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A sensitively non-enzymatic amperometric sensor and its application in living cell superoxide anion radical detection

Xinhe Liu, Miaomiao Ran, Guoan Liu, Xiuhui Liu*, Zhonghua Xue, Xiaoquan Lu**

Key Laboratory of Bioelectrochemistry & Environmental Analysis of Gansu Province, College of Chemistry & Chemical Engineering, Northwest Normal University, Lanzhou 730070, PR China

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ABSTRACT

Here, we report a nanocomposite composed of silver nanoparticles and multi-walled carbon nanotubes (AgNPs/MWNTs) utilized as an efficient electrode material for sensitive detection superoxide anion ($O_2^{\bullet -}$). The procedure to synthesize AgNPs/MWNTs nanocomposites was green and facile. In the presence of functionalized multi-wall carbon nanotubes (MWNTs), silver nanoparticles (AgNPs) were in situ generated by chemical reduction of silver nitrate with glucose as a reducing and stabilizing agent to give the desired AgNPs/MWNTs nanocomposites. The nanocomposites can be easily used for the construction of an electrochemical sensor on glassy carbon electrode (GCE). The characterization of sensor and experimental parameters affecting its activity were investigated employing scanning electron microscopy (SEM), energy-dispersive X-ray spectrometer (EDS), X-ray diffraction (XRD), and cyclic voltammetry (CV). The resulted sensor exhibited favorable electrochemical performance for $O_2^{\bullet -}$ sensing with a low detection limit of 0.1192 nM and wide linear range of 6 orders of magnitude, which guarantees the capacity of sensitive and credible detection of $O_2^{\bullet -}$ released from living cells. Notably, a simulation experiment indicated the capacity to resist oxidative stress is limited in biological milieu. Thus this work has great potential for further applications in biological researches.

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the by-products resulting from the cellular metabolic processes. These reactive species play a dual role in human as both deleterious and beneficial compounds, and the delicate balance between their two opposite effects is undoubtedly a key aspect of life [1–3]. Superoxide anion ($O_2^{\bullet -}$), one of the most active ROS in micro-environment of the human body, has significant functions as a signaling mediator in the regulation of a variety of biological processes at low levels [4,5]. By contrast, excessive $O_2^{\bullet -}$ may cause oxidative damage to proteins, nucleic acids and lipids, resulting in mutagenesis, cell destruction and altered signaling pathways [6,7]. Oxidative stress, which is the result of an imbalance between ROS production and scavenging systems in cellular milieu, is involved in many human disease pathogenesis, such as neurological disorders, cardiovascular disease, autoimmune diseases and cancer progression [8,9]. In this context, developing sensitive and selective superoxide sensors has attracted increasing attention in recent years, with application for determining $O_2^{\bullet -}$ in living systems.

Up to date, all kinds of techniques and methods including spectrophotometry [10], electron spin resonance trapping [11], chemiluminescence [12], and fluorimetry [13] have been carried out for reliable detection of $O_2^{\bullet -}$. Besides these techniques, electrochemical technique which is also a fine detecting method has received extensive attention on account of the possibility for direct, real-time detection, simplicity and capability of in vivo detection [14,15]. Electrochemical sensors based on the immobilization of superoxide dismutase (SOD) on various substrates have been the topics of the most previous studies on this subject. These enzymatic sensors showed good selectivity and high sensitivity for the detection of $O_2^{\bullet -}$ [16–18]. Although these sensors have achieved a better analytical performance, some drawbacks still exist, for instance, chemical and thermal instabilities, poor reusability, and critical operating situation, which limited their further application in living systems [19]. Thus, non-enzymatic $O_2^{\bullet -}$ sensors have elicited much interest and need to be developed rapidly recently.

Electrocatalytic reduction of $O_2^{\bullet -}$ at non-enzymatic electrode seems

* Correspondence to: Northwest Normal University, No. 967 Anning East Road, Lanzhou, Gansu 730070, PR China.
** Corresponding author.
E-mail addresses: liuxh@nwnu.edu.cn (X. Liu), luxq@nwnu.edu.cn (X. Lu).

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to be an attractively alternative technique free from above-mentioned drawbacks. In general, non-enzymatic sensor uses catalytic activity materials to catalyze related substrates and carries on the qualitative and quantitative detection of sensing device. Various nanomaterials, such as carbon materials [20,21], metals [22,23] and metal oxide [24] had been applied in the field of non-enzymatic sensors because of their high surface reactivity, good biocompatibility, excellent electrical signal amplification and high catalytic performances.

Carbon nanotubes (CNTs), as typical nanomaterial, have recently captured considerable interest in electrochemical sensing since they possess excellent electric performances [25,26]. CNTs were used as a robust catcher for nanoparticles to form hybrid materials with improved properties. The decoration of CNTs with metal nanoparticles can effectively accelerate the electron transfer between electrode and detection molecules leading to a more rapid and sensitive current response [27]. Recently, many efforts have been focused on the design and preparation of CNTs/metal composites not only because the CNTs can improve the electric properties of composites, but also because the composites possess the property of a synergistic effect [28,29]. Among many metal nanoparticles, silver nanoparticles (AgNPs) exhibit outstanding catalytic performance for $O_2^{\bullet -}$ reduction, which have been used as an electrode material for the detection of $O_2^{\bullet -}$ [30,31].

In the present study, by integrating the advantages of carbon nanotubes and AgNPs, AgNPs/MWNTs nanocomposites were prepared by a simple one-pot synthesis method using glucose as reducing and stabilizing agent. The synthetic process was carried out only in aqueous solution, which is versatile and environmentally friendly. The AgNPs/MWNTs composites were then used to construct a novel electrochemical sensor, which could be applied to measure the release of $O_2^{\bullet -}$ from living cells under the stimulating of Zymosan A (Scheme 1). Considering the wide linear range, low detection limit, and fast response time of the sensor, we can rapidly determine the release of $O_2^{\bullet -}$ from living cells as potentially use for physiological and pathological studies.

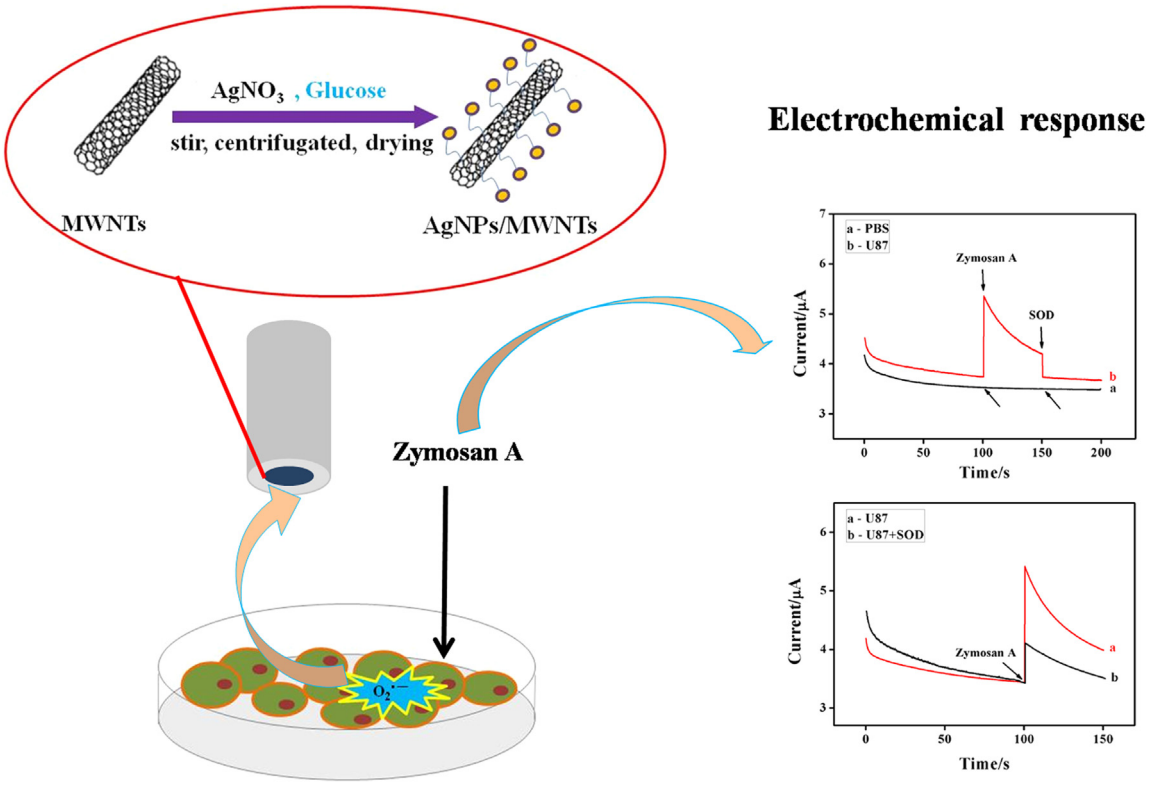
2. Materials and methods

2.1. Instruments

Scanning electron microscopy (SEM) images were acquired from JSM-6701F field emission SEM system (JEOL, Japan). Surface elemental composition of the synthesized samples was characterized by an energy-dispersive X-ray spectrometer (EDS). X-ray diffraction (XRD) patterns were recorded on a Rigaku x-ray diffractometer D/MAX-2400 (Rigaku, Japan). Electrochemical measurements were carried out on a CHI660C electrochemical workstation (Austin, TX, USA) with conventional three-electrode system. A bare or modified glassy carbon electrode (GCE, d = 3.0 mm) was employed as working electrode. A saturated calomel electrode (SCE) and a platinum electrode were used as the reference and counter electrodes, respectively. All potentials given in this paper were referred to the SCE. Electrochemical impedance spectroscopy (EIS) experiments were carried out on Multi-potentiostat (VMP2, Princeton Applied Research, USA). Ultraviolet-Visible spectrophotometer (UV–vis, EVOLUTION 220, Thermo Scientific) was used to detect the concentration of $O_2^{\bullet -}$ obtained from the KO_2 stock solution. Before each electrochemical measurement, solutions were thoroughly deoxygenated by bubbling nitrogen through the solution for at least 20 min to remove dissolved oxygen.

2.2. Reagents

The multi-walled carbon nanotubes (MWNTs) used (diameter: 20–40 nm, length: < 5 μm, purity: ≥ 96%) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). Potassium superoxide (KO_2) was obtained from Aladdin Industrial Inc. DMSO was purchased from Beijing Chemical Works (Beijing, China). 18-crown-6 was bought from Energy Chemical (Shanghai Chemical Industries, Ltd.). 4 Å molecular sieve was obtained from Tianjin Kermel Chemical Industries, Ltd (Tianjin, China). Zymosan A, 3-[(3-cholamidopropyl)



Scheme 1. Schematic of the AgNPs/MWNTs modified GCE used for detecting $O_2^{\bullet -}$ release from cells stimulated with Zymosan A.