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Non-enzymatic electrochemical biosensor based on Pt NPs/RGO-CS-Fc nano-hybrids for the detection of hydrogen peroxide in living cells

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ABSTRACT

A highly sensitive non-enzymatic electrochemical sensor based on platinum nanoparticles/reduced graphene oxide-chitosan-ferrocene carboxylic acid nano-hybrids (Pt NPs/RGO-CS-Fc biosensor) was developed for the measurement of hydrogen peroxide (H_2O_2). The RGO-CS-Fc nano-hybrids was prepared and characterized by UV–vis spectrum, Fourier transform infrared spectroscopy, transmission electron microscopy, Raman spectrometer and electrochemical impedance spectroscopy. Under optimal experimental conditions, the Pt NPs/RGO-CS-Fc biosensor showed outstanding catalytic activity toward H_2O_2 reduction. The current response of the biosensor presented a linear relationship with H_2O_2 concentration from 2.0×10^{-8} M to 3.0×10^{-6} M with a correlation coefficient of $R^2=0.9968$ and with logarithm of H_2O_2 concentration from 6.0×10^{-6} M to 1.0×10^{-2} M with a correlation coefficient of $R^2=0.9887$, the low detection limit of 20 nM was obtained at the signal/noise (S/N) ratio of 3. Moreover, the Pt NPs/RGO-CS-Fc biosensor exhibited excellent anti-interference capability and reproducibility for the detection of H_2O_2. The biosensor was also successfully applied for the detection of H_2O_2 from living cells containing normal and cancer cells. All these results prove that the Pt NPs/RGO-CS-Fc biosensor has the potential application in clinical diagnostics to evaluate oxidative stress of different living cells.

1. Introduction

Reactive oxygen species (ROS) is deemed to be the important intracellular signaling molecules which can regulate protein synthesis, DNA damage, cell apoptosis, and other living activities. It also participates in several physiology events such as signal transition and immunity activity (Chang et al., 2013; Zhang et al., 2014). However, accumulation of excess of ROS in cells result in sabotage of intracellular activity and generate oxidative stress which is considered as the root in diseases and caducity, giving rise to damaging the balance of normal cells and tissues (Wu et al., 2011; Le et al., 2015; Khodade et al., 2010). Hydrogen peroxide (H_2O_2) is the most common representative of ROS studied in cellular environments since H_2O_2 can access and distribute into cellular compartments for long lifetime to induce various harmful biological modifications (Borgmann, 2009). However, the concentration of H_2O_2 is rather low under normal physiological and can increase to $\sim10^{-3}$ M under pathological conditions such as traumatic brain injury ischemia-reperfusion, environmental stresses, and so on (Luo et al., 2009; Yu et al., 2015).

Therefore, the accurate detection of H_2O_2 in cells and measurements of its dynamic release process from living cells are important to clarify the mechanism of regulating signal transduction pathways and further exploit the potential application in clinical pathological diagnosis. So far, many techniques have been developed for the determination of H_2O_2, including colorimetry (Chen et al., 2014; Lin et al., 2015), fluorescence (Wen et al., 2011; Shi et al., 2014), chromatography (Gimeno et al., 2015), chemiluminescence (Yu et al., 2016), electrochemistry (Ma et al., 2015; Li et al., 2012; Chakraborty and Raj, 2009), and so on. Among these strategies, electrochemical techniques, especially the enzyme-based sensors, have been developed for the monitoring of H_2O_2 due to their high sensitivity and excellent selectivity. However,

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natural enzymes are sensitive to the environmental conditions and the instability of enzyme-based biosensors is inevitable.

Over the past few decades, great attention has been paid to the development of non-enzymatic electrochemical sensors because of their simplicity, high reliability, sensitivity, low cost and detect under the condition of without enzyme directly (Bianchi et al., 2014; Chen et al., 2010). In general, non-enzymatic sensor uses catalytic activity materials to catalyze related substrates and carries on the qualitative and quantitative detection of sensing device (Lee and Perk, 2010). Various nanomaterial, for example, metals (Chen et al., 2015; Qin et al., 2015), metal oxide (Lu et al., 2015) and carbon materials (Zhang et al., 2013; Sahin, 2015) had been used to fabricate non-enzymatic sensors due to their high surface reactivity, efficient charge transfer and excellent catalytic performances.

It is well known that noble metals exhibit outstanding electrocatalytic activity toward H_2O_2 redox reactions (Han et al., 2015). Particularly, Pt nanoparticles (Pt NPs) have been widely used as an excellent electrocatalyst for the detection of H_2O_2 since the presence of Pt NPs is able to trigger a strong electrocatalytic current for the evolution of hydrogen peroxide and lower the H_2O_2 oxidation/reduction overvoltage efficiently, which can easily avoid the anodic interferents like AA, UA (Chen et al., 2010). Moreover, some methods which decorating Pt NPs onto a second nanomaterials, such as Au, graphene, and so on, had been reported to improve the electrocatalytic activity and provide more active site for the anchoring of substrate in recent years (Le et al., 2015; Zhang et al., 2014; Dey and Raj, 2010).

Graphene, a novel two-dimensional graphitic carbon system, have attracted enormous attention in non-enzymatic sensor because of its large specific surface area, fast heterogeneous electron-transfer rate at the graphene sheet edges, and good biocompatibility (Xiong et al., 2014; Zhang et al., 2015). Especially, reduced graphene oxide (RGO), composited through the reduction of graphene oxide, has some functional groups such as carboxyl (–COOH) and hydroxyl (–OH) groups giving rise to the better conductivity (Liu et al., 2014). It is widely used in electrochemical catalysis of H_2O_2. However, due to the strong π–π stacking among the molecular, RGO would easily generate the reunion and interlayer, which will affect the dispersion and hinder the further applications (Wang et al., 2015). Recently, surface modification of RGO by coating of chitosan have gained increasing interest due to not only the better tenability of particle size, shape and high specific surface area for large loading of guest molecules, but also assisting the dispersion of RGO in aqueous solution (Qiu et al., 2011).

Ferrocene and its derivatives, a type of organometallic chemical compound consisting of two cyclopentadienyl rings bound on opposite sides of a central ferrum atom, are widely used to construct an electrochemical biosensor as electrochemical active tag for the assay of bio-molecules (Shen et al., 2015). In our previous work, we had successfully developed an electrochemical genotyping technique via gap ligation reaction and surface hybridization detection using ferrocene carboxylic acid, a derivative of ferrocene, as electrochemical probe (Huang et al., 2009). In addition, ferrocene and its derivatives are also used as electron transfer mediators in all kinds of sensors owing to their reversible redox behavior of Ferrocene → Ferrocene$^+$ (Saleem et al., 2015; Xie et al., 2015; Ghosh et al., 2015).

Herein, we presented a highly sensitive non-enzymatic biosensor for the detection of H_2O_2 based on the multi-lays of platinum nanoparticles/reduced graphene oxide-chitosan-ferrocene carboxylic acid nano-hybrids (Pt NPs/RGO-CS-Fc nano-hybrids). Taking advantage of the high electrocatalytic efficiency of Pt NPs, high electronic conductivity of RGO and the reversible electrochemical behavior of Fc, the integration of different ingredients combined merits of each component and yielded enhanced properties via a synergistic effect. To the best of our knowledge, this is the first report on the application of Pt NPs/RGO-CS-Fc nano-hybrids for the construction of a non-enzymatic electrochemical H_2O_2 biosensor. This non-enzymatic biosensor was further used to detect the H_2O_2 concentrations released from different kinds of cells stimulated by extracellular matters and to evaluate the oxidative stress of different living cells.

2. Experimental

2.1. Reagents

Graphene oxide (GO) was purchased from Xianfeng Nano Materials Tech Co. Ltd. (Nanjing, China). ferrocene carboxylic acid (Fc), N-hydroxysulfosuccinimide sodium salt (Sulfo-NHS), N-(3-dimethylaminopropyl)-N-ethyl-carbodiimide hydrochloride (EDC), mercaptopropionic acid (MPA), $RuCl_3 \cdot XH_2O$, glutaraldehyde, $HAuCl_4 \cdot 4H_2O$, $H_2PtCl_6 \cdot 6H_2O$, $Pd(NO_3)_2 \cdot XH_2O$, chitosan (CS), ascorbic acid (AA), uric acid (UA) and β-D (+)-glucose were purchased from Sigma (St. Louis, MO, USA), Human hepatocarcinoma cell lines (HepG2), human normal liver cell (LO2) and human lung cancer cell line (A549) were purchased from the Chinese Academy of Medical Sciences (Beijing, China). All other reagents were of analytical grade and used without further purification. All solutions were prepared with ultra-pure water of 18 MΩ purified from a Milli-Q purification system (Milli-Pore, Bedford, MA, USA).

2.2. Instruments

The morphologies and surface structures were characterized with scanning electron microscope (SEM, Zeiss Ultra55, Germany) and transmission electron microscopy (TEM, JEM-2100, Japan). Raman spectra were measured on a DXR Raman microscope (Thermo Fisher Scientific, USA). The Fourier transform infrared (FT-IR) spectra were recorded on a FT-IR spectrometer (Bruker Tensor27, Germany) using KBr pellets.

All electrochemical measurements were performed on a CHI 660 electrochemical analyzer (Shanghai Chenhua Instrument Corporation, China) at room temperature. A conventional three-electrode system was used with modified gold electrode (GE) as the working electrode, saturated calomel electrode (SCE) as the reference electrode, and platinum (Pt) electrode as the auxiliary electrode. The electrochemical measurements were carried out in the electrolyte solution of phosphate buffer saline (PBS, pH 7.4, 0.02 M Na_2HPO_4/KH_2PO_4 and 0.1 M NaCl) and the electrolyte was bubbled with highly purity nitrogen for 15 min prior to use. Cyclic voltammetric (CV) was carried out in PBS containing 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ from −0.2 to 0.6 V with a 100 mV/s scanning rate or 1 mM H_2O_2 from −0.4 to 0.8 V with a 100 mV/s scanning rate. Electrochemical impedance spectroscopy (EIS) was carried out in PBS containing 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ at 0.24 V (versus SCE) with a frequency range of 0.1–100 kHz. All amperometric measurements were regularly carried out at −0.05 V in PBS with stirring.

2.3. Preparation of RGO-CS-Fc nona-hybrids

RGO was prepared according to the previous literation reports with minior modifications (Guo et al., 2011). Briefly, 30 μl hydrazine solution was added to 10 ml GO suspension (0.5 mg/ml) and shaken for 10 min vigorously. Then the mixture was incubated in water bath at 60 °C for 4 h To isolate the RGO from the liquid, the mixed solution was centrifuged for 30 min at 20,000 r.p.m. Following the removal of the supernatant, the black oily precipitate, that is RGO, was obtained and then washed twice with water. Finally, the RGO