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Electrochemical biosensor based on nanoporous gold electrode for detection of PML/RARα fusion gene

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ABSTRACT

In this study, a kind of nanoporous gold electrode (NPG) prepared with repetitive square-wave oxidation reduction cycle (SWORC) was reported. The active surface area of the proposed NPG electrode was 9.9 times larger than that of a bare flat one characterized by cyclic voltammetry (CV). An electrochemical DNA biosensor based on NPG electrode was fabricated for detection of promyelocytic leukemia/retinoic acid receptor α (PML/RARα) fusion gene in acute promyelocytic leukemia (APL) by using Methylene Blue (MB) as an electroactive indicator. Differential pulse voltammetry (DPV) was employed to monitor the hybridization reaction on the probe modified electrode. The decrease of the peak current of MB was observed upon hybridization of the probe with target DNA. The results indicated that the peak current was linear with the concentration of complementary strand in the range of 60 pM to 220 pM with a detection limit of 6.7 pM. This new biosensor exhibited excellent sensitivity and selectivity and had been used for an assay of PCR real sample with a satisfactory result.

1. Introduction

Electrochemical DNA biosensor, as a monitoring technique, is widely considered to be a promising way for diagnosis of genetic diseases and other biological analysis due to their rapid and sensitive response as well as the simple and convenient operation (Bagni et al., 2006; Lee et al., 2008; Liu et al., 2008). The introduction of nanomaterials and nanotechnologies has greatly improved the sensitivity and selectivity of electrochemical DNA biosensors (Hahm and Lieber, 2004). In recent years, high-surface-area nanoporous gold (NPG) has attracted increasing attention due to its fascinating properties such as superior conductivity, large surface area, high stability and biocompatibility. Several methods such as electrochemical deposition (Shin et al., 2003; Li et al., 2011), a liquid-crystal template technique (Luo et al., 2004), organic templating (Walsh et al., 2003; Zhang et al., 2004), "direct freezing" (Zhang et al., 2005), a voltage-induced dimension change method (Weissmuller et al., 2003), "dealloying process" (Jia et al., 2007) and repetitive square-wave oxidation reduction cycle (SWORC) (Gamero et al., 2010) were reported to prepare nanoporous gold. Among these methods, SWORC is very attractive due to its easy, time-saving and controllable preparation for nanoporous gold.

SWORC is an important method for the surface pretreatment which can easily and quickly obtain the roughening surface. Bilmes et al. (1989) reported the application of SWORC in the formation of roughened platinum electrode which was used for the enhancement of surface-enhanced Raman scattering (SERS). Cai et al. (1998) prepared roughened platinum electrode with different roughness factors by using repetitive SWORC and investigated the influences of roughening pretreatment on surface Raman signals, enhancement factors and surface homogeneity at roughened Pt electrode. Recently, Gamero et al. (2010) developed a biosensor for the detec-

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tion of lactate using a nanostructured rough gold surface prepared with repetitive SWORC as transducer.

Acute promyelocytic leukemia (APL) is a clonal expansion of hematopoietic precursors blocked at the promyelocytic stage (Raymond et al., 1993). APL is characterized by a 15;17 chromosome translocation with breakpoints within the retinoic acid alpha receptor α (RARα) gene on chromosome 17 and the promyelocytic leukemia (PML) gene, which encodes a putative transcription factor, on chromosome 15. The translocation creates a PML/RARα fusion gene which produces a chimeric protein. This fusion protein binds with enhanced affinity to sites on the cell's DNA, blocking transcription and differentiation of granulocytes (Goddard et al., 1991; de The et al., 1990; Pandolfi et al., 1991; Wang et al., 2011). Thus, the PML/RARα fusion gene is a biomarker of APL, and is also a gold standard in clinical diagnosis. The PML/RARα fusion gene has become diagnostic for APL, as it is present in almost 100% of cases (Borrow et al., 1990; Wei et al., 2009). Early diagnosis of this disease is essential because of the associated life-threatening coagulopathy and the unique response of the disease to all-trans retinoic acid (ATRA) therapy. The detection of this PML/RARα fusion gene will afford an early diagnosis and monitor of APL. The reported monitoring methods of clinical diagnosis and prognosis about PML/RARα fusion gene included chromosome analysis (Yoo et al., 2006), fluorescence in situ hybridization (FISH) (Amare et al., 2001), flow cytometry (FCM) (Tirado et al., 2003), real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) (Han et al., 2007), etc. But there were some limitations in these methods, such as time consuming, poor precision, and expensiveness. Thus, it was very significant to develop a new effective method to detect the PML/RARα fusion gene.

In the present work, an electrochemical DNA biosensor based on the nanoporous gold electrode prepared with repetitive SWORC was fabricated for the detection of PML/RARα fusion gene existed in APL. The nanoporous gold electrode was first covalently assembled with the probe DNA, followed by the hybridization with the PML/RARα fusion gene in APL. DPV was employed to monitor the variation of pre and post hybridization by using MB as electroactive indicator. This new pattern showed excellent sensitivity and specificity and had been used for assay of the PCR real sample with a satisfactory result.

2. Materials and methods

2.1. Materials and chemicals

All synthetic oligonucleotides designed according to the PML/RARα fusion gene were purchased from TaKaRa Biotechnology (Dalian, China) Co., Ltd. Their base sequences were as follows: immobilized probe (22-base sequence S1)-5′-SH-GGT CTC AAT GGC TGC CTC CCC G-3′; target DNA (22-base sequence S2)-5′-CGG GGA GGC AGC CAT TGA GAC C-3′; single-mismatched DNA (22-base sequence S3)-5′-CGG GGA GGC ACC CAT TGA GAC C-3′; partial PML (22-base sequence S4)-5′-CGG GGA GGC AGA GGA ACG CGT T-3′; partial RARα (22-base sequence S5)-5′-ATC CCC AGC CAC CAT TGA GAC C-3′. Ethylenediamine-tetraacetic acid (EDTA) and mercaptohexanol (MCH) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Shanghai Sangon Biological Engineering Technology Services Reagents Co., Ltd. (China). Methylene Blue (MB) was purchased from Aldrich. All other chemicals were of analytical grade.

Stock solutions of MB (1.0 mM) were prepared with deionized water. The buffers involved in this work were as follows: DNA immobilization buffer, 10 mM Tris–HCl, 1.0 mM EDTA, 10 mM TCEP (pH 7.4) and 1.0 M NaCl; hybridization buffer was 1 M NaCl and 10 mM PBS buffer (pH 7.4); washing buffer was 0.1 M NaCl and 10 mM PBS buffer (pH 7.4). All solutions were prepared with deionized water (18.2 MΩ cm resistivity) from a Millipore system.

2.2. Apparatus

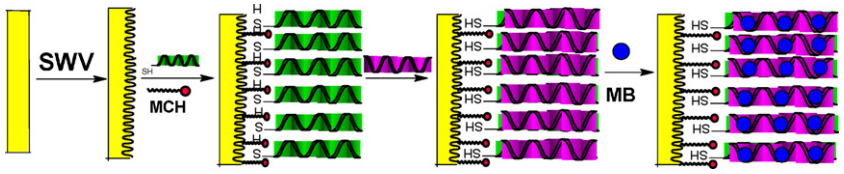
Electrochemical measurements were performed on a CHI 760D Electrochemical Workstation (CH Instrument, USA) using a traditional three-electrode system. The nanoporous gold electrode was used as working electrode, a platinum wire and Ag/AgCl electrode were used as counter electrode and reference electrode, respectively. All potentials herein were referred to this reference electrode. Differential pulse voltammetry (DPV) was carried out from −0.40 to 0.00 V with an amplitude of 50 mV. SEM images were obtained from XL-30E scanning electron microscopy (Philips, The Netherlands).

2.3. Fabrication of nanoporous gold electrode

A schematic representation of the novel sensor fabrication procedure was illustrated in Scheme 1. A gold electrode (2 mm in diameter) was polished before each experiment with 1.0, 0.3 and 0.05 μm alumina powder, respectively, and sonicated in ethanol and deionized water thoroughly. After electrochemically cleaning in fresh 0.5 M H_2SO_4 solution to remove any possible impurities on the surface (Fan et al., 2003), the gold electrode was then subjected to a repetitive SWORC procedure for its surface to be roughened. The procedure was carried out in two consecutive stages as follows: Firstly, a relatively thick hydrous gold oxide layer was accumulated on the gold electrode immersed in 0.5 M sulphuric acid by using repetitive square wave potential between −0.8 V and 2.5 V (versus Ag/AgCl electrode) at 2000 Hz for a time of 5 min. Subsequently, the potential was held at −0.8 V until the complete electroreduction of the hydrous gold oxide layer was accomplished (Bilmes et al., 1989), then the resulting nanoporous gold electrode was sonicated in deionized water for 1 min and cycled at 0.1 V/s between −0.35 and 1.5 V in 0.5 M H_2SO_4 solution to clean the surface of the electrode, and check variations in the electroadsorption charge of the O adatoms between the rough Au electrode and flat Au electrode.

2.4. Preparation of DNA biosensor based on NPG electrode

The cleaned NPG electrode was used for the preparation of the DNA biosensor by incubation in an immobilization buffer containing 10 μM capture probe modified with thiolate for 2 h at room



Scheme 1. Schematic representation of the fabrication procedure of DNA biosensor based on NPG-electrode.