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Real-time and in situ enzyme inhibition assay for the flux of hydrogen sulfide based on 3D interwoven AuPd-reduced graphene oxide network

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ABSTRACT

A highly sensitive enzyme inhibition analytical platform was established firstly based on paper-supported 3D interwoven AuPd-reduced graphene oxide (rGO) network (NW) for real-time and in situ analysis of H_2S released from cancer cells. The novel paper working electrode (PWE) with large electric conductivity, effective surface area and unusual biocompatibility, was fabricated via controllably assembling rGO and AuPd alloy nanoparticles onto the surface of cellulose fibers and into the macropores of paper, which was employed as affinity matrix for horseradish peroxidase (HRP) loading and cells capture. It was the superior performances of AuPd-rGO-NW-PWE that made the loaded HRP exhibit excellent electrocatalytic behavior to H_2O_2, bring the rapid enhancement of current response. After releasing H_2S, the current response would be obviously decreased due to the efficient inhibition effect of H_2S on HRP activity. The inhibition degree of HRP was directly proportional to the amount of H_2S, and so, the flux of H_2S released from cells could be recorded availably. Thus, this proposed enzyme inhibition cyto-sensor could be applied for efficient recording of the release of H_2S, which had potential utility to cellular biology and pathophysiology.

1. Introduction

Hydrogen sulfide (H_2S), as the third endogenously produced gaseous signaling molecule, plays an increasingly crucial role in a variety of physiological processes. (Yuan et al., 2015; Tang et al., 2014; Yue et al., 2015). The abnormal H_2S level in cells has been associated with diseases such as liver cirrhosis, Down's syndrome, Alzheimer's disease (Mao et al., 2013; Liu et al., 2016a; Zhao et al., 2014). Although several conventional methods, including colorimetric, electrochemiluminescence and fluorescence spectroscopy, have been proposed for the detection of H_2S at trace quantity levels (Choi et al., 2009; Li et al., 2015; Wang et al., 2013), it is urgent to develop a ultrasensitive, facile and rapid detection method for realizing in situ and reliable detection of H_2S in consideration of its high volatility and fast catabolism.

In comparison with traditional detection methods, electrochemical (EC) assay is more attractive due to its high sensitivity, low cost, simple instrumentation and good portability (Wang et al., 2014; Yan et al., 2016; Xie et al., 2015; Han et al., 2013; Liu et al., 2016b). To amplify the detection signal, enzyme, as intrinsic signal amplifier, is often considered because of their efficient catalytic property (Si et al., 2014). In enzyme inhibition sensor, the analytical signals can be amplified in an exponential manner by harnessing the remarkable catalytic ability of enzyme (Xianyu et al., 2013). Horseradish peroxidase (HRP) is extensively employed due to its exceptional performances (rapid response, high catalytic efficiency, good stability and biocompatibility) (Lai et al., 2011). Quantitative detection sulfides based on their inhibition effect on the catalytic ability of HRP is of considerable interest (Shan et al., 2010). Inspired by this detection method, a ultrasensitive, facile and rapid enzyme inhibition analytical format for detecting the release of H_2S from tumor cells (using MCF-7 cells as a model analyte) was proposed.

To further perform high-performance, sensitivity enhanced enzyme inhibition assay for H_2S, attention has been focused on the substrate of loading biomolecules and cells. Microfluidic paper-based analytical devices (lab-on-paper) have been widely used in cytological tests or histological studies (Li et al., 2015; Deiss et al., 2014). As we all know, paper is composed of interconnected cellulose fibers (CFs) network (NW) with macroporous structure. The

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abundant functional groups on CFs surface can provide strong interactive sites for binding carbon materials, noble metals and metal oxides (Hu et al., 2012; Liu et al., 2014; Zhang et al., 2014). However, the functional materials were only attached on the surface of CFs and the macropores of paper were not effectively utilized. To sufficiently utilize the specific texture of paper, a novel paper working electrode (PWE) was fabricated (details in Supporting information) on EC lab-on-paper cyto-device (ELPCD) (Scheme 1A and B) by controllably assembling reduced graphene oxide (rGO) and AuPd alloy nanoparticles (NPs) onto the surface of CFs NW and into the macropores of paper. The formed 3D interwoven NW structure and synergistic effects of rGO and AuPd NPs provided the PWE with superior performances in the analytical application.

In this work, a highly sensitive enzyme inhibition cyto-sensor was constructed based on 3D interwoven AuPd-rGO NW, and applied to measure the release of H_2S from cancer cells. The obtained AuPd-rGO-NW-PWE successfully integrating the specific texture of paper and the fascinating properties of rGO and AuPd alloy NPs, which was employed as affinity matrix for HRP loading and cells capture. When H_2S was generated upon the excitation of vascular endothelial growth factor (VEGF), the reduction current of HRP to H_2O_2 would be obviously decreased due to the efficient inhibition effect of H_2S on HRP activity. The inhibition degree of HRP was directly proportional to the amount of H_2S, and thus, the flux of H_2S could be recorded availably. Therefore, this work provided highly sensitive enzyme inhibition analytical platform for real-time and in situ assay of H_2S released from cells, which had potential application prospect in clinical assay of cellular H_2S.

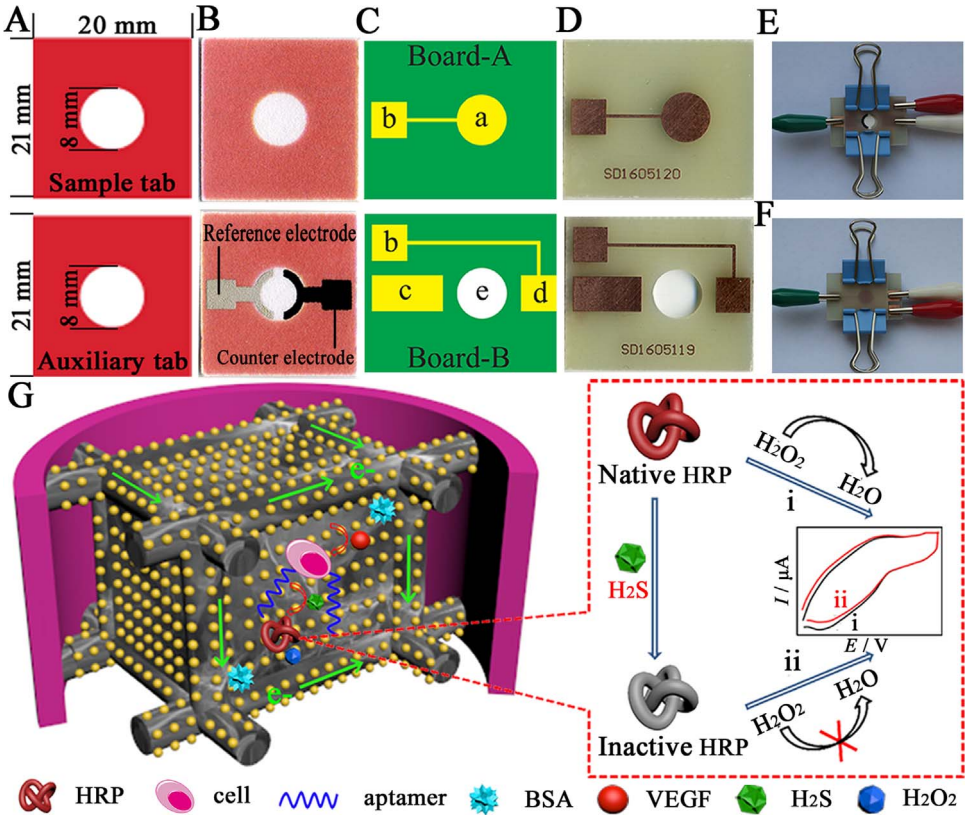
2. Experimental section

2.1. Fabrication of the ELPCD

The fabrication process of the ELPCD was shown in Scheme 1G. To begin with, 10 μL HRP (2 mg mL^{-1}) were applied into the AuPd-rGO-NW-PWE, and reacted at room temperature for 30 min, followed by washing to remove the physically absorbed excess HRP. Subsequently, 10 μL 4.0 μM aptamers were dropped onto the HRP/AuPd-rGO-NW-PWE and left for 30 min, and then 10 μL 1% bovine serum albumin (BSA) were added into the aptamer/HRP/AuPd-rGO-NW-PWE to block nonspecific active sites. After washing with phosphate buffered solutions (PBS), 10 μL homogeneous cells suspension at a certain concentration was dropped into the BSA/aptamer/HRP/AuPd-rGO-NW-PWE and incubated at 37 °C for 35 min to capture the cells. The obtained MCF-7 cell/BSA/aptamer/HRP/AuPd-rGO-NW-PWE was carefully rinsed with PBS to wipe out the noncaptured cells, and ready for subsequent characterizations and assays.

2.2. Inhibitive assay procedure of the ELPCD

During inhibited assay procedure, firstly, 20 μL PBS solution (pH 7.0) containing H_2O_2 (0.5 mM), as substrate electrolyte, was added into the paper EC cell through the hole in Board-B (Scheme 1E) to record the initial current response. Up the addition of VEGF into the substrate electrolyte, cells were stimulated by VEGF to release H_2S and the current response would be decreased due to the enzyme inhibition effect of H_2S. The flux of H_2S was measured by recording the degree of inhibition (Inhibition %) (Shan et al., 2010) variation, which was calculated by making a comparison between the current responses with and without addition of VEGF.



Scheme 1. (A) The schematic representation, size and shape of lab-on-paper device, (B) The picture of lab-on-paper device with screen-printed electrodes, (C) Scheme representation and (D) picture of home-made circuit boards, (E, F) The ELPCD was assembled with circuit boards, (G) Schematic representation of the fabrication procedures and EC analytical principle of this ELPCD.