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Molecularly imprinted polymers-based electrochemical DNA biosensor for the determination of BRCA-1 amplified by SiO_2@Ag

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ABSTRACT

A novel electrochemical DNA (E-DNA) biosensing strategy was designed and used for the detection of breast cancer susceptibility gene (BRCA-1). The biosensor was based on gold nanoparticles-reduced graphene oxide (AuNPs-GO) modified glass carbon electrode (GCE) covered with the layer of molecularly imprinted polymers (MIPs) synthesized with rhodamine B (RhB) as template, methacrylic acid (MAA) as the monomer, and Nafion as additive. The signal amplification tracing tag SiO_2@Ag NPs were prepared by covering AgNPs on the surface of SiO_2 nanoparticles in situ, and then DNA probes were modified on AgNPs by Ag-S bond, forming the composites SiO_2@Ag/DNA. In presence of target DNA (T-DNA), homogeneous hybridization was performed with SiO_2@Ag/DNA and RhB labeled DNA, and the resulting SiO_2@Ag/dsDNA/RhB was specifically recognized by MIPs via the interaction between imprinting cavities and RhB. Under optimal conditions, the proposed biosensor exhibited wide linear range from 10 fM to 100 nM, low detection limit of 2.53 fM (S/N = 3), excellent selectivity, reproducibility, stability, and feasibility in serum analysis. Overall, these findings suggest the promising prospects of the proposed biosensing strategy in clinical diagnostics.

1. Introduction

In the past decades, DNA detection methods have attracted tremendous attention due to their pertinent applications, notably in molecular diagnostics and early diagnosis of different diseases, such as genetic disorders, cancer, viral infection, and chronic diseases (Farjami et al., 2011; Mahshid et al., 2015; Saito et al., 2012). Varieties strategies have recently been employed for the manufacturing of DNA sensing platforms (Du and Dong, 2017; Zhao et al., 2015), including optical (Wang et al., 2013), electrochemical (Ling et al., 2015), mass spectrometric (Tretyakova et al., 2013), chromatographic (Nagai et al., 2016) and microgravimetric analysis (Becker and Cooper, 2011). Among these, electrochemical DNA sensing has attracted increasing attention due to its high sensitivity and selectivity, rapid response, amenability to miniaturization, simple instrumentation, and low cost (Diculescu et al., 2016; Palecek and Bartosik, 2012). However, most of the E-DNA sensing approaches require immobilization of the DNA probes on the electrode surface (Drummond et al., 2003; Xiao et al., 2009), which undoubtedly results in lower recognition efficiency and speed with targets when compared to homogeneous recognition. Therefore, homogeneous electrochemical aptasensing strategies were used to take full advantage of their high recognition efficiencies, configurational freedom of probes, and preservation of DNA under physiological conditions (Zhang et al., 2016, 2013). On the other hand, the electroactive substances in most homogeneous E-DNA detection are freely dispersed in solutions, leading to limited sensitivities.

To enhance response signals in homogeneous E-DNA biosensor, several approaches have been developed to capture the electroactive substances from solution to the electrode surface, including host-guest interaction (Cui et al., 2014), π-π stacking interaction (Wang et al., 2015), thiol self-assembly (Hong et al., 2017), biotin-avidin interaction (Zhang et al., 2005), and molecular imprinting adsorption (Tiwari et al., 2012). Among these techniques, molecular imprinting adsorption offers a promising approach to improve sensitivity and selectivity of homogeneous E-DNA biosensors. This is due to recognition of target molecules by MIPs through the specific size, shape, and functionality of three-dimensional imprinting cavities (Yoshikawa et al., 2016), displaying the outstanding affinity. Moreover, MIPs often exhibit better stability than natural biomolecules (Schirhagl, 2014; Wackerlig et al., 2016), coupled with easy preparation and extensive application in biosensing.

In this study, a novel homogeneous electrochemical DNA biosensing strategy with high sensitivity and selectivity was developed based on the specific recognition of MIPs and the signal amplification using SiO_2@Ag nanoparticles. As illustrated in Scheme 1, the target DNA homogeneously hybridized with SiO_2@Ag modified DNA and RhB

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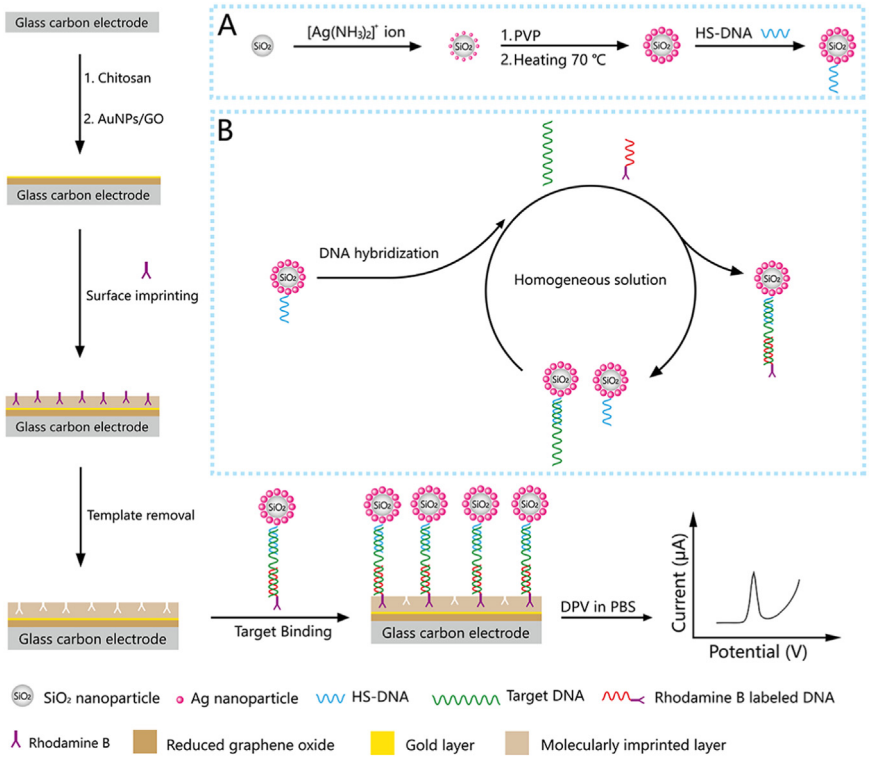

Scheme 1. Schematic illustration of the MIPs-based E-DNA biosensing. Inset of (A) and (B) display the preparation of SiO_2@Ag/DNA and homogeneous DNA hybridization.

labeled DNA, forming the recognizable nanocomposite SiO_2@Ag/dsDNA/RhB. This resulting nanocomposite was transferred from solution to the MIPs modified electrode surface thanks to the recognition of imprinting cavities to RhB. This yielded an amplified electrochemical signal with a detection limit down to 2.53 fM (S/N = 3), coupled with high selectivity and promising applicability in real samples.

2. Experimental sections

2.1. Materials and reagents

Silver nitrate ($AgNO_3$), sodium dodecyl sulfate (SDS), trisodium citrate, chloroauric acid ($HAuCl_4{\cdot}4H_2O$), L-ascorbic acid (AA), sodium borohydride ($NaBH_4$), graphite powder (analytical grade), rhodamine B, ethanol, n-hexanol, cyclohexane, tetraethoxysilane (TEOS), $NH_3{\cdot}H_2O$, Triton X-100, N, N′-methylenebis(acrylamide) (MBA), methacrylic acid, methacrylamide (MAC), poly(acrylamide) (PAA, MW = 3 000 000), aqueous ammonia solution (28 wt%), poly(ethylene glycol) (PEG, MW = 2000), and azobisisobutyronitrile (AIBN) were all purchased from Sinopharm Chemical Reagent (Shanghai, China). Tris(2-carboxyethyl)phosphine (TCEP), polyvinylpyrrolidone (PVP, MW = 55,000), poly(diallyldimethylammonium chloride) solution (PDDA, MW: 200,000–350,000, 20 wt%), 2-trifluoromethacrylic acid (TFMAA), 4-vinylpyridine (4-VPY), Nafion 117 solution (5%), and ethylene glycol dimethacrylate (EGDMA) were provided by Sigma-Aldrich (St. Louis, MO). The RhB labeled DNA probe was obtained from TaKaRa Biotechnology (Dalian, China), and the other DNA sequences with HPLC purification (Table S1) were obtained from Sangon Biotechnology (Shanghai, China). MAA was distilled under reduced pressure to remove the polymerization inhibitor. Clinical human serum samples were collected from a local pathology laboratory and stored at 4 °C. Ultrapure water obtained by Millipore water purification system (≥ 18 MΩ cm, Milli-Q, Millipore) was used throughout the experiments. Phosphate buffer saline (PBS, 0.1 M, pH = 7.4) was prepared with ultrapure water and employed as the supporting electrolyte. The other chemicals and reagents were all of analytical grade and used as received without further purification.

2.2. Instruments

The surface morphologies were observed with scanning electronic microscopy (SEM, HITACHI S-4800, Hitachi, Tokyo, Japan), transmission electron microscopy (TEM, JEOL JEM-2100F), and atomic force microscopy (AFM, Veeco Nanoscope IIIa MultiMode) in tapping mode. The UV–visible (UV–vis) absorption spectra were obtained with UV-1800 spectrophotometer (Shimadzu, Japan). The electrochemical measurements were performed using a CHI 820B electrochemical workstation (CH Instruments, Shanghai, China) with a conventional three-electrode system in 10 mL of glass cell, composed of a modified or bare glassy carbon electrode (GCE, 3 mm in diameter) as working electrode, an Ag/AgCl with saturated KCl solution as reference, and a platinum wire as auxiliary electrode.

2.3. Preparation of SiO_2@Ag/DNA

The preparation procedures of SiO_2@Ag/DNA are illustrated in Scheme 1A. Monodisperse silica nanoparticles were synthesized according to previous literature with some modifications (Fan et al., 2013). The details were provided in the Supporting information. The SiO_2@Ag was prepared according to reported literature (Deng et al., 2007), where 1.0 mL of 0.1 g/mL SiO_2 nanoparticles in aqueous solution was quickly added to 10 mL of freshly prepared $[Ag(NH_3)_2]^+$ solution under magnetic stirring at room temperature. $[Ag(NH_3)_2]^+$ was allowed to absorbed for 1 h onto SiO_2 nanoparticles surface via electrostatic attractions between $[Ag(NH_3)_2]^+$ and negatively charged Si-OH groups. The resulting dispersion was mixed with 50 mL of 5×10^{-4} M PVP ethanol solution placed in a 250 mL three-neck flask under vigorous magnetic stirring at 70 °C for 7 h. The obtained product was then collected by centrifugation (10,000 rpm) and washed at least three times with ultrapure. The resulting SiO_2@Ag was further resuspended