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Graphite paper-based bipolar electrode electrochemiluminescence sensing platform

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ABSTRACT

In this work, aiming at the construction of a disposable, wireless, low-cost and sensitive system for bioassay, we report a closed bipolar electrode electrochemiluminescence (BPE-ECL) sensing platform based on graphite paper as BPE for the first time. Graphite paper is qualified as BPE due to its unique properties such as excellent electrical conductivity, uniform composition and ease of use. This simple BPE-ECL device was applied to the quantitative analysis of oxidant (H_2O_2) and biomarker (CEA) respectively, according to the principle of BPE sensing−charge balance. For the H_2O_2 analysis, Pt NPs were electrodeposited onto the cathode through a bipolar electrodeposition approach to promote the sensing performance. As a result, this BPE-ECL device exhibited a wide linear range of 0.001–15 mM with a low detection limit of 0.5 μM (S/N=3) for H_2O_2 determination. For the determination of CEA, chitosan-multi-walled carbon nanotubes (CS-MWCNTs) were employed to supply a hydrophilic interface for immobilizing primary antibody (Ab_1); and Au@Pt nanostructures were conjugated with secondary antibody (Ab_2) as catalysts for H_2O_2 reduction. Under the optimal conditions, the BPE-ECL immunodevice showed a wide linear range of 0.01–60 ng mL^{-1} with a detection limit of 5.0 pg mL^{-1} for CEA. Furthermore, it also displayed satisfactory selectivity, excellent stability and good reproducibility. The developed method opened a new avenue to clinical bioassay.

1. Introduction

Recently, bipolar electrode (BPE) has attracted considerable attention due to its great practical value and wide applications, such as bipolar plate technology (Kim et al., 2016; Rafi-ud-din et al., 2016), asymmetric electrodeposition of materials (Bradley et al., 2005; Warakulwit et al., 2008), biosensing (Zhang et al., 2016), multi-component screening of electrocatalysts (Guerrette et al., 2013) and so on. BPE is an electronic conductor that can promote redox reactions at its two opposite poles without a direct ohmic contact (Fosdick et al., 2013). When a sufficient external potential is applied across the electrolyte solution where a BPE is immersed, a linear electric field forms in the electrolyte, and the interfacial potential difference between the solution and BPE triggers the faradic electrochemical reactions at the two poles (Liu et al., 2016). As no electron accumulation is allowed for BPE, the current passing through the two poles of BPE must be equal (Shi et al., 2014), and thus a quantitative relationship between the two reactions exists in this system, laying a foundation for analytical determination. Meanwhile, no requirement for a direct electrical connection confers many attractive advantages upon BPE technology, such as ease of integration into miniaturized devices and feasibility of controlling a large-scale bipolar array in parallel for high-throughput analysis (Zhai et al., 2016a).

In the field of chemical and biological analysis, bipolar electrochemistry is usually combined with electrochemiluminescence (ECL) (Shi et al., 2016), fluorescence (Oja and Zhang, 2014) and anodic dissolution (Chow et al., 2010) for signal readout. Among them, BPE-ECL analytical device has some overwhelming advantages, like high sensitivity with low background interference, simple operation process and low cost of instrument (Zhang and Ding, 2017). The miniaturized BPE device could also reduce the consumption of ECL luminophore, co-reactant and analyte. Moreover, BPE-ECL imaging is an important part in bioanalysis (Wu et al., 2013). In some cases, ECL luminophore/co-reactant may be incompatible with target analyte and cause mutual inference. Closed BPE-ECL is a good choice for it can physically separate them into two isolated compartments (Wu et al., 2016). Besides, as a powerful device, a closed BPE possesses high current efficiency (Zhai et al., 2016b).

To date, indium tin oxide (ITO) coated aluminosilicate glass slide, as a traditional BPE substrate, is widely used in BPE-ECL sensing platform owing to its excellent conductivity and superior transparency (Wu et al., 2015; Zhang et al., 2014). But the ITO band is constructed

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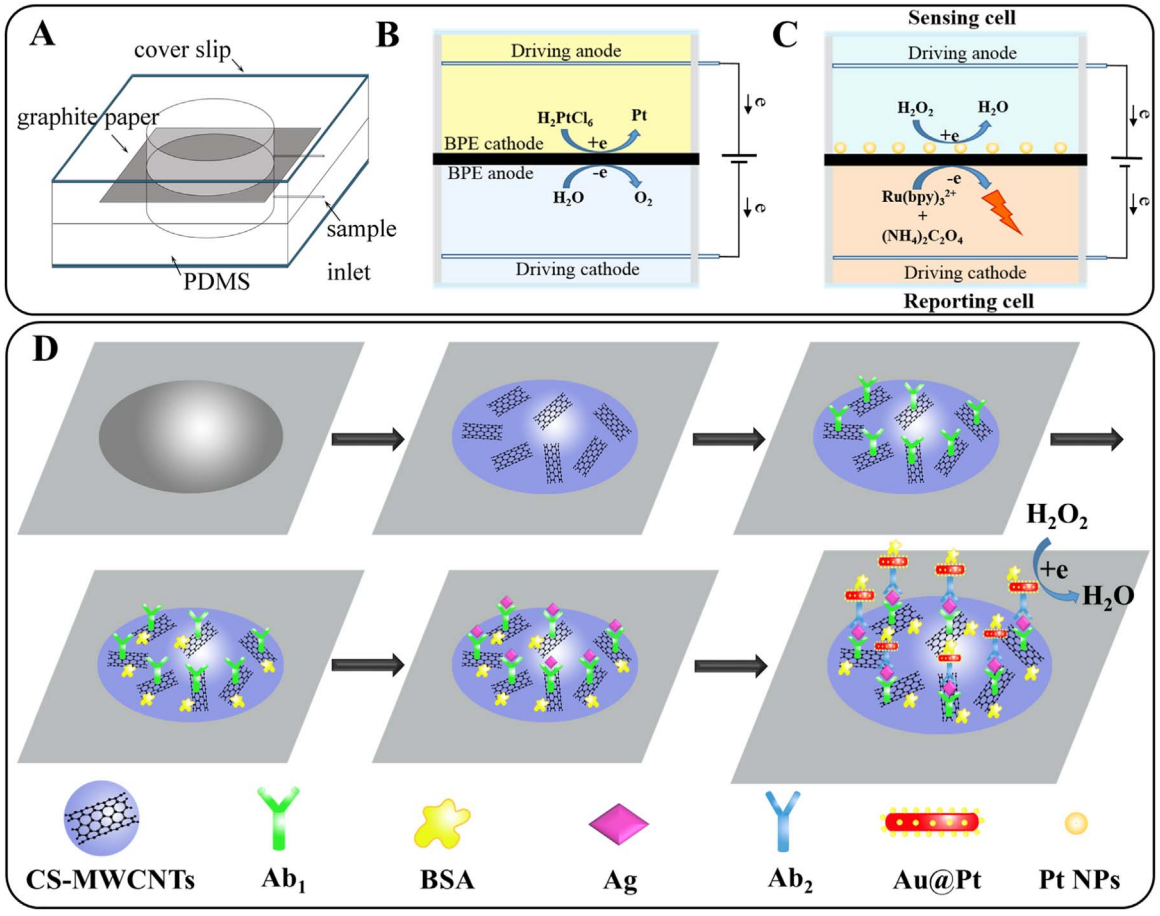
by photolithography and wet chemical etching techniques, which are complicated and time-consuming. In addition, SnO_2 at ITO is easily reduced under a relatively high driving voltage, causing the damage of ITO (Zhang et al., 2014, 2016). Au-based BPE is also favored due to its stability and reflectivity (Chow et al., 2009; Qi et al., 2016a, 2016b; Zhai et al., 2016b; Zhang et al., 2015), but its design is uneconomic and complicated, which limits its usage in routine application. To break the mentioned limitation, scholars have paid more and more attention on cheap and stable alternative—carbon substrate. In 2013, Guerrette et al. first fabricated a large-scale BPE array consisting of 1, 000 carbon fibers to achieve direct imaging of heterogeneous electrochemical process by fluorescence-enabled electrochemical microscopy (FEEM) (2013). Besides, conductive carbon ink was used in the fabrication of paper-based BPE-ECL biosensor by screen printing or inkjet printing (Feng et al., 2014; Liu et al., 2016). Molded carbon materials, like carbon rod and pencil core, were also applied in this field because of their satisfactory stability, good convenience and low cost (Eßmann et al., 2015; Lu et al., 2016). Recently, Rusling's group used a thin pyrolytic graphite wafer as substrate to design a microwell array for ECL detection of cancer biomarker protein, and even achieved automated microfluidic immunoarray (Kadimisetty et al., 2015; Sardesai et al., 2011). Inspired by these ingenious designs, we decided to find a new carbon substrate to fabricate a disposable, wireless, sensitive, and low-cost BPE sensing platform. Graphite paper (graphite foil) is a commercially available material made from compressed exfoliated mineral graphite, and it has been used as the matrix material in electrochemical energy storage (Jiao et al., 2016; Lee et al., 2016a, 2016b; Song et al., 2014). Cercado (2016) has reported graphite paper is a promising alternative that could be used for constructing bioelectrodes. However, little attention has been paid to the potential application of graphite paper in the fabrication of BPE device. Actually, graphite paper is suitable to prepare BPE device since its high uniformity and smoothness could boost the reproducibility of the analytical device. Meanwhile, high carbon content endows the flexible graphite paper with good electrical conductivity. In addition, it could be cut into required patterns easily just by scissors. More importantly, the electrical conductivity of double sides is favorable for the design of BPE sensing interface.

In this paper, a novel closed BPE-ECL sensing device was fabricated, which was made of graphite paper, PDMS and cover slip (Scheme 1A). Fabrication of the device only costs US $ 0.43 each, and the materials are commonly available in most research laboratories. As-prepared BPE device was then applied in the detection of different analytes (H_2O_2 and CEA). Prior to H_2O_2 detection, Pt NPs were electrodeposited onto the cathode to promote the sensing performance (Scheme 1B). According to the charge balance, the reduction rate of H_2O_2 at the BPE cathode equals to the oxidation rate of ECL luminophore and coreactant at the BPE anode, so H_2O_2 in the sensing cell could be quantitatively analyzed by measuring the ECL emission in the reporting cell (Scheme 1C). Visual detection could also be realized by this BPE device. A CEA sensor was also designed by constructing a sandwich immuno-structure at the cathode (Scheme 1D). Based on the excellent film-forming ability of chitosan, chitosan-multi-walled carbon nanotube (CS-MWCNT) composites were modified on the BPE cathode easily, supplying a hydrophilic interface for effective immobilization of primary antibody (Ab_1). And secondary antibody (Ab_2) was labeled with Au@Pt nanostructures, which possess excellent electrocatalytic activity for H_2O_2 reduction. In the presence of target CEA, Au@Pt-Ab_2 could be caught by CEA, and Au@Pt nanostructures could be attached to cathode surface. Au@Pt nanostructures



Scheme 1. Schematic representations of the graphite paper-based BPE device (A); Pt electrodeposition on the cathodic pole of BPE (B); H_2O_2 detection with Pt-graphite paper hybrid BPE device (C); and fabrication process at the cathodic pole of BPE for immunoassay (D).