Sensors and Actuators B 221 (2015) 148–154

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Amplified electrochemical detection of *mecA* gene in methicillin-resistant *Staphylococcus aureus* based on target recycling amplification and isothermal strand-displacement polymerization reaction

Ting Wang[a], Zhang Zhang[a], Yiyan Li[b], Guoming Xie[a,*]

[a] *Key Laboratory of Laboratory Medical Diagnostics, Ministry of Education, Department of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, PR China*
[b] *College of Engineering, University of Nevada, Las Vegas, NV 89154-4026, USA*

ARTICLE INFO

Article history:
Received 27 February 2015
Received in revised form 19 May 2015
Accepted 10 June 2015
Available online 28 June 2015

Keywords:
Methicillin-resistant *Staphylococcus aureus*
mecA gene
Target recycling
Strand displacement
Polymerization reaction
Electrochemical DNA sensor

ABSTRACT

Rapid detection of drug-resistant bacteria can facilitate the early diagnosis of various infections and provide essential guidance for appropriate usage of antibiotics. An ultrasensitive electrochemical DNA (E-DNA) sensor based on isothermal strand-displacement polymerization reaction (ISDPR) for detection of *mecA* gene in methicillin-resistant *Staphylococcus aureus* (MRSA) was proposed in this study. Methylene blue (MB)-labeled hairpin probes were self-assembled on a gold electrode to confine MB molecules close to the electrode surface for efficient electron transfer. The hairpin probes were then hybridized with complementary target DNA and undergone conformational changes, which led to a decreased electrochemical response. The primers annealed with the opened stems of hairpin probes were extended by DNA polymerase, which in turn released the target DNA to trigger the next polymerization cycle. Finally, each target DNA underwent through many rounds, resulting in a mass of MB molecules moved away from the electrode surface, which generated significant amplified current suppression for *mecA* gene detection. With this target recycling amplification strategy, the proposed DNA biosensor could offer excellent analytical performance for the detection of *mecA* gene and provide a new electrochemical method for the early diagnosis of drug-resistant bacteria.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of human bacterial infections worldwide, some of which are life threatening, such as sepsis, necrotizing pneumonia, and toxic shock syndrome [1–3]. Clinical evidence has shown that rapid diagnosis and appropriate early treatment of MRSA infections have positive influence on making infection control strategies and decreasing the morbidity and mortality rate [4]. So far, the existing assays for MRSA identification are mainly standard microbiology methods and real-time polymerase chain reaction (PCR). However, these culture-based methods for MRSA detection are insensitive, cumbersome, and time-consuming (24–72 h) [5]. Although PCR is regarded as the "golden standard" method because of its high sensitivity and promptness, it also suffers from several drawbacks like labor-intensive, expensive, and unportable [6–8]. Therefore, new methods for sensitive, rapid, and convenient determination of MRSA need to be developed.

The mechanism underlying resistance to a family of antibiotics is reportedly based on the acquisition of a chromosomal *mecA* gene [9,10]. The *mecA* gene encodes penicillin-binding protein (PBP2a or PBP2′) and permits methicillin resistance [11,12]. Hence, detection of *mecA* gene is tantamount to methicillin resistance detection. Currently, a number of optical [13,14], electrochemical [15–17], and piezoelectric [18] approaches based on DNA hybridization of *mecA* gene have been developed to investigate MRSA. Among these sensing devices, electrochemical biosensor has been proven to be a particularly promising approach because of the following advantages: convenience, sensitivity, speed, and portability. Thus, this sensing device can meet the requirements in conventional MRSA analysis.

* Corresponding author at: Key Laboratory of Laboratory Medical Diagnostics, Ministry of Education, Department of Laboratory Medicine, Chongqing Medical University, No. 1, Yi Xue Yuan Road, Chongqing 400016, PR China. Tel.: +86 23 68485239; fax: +86 23 68485239.
E-mail address: guomingxie@cqmu.edu.cn (G. Xie).

http://dx.doi.org/10.1016/j.snb.2015.06.057
0925-4005/

Since the electrochemical DNA (E-DNA) sensor was first developed by Fan et al. for nucleic acid detection [19], it has received substantial attention [20–22]. In this method, the target binding-induced conformational changes of the redox tag-labeled probes can be monitored by detecting the distance-dependent electron transfer between the attached signal molecules and the electrode. This E-DNA sensor does not need target labeling, so the detection process is fast and simple [23]. In addition, the application of hairpin probes provides high selectivity [24]. However, the sensitivity of this biosensor for DNA detection is limited by the lack of signal amplification recycling. To overcome this issue, an effective amplification strategy is needed to improve the sensitivity of the traditional E-DNA sensor.

In recent years, isothermal strand-displacement polymerization reaction (ISDPR) has been widely used for ultrasensitive nucleic acid detection [25,26]. In ISDPR, the hybridization between the hairpin probe and target DNA can induce polymerization reaction in the presence of a primer. The polymerization reaction produces a new strand to replace the target strand. The released target DNA opens another hairpin probe to initiate next polymerization reaction, thereby resulting in target DNA recycling and achieving signal amplification. Compared with other signal amplification techniques such as PCR [27], exonuclease-based methods [28,29], and rolling circle amplification [30,31], ISDPR does not need repeated thermal cycling, a specific recognition site, and a specially designed circular template. Thus, ISDPR can be conveniently used to fabricate DNA biosensors. Hu's group reported a portable and sensitive personal glucose meter to analyze a single DNA strand by using ISDPR [32]. Feng and Qiu constructed an optical bifunctional signaling probe via a target recycling signal enhancement technique to detect different disease markers in parallel [33]. Li and Zhou developed a label-free fluorescence DNA biosensor for nucleic acid detection combining polymerization reaction with graphene oxide with a low detection limit of 4 pM [34]. The ISDPR advantages mentioned above prove that this target recycling method has great potential to improve the detection sensitivity of electrochemical biosensor.

In this work, we developed a novel sensing system for ultrasensitive detection of *mecA* gene in MRSA. The electrochemical signal was transmitted by an E-DNA sensor and amplified by ISDPR. After target DNA hybridization with the hairpin probe, the primer released the target DNA to go through a new hybridization and displacement cycle on the biosensor's surface, thereby resulting in successful target recycling. The detection mechanism of this DNA biosensor involved distance variation between the redox tags and the gold electrode, which was associated with target-induced conformational changes of hairpin probe. The subtle current changes can be directly monitored by electrochemical measurements. The proposed electrochemical biosensor exhibited good analytical performance in *mecA* gene detection, thereby providing a rapid and convenient sensing platform for nucleic acid detection.

2. Experimental

2.1. Reagents and materials

The *mecA* gene-related oligonucleotides were synthesized and HPLC-purified by Sangon Biotechnology Inc. (Shanghai, China), and their base sequences were listed (supplementary information, Table 1). Polymerase Klenow fragment exo$^-$ (KF exo$^-$) and deoxynucleotide solution mixtures (dNTPs) were obtained from Fermenta Biotechnology Co., Ltd. (Ontario, Canada). Tris–HCl and 6-mercapto-1-hexanol (MCH) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Premix Taq Version 2.0, DL500 DNA Marker, and agarose were purchased from Takara Co. (Dalian, China). Ethylenediaminetetraacetic acid (EDTA) and Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were supplied from Sigma–Aldrich Co. (St. Louis, USA). All other reagents were of analytical grade and were used as received. The buffer solutions used in this work were in supplementary information.

2.2. Apparatus and measurements

All electrochemical measurements were performed on a CHI660D electrochemical workstation (Shanghai CH Instruments Co., China). A conventional three-component electrochemical cell was used in all experiments. A platinum wire was used as the auxiliary electrode; a KCl-saturated Ag/AgCl electrode was used as the reference electrode; and a modified gold electrode was used as the working electrode. EIS and cyclic voltammetry (CV) measurements were used to monitor the interface properties of the modified electrode. Square wave voltammetry (SWV) measurements were obtained in working buffer within the potential range from −0.5 to 0.1 V under pulse amplitude of 25 mV and a frequency of 25 Hz. All electrochemical measurements were carried out at room temperature (RT, 25 ± 2 °C). An atomic force microscope (AFM) NanoWizard II (JPK Instruments Inc., Germany) was employed to observe the images of different modified electrodes.

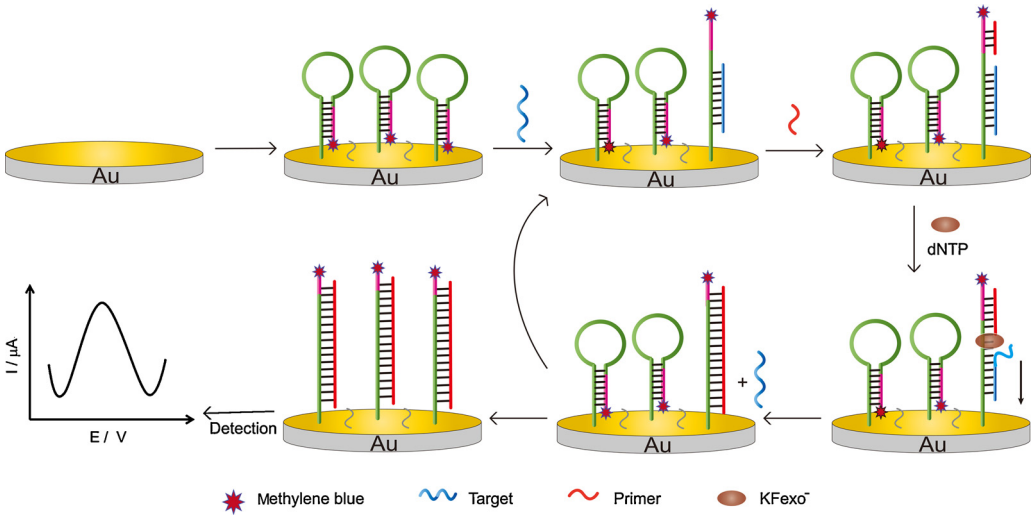
2.3. Preparation of the electrochemical biosensor

First, the gold electrode (3 mm in diameter) was mechanically polished to a mirror-like surface using alumina powder of 0.3 and 0.05 μm, followed by successive ultrasonic treatment for 10 min each in ultrapure water, ethanol, and ultrapure water. The electrode was then immersed in freshly prepared piranha solution (98% H_2SO_4: 30% H_2O_2, 3:1, v/v) for 10 min (*Caution: Piranha solution dangerously attacks organic matter*!) and further electrochemically cleaned by scanning in 0.1 M H_2SO_4 between −0.2 and 1.6 V until a stable cyclic voltammogram was obtained. Finally, the gold electrode was thoroughly washed with ultrapure water and dried with nitrogen gas.

Prior to modification, the thiol-modified hairpin probes were reduced for 1 h at RT by mixing 100 μL of 10 μM of the hairpin probes with 1 μL of 100 mM TCEP and subsequently diluted to 1 μM with TE buffer [35]. Then, the mixture was annealed by incubating at 95 °C for 5 min and slowly cooled to RT to form the hairpin structure. The cleaned gold electrode was immersed in the above solution for 8 h at RT in the dark. After rinsing with ultrapure water, the electrode was incubated with 1 mM MCH solution for 1 h at 37 °C to block the remaining bare area. The electrode was then washed and ready for the next modification.

2.4. Electrochemical detection of mecA gene

The modified electrode was immersed in 10 μL hybridization buffer, which contained various concentrations of target DNA. This process was performed in the dark at 37 °C for 2 h and was terminated by thorough washing. Subsequently, target recycling based on ISDPR was carried out by dipping 10 μL of the polymerase reaction mixture (5 U KF exo$^-$, 500 μM dNTPs, 100 nM primer, and 1 × KF buffer) on the as-prepared DNA biosensor surface for 2 h at 37 °C. The electrode was then washed three times with ultrapure water. Finally, SWV measurements of the resulting DNA biosensor were recorded in working buffer solution. All analyses were made in triplicate.



Scheme 1. Schematic illustration of the electrochemical DNA biosensor fabrication process.

3. Results and discussion

3.1. Principle of this sensing system

The principle of this sensing mechanism was depicted in Scheme 1. A hairpin-structured probe, dually labeled with 5′-SH and 3′-MB, possessed a stem of 10 base pairs enclosing a 24 unpaired nucleotide loop. The loop region was complementary to the target strand, whereas the 3′ terminal of the stem portion was complementary to the primer. The hairpin probe was first self-assembled on the gold electrode through Au–S binding, and was kept upright due to the immobilized MCH. The two terminals on the stem of the hairpin probe were aligned. Hence, the MB molecule, which has efficient electrochemical activity, can be restricted close to the gold electrode surface and produced a large Faradaic current.

When challenged with the *mecA* gene, the hairpin probe partly hybridized with target strand and underwent a conformational change. The conformational change led to stem separation, which altered the distance between the MB molecule labeled at the 3′ end of the hairpin probe and the gold surface. The distance variation generated a decreased current response. Then, the primer was annealed with the opened stem and was extended by the DNA polymerase, which enhanced the release of the target DNA. The replaced target strand was then bound with another hairpin probe to trigger the second polymerization cycle. Eventually, each *mecA* gene underwent many cycles, and more MB molecules then moved away from the electrode surface, which led to a dramatically decrease of the electrochemical signal.

Moreover, the stem of hairpin probe was designed long enough to ensure primer failure in initiating the polymerization reaction without target DNA. Therefore, the background signal was very weak. In conclusion, this electrochemical biosensor produced a readily measured current suppression that could be related to the presence and concentration of the *mecA* gene.

3.2. Characterization of the DNA biosensor

EIS is an effective and convenient tool to monitor the stepwise modification of the gold electrode. In an impedance spectrum, the larger semicircle diameter represents larger electrode-transfer resistance (R_{et}) of the modified electrode. All EIS tests were carried out under open circuit potential. EIS data were fitted by an equivalent electrical circuit (inset in Fig. 1A). The EIS of different surface-modified biosensors is exhibited in Fig. 1A. The bare

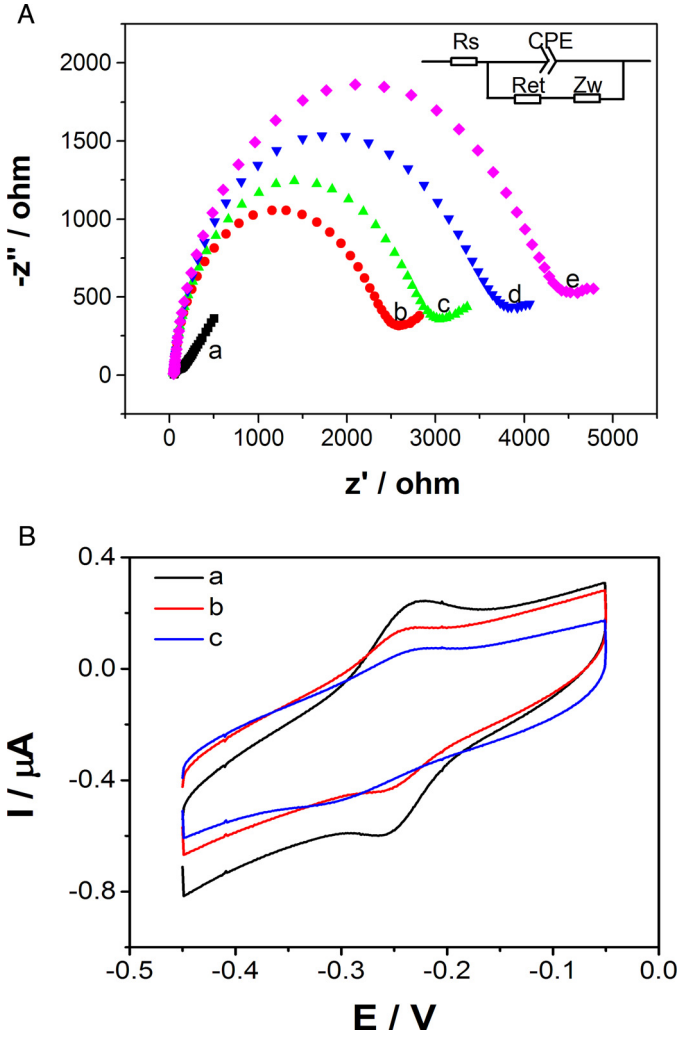


Fig. 1. Electrochemical characterization of the proposed biosensor. (A) EIS responses of: (a) bare gold electrode; (b) hairpin probe/gold electrode; (c) MCH/hairpin probe/gold electrode; (d) *mecA* gene/MCH/hairpin probe/gold electrode; and (e) after ISDPR in phosphate buffer solution (pH 7.0) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl. (B) CVs of: (a) hairpin probe/gold electrode; (b) *mecA* gene/MCH/hairpin probe/gold electrode; and (c) after ISDPR in Tris–HCl buffer solution (20 mM, pH 7.4) containing 140 mM NaCl and 5 mM $MgCl_2$.

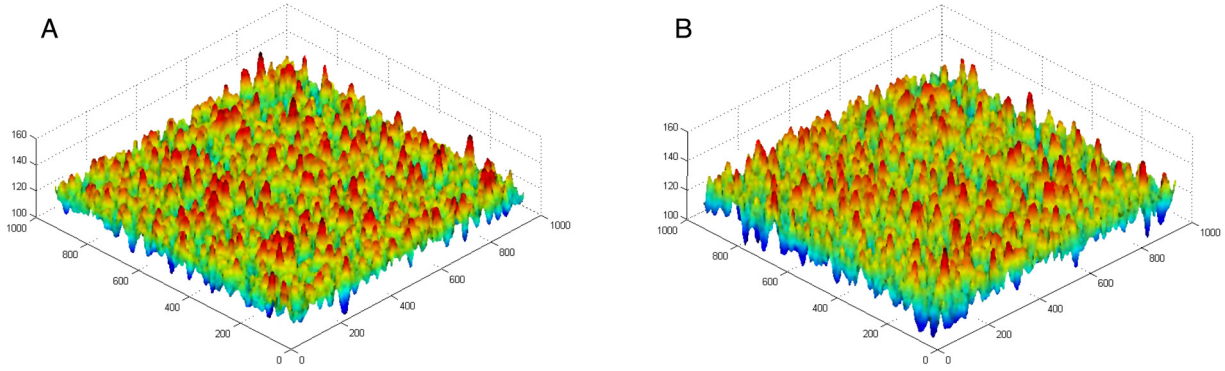


Fig. 2. AFM images for the changes of interfacial properties of each immobilization step: (A) hairpin probe/gold electrode and (B) after *mecA* gene hybridization with self-assembled hairpin probe in the presence of a certain polymerase reaction mixture.

gold electrode showed a very small semicircle domain (curve a), thereby suggesting an excellent electron transfer process at the gold electrode surface. After immobilization of hairpin probes, the impedance increased (curve b) because the negatively charged phosphoric acid backbones produced an electrostatic repulsion force to anionic $[Fe(CN)_6]^{3-/4-}$. Subsequently, the blocking of MCH increased the diameter of the semicircle (curve c), implying that the immobilized MCH inhibited electron transport to the electrode surface with nonconductive properties. In the presence of *mecA* gene, the value of R_{et} further increased (curve d) because of the accumulation of negative charges on the electrode upon the formation of double-stranded DNA. When the electrode was incubated with polymerase reaction mixture, strand displacement and polymerase reaction were initiated and the R_{et} value increased significantly (curve e), because the target circulation produced a large amount of double-stranded DNA. Therefore, these observations showed the successful preparation of the sensing interface.

CV measurement was also employed to characterize the changes of interfacial properties during the E-DNA sensor fabrication. As shown in Fig. 1B, a couple of strong redox peaks were observed at −0.24 and −0.26 V in the potential window (curve a). The MB-modified stem-loop probes were immobilized on the surface of gold electrode. Upon the addition of the complementary target strand, the redox current decreased (curve b), which indicated that the target DNA was loaded onto the electrode surface. With the primers hybridized with the opened hairpin probes in the presence of DNA polymerase, an obvious decline in redox current was obtained (curve c). This finding implied that many MB molecules moved away from the electrode surface to generate strong current suppression. Similarly, the CV results demonstrated the success of the stepwise modification.

AFM was utilized to further verify the working electrode modification processes [36]. The three-dimensional height maps of the modified probes in the absence or presence of *mecA* gene with a certain polymerase reaction mixture are shown in Fig. 2A and B. A rough and blunt appearance was obtained after modification of the hairpin-structured probes (Fig. 2A). The surface mean roughness (Sa) of this electrode was 2.465 nm, and the surface root mean square roughness (Sq) was 3.185 nm. In addition, a more intensive and towering image was observed upon hybridization and polymerase reaction (Fig. 2B). The Sa of this electrode was 4.774 nm and the Sq was 6.619 nm. Thus, DNA hybridization and ISDPR smoothly occurred on the electrode surface.

3.3. Feasibility of the electrochemical biosensor

The viability of this DNA detection system was investigated using SWV method. As exhibited in Fig. 3, no electrochemical

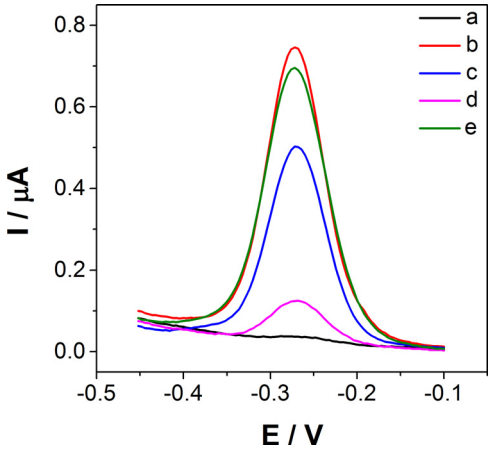


Fig. 3. Feasibility investigation of the electrochemical biosensor. SWVs of: (a) bare gold electrode; (b) hairpin probe/gold electrode; (c) *mecA* gene/MCH/hairpin probe/gold electrode; and (d and e) after ISDPR in the presence and absence of target DNA respectively. The target DNA concentration was 200 pM. All data were recorded in Tris–HCl (20 mM, pH 7.4) buffer solution containing 140 mM NaCl and 5 mM $MgCl_2$.

response was measured onto the bare gold electrode (curve a), since no redox indicator was introduced to the gold electrode surface. After immobilization of redox-labeled hairpin probes, a remarkable Faradaic current can be observed and a well-defined current peak of MB appeared at about −0.272 V (curve b), which can be attributed to the stem-loop probes holding a large amount of MB molecules around the electrode, thereby enabling efficient electrode transfer. Upon hybridization with the *mecA* gene, an obvious decrease in response current was observed (curve c). The double-stranded conformation of hairpin probe moved a number of MB molecules away from the electrode surface, which suppressed electron transfer. By contrast, a sharper decrease in current signal was obtained when the biosensor was incubated with the polymerase reaction mixture (curve d). Obviously, the detectable reduction in redox current was caused by target recycling and strand displacement, which forced MB molecules to continuously move away from the electrode. However, the current intensity only changed slightly (curve e) upon the addition of the polymerase reaction mixture without DNA, which suggested that the primers were unable to open the stem of hairpin probes because the stem hybridization affinity was stronger than the hybridization affinity with the primers. Because of the target recycling amplification, a small amount of target DNA can induce a highly suppressed current. These results showed that the developed E-DNA sensor with the amplification of ISDPR could be carried out to quantitatively analyze the concentration of *mecA* gene.

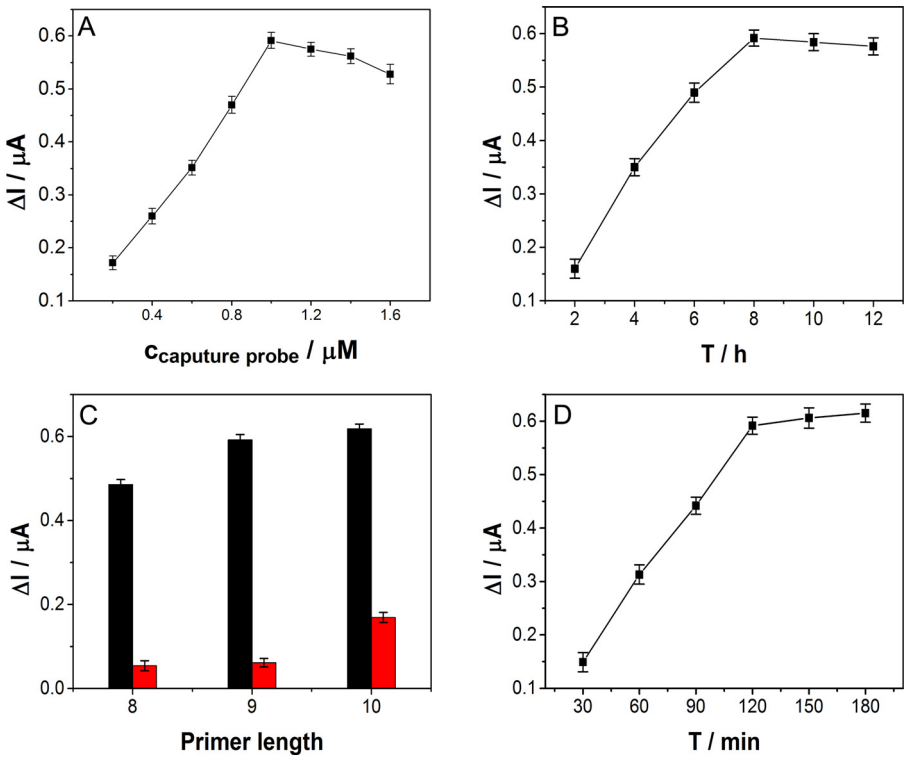


Fig. 4. Effects of (A) hairpin probe concentration; (B) hairpin probe incubation time; (C) the length of the primer; and (D) polymerase reaction time on the current suppression. The black pillar represents the current signal variation of target DNA and the red pillar stands for the blank. When one parameter changed, the others kept on their optimal conditions. The target DNA concentration was 200 pM. Error bars represent standard deviations of the measurements ($n = 3$).

3.4. Optimization of the experimental conditions

To achieve the perfect assay performance for this electrochemical biosensor, various crucial experimental factors including the density of MB-labeled probe, the length of the primer, and the reaction time of the polymerization reaction were selectively optimized.

Previous studies indicated that the probe density dependence of E-DNA sensor is complicated [37]. To an E-DNA signaling system, the hybridization efficiency may be reduced as the probe density increases, but higher signal suppression will be observed with a higher probe density [38]. Hence, the concentration and the incubation time of hairpin probe, which were related to the density of the hairpin probe on the gold electrode, should be tested. As shown in Fig. 4A, ΔI (SWV current decrease, the peak current variation between the absence and presence of *mecA* gene fragment with a certain polymerase reaction mixture) increased with hairpin probe concentration from 0.2 μM to 1.0 μM. Further increase in the concentration of hairpin probe resulted in a slight decrease in the signal suppression. Therefore, 1.0 μM of hairpin probe was used for the following experiments. The dependence of the ΔI on the self-assembly time was studied in the range of 2–12 h (Fig. 4B). The results showed that ΔI increased gradually with increasing time and stabilized after 8 h. Thus, 8 h was selected as the optimal probe incubation time for this electrochemical biosensor.

The length of the primer was a key factor affecting the reaction efficiency of strand displacement, three different lengths of primers were designed to maximize the signal-to-noise ratio and the detection sensitivity. As illustrated in Fig. 4C, the black pillar represents the current variation obtained by applying target DNA hybridization and ISDPR. The red pillar stands for the electrochemical signal obtained by straightforward ISDPR without introducing the target DNA. Obviously, the current responses of the target strand increased as the length of primer increased. However, the background signal also increased with increasing length of the primer sequence. Therefore, the 9-nucleotides primer which induced the maximum signal-to-noise ratio was selected for further research.

Based on the optimization strategy mentioned above, polymerase reaction time was also investigated. As displayed in Fig. 4D, ΔI increased remarkably with increased polymerase reaction time before reaching the saturation condition at 120 min. The results suggested that a polymerase reaction time of 120 min was the ideal reaction time for subsequent studies.

3.5. Analytical performance of the DNA biosensor

Under the optimized experimental conditions, the performances of the integrated biosensor were evaluated by SWV measurement. Fig. 5 presents the steady-state response of the proposed biosensor for different concentrations of *mecA* gene. As shown in Fig. 5A, the addition of *mecA* gene resulted in a significant decrease in the peak current of MB compared with the blank (curve 1). Meanwhile, with the increasing concentration of the target DNA, more MB molecules moved away from the electrode, thereby increasing the suppression of current response (curves 2–14). Fig. 5B illustrates the dependence of the current response upon the *mecA* gene concentration range from 0 pM to 400 pM. Moreover, the calibration curve exhibited a good linear relationship between ΔI and the concentration of target strand over the detection range of 0.075–200 pM (inset in Fig. 5B). The regression equation can be expressed as $\Delta I\,(\mu A) = 1.875 + 0.132 \log C\,(M)$ with a coefficient of determination (R^2) of 0.997, where C is the *mecA* gene concentration ($n = 3$). The detection limit was estimated at 63 fM (S/N = 3), thereby indicating that a small amount of *mecA* gene can result in a measurable suppressed current because of the target recycling amplification. The analytical performance of the

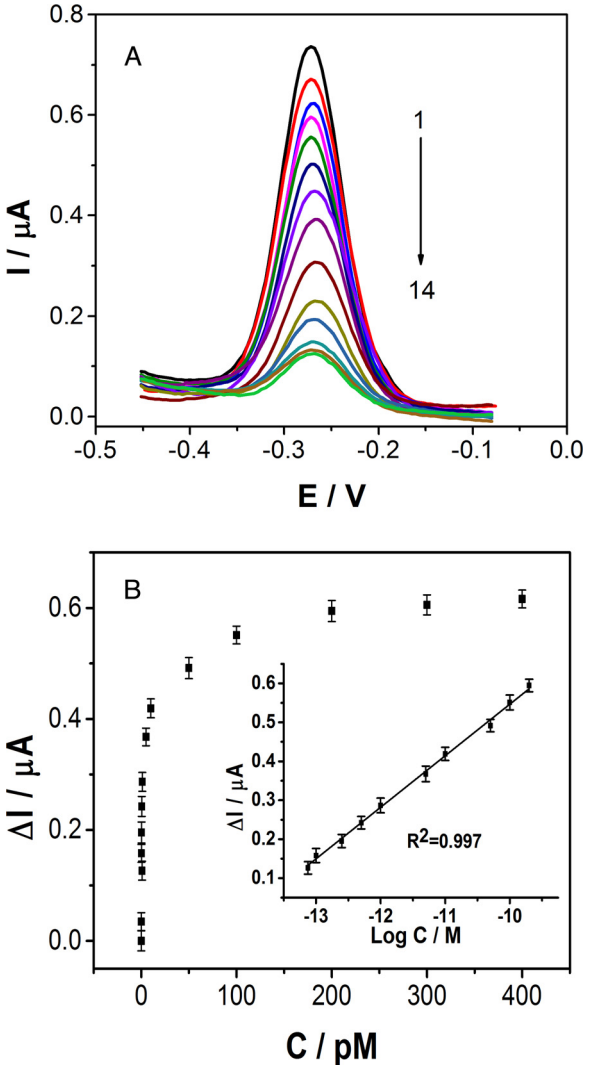


Fig. 5. (A) SWV responses of the electrochemical biosensor with the increasing concentrations of complementary DNA (from 1 to l4: 0, 0.05, 0.075, 0.1, 0.25, 0.5, 1, 5, 10, 50, 100, 200, 300 and 400 pM, respectively). (B) Relationship between the current response suppression and *mecA* gene concentration. Inset: calibration curve of the SWV current decrement versus the logarithm of complementary DNA concentration. Error bars represent standard deviations of the measurements ($n = 3$).

proposed method for the detection of *mecA* gene was compared with the performance of previously reported methods (supplementary information, Table 2). Obviously, the proposed method in this study showed good performance in terms of analytical range and detection limit.

3.6. Specificity, reproducibility, and stability

The specificity of the proposed biosensor was verified by exposing the biosensor to four types of target sequences including perfectly complementary DNA (T1), one-base mismatched DNA (T2), three-base mismatched DNA (T3), and non-complementary DNA (T4) (inset in Fig. 6). Among the four types of DNA sequence, the current change of complementary strand was much greater than those of the other mismatched sequences. As shown in Fig. 6, the corresponding suppression of T2 and T3 was much less than that of T1. Thus, the electrochemical biosensor was effective in discriminating the complementary sequence from the one-base and three-base mismatched DNA. Moreover, the detection of T4 showed an even smaller current decrease (13.6%, similar to T1), and this

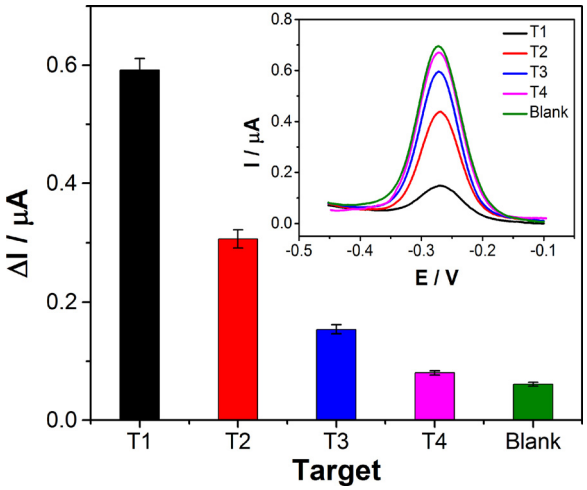


Fig. 6. Specificity of the proposed electrochemical biosensor: (T1) complementary DNA; (T2) one-base mismatch DNA; (T3) three-base mismatch DNA; (T4) non-complementary DNA; and the blank. Inset: the SWV responses of different DNA sequences and the blank. The DNA concentration was 200 pM. The current signals were measured in Tris–HCl buffer solution (20 mM, pH 7.4) containing 140 mM NaCl and 5 mM $MgCl_2$. Error bars represent standard deviations of the measurements ($n = 3$).

percentage was nearly the same as blank ground current. These current responses indicated that the present DNA sensing strategy has a high electrochemical specificity.

The electrode-to-electrode reproducibility was also evaluated. Five integrated biosensors prepared with the same procedure were tested in multiple assay concentrations at 0.25 pM, 1 pM, and 10 pM. The relative standard deviations (RSD) were 4.8%, 5.6%, and 6.0% respectively. The excellent reproducibility strongly confirmed that the label-free signal amplification method remarkably minimized the biosensor-to-biosensor deviation. Stability of the fabricated biosensor was also examined. After the biosensor was stored at 4 °C for over 2 weeks, no obvious changes in electrochemical response were obtained, thereby indicating a high stability.

3.7. Real sample detection

To prove whether the fabricated DNA biosensor could detect actual samples sensitively, the *mecA* gene extractive of standard *mecA*-positive strains of *S. aureus* (SA0129) was collected as the detecting targets (see in supplementary information).

4. Conclusions

An ultrasensitive E-DNA sensor based on target recycling signal amplification for the detection of *mecA* gene in MRSA is presented in this study. With the help of the amplification capability of ISDPR, the proposed electrochemical biosensor exhibited remarkable analytical capability toward the *mecA* gene. A sensitive detection limit of 63 fM and a wider linear range from 0.075 pM to 200 pM were achieved. The E-DNA sensor provided a simple signal transduction manner that greatly simplified the detection process. Meanwhile, combining target recycling with the label-free electrochemical detection method, the detection sensitivity is improved. Repeated thermal cycling, specific recognition site, and extra instrumentation were not required in this method, which is good for point-of-care test. More importantly, this electrochemical biosensor could be conveniently extended to detect other nucleic acids simply by altering the corresponding DNA sequence. Results showed that the proposed biosensor has great potential

for application in the fields of food safety, disease diagnosis, and environmental monitoring.

Acknowledgment

This work was financially supported by Natural Science Foundation of China (No. 81171415).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2015.06.057

References

[1] H. Grundmann, M. Aires-de-Sousa, J. Boyce, E. Tiemersma, Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat, Lancet 368 (2006) 874–885.
[2] F.R. DeLeo, M. Otto, B.N. Kreiswirth, H.F. Chambers, Community-associated meticillin-resistant *Staphylococcus aureus*, Lancet 375 (2010) 1557–1568.
[3] M.Z. David, R.S. Daum, Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic, Clin. Microbiol. Rev. 23 (2010) 616–687.
[4] S. Beekmann, D. Diekema, K. Chapin, G. Doern, Effects of rapid detection of bloodstream infections on length of hospitalization and hospital charges, J. Clin. Microbiol. 41 (2003) 3119–3125.
[5] P.D. Stamper, M. Cai, T. Howard, S. Speser, K.C. Carroll, Clinical validation of the molecular BD GeneOhm StaphSR assay for direct detection of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* in positive blood cultures, J. Clin. Microbiol. 45 (2007) 2191–2196.
[6] A. Huletsky, R. Giroux, V. Rossbach, M. Gagnon, M. Vaillancourt, M. Bernier, F. Gagnon, K. Truchon, M. Bastien, F. Picard, New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci, J. Clin. Microbiol. 42 (2004) 1875–1884.
[7] P. Francois, D. Pittet, M. Bento, B.a. Pepey, P. Vaudaux, D. Lew, J. Schrenzel, Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay, J. Clin. Microbiol. 41 (2003) 254–260.
[8] L.G. Bode, P. van Wunnik, N. Vaessen, P.H. Savelkoul, L.C. Smeets, Rapid detection of methicillin-resistant *Staphylococcus aureus* in screening samples by relative quantification between the *mecA* gene and the SA442 gene, J. Microbiol. Methods 89 (2012) 129–132.
[9] T. Ito, K. Okuma, X.X. Ma, H. Yuzawa, K. Hiramatsu, Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC, Drug Resist. Updates 6 (2003) 41–52.
[10] L.I. Llarrull, J.F. Fisher, S. Mobashery, Molecular basis and phenotype of methicillin resistance in *Staphylococcus aureus* and insights into new β-lactams that meet the challenge, Antimicrob. Agents Chemother. 53 (2009) 4051–4063.
[11] M. Ender, B. Berger-Bächi, N. McCallum, A novel DNA-binding protein modulating methicillin resistance in *Staphylococcus aureus*, BMC Microbiol. 9 (2009) 15.
[12] A. Villegas-Estrada, M. Lee, D. Hesek, S.B. Vakulenko, S. Mobashery, Co-opting the cell wall in fighting methicillin-resistant *Staphylococcus aureus*: potent inhibition of PBP 2a by two anti-MRSA β-lactam antibiotics, J. Am. Chem. Soc. 130 (2008) 9212–9213.
[13] W.C. Lindsey, E.S. Woodruff, D. Weed, D.C. Ward, R.D. Jenison, Development of a rapid diagnostic assay for methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative Staphylococcus, Diagn. Microbiol. Infect. Dis. 61 (2008) 273–279.
[14] J. Klonoski, R. Mondesire, L. Rea, D.C. Ward, R.D. Jenison, Enhanced detection of staphylococcal genomes in positive blood cultures using a polymeric enzyme complex, Anal. Biochem. 396 (2010) 284–289.
[15] Z. Wang, J. Zhang, P. Chen, X. Zhou, Y. Yang, S. Wu, L. Niu, Y. Han, L. Wang, F. Boey, Label-free, electrochemical detection of methicillin-resistant *Staphylococcus aureus* DNA with reduced graphene oxide-modified electrodes, Biosens. Bioelectron. 26 (2011) 3881–3886.
[16] D.K. Corrigan, H. Schulze, G. Henihan, I. Ciani, G. Giraud, J.G. Terry, A.J. Walton, R. Pethig, P. Ghazal, J. Crain, Impedimetric detection of single-stranded PCR products derived from methicillin resistant *Staphylococcus aureus* (MRSA) isolates, Biosens. Bioelectron. 34 (2012) 178–184.
[17] M. Liu, H. Xiang, E. Hua, L. Wang, X. Jing, X. Cao, S. Sheng, G. Xie, Ultrasensitive electrochemical biosensor for the detection of the *mecA* gene sequence in methicillin-resistant strains of *Staphylococcus aureus* employing gold nanoparticles, Anal. Lett. 47 (2014) 579–591.
[18] S. Tombelli, M. Minunni, A. Santucci, M. Spiriti, M. Mascini, A DNA-based piezoelectric biosensor: strategies for coupling nucleic acids to piezoelectric devices, Talanta 68 (2006) 806–812.
[19] C. Fan, K.W. Plaxco, A.J. Heeger, Electrochemical interrogation of conformational changes as a reagentless method for the sequence-specific detection of DNA, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 9134–9137.
[20] W. Yang, R.Y. Lai, A dual-signalling electrochemical DNA sensor based on target hybridization-induced change in DNA probe flexibility, Chem. Commun. 48 (2012) 8703–8705.
[21] I. Grabowska, K. Malecka, A. Stachyra, A. Goíra-Sochacka, A. Sirko, W. Zagoírski-Ostoja, H. Radecka, J. Radecki, Single electrode genosensor for simultaneous determination of sequences encoding hemagglutinin and neuraminidase of avian influenza virus type H5N1, Anal. Chem. 85 (2013) 10167–10173.
[22] L. Wu, X. Zhang, W. Liu, E. Xiong, J. Chen, Sensitive electrochemical aptasensor by coupling "Signal-on" and "Signal-off" strategies, Anal. Chem. 85 (2013) 8397–8402.
[23] E. Farjami, L. Clima, K. Gothelf, E.E. Ferapontova, Off–on electrochemical hairpin-DNA-based genosensor for cancer diagnostics, Anal. Chem. 83 (2011) 1594–1602.
[24] D. Li, S. Song, C. Fan, Target-responsive structural switching for nucleic acid-based sensors, Acc. Chem. Res. 43 (2010) 631–641.
[25] F. Wang, C.H. Lu, I. Willner, From cascaded catalytic nucleic acids to enzyme-DNA nanostructures: controlling reactivity, sensing, logic operations, and assembly of complex structures, Chem. Rev. 114 (2014) 2881–2941.
[26] H. Zhang, F. Li, H. Chen, Y. Ma, S. Qi, X. Chen, L. Zhou, AuNPs colorimetric sensor for detecting platelet-derived growth factor-BB based on isothermal target-triggering strand displacement amplification, Sens. Actuators B 207 (2015) 748–755.
[27] N.C. Cady, S. Stelick, M.V. Kunnavakkam, C.A. Batt, Real-time PCR detection of Listeria monocytogenes using an integrated microfluidics platform, Sens. Actuators B 107 (2005) 332–341, Q.
[28] D.M. Chen, Z.F. Gao, J. Jia, N.B. Li, H.Q. Luo, A sensitive and selective electrochemical biosensor for detection of mercury (II) ions based on nicking endonuclease-assisted signal amplification, Sens. Actuators B 210 (2015) 290–296.
[29] F. Xuan, X. Luo, I.-M. Hsing, Ultrasensitive solution-phase electrochemical molecular beacon-based DNA detection with signal amplification by exonuclease III-assisted target recycling, Anal. Chem. 84 (2012) 5216–5220.
[30] M. Liu, J. Song, S. Shuang, C. Dong, J.D. Brennan, Y. Li, A graphene-based biosensing platform based on the release of DNA probes and rolling circle amplification, ACS Nano 8 (2014) 5564–5573.
[31] C. Yao, Y. Xiang, K. Deng, H. Xia, W. Fu, Sensitive and specific HBV genomic DNA detection using RCA-based QCM biosensor, Sens. Actuators B 181 (2013) 382–387.
[32] X. Xue-tao, L. Kai-yi, Z. Jia-ying, Portable, sensitive quantitative detection of DNA based on personal glucose meters and isothermal circular strand-displacement polymerization reaction, Biosens. Bioelectron. 64 (2015) 671–675.
[33] K. Feng, L.-P. Qiu, Y. Yang, Z.-S. Wu, G.-L. Shen, R.-Q. Yu, Label-free optical bifunctional oligonucleotide probe for homogeneous amplification detection of disease markers, Biosens. Bioelectron. 29 (2011) 66–75.
[34] Z. Li, W. Zhu, J. Zhang, J. Jiang, G. Shen, R. Yu, A label-free amplified fluorescence DNA detection based on isothermal circular strand-displacement polymerization reaction and graphene oxide, Analyst 138 (2013) 3616–3620.
[35] Y. Qian, D. Tang, L. Du, Y. Zhang, L. Zhang, F. Gao, A novel signal-on electrochemical DNA sensor based on target catalyzed hairpin assembly strategy, Biosens. Bioelectron. 64 (2015) 177–181.
[36] D.Z. Wang, W. Tang, X.J. Wu, X.Y. Wang, G.J. Chen, Q. Chen, N. Li, F. Liu, Highly selective detection of single-nucleotide polymorphisms using a quartz crystal microbalance biosensor based on the toehold-mediated strand displacement reaction, Anal. Chem. 84 (2012) 7008–7014.
[37] F. Ricci, R.Y. Lai, K.W. Plaxco, Linear, redox modified DNA probes as electrochemical DNA sensors, Chem. Commun. 36 (2007) 3768–3770.
[38] F. Ricci, R.Y. Lai, A.J. Heeger, K.W. Plaxco, J.J. Sumner, Effect of molecular crowding on the response of an electrochemical DNA sensor, Langmuir 23 (2007) 6827–6834.

Biographies

Ting Wang is a currently postgraduate of Chongqing Medical University majoring in clinical laboratory diagnostics.

Zhang Zhang is a currently postgraduate of Chongqing Medical University majoring in clinical laboratory diagnostics.

Yiyan Li is currently a Ph.D. student in electrical and computer engineering at University of Nevada Las Vegas. He received his B.S. degree in biomedical engineering from Henan University of Science and Technology in 2009, and his M.S. degree in biomedical engineering from Chongqing University in 2012. His research interest includes the development of high throughput automated bio-chips for immunohistochemistry experiments, nanomaterial fabrication, analog to digital converters and electrical stimulation for neural disorders.

Guoming Xie is a Professor in the College of Clinical Laboratory Diagnostics, Chongqing Medical University, China. He received his B.S. degree in electron engineering from Chongqing University in 1989, and his M.S. degree in biomedical engineering from Chongqing University in 1997. During 2013–2014, he was a visiting scholar in University of Illinois at Urbana-Champaign and University of Nevada, Las Vegas. He has published more than 30 papers on various aspects of electrochemical biosensors in reputed national and international journals. His research is focused on point of care testing and electrochemical biosensor.