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Short Communication

A Facile and Effective Electrochemical DNA Biosensor for the Detection of GardnerellaVaginalis Based on One-Step BSA Blocked Electrode

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An electrochemical biosensor based on bovine serum albumin blocked functional electrode (BSA-E) for the precise detection of specific gene of GardnerellaVaginalis was developed in this work. BSA assembled gold electrode was used for constructing the electrochemical sandwich-type DNA biosensor to achieve excellent sensitivity, good reproducibility. And a low detection limit of 0.5 pM and a wide linear range were obtained under optimized condition. The wonderful performance of the proposed sensor was attributed to the application of BSA which could effectively to prevent the possible nonspecific adsorption of target DNA and protein (avidin-HRP) on the surface of Au electrode. The proposed approach provides another effect choice for the determination of GardnerellaVaginalis.

Keywords: GardnerellaVaginalis; Electrochemical DNA biosensor; Amperometric i-t curve; Bovine serum albumin; Gold electrode

1. INTRODUCTION

Gardnerellavaginalis is classified under the family Bifidobacteriaceae [1] and often produces a cholesterol-dependent cytolysin and vaginolysin [2]. This bacterium is one of the most prevalent bacterium in the biofilm of bacterial vaginosis (BV) [3]. BV may result in vaginal discomfort and discharge as well as far-reaching consequences such as preterm delivery and an increased rate of sexually transmitted infection. It is considered the most common vaginal infection in women of childbearing age [4]. Therefore, precise and early indntification of the pathognomonic pathogen is important for the diagnosis of BV. Recently, several effective methods, such as isolation, culture and

identification, Gram's staining and Acridine orange staining immunofluorescence assay [5], for the clinical diagnosis or detection of bacterial vaginosis caused by Gardnerellavaginalis have been used. However, current routines are time-consuming and lack of accuracy. Developing some special methods with high specificity and sensitivity for the detection of Gardnerellavaginalis is very demanding.

In recent years, the diagnosis of Gardnerellavaginalis based on the sensing of DNA of causative agent, such as qRT-PCR and DNA biosensors, have attracted more and more attention [6-7]. Among existing diagnostic techniques for gene, DNA biosensor owes the characteristic of simplicity, speed, economy and sensitivity [7]. DNA biosensors were widely used for DNA diagnostics, gene analysis, fast detection of biological warfare agents, and forensic applications [8-9]. Electrochemical measuring technique often employed to develop DNA biosensors due to its high sensitivity, small dimensions, low cost, and compatibility with micromanufacturing technology [9-13]. The core of DNA biosensors is surface-confined capture probes (single-stranded DNA, ssDNA) that are tethered to a substrate for recognition of their target DNA (complementary sequence) by hybridization. The immobilization of capture probes on the special electrode surface influences the performance of the resulting biosensors. Thus, an effective and facile approach to control of probes immobilization on electrode is significant. While, capture probes with end-modified thiol self-assembled onto gold electrode via the thiol-gold chemistry is the famous and effective method [14]. However, the assembly of DNA on gold electrode generally needs the assistance and blocking effect of MCH and bovine serum albumin (BSA) to fabricate the complete sandwich structure DNA biosensors [14]. While this protocol improves the performance of the sensing, it remains challenging of the complex multistep assembly and reactions.

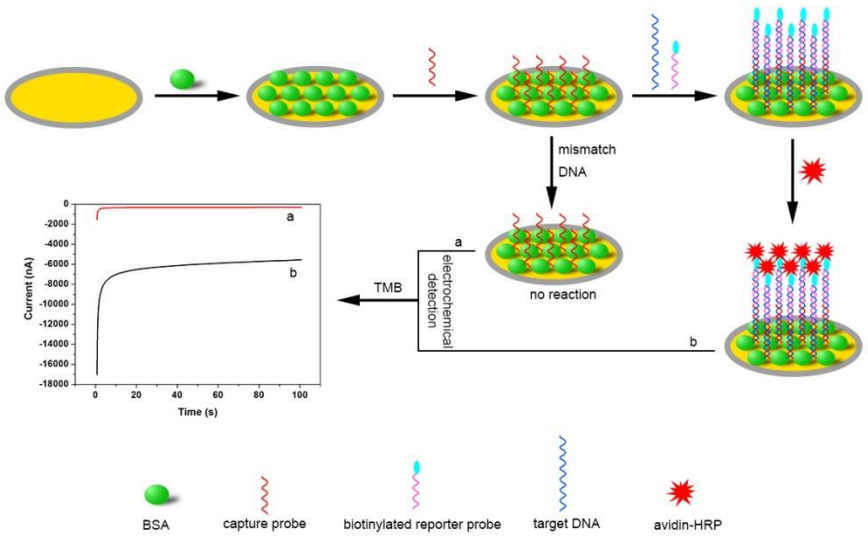

Figure 1. Schematic diagram of the proposed approach for the detection of target DNA of Gardnerellavaginalis.

In this paper, we proposed a DNA probe carrier platform based on the application of BSA to control the interactions between probe and target DNA on the sensor surface. The fabricating process

of the DNA biosensor only needs one-step and initial assemble process of BSA on gold surface with the following self-assemble of thiol modified capture probes. And then, the target gene of gardnerellavaginalis was detected sensitively and selectively through the induced signal based on the introduced avidin-HRP through avidin-biotin interaction based on the biotinylated reporter probe, which likes the sandwich type biosensor [15], as shown in figure 1. The different concentrations of target DNA can introduce variable number HRP, which catalyze and amplify the redox reaction between TMB and H_2O_2 to generating signal. The chronoamperometry was applied to monitor the electrochemical current of oxidized TMB to quantify the concentrations of target DNA [16]. Such biosensing platform is more facile and more time-saving, compared with other detection methods of Gardnerellavaginalis.

2. EXPERIMENTAL

2.1. Reagents and apparatus

All the oligonucleotide sequences were purchased from Takara Biotechnology Co., Ltd (Dalian, China). TMB substrate (TMB is 3,3',5,5'tetramethylbenzidine; Neogen K-blue low activity substrate) was from Neogen (USA). HRP (Streptavidin-horseradish peroxidase) was from Roche Diagnostics. The DNA sequences are listerd as follow:

Capture probe: 5'-HS-$(CH_2)_6$-T TTT TTT TTTGCA GCC CGT CAC GTC CTT CA-3'

Reporter probe: 5'-TCG GTC CTG TCT ACC AAG GCA TGC-$(CH_2)_6$-biotin

Target DNA: 5'- GCC TTG GTA GAC AGG ACC GAT GAA GGA CGT GAC GGG CTG C-3'

One-mismatch DNA: 5'-GCC TTG GTA GAC AGG ACC GAT G*A*A GGA CGT TAC GGG CTG C-3'

Two-mismatch DNA: 5'-GCC TTG GTA G*C*C AGG ACC GAT G*A*A GGA CGT TAC GGG CTG C-3'

Three-mismatch DNA: 5'-GCC TTG GTA G*C*C AGG ACC GAT *TA*A GGA CGT TAC GGG CTG C-3'

Non-complemenary DNA: 5'-GTA ATG GTC TGA TTT GTA AGC GCG ATG TTA CTG CCT-3'

Ethylenediamine-tetraacetic acid, potassium hexacyanoferratetrihydrate, potassiumhexacyanoferrate, absolute ethyl alcohol, NaOH, NaCl, HCl, H_2SO_4, $Na_2HPO_4 \cdot 10H_2O$ and $NaH_2PO_4 \cdot 2H_2O$ were purchased from Sinopharm Chemical Reagent Co., Ltd. These reagents were of analytical reagent grade. Alumina powder (AlfaAesar, USA). The purified water with a specific resistance of 18.2 MΩ·cm was prepared with Milli-Q-purified system (Millipore, USA). Au electrode (AuE) (Φ=2 mm) was obatined from Shanghai ChenHua Instruments Co. Ltd.

Phosphate buffer solution (PBS, pH=7.4) acted as the hybridization buffer containing 100 mM NaH_2PO_4-Na_2HPO_4 and 100 mMNaCl and adjusting its pH with 50mM H_3PO_4 solution and NaOHsolution. TE buffer (pH=8.0) was used to dilute the capture DNA, containing 10 mMTris, 1.0

mM EDTA and 1 MNaCl and pH of the solution was adjusted with 50 mMHCl. All the experimental solution was stored in a refrigerator (4℃) when not used.

The measurements of amperometric i-t curve were carried out on CHI 660C electrochemical workstation (Shanghai ChenHua Instruments Co., China). Electrochemical impedance spectroscopy (EIS) were performed by Autolab PGSTAT302F system (Eco Chemie, The Netherlands) in a solution containing 1 mol/L KCl and 10 mmol/L $[Fe(CN)_6]^{3-/4-}$ from 0.05 Hz to 10^5 Hz. Three-electrode system composing of modified Au electrode as the working electrode, Ag/AgCl electrode as the reference electrode and a platinum wire as the counter electrode was applied to the electrochemical detection.

2.2. Pretreatment of gold electrode and fabrication of electrochemical DNA biosensor

Prior to modification, the bare AuE was first pretreated according to the following methods. The AuE was first polished in the order with 0.3 μm and 0.05μm alumina powder suspension on microcloth pad and then thoroughly sonicated in HNO_3 solution (volume ratio of water to HNO_3 is 1:1), ethanol and ultrapure water for 5 min, respectively. After drying with nitrogen, the polished AuE was electrochemically cleaned to remove any possible impurities by cyclic voltammetry in fresh-prepared 0.5 M H_2SO_4 solution between -0.2 and 1.55 at 100 $mV \cdot s^{-1}$ until steady redox waves observed.

The treated AuE was incubated in 10 mM PB (pH=7.4) containing 5 $mg \cdot mL^{-1}$ BSA for 15 min at room temperature to obtain BSA/AuE. After thoroughly rinsed with PBS and water in sequence, 3 μL of capture probe (1 μM) was gently dropped on the surface of BSA/AuE for 1 h at room temperature to get ssDNA/BSA/AuE. Finally, the prepared ssDNA/BSA/AuE was thoroughly rinsed with PBS and purified water in sequence and dried under nitrogen.

2.3. DNA detection assay

A series of different concentrations target DNA sequence were mixed with the biotinylated reporter probe (20 nM) to be hybridization solution. Then, the prepared ssDNA/BSA/AuE was incubated in 100 μL of the hybridization solution for 1 h at 50 ℃ to obtain dsDNA/BSA/AuE. After being thoroughly rinsed with PBS and purified water in sequence, the electrode with the biotinylated reporter DNA was incubated in 200 μL of the avidin−HRP for 15 min at room temperature to get HRP/dsDNA/BSA/AuE. After thoroughly rinsed with PBS and purified water in sequence, the modified electrode subjected to electrochemical measurement to record an amperometric i-t curve in TMB solution.

3. RESULTS AND DISCUSSION

3.1.Electrochemical impedance spectroscopy of the electrodes in a different stage

Electrochemical impedance spectroscopy (EIS) is based on the change of the charge transfer resistance using a redox couple ($[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$) [17]. EIS is an effective tool for the

characterization and even quantification of the bio-functionalized electrodes and the varied assembly of the electrode using biological materials [18]. EIS is used to investigate the different stages of the electrochemical sensor preparation to evaluate and characterize the modified electrodes, as shown in Fig. 2. The insert in Fig. 2 revealed that a Randles equivalent circuit is applied to fit all the electrical parameters of EIS results of the modified electrode in a different stage [19]. The electron transfer resistance (Ret) inducing by the diffusion of ions from the electrolyte to the working electrode interface can illustrate the variable stage of the construction of DNA biosensor. The different Ret detected by EIS can be applied to confirm the assembly of protein, immobilized DNA probe and the hybridization process of DNA on the surface of electrode [18, 20]. The EIS of the bare gold electrode exhibited a straight line (curve a), and the corresponding Ret was almost 0. After the self-assembly of BSA was on the bare gold electrode, the EIS of BSA/AuE showed an obvious semicircle domain (curve b) and the corresponding Ret was about 2757Ω. After the capture DNA was immobilized on the BSA/AuE, the semicircle domain of EIS was bigger and the Ret of ssDNA/BSA/AuE was about 6021Ω, indicating that the immobilization of capture DNA on the electrode was successful. After the hybridization reaction on the ssDNA/BSA/AuE, the EIS had significant change, and the Ret of dsDNA/BSA/AuE increased to be about 8601 Ω. Finally, the Ret of the HRP/dsDNA/BSA/AuE (curve e) reached 10279 Ω. The experimental results revealed that the Ret in the whole stages gradually increased. This reason was attributed to the fact that BSA, DNA and HRP on the electrode being non-conductive material went against $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ to spread to the electrode surface. The changes of EIS demonstrated that the different stages of the proposed sensor preparation were successful [17-18].

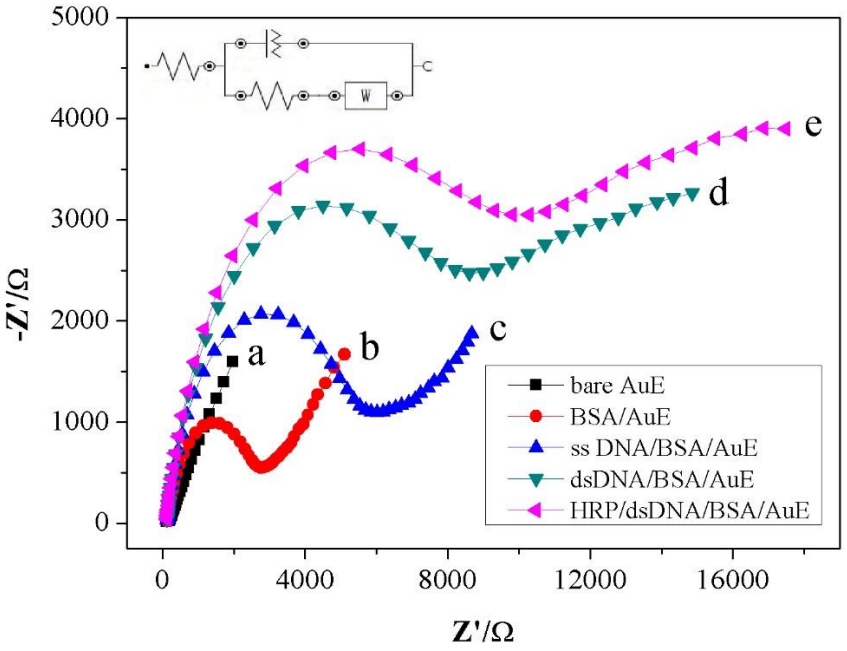

Figure 2. EIS of bare AuE (a), BSA/AuE (b), ssDNA/BSA/AuE (c), dsDNA/BSA/AuE (d) and HRP/dsDNA/MCH/AuE (e) in 10 mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ and 1 MKCl from 0.05 Hz to 100 kHz. Insert displays the equivalent electrical circuit of work electrode.

3.2. Optimization of experimental conditions

The hybridizing efficiency of DNA is closely concerned with temperature. Thus, the optimized temperature of hybridization assay was discussed detailed. For this purpose, hybridization assay was executed at different temperature and in constant concentration of target DNA for 60 min. As shown in Fig. 3A, the current value increased in the range of 40 ℃ to 50 ℃. However, the current value decreased with the increase of temperature from 50 ℃ to 60 ℃. When the hybridization temperature was 50 ℃, the current value reached a maximum. Accordingly, the optimum temperature of hybridization assay was obtained as 50 ℃.

Furthermore, the hybridization time was investigated in this work, when the hybridization assay was done at 50 ℃ with the same concentration of target DNA. As shown Fig. 3B, the current value rapidly increased with the extension of hybridization time from 20 min to 60min. After the hybridization time was more than 60 min, the current value reached to a plateau. Hence, 60 min was obtained as the optimal hybridization time.

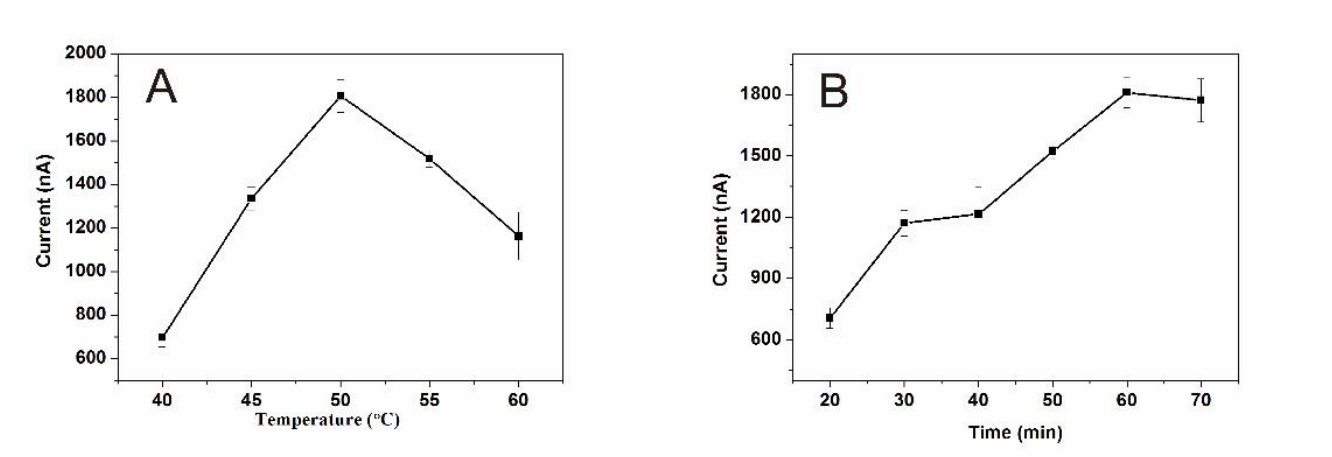

Figure 3. The influence of temperature (A) and time (B) of the proposed sensor for the testing of the concentration of DNA with 1 nM. Error bars show the standard deviations of results obtained from independent experiments (n=3).

3.3. Specificity of the DNA electrochemical biosensor

The specificity is one of the most significant aspects of the electrochemical DNA sensor. In order to investigate the specificity of the proposed sensor, we composed the response of target DNA with non-complementary DNA including single-base mismatch, double base mismatch, three base mismatch and complete mismatch.

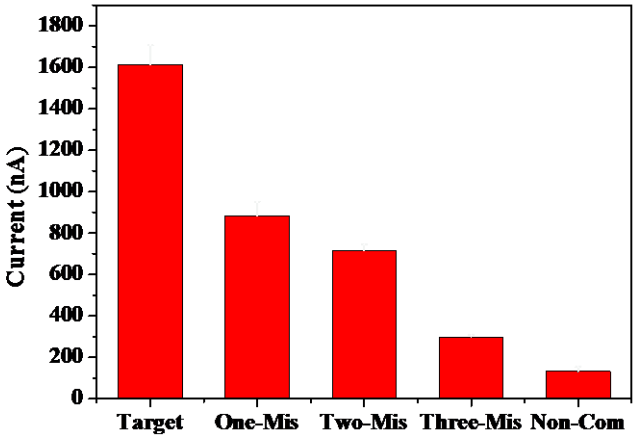

Figure 4. The histograms of the response currents of the proposed sensor for target DNA (1nM) and different mismatch DNA (10nM). Error bars show the standard deviations of results obtained from independent experiments (n=3).

As can be seen from Fig. 4, the current value of target DNA was obviously greater than non-complementary DNA, although the concentration of target DNA is one-tenth of the mismatched sequence. And the Fig. 4 clearly showed that the response current decrease with the increasing number of mismatch bases. As a consequence, the proposed sensor possessed an acceptable specificity toward the target DNA.

3.4. Quantification detection and reproducibility of target DNA

The detection performance of proposed DNA sensor was evaluated by detecting a series of different concentrations of target DNA under optimal conditions. The Fig.5 showed that the response current of the amperometric i-t curve of the proposed sensor increased correspondingly with the increment of the target DNA concentrations from 5×10^{-12} M to 5×10^{-9} M. The response current gradually increased with the increased concentrations of target DNA. The inset of Fig.5 showed the calibration curve between target DNA concentration and response current. The linear regression equation of the proposed sensor was $I(nA)=419.6+1.129\times10^{12}C(M)$ ($R^2=0.9715$). Additionally, an estimated detection limit of 0.5×10^{-12} M (0.5 pM, S/N=3) for target DNA was obtained. The result indicated that the proposed method was a sensitive, reliable and promising way for quantitative recognition of target DNA. It can be seen that the proposed method performed well with the low limit of detection (LOD) and the wide linear range. The LOD was lower than previously reported electrochemical methods based on peptiede nucleic acid as probe [17], neutral cobalt complex as indicator [21], and eriochrome cyanine R (ECR) as active modified material [22]. Furthermore, the magnitude of the linear range of this work is wider than reported approaches based on electrochemical routes [21-23]. The reproducibility of the electrochemical DNA biosensor was evaluated through intraassay relative standard deviation (RSD) values of the detection of 5 nM, 0.1 nM and 0.01 nM of target sequence under the same conditions. Each assay was repeatedly performed using 6 parallel

independent electrodes. The intraassay RSD values of the measurements were 7.3%, 8.3% and 4.5% for 5 nM, 0.1 nM and 0.01 nM of target DNA sequence,respectively, illustrating the excellent repeatability of the prepared DNA biosensor.The proposed approach in the present study may be another alternative for the detection of gardnerellavaginalis.

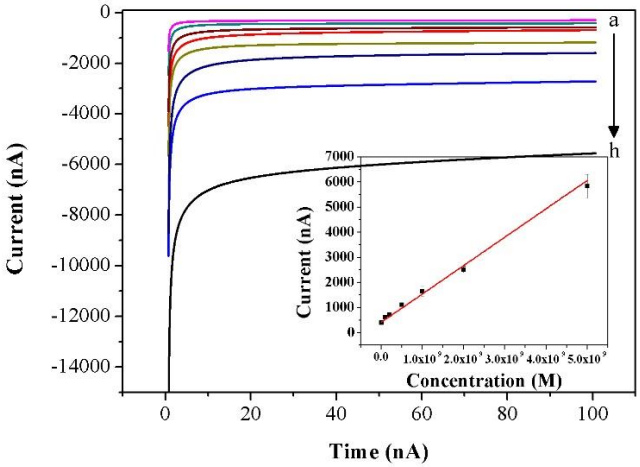

Figure 5. Amperometric i-t curves of target DNA different concentrations (from a to h: 0, 5×10^{-12} M, 1×10^{-11} M, 1×10^{-10} M, 2×10^{-10} M, 5×10^{-10} M, 1×10^{-9} M, 2×10^{-9} M and 5×10^{-9} M) on the proposed sensor; the insert showed the linear relation between the response current values and the target DNA concentrations, error bars show the standard deviations of results obtained from independent experiments (n=3).

4. CONCLUSIONS

In summary, an electrochemical DNA biosensor based on the BSA assemble Au electrode was developed and used for the detection of target DNA (gardnerellavaginalis). The proposed sensor possessed several obvious features to give rise to a markedly elevated performance. The proposed approach had the advantages of wild linear range (5×10^{-12} M~5×10^{-9} M) and low detection limit (0.5 pM). The reason for the commendable reproducibility and great sensitivity of the DNA biosensor was that the assembly of BSA can not only improved spatial positioning range and accessibility of the capture probe on the surface of the electrode but also prevented the nonspecific adsorption of avidin-HRP to decrease the background signal.Meanwhile, the sensor based on BSA possessed high protein resistance ability, so the sensor can be used to detect target DNA in the biological fluids. The electrochemical DNA sensor provides another choice for the detection of gardnerellavaginalis.

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