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Low fouling label-free DNA sensor based on polyethylene glycols decorated with gold nanoparticles for the detection of breast cancer biomarkers

Wenting Wang [a], Xiaojian Fan [b], Shenghao Xu [a], Jason J. Davis [c,*], Xiliang Luo [a,*]

[a] Key Laboratory of Sensor Analysis of Tumor Marker, Ministry of Education, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China
[b] Department of Breast Surgery, The Eighth Peoples' Hospital of Qingdao, Qingdao 266100, China
[c] Department of Chemistry, University of Oxford, Oxford OX1 3QZ, UK

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ABSTRACT

A label-free and low fouling biosensor based on functional polyethylene glycols selective for breast cancer susceptibility gene (BRCA1) is reported. Sensory interfaces were prepared through the modification of a glassy carbon electrode with highly cross-linked polyethylene glycol (PEG) film containing amine groups, followed by the self-assembly of gold nanoparticles and the immobilization of BRCA1 complementary single-strand 19-mer oligonucleotides. In the presence of a specific BRCA1 sequence capture and hybridization results in interfacial change sensitively monitored using electrochemical impedance spectroscopy. The combined utilization of a PEG polymer film and gold nanoparticle mixed interface enables very high levels of sensitivity and a highly effective assaying in patient samples. Assay linear range was from 50.0 fM to 1.0 nM, with a limit of detection of 1.72 fM. Furthermore, this label-free DNA sensor has been used for assaying BRCA1 in serum samples, showing its feasible potential for diagnostic applications in clinical analysis of breast cancer gene BRCA1. Foreseeable, this sensor made on this basis undoubtedly provide the most effective and sensitive detection for BRCA1.

1. Introduction

Recently, genome-wide association studies have led to the identification of numerous genes associated with an increased cancer risk (Li et al., 2012). Most human breast cancers are associated with specific mutations in the BRCAl anti-oncogene sequence on chromosome 17 at band 21 (Miki et al., 1994; Venkitaraman, 2004) responsible for the production of tumor suppressor proteins. As one of the three most common invasive cancer in females (Parkin et al., 2005), breast cancer mainly takes place in the inner lining of the milk ducts or lobules with different spread, aggressiveness and genetic makeup, and it is one of the leading causes of cancer mortality among women worldwide (Jemal et al., 2009). The value of identifying BRCA1 gene mutations in facilitating early diagnosis and intervention is well known (Sega and Low, 2008; Yucai et al., 2009) and the subject of intensive study and technological development (Balapanuru et al., 2010; Swierczewska et al., 2012).

The sequence-specific detection of nucleic acid targets is, of course, relevant to a multitude of research areas including diagnostics and forensics (Sassolas et al., 2008; Shuang et al., 2009). In the past few years, a number of methods have been specifically applied to the determination of BRCAl, including high performance liquid chromatography (Arnold et al., 1999), fluorescence conformation sensitive gel electrophoresis (Ganguly et al., 1998), single-strand conformation polymorphism assay (Rashid et al., 2006) and RNA/DNA sequencing (Jakubowska et al., 2001). However, there are certain limitations such as low sensitivity, high cost, time-consuming and complex procedures that prevent the broad practical application of these techniques.

Label-free DNA hybridization biosensors have received great attention in the past because of the simple fabrication procedure, rapid assay time and low cost. Typically, the fabrication of these involves the immobilization of a capturing single strand DNA (ssDNA) probe on a transducer surface (Liu et al., 2014; Lu et al., 2008), complementary sequence hybridization with the target probe, and an analysis based on fluorescence (Gerion et al., 2003; Taton et al., 2001), mass (quartz crystal microbalance) (Patolsky et al., 2001), local dielectric (surface plasmon resonance) (D'Agata et al., 2010; He et al., 2000) or steric bulk/electrostatics/conformational change (electrochemistry) (Ferapontova et al., 2010;

* Corresponding author. Fax: +86 53284022681.
E-mail addresses: jason.davis@chem.ox.ac.uk (J.J. Davis), xiliangluo@hotmail.com (X. Luo).

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Xia et al., 2010). The latter were initially explored by Millan and Mikkelsen (1993), and have subsequently attracted considerable attention by virtue of low associated assay cost and time and high levels of sensitivity (Kimmel et al., 2012; Tiwari and Gong, 2009). One key parameter in the efficacy of these approaches is the quantity of immobilized and available capture sequence (Du et al., 2009). Gold nanoparticles (AuNPs) have often been presented as accessible, chemically stable and effective entities in supporting high levels of biomolecular immobilization (Daniel and Astruc, 2004; Dreaden et al., 2012; Niu et al., 2011), including that associated with thiol-functionalized DNA in underpinning sequence-specific DNA assays (Ai et al., 2009; Jans and Huo, 2012; Pei et al., 2012). Analysis in real biological samples like serum, where plasma proteins and salts will typically initiate severe nonspecific adsorption and particle aggregation is, though, challenging (Henry et al., 2010; Kim et al., 2007; Zhang et al., 2009). PEG, a nontoxic and hydrophilic polymer (Ekblad et al., 2008; Goodwin et al., 2009), has been extensively applied in minimizing the nonspecific adsorption of proteins (Otsuka et al., 2003; Samantha et al., 2010; Zhang et al., 2009), within a range of biosensor configurations (Eck et al., 2008; Simpson et al., 2011).

Here, we report a label-free DNA hybridization biosensor for the detection of BRCA1 related sequence. In this biosensor system, highly cross-linked PEG films containing amine groups was used as the antifouling substrate, and AuNPs were then self-assembled onto the surface of the PEG film for the further immobilization of the DNA recognition probe. Faradaic electrochemical impedance spectroscopy (EIS), a non-destructive, label free and highly sensitive technique that has been used for the detection of a number of disease markers (Luo and Davis, 2013; Xu et al., 2013), was utilized herein as the target recruiting transducer. The marriage of hybrid PEGylated polymer–gold nanoparticle interface with EIS offers the biosensor several advantages such as facile fabrication, high sensitivity and selectivity, and acceptable reproducibility, and the biosensor is thus of great potential for future clinical application.

2. Experimental

2.1. Materials and apparatus

The 19-mer synthetic oligonucleotides related to BRCA1 breast cancer gene (Mohan et al., 2010) were supplied by Sangon Biotech (Shanghai, China) Co., Ltd., purified by high-performance liquid chromatography and used as received. And their sequences were as follows:

Immobilized probe (S_1): 5′ GAT TTT CTT CCT TTT GTT C 3′-SH
Target probe (Complementary) (S_2): 5′ GAA CAA AAG GAA GAA AAT C 3′
One-base mismatch (S_3): 5′ CAA CAA AAG GAA GAA AAT C 3′
Three-base mismatch (S_4): 5′ CAA CAA AAG CAA CAA AAT C 3′
Non-complementary (S_5): 5′ CCT TGT TGG ACT CCC TTC T 3′

Stock solutions of oligonucleotides were prepared with PBS (0.2 M, pH 7.4, we use NaCl in the buffer considering the ionic strength of the buffer) and stored in a freezer at 4 °C before use. 4-armed PEG-epoxide and 4-armed PEG-amine with molecular weights of 2000 g/mol were purchased from Creative PEGWorks USA, were separately dissolved in chloroform with concentrations 30 mg/mL. AuNPs (diameter of ~20 nm) and bovine serum albumin (BSA) were purchased from Aladdin Reagents (Shanghai, China). All other reagents were of analytical reagent grade and used directly without further purification. Millipore water from a Milli-Q water purifying system was used throughout. All experiments were performed at ambient room temperature.

Electrochemical experiments, which mainly including EIS, was performed with a CHI 760D electrochemical workstation (Shanghai Chenhua Instrument Co. Ltd., China). All experiments were carried out in a conventional three-electrode system consisting of a platinum wire counter electrode, a silver/silver chloride (Ag/AgCl, filled with 3.0 M KCl) reference electrode and a glassy carbon working electrode (GCE, diameter 3.0 mm). EIS measurements were recorded in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl with the direct current potential set at 0.25 V. The amplitude of the applied sine wave potential was 5 mV, the frequency range was from 0.01 Hz to 100 kHz. All experiments were conducted at ambient temperature. Field emission scanning electron microscope (SEM) was performed with a JEOL JSM-7500 F SEM instrument (Hitachi High-Technology Co., Ltd., Japan).

2.2. Sensor surface modification

The preparation process is schematically summarized in Fig. S1. GCE was polished, cleaned and electrochemically pretreated in phosphate buffered saline (PBS) according to a previous report (Luo et al., 2007). The pre-treated GCEs were then drop-coated with 2.0 μL chloroform solution containing 1.0 mg/mL 4-armed PEG-amine and 0.6 mg/mL 4-armed PEG-epoxide by pipette prior to gentle heating in an oven at 80 °C for ~3 h under nitrogen atmosphere. A highly non-fouling cross linked hydrated polymeric network is formed across the electrode by this (catalyst and by-product-free) process (Meyerbröker et al., 2013). After washing and soaking in PBS (0.2 M, pH 7.4) for 12 h to remove non-cross-linked material and equilibrate the PEG film. PEG/AuNPs composite films were prepared by immersion of the PEG films into a freshly prepared (stored at 4 °C) solution of citrate-stabilized AuNPs for 6 h, followed by extensive rinsing with water and drying in a nitrogen stream. The obtained electrodes were denoted as PEG/AuNPs/GCEs. The PEG/AuNPs/GCEs were then incubated in 1.0 μM thiol-functionalized oligonucleotides solution (0.2 M PBS, pH 7.4) for 12 h to allow the covalent attachment of oligonucleotides to the surface of the AuNPs via the formation of Au–S bond. The obtained electrodes were denoted as S_1/PEG/AuNPs/GCEs.

2.3. Hybridization studies

The hybridization experiments were carried out by immersing the S_1/PEG/AuNPs/GCEs in PBS (0.2 M, pH 7.4) containing different concentrations of target analyte (S_2, S_3, S_4, S_5, BSA) for 2 h at ambient room temperature. And then, the hybridized electrode was next rinsed with PBS (0.2 M, pH 7.4) for several times to remove the non-specifically bound target analyte (S_2, S_3, S_4, S_5, BSA). The electrodes thus obtained were denoted as S_1–S_2 (S_3, S_4, S_5, BSA)/PEG/AuNPs/GCEs.

3. Results and discussion

3.1. SEM characterization of the modified electrodes

The morphology and microstructures of the prepared films (PEG and PEG/AuNPs) were characterized by SEM. As shown in Fig. 1, a layer of PEG was formed on the electrode, and the surface of the PEG film (Fig. 1a and b) was relatively uniform and smooth. After the self-assembly of AuNPs, a layer of nanoparticles with a diameter of approximately 20 nm were homogeneously distributed among the PEG film (Fig. 1c and d), indicating successful attachment of AuNPs onto the PEG film. Due to the rich terminal amine groups of the PEG film formed on the electrode, AuNPs can easily attach to the electrode surface through their interaction with the amine groups, providing a considerably large surface area