochemical $O_2^{\bullet-}$ sensors [21].

In previous researches about non-enzymatic electrochemical $O_2^{\bullet-}$ sensors, several kinds of enzyme-mimic inorganic catalysts have been developed mainly include metal oxide semiconductors [22], transfer metal phosphates [23] and noble metals [24]. However, the above mentioned catalysts are mostly limited by some unavoidable defects such as poor conductivity, time-consuming synthesis process and high costs. Apart from the above research, some carbon-based materials, such as carbon spheres and fullerene, have been demonstrated as exhibiting SOD-mimic activity with good electron transfer ability as well as satisfying biocompatibility [25,26]. Nevertheless, the design and synthesis of novel carbon-based catalysts for $O_2^{\bullet-}$ sensing is still highly required. Recently, some research have developed graphene-based catalysts for electrochemical sensing of different chemicals [27]. Based on these investigations, we further employed multi-walled carbon nanotubes (MWCNTs), which is another kind of widely-used carbon materials, to fabricate novel CNT-based electrochemical catalyst with enhanced conductivity and electrochemical catalysis activity for $O_2^{\bullet-}$ sensing in cellular environment.

In this work, phytic acid (PA) induced CNT-based foam (PACNTF) was successfully obtained through a one-step hydrothermal process. After treated with PA, the morphology of PACNTF was revealed by TEM to be porous stacked foam-like aggregation. With enhanced degree of defects and disorders in its 3D structure, the PACNTF was demonstrated to present better electron transfer ability and catalytic activity toward $O_2^{\bullet-}$ reduction when compared with CNT precursor and CNTF without PA treatment. The PACNTF was modified on home-made screen printed electrodes (SPCE) to detect $O_2^{\bullet-}$ in vitro with high sensitivity, excellent anti-interference and long-term stability. Furthermore, the PACNTF/SPCE was also successfully employed in the dynamic detection of drug-induced cellular $O_2^{\bullet-}$ from Hela cells.

2. Experimental

2.1. Chemicals and apparatus

Multi-walled carbon nanotubes (MWCNTs) were commercially pursued from Shenzhen Nanotech Port Co. Concentrated HNO_3 and H_2SO_4 were bought from Shanghai Lingfeng Chemical Reagent Co., Ltd. Phytic acid (70%), anhydrous dimethyl sulfoxide (DMSO), 18-crown-6 and KO_2 powder was obtained from Aladdin Industrial Inc. H_2O_2(30%), KH_2PO_4, K_2HPO_4, KCl, $NaNO_3$, K_2SO_4, $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were purchased from Sinopharm Chemical Reagent Co., Ltd. D-glucose, ascorbic acid (AA), uric acid (UA), 4-acetamidophenol (AP) and Zymosan A were pursued from Sigma-Aldrich. Dulbecco's modified Eagle's medium (DMEM) cell culture medium, penicillin/streptomycin, trypsin and fetal bovine serum (FBS) were provided by Gibco Invitrogen. All water used during the experiments was ultrapure water produced by a Laboratory Water Purification System (18.2 M cm). All the above chemicals were directly used without any further purification.

The morphologies of all products were characterized by a JEM-1400 transmission electron microscope (JEOL, Japan) with an accelerating voltage of 100 kV. An ESCALAB 250Xi X-ray photoelectron spectrometer (ThermoFisher, USA) was applied to reveal the elements of synthesized materials. Raman spectra were acquired using an InVia-Reflex Laser Micro-Raman Spectrometer (Renishaw). Ultraviolet-visible spectroscope (UV-Vis, CARY 300, Agilent Technologies) was used to determine the concentration of $O_2^{\bullet-}$in the KO_2 stock solution.

2.2. Synthesis of carbon-nanotubes-based foam

The commercially purchased MWCNTs were firstly pretreated with HNO_3 and H_2SO_4 before the synthesis of PACNTF [28–30]. Typically, 2 g CNT were dispersed in a mixture of 60 mL HNO_3 and H_2SO_4 (v/v, 1:1). Then, the above liquid was heated to 80 °C and refluxed for 2 h. After cooled down to room temperature, the mixture was carefully transferred into 2 L H_2O and stand still overnight. Finally, the supernatant was abandoned and the solid washed by using H_2O through filtration for several times. The obtained product was freeze-dried for further use.

The PACNTF catalyst was prepared according to the previous reference with some modification as shown in Scheme 1 [31]. The CNT contains abundant oxygen-containing groups which could connect with the phosphate groups of PA, forming compact 3D structures during the hydrothermal process. In detail, 0.12 g above treated CNT was dispersed in 60 mL H_2O and sonificated for 30 min. After that, 2.86 mL PA was added and the mixture was sonificated for another 40 min. Then, the dispersion was transferred into a 100 mL Teflon-lined autoclave and heated to 180 °C. After reacted for 12 h, the mixture was cooled down and washed by water and ethanol for several times and freeze dried for further use. As comparison, the CNTF was synthesized using the same procedure without the addition of PA.

2.3. Fabrication of modified electrodes

10 mg PACNTF was dispersed in a mixture of 6 mL water and 4 mL ethanol. After sonificated for 40 min, 6 μL of the dispersion

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