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Paper–based analytical device for detection of extracellular hydrogen peroxide and its application to evaluate drug–induced apoptosis

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ABSTRACT

Developing cost-effective and simple analysis tools is of vital importance for practical applications in bioanalysis. Here, a disposable paper-based analytical device based on Au nanoparticles modified indium tin oxide electrode has been designed, which was applied for the reliable and non-enzymatic detection of H_2O_2. Due to the excellent electrocatalytic activity of Au nanoparticles, the disposable electrode exhibited favorable performance toward H_2O_2 reduction in the linear concentration range from 0.1 to 15 μM. The detection limit has been estimated to be 0.08 μM, which was lower than certain enzymes and other metal nanomaterials-based sensors. Because of these analytical advantages, the constructed device was used to study the extracellular H_2O_2 release from NB4 cells and further applied to evaluate sodium selenite induced apoptosis. The results obtained by electrochemical method are correlated well with the results of MTT assays. The developed paper-based sensor is easy-to-fabricate and portable, providing an effective platform for cellular H_2O_2 sensing and can be used to study the dynamic biological process involving H_2O_2 in biological and biomedical applications.

1. Introduction

Developing a rapid and accurate detection assay of hydrogen peroxide (H_2O_2) in biological samples is of broad interest in the fields of biology and biomedicine [1–3]. As a major reactive oxygen species in living organisms, H_2O_2 is produced by almost all oxidases in mitochondria and its appropriate level is essential for intracellular signaling transduction and normal cell functions [4,5]. But the excessive H_2O_2 quantity can induce different kinds of biological damages connected with lipid peroxidation [6], DNA damage [7] and tumor promotion [8], etc. Therefore, quantitative detection of H_2O_2 in cellular environment and monitoring its dynamic release process are essential to fully understand its roles in cellular physiology, and can further provide reliable diagnosis of pathological conditions.

Over the past years, considerable efforts have been paid on the development of electrochemical methods for determination of H_2O_2 in living cells [9,10]. Enzyme-based electrochemical biosensors have received considerable attention due to their good selectivity and sensitivity [11,12]. However, the enzyme-based biosensors are limited by some serious disadvantages, such as environmental instability, high cost, and a complicated immobilization procedure. Besides, the majority of other sensors for H_2O_2 detection usually use a glassy carbon electrode or Au electrode as working electrodes in a standard three-electrode system [13–15]. Unfortunately, these electrodes are limited by some serious disadvantages for analyzing biological samples. For example, the surface of electrodes could be easily contaminated during studies and the electrodes should be polished physically to obtain a clean and refreshed surface for the following detection. In addition, the treatment process requires complex operation and is time-consuming, which further hinders the practical applications of the sensors. To overcome these obstacles, simplified and disposable paper-based analytical devices could be explored as alternative to construct sensors for H_2O_2 detection.

Paper is an attractive substrate for the development of cheap, point-of-care sensors because it is easily available, portable, disposable and biocompatible [16–19]. The first paper-based sensor was reported in 1883 [20], and since that time the field has evolved from lateral flow assays to three-dimensional origami devices [21,22]. Researchers have put significant efforts into developing highly sensitive paper-based sensors for biological analysis. Crooks's group reported a self-powered aptamer-based

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origami paper analytical device for electrochemical detection of adenosine [23]. Noiphung et al. developed electrochemical paper-based analytical devices with integrated plasma isolation for determination of glucose from whole blood samples [24]. So far, it is believed that the paper-based electrochemical sensors could open more opportunities for real−time analysis of biological analytes that requires low sample volumes.

In the present work, we describe a paper-based electrochemical sensor for detection of extracellular H_2O_2 and its application to evaluate drug-induced apoptosis in NB4 cells. Indium tin oxide (ITO) was selected as the substrate electrode, which was further modified by Au nanoparticles (AuNPs) through electrochemical deposition. By coupling the AuNPs/ITO electrode in a paper-based analytical device, the electrochemical sensing of H_2O_2 can reach as low as 0.08 μM. Subsequently, the fabricated device was used to monitor H_2O_2 release from NB4 cells and further applied to evaluate sodium selenite induced apoptosis. The cellulose paper used here provided an effective strategy for cell-based electrochemical sensors. Living cells could be well trapped by the fiber matrix of paper and thus cells could be maintained in good conditions conveniently. In addition, the porous structure of paper allows external materials, such as stimuli, to reach cells without disturbance so that responses from cells could be well investigated. In this way, the developed paper-based sensor provided a useful platform for in vitro electrochemical sensing of H_2O_2 in living cells with high sensitivity, highlighting the potential application of such devices in cell biology study and drug screening.

2. Experimental

2.1. Materials and reagents

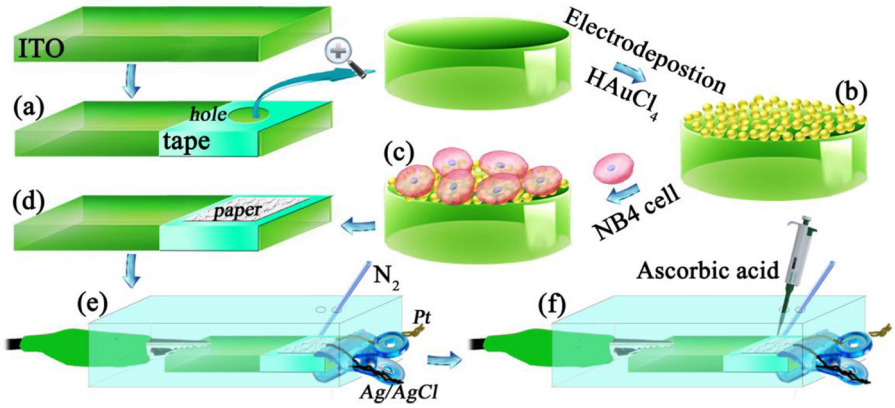
Hydrogen tetrachloroaurate trihydrate ($HAuCl_4$, 99%), ascorbic acid (AA, ≥99%) and sodium selenite (purity 98%) were purchased from Sigma (St. Louis, MO, USA) and used without further purification. H_2O_2 (30%), glucose, dopamine and urine was purchased from Shanghai Chemical Reagent Company and was freshly prepared before being used. Indium tin oxide (ITO) conductive glass (355.6 × 406.4 × 1.1 mm STN, 10 Ω/cm²) was purchased from Nanbo Display Technology Co. LTD (Shenzhen, China). The qualitative filter papers (Whatman No.1) were from Whatman International Ltd. (Maidstone, United Kingdom). Phosphate buffer saline (PBS, pH 7.4) containing 87.2 mM Na_2HPO_4, 14.1 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl was used as the electrolyte. A fresh solution of H_2O_2 was prepared daily. Other chemicals were of analytical grade. All the solutions were prepared with doubly distilled water.

2.2. Design and fabrication of the disposable electrode

ITO plate was cut into pieces of 20 mm × 10 mm to fabricate disposable electrodes. The ITO glasses were ultrasonically cleaned sequentially with acetone, absolute ethanol, and deionized water for 5 min, respectively, and dried with nitrogen gas. Subsequently, a section of plastic adhesive tape was punched with a circle hole (4 mm diameter) and attached on the ITO surface (Scheme 1a) to provide an identical detection area. Subsequently, the Au nanoparticles (AuNPs) were electrochemically deposited on the prepared ITO glass by cyclic voltammetry scanning from 0.2 V to −1.0 V in 0.1 M KCl solution containing 0.5 mM $HAuCl_4$ at a scan rate of 50 mV s^{-1} for 10 cycles (Scheme 1b). After rinsed clearly with twice-distilled deionized water, AuNPs/ITO electrode was developed and allowed to dry at room temperature. The UV–vis spectra were recorded on an UV-2450 spectrophotometer (Shimadzu, Japan). The scanning electron microscopy (SEM) images were obtained with a JSM-6510 scanning electron microscope (JEOL Ltd., Japan). Electronic dispersive spectroscopy (EDS) analysis was obtained by using a Hitachi S3400 SEM (Hitachi, Japan).

2.3. Electrochemical measurements

The electrochemical experiments were performed with a CHI1230B workstation (CH Instrumentation, Shanghai, China). A three-electrode system comprised the AuNPs/ITO as the working electrode, a platinum wire as the auxiliary electrode, and Ag/AgCl as the reference electrode. Sample solutions in pH 7.4 PBS with the volume of 10 μL was dropped on the electrode region of the AuNPs/ITO (Scheme 1c). Then a piece of Whatman filter paper with 6 mm long and 6 mm wide was put on the surface of the electrode region (Scheme 1d). The paper used here is to construct a thin-layer electrochemical cell based on its porous structure. It is notable that the paper could not only store the reagent solution but also electrically connect working, reference and counter electrodes for electrochemical detection. By coupling the three-electrode system in a cover made of elastomeric material, PDMS (polydimethylsiloxane), the paper-based analytical device was developed for voltammetric analysis (Scheme 1e). Cyclic voltammetry (CV) was measured with scan rate at 0.1 V/s. Differential pulse voltammetry (DPV) was performed with amplitude at 25 mV, pulse width at 0.2 s, pulse



Scheme 1. Schemes of stepwise fabrication of the analytical device. (a) A piece of punched adhesive tape with a circle hole (diameter: 4 mm) was attached on the ITO glass. (b) Electrodeposition of AuNPs on the electrode region. (c) NB4 cell suspension in pH 7.4 PBS with the volume of 10 μL was dropped on the electrode region. (d) A piece of filter paper with 6 mm long and 6 mm wide was covered on the hole. (e) An Ag/AgCl wire and a Pt wire were integrated with the ITO glass to form the electrochemical detection system. (f) Electrochemical analysis after addition of the stimuli.