Biosensors and Bioelectronics 77 (2016) 19–25

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A facile and pragmatic electrochemical biosensing strategy for ultrasensitive detection of DNA in real sample based on defective T junction induced transcription amplification

Rui Yuan [a,b], Shijia Ding [b], Yurong Yan [b], Ye Zhang [b], Yuhong Zhang [a], Wei Cheng [a,*]

[a] The Center for Clinical Molecular Medical detection, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, PR China
[b] Key Laboratory of Clinical Laboratory Diagnostics (Ministry of Education), College of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, PR China

ARTICLE INFO

Article history:
Received 26 June 2015
Received in revised form
25 August 2015
Accepted 4 September 2015
Available online 8 September 2015

Keywords:
Electrochemical DNA biosensor
Transcription amplification
Group B Streptococci
Signal amplification

ABSTRACT

A novel and pragmatic electrochemical sensing strategy was developed for ultrasensitive and specific detection of nucleic acids by combining with defective T junction induced transcription amplification (DTITA). The homogeneous recognition and specific binding of target DNA with a pair of designed probes formed a defective T junction, further triggered primer extension reaction and in vitro transcription amplification to produce numerous single-stranded RNA. These RNA products of DTITA could hybridized with the biotinylated detection probes and immobilized capture probes for enzyme-amplified electrochemical detection on the surface of the biosensor. The proposed isothermal DTITA strategy displayed remarkable signal amplification performance and reproducibility. The electrochemical DNA biosensor showed very high sensitivity for target DNA with a low detection limit of 0.4 fM (240 molecules of the synthetic DNA), and can directly detect target pathogenic gene of *Group B Streptococci* (GBS) from as low as 400 copies of genomic DNA. Moreover, the established biosensor was successfully verified for directly identifying GBS in clinical samples. This proposed strategy presented a simple and pragmatic platform toward ultrasensitive and handy nucleic acids detection, and would become a potential tool for general application in point-of-care setting.

1. Introduction

Highly sensitive detection of sequence-specific DNA plays essential roles in early clinical diagnosis (Debouck and Goodfellow, 1999), environmental monitoring (Palchetti and Mascini, 2008), forensic analysis (Divne and Allen, 2005) as well as counter-terrorist (Varma-Basil et al., 2004). Especially, point-of-care testing (POCT) for nucleic acid detection is attracting considerable interest owing to its general application in the setting where suitable facilities are unavailable and a rapid answer is required (Niemz et al., 2011). Over the past decades, PCR-based methods have facilitated rapid and accurate identification of DNA in central laboratories (Park et al., 2013; de-Paz et al., 2014). However, requirements of rigid control of temperature cycling, sophisticated and expensive equipments, and highly trained analysts limit its application in point-of-care (de-Paz et al., 2014; Tanaka et al., 2015). Thus, it is still urgent to develop highly sensitive, low-complexity, handy methods for DNA detection in point-of-care setting.

Electrochemical biosensor has attracted substantial attention as a potential POCT platform owing to its high sensitivity, easy to use, rapid response, low cost and inexpensive instrumentation (Wang, 2002; Dungchai et al., 2009; Lu et al., 2012). To explore the development of highly sensitive electrochemical DNA sensor, several isothermal amplification techniques have been used. These isothermal DNA amplification strategies, such as the strand displacement reaction (SDR) (Gao et al., 2014; Cheng et al., 2014), the hybridization chain reaction (HCR) (Gao et al., 2013; Li et al., 2014), rolling circle amplification (RCA) (Jiang et al., 2014; Deng et al., 2014), do not require special laboratory conditions for thermal cycling and is highly compatible to biosensor systems. But most of them trended to perform DNA target recycle or amplification directly on the electrode surface (Chen et al., 2011). The inherent features of heterogeneous formats, including the steric hindrance, restricted configurational freedom and the variant chemical microenvironment, lead to relatively low hybridization efficiency and enzyme kinetics (Vijayendran and Leckband, 2001). These disadvantages counteracted the sensitivity and reproducibility of the heterogeneous DNA amplification-based biosensor, especially in the setting of detection a low abundance target gene from matrix genomic DNA in real sample (Miranda-Castro et al., 2012).

* Corresponding author. Fax: +86 23 89011816.
E-mail address: chengwei@cqmu.edu.cn (W. Cheng).

http://dx.doi.org/10.1016/j.bios.2015.09.009
0956-5663/

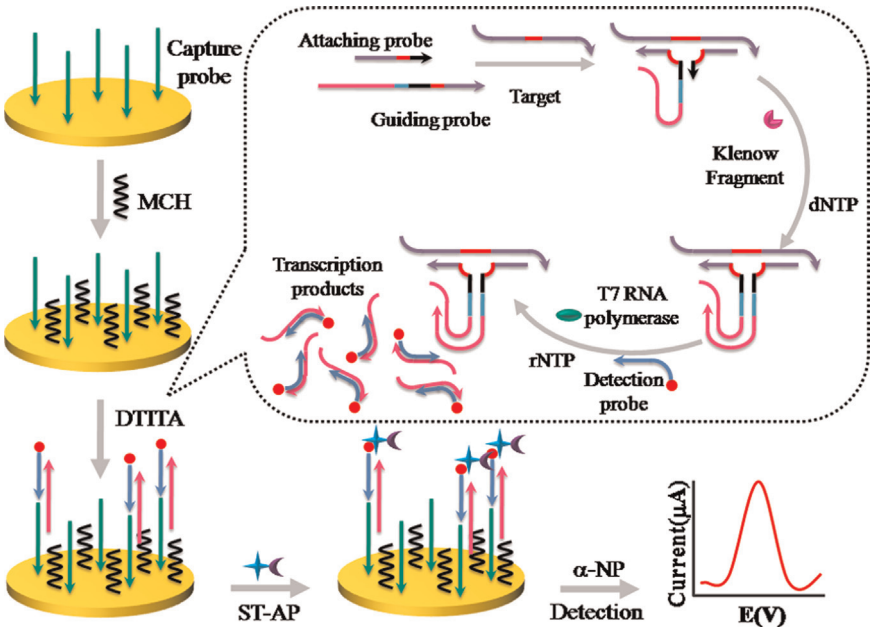

Fig. 1. Schematic representation of the DITIA strategy for electrochemical DNA biosensing.

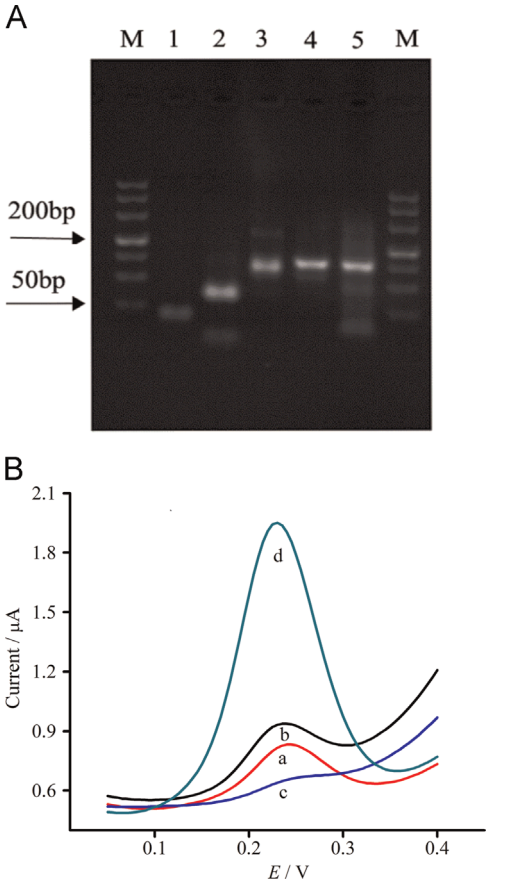

Fig. 2. (A) gel electrophoresis analysis of 2 μM target DNA (lane 1), 2 μM attaching probe and 2 μM guiding probe (lane 2), hybridization of 0.75 μM target DNA with 0.75 μM attaching probe and 0.75 μM guiding probe (lane 3), products of target DNA and two probes after primer extension reaction (lane 4) and transcription amplification (lane 5). (B) Typical DPV curves of designed biosensor responding to blank control (a), 1 nM target DNA without primer extension reaction (b), without transcription amplification (c), and with integrity DTITA (d), respectively.

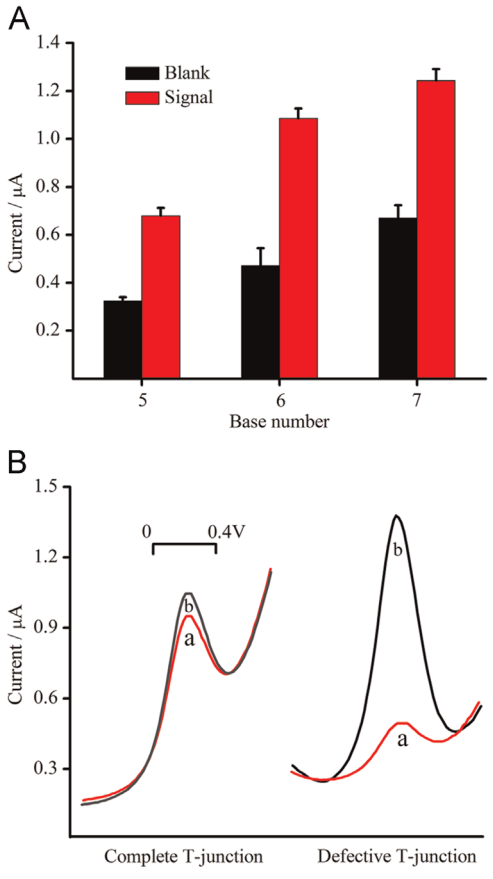

Fig. 3. (A) DPV signals of the designed biosensor by using complete T-junction in responding to blank and 1 nM target DNA with different base numbers for inter-hybridization of two probes, respectively. Error bars are standard derivation obtained from three independent experiments. (B) Typical DPV curves of the designed biosensor by using complete T-junction and defective T-junction in responding to blank (a) and 1 pM target DNA (b).

T7 RNA polymerase, a class of DNA-dependent RNA polymerase, only recognizes the specific promoter sequence and transcribes its downstream DNA sequence at approximate rate of

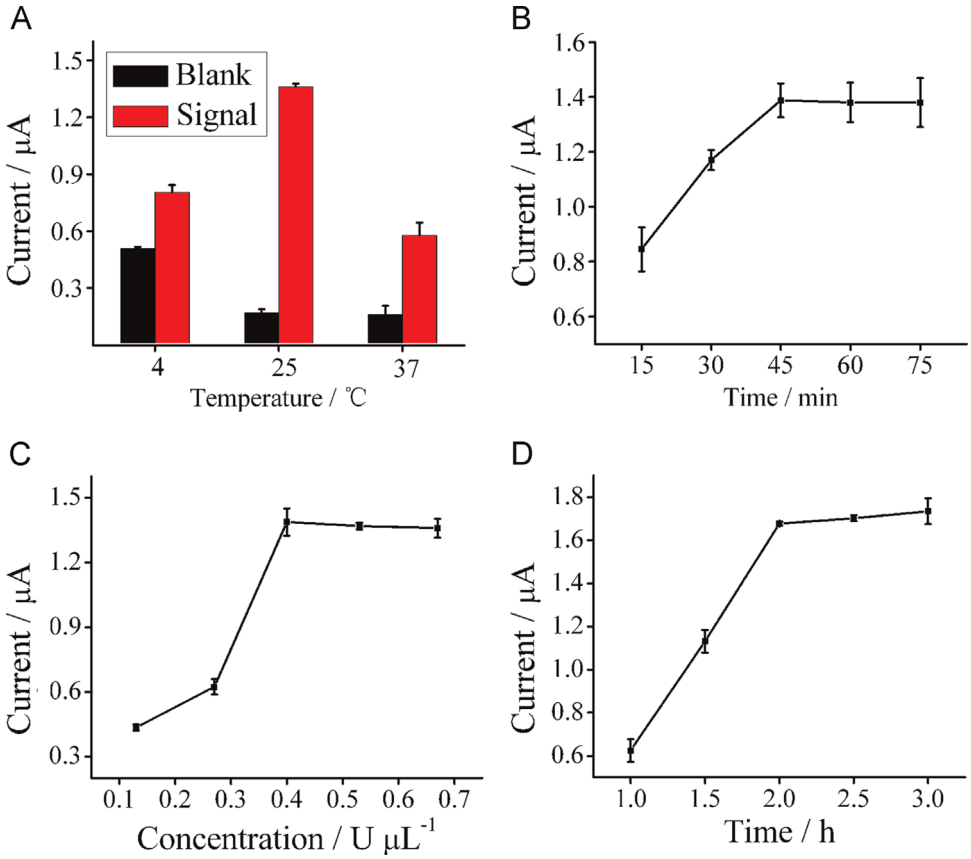

Fig. 4. Dependences of DPV peak currents on the temperature for hybridization of RNA products with detection and capture probes (A), the reaction time of primer extention (B), concentration of T7 RNA polymerase (C) and reaction time for transcription amplification (D). When one parameter changed and the others were under their optimal conditions, except the time for transcription amplification was 100 min in A, B and C. Error bars are standard derivation obtained from three independent experiments.

100 bp per second. The RNA transcription products only depend on the quantity of template DNA (Zhang et al., 2001). Compared with isothermal DNA amplification such as RCA, SDA, the transcription amplification based on T7 polymerase is highly compatible to target-dependent signal amplification and holds higher flexibility for the design of amplification products (Zhang et al., 2006; Kattah et al., 2008). So, T7-based transcription amplification has been attempted to develop sensitive and multianalyte assays for cell surface molecules, DNA binding proteins and nucleic acids (Zhao et al., 2012; Ma et al., 2014; Yu et al., 2014; Yin et al., 2015).

Herein, aiming at further improving the efficiency and practicality of electrochemical DNA biosensor for POCT, a simple, highly sensitive, and specfic electrochemical biosensing methodology was developed by combining with a new target-dependent isothermal signal amplification, named defective T junction induced transcription amplification (DTITA). The homogeneous recognition and specific binding of target DNA with a pair of designed probes formed a defective T junction which contained an artificial vacuole at the junction site and further triggered in vitro transcription amplification to produce numerous single-stranded RNA. These RNA products could hybridized with the biotinylated detection probes and immobilized capture probes for enzyme-amplified electrochemical readout on the surface of the biosensor. Compared with the classic T junction DNA framework, the designed defective T junction proved more conformational stability and homogenicity, and could remarkably improve the signal-to-noise ratio and reproducibility of the proposed target-dependent transcription amplification. A pathogenic gene of *Group B Streptococci* (GBS) was used as a model target DNA to verify the practicability of the designed strategy. GBS is an important pathogen in maternal and newborns infection at perinatal stage (Johri et al., 2006). Prenatal or intrapartum screening of GBS at point-of-care plays vital role in guiding reasonable antibiotic prophylaxis (Verani et al., 2010). The proposed DTITA-based electrochemical DNA biosensor showed very high sensitivity and selectivity and was successfully applied to directly screening GBS from clinical vaginal/anal samples. Thus, the designed electrochemical biosensing strategy presented a simple and pragmatic platform toward ultrasensitive nucleic acids detection, and a potential tool for general application in POCT.

2. Materials and methods

2.1. Materials and reagents

Klenow Fragment (3′→5′exo$^-$) (KF exo$^-$), 10 × KF buffer (500 mM Tris–HCl, 50 mM $MgCl_2$, 10 mM DTT, pH 8.0), T7 RNA Polymerase and 5 × Transcription buffer (200 mM Tris–HCl, 30 mM $MgCl_2$, 50 mM DTT, 50 mM NaCl, 10 mM spermidine, pH 7.9) were obtained from Fermentas (Lithuania). 6-Mercapto-1-hexanol (MCH), α-naphthyl phosphate (α-NP), streptavidin–alkaline phosphatase (ST–ALP), deionized formamide and polyethylene glycol sorbitan monolaurate (Tween-20) were purchased from Sigma-Aldrich (USA). Gold-view, DL500 DNA Marker and recombinant RNase inhibitor, diethypyrocarbonate (DEPC), dNTP mixture solution and NTP mixture solution were purchased from Takara (Dalian, China). The Group *B Streptococus* Nucleic Aid Detection Kit (Fluorescent PCR) was purchased from Taipu Bioscience Co., Ltd. (Fujian, China). All DNA oligonucleotides were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. The gene of GBS surface immunogenic protein (GenBank Accession no. 1012782),

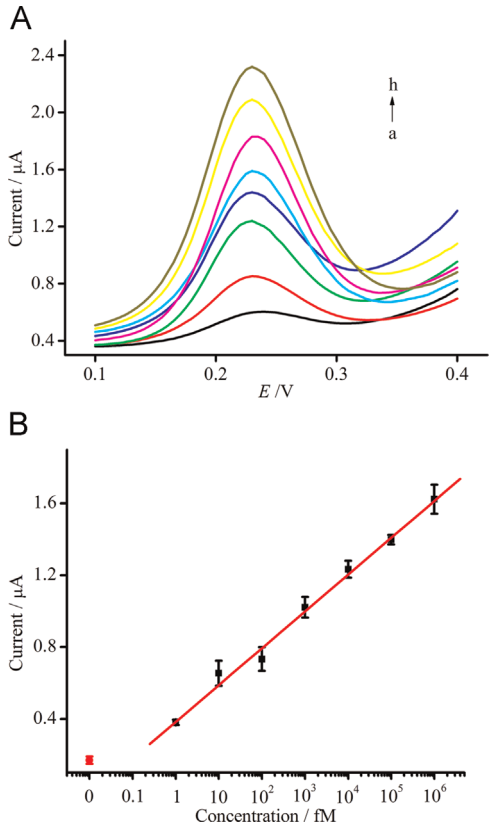

Fig. 5. (A) Typical DPV curves of the designed method responding to 0, 0.001, 0.01, 0.1, 1, 10, 100 pM and 1 nM of synthetic target DNA (from a to h), respectively. (B) Calibration plot of DPV peak current vs. logarithm of target DNA concentration. The error bars represent the standard deviations in three different measurements for each concentration.

which exist in all GBS was used as target. The specificity of probes has been verified in GenBank. The detailed sequences are listed in Table S1. All other reagents were of analytical grade and solutions were prepared and diluted by RNase-free water.

2.2. Apparatus

All electrochemical measurements were performed on a CHI660D electrochemical workstation (Shanghai Chenhua Instruments Co. Ltd., China) with a conventional three-electrode system composed of platinum wire as auxiliary, Ag/AgCl electrode as reference, and a 3-mm diameter gold electrode (GE) as working electrode.

2.3. DNA extraction from GBS culture and clinical specimens

GBS strain was obtained from Chongqing Municipal Center for Disease Control and Prevention. The pure culture of GBS was grown in Luria-Bertani medium at 37 °C for 16 h with shaking. The culture was then washed twice in sterile ultrapure water by centrifugation at 12,000 rpm for 10 min and resuspended in sterile ultrapure water. Viable counts were performed by plating 100 μL of appropriate 10-fold dilution in sterile ultrapure water onto plate count agar. After incubating the plates at 37 °C for 24 h, the culture colonies were counted to estimate forming unit per milliliter (CFU mL^{-1}) on the plates.

The vaginal/anal samples of pregnant women for prenatal or intrapartum GBS screening were collected from The First Affiliated Hospital of Chongqing Medical University. Genomic DNA was extracted from 2 mL of different concentration cultures using the TaKaRa MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 according to the instructions and resuspended in 50 μL of sterile ultrapure water, so did the clinical samples. All prepared DNA were stored at −20 °C for further use.

2.4. Sensor fabrication

The gold electrode was polished to a mirror sequentially with 0.3 μm and 0.05 μm alumina powder, followed by ultrasonic cleaning with water, ethanol and water. Then, the electrode was soaked in the piranha solution (H_2SO_4:H_2O_2=3:1) for 10 min, followed by rinsing thoroughly with ultrapure water to eliminate other substances. 10 μL 200 nM thiolated capture probe was dropped on the prepared electrode surface and incubated overnight at 4 °C. After washing with Tris–HCl buffer solution (50 mM Tris–HCl, 6 mM $MgCl_2$, 10 mM NaCl, pH 7.5), the resulting electrode was immersed into 1 mM MCH solution for 1 h to occupy the left bare sites on electrode surface and obtain well-aligned DNA monolayer. Then the electrode was rinsed with Tris–HCl buffer and further treated with 2% BSA containing 125 mg/mL salmon sperm DNA for 30 min to block the nonspecific binding sites on its surface. The electrochemical biosensor was rinsed with the washing buffer and used for following operation.

2.5. DNA detection protocol

3 μL target DNA was added to a 5 μL mixture whihch contained 2 nM guiding probe, 2 nM attaching probe and 15% deionized formamide in Tris–HCl buffer solution. Then, the mix was heated at 95 °C for 5 min in a water bath and cooled down slowly to the room temperature to ensure denaturing of genomic DNA and forming of the T-DNA complex molecular structure. Hereafter, 2 μL

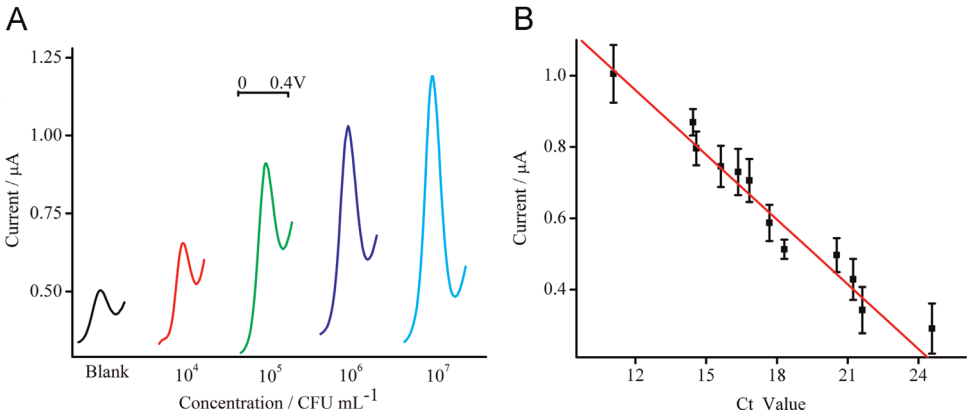

Fig. 6. (A) Typical DPV curves responding to different concentrations of GBS. (B) Comparison between the DPV peak currents of proposed biosensor and *Ct* value of RT-PCR assay for GBS positive clinical samples. Error bars are standard derivation obtained from three independent experiments.

extension solution containing 500 nM each dNTP, 0.5 unit μL^{-1} KF exo$^-$ in KF buffer were added to the mixture and incubated at 41 °C for 45 min for extension reaction. KF exo$^-$ was inactivated by heating at 75 °C for 10 min. Then 20 μL transcription solution including 1 mM rNTPs, 50 nM biotinylated detection probe, 0.4 unit μL^{-1} T7 RNA polymerase, 0.8 unit μL^{-1} RNase inhibitor in transcription buffer were added and the mix was incubated at 37 °C for 2 h for transcription amplification.

Then, 10 μL of resulted reaction mixture was dropped on the above prepared electrodes and incubated at room temperature for 1 h. The electrode was then rinsed with diethanolamine buffer solution (DEA, 100 mM diethanolamine, 1 mM $MgCl_2$, 100 mM KCl, pH 9.62). Subsequently, 10 μL of DEA buffer containing 1.25 μg/mL ST-ALP and 10 mg mL^{-1} BSA was dropped on the sensor surface and incubated for 30 min at room temperature. The electrochemical sensor was washed thoroughly with DEA buffer containing 0.05% Tween-20. The electrochemical measurement was performed in DEA buffer containing 1 mg mL^{-1} of α-NP substrate with modulation time of 0.05 s, interval time of 0.017 s, step potential of 5 mV, modulation amplitude of 70 mV and potential scan from 0.0 to+0.5 V.

2.6. Gel electrophoresis

A 2% agarose gel electrophoresis analysis of the products via the polymerization and transcription reaction was carried out in 1 × TBE buffer (90 mM Tris–HCl, 90 mM boric acid, 2 mM EDTA, pH 7.9) at 120 mV for about 40 min. The gel was photographed by Bio-Rad digital imaging system.

3. Results and discussion

3.1. Design of DTITA and electrochemical DNA sensing strategy

As shown in Fig. 1. A pair of single-strand probes, named attaching and guiding probes, were designed to contain target recognition regions and short inter-hybridization regions, respectively. Furthermore, the guiding probe also included T7 polymerase promoter and downstream template sequence (see Table S1 for the detailed sequence information). In the presence of the target DNA, two probes specifically bound to target DNA with their target recognition regions and hybridized with each other for forming a defective T junction by manufacturing an artificial vacuole at the junction region. Followed the T-junction DNA molecular formation, primer extension reaction was performed in the presence of KF exo- and dNTPs, producing a DNA duplex. Then T7 RNA polymerase recognized the double-stranded T7 promoter and triggered transcription amplification, resulting in numerous single-stranded RNA products. These RNA products of DTITA were hybridized with the biotinylated detection probes and immobilized capture probes on the surface of the biosensor. ST-ALP was then labeled on the sensor surface by the specific recognition of streptavidin and biotin, and implemented enzyme-amplified electrochemical readout for quantitative detection of target DNA.

3.2. Verification of the designed strategy

In order to validate the designed DTITA strategy, several reaction products were characterized by agarose gel electrophoresis. As shown in Fig. 2A, the hybridization product of the attaching, guiding probes with synthetic DNA target exhibited one well-defined band with relative less migration than that of separate target and probes, proving the formation of the expected T junction (Zhu et al., 2013). After the primer extension reaction, the corresponding band showed a slightly slower migration, owing to the implement of polymerization reaction (Zhang et al., 2014). When the T7 RNA polymerase and rNTPs were added, a new dispersion band about 40 bp was obviously observed, which account for the RNA products of transcription amplification. These results indicated the feasibility of the designed DTITA strategy directly.

To further characterize the feasibility of the combination of DTITA with electrochemical DNA biosensor for target DNA detction, DPV measurements were performed with or without the integrity DTITA (Fig. 2B). The DPV curves showed a well-defined oxidation peak for target DNA, which corresponded to the oxidation of a-naphthyl, the ALP-catalyzed product of a-NP. Compared with the controls of the absent of the polymerization or transcription reaction, The DPV signal corresponding to 1 nM target DNA showed remarkable increase with the introduction of integrity DTITA, indicating that the target-dependent DTITA formed a lot of RNA products for electrochemical DNA biosensing. A slight DPV signal for blank was observed with DTITA, which was likely attributed to that a little part of the transcription due to a weak nonspecific binding of two probes.

3.3. Probes design

The design of attaching and guiding probes was critical for the signal amplification performance of T junction induced transcription amplification. Firstly, the attaching and guiding probes were designed to completely match with target and each other for forming complete T-junction without any vacuole at the junction site. We attempted to improve the signal-to-noise ratio through optimization of the base number of inter-hybridization regions. Though signal-to-noise ratio of 6-base complementary sequences was higher than that of 5 and 7-base, it could not ensure high performance of target-dependent transcription amplification (Fig. 3A).

Chemical linker molecule such as hexaethylene glycol has been applied to incorporate into the guiding probe at the junction site and improved the signal-to-noise ratio (Wharam et al., 2001). But interpolation of non-nucleotide linker into oligonucleotides may increase the cost and difficulty for probes preparation. Herein, by designing a pair of probes containing two unpaired nucleotides in the junction site respectively, a vacuole at the junction region with target was artificially manufactured, forming a defective T junction to perform target-dependent transcription amplification. As showing in Fig. 3B, the signal of defective T junction responding to 1 pM of target DNA was 6 times as much as that of background, displaying remarkable signal-to-noise ratio by both increasing the signal and repressing the background. Meanwhile, the signal of complete T-junction at 1 pM of target DNA showed little difference with that of background. Furthermore, the variation coefficients of signal of the defective T-junction were calculated to be 5.6%, which was obviously lower than 15% of that of complete T-junction. These results attributed to that the defective T junction held more conformational stability and homogenicity (Leontis et al., 1991), and clearly proved that the designed defective T junction was contributed to improve the signal-to-noise ratio and reproducibility of the proposed target-dependent transcription amplification and electrochemical DNA sensing strategy.

3.4. Optimization of detection conditions

In order to achieve optimal sensing performance, several important experimental parameters were investigated. The peak currents of DPV were used to evaluate the performance of the proposed approach. The hybridization temperature of the RNA products with the detection probe and capture probe had an great influence on the DPV current response. For implement reaction on the biosensor surface at near room temperature, the detection and

capture probes were designed to hold 20 bases of complementary sequences with transcription products, respectively. As shown in Fig. 4A, the maximum signal-to-noise ratio was indeed achieved at 25 °C. At lower temperature, the probes and RNA products could not get a sufficient collision probability that significantly reduced the formation of three strands complexes as well as increased some non-specific binding. On the other hand, at higher temperature than melting temperature, the hybridization was not sufficient.

The primer extension process of T-junction structure could be affected by the reaction time of KF exo$^-$. The peak current increased with the increasing reaction time and tended to a constant value at 45 min (Fig. 4B). Therefore, 45 min was chosen as the optimal time for primer extension reaction. The effect of the concentration of T7 RNA polymerase for transcription amplification was depicted in Fig. 4C. The peak current increased until the amount of T7 RNA polymerase exceeded 0.4 unit μL^{-1} which was selected as the optimal concentration. The transcription reaction time was then investigated (Fig. 4D). With the increasing reaction time, the obtained peak current increased rapidly and then tended to reach the platform at 2 h. Thus, 2 h was selected as the optimum reaction duration for transcription process.

3.5. Analytical performance of biosensor

Under the optimal experimental conditions, the DPV current response gradually increased with the elevated concentration of the synthetic target oligonucleotides (Fig. 5A). The plot of the response vs. the logarithm of target DNA concentration showed a strong linear relationship in the range from 1 fM to 1 nM with a correlation coefficient of 0.999 (Fig. 5B). The limit of detection (LOD) was calculated to be 0.4 fM in a 3σ rule. The obtained LOD was superior sensitivity than previous reported assays with T7 RNA polymerase-aided signal amplification (Sendroiu et al., 2011; Yu et al., 2014; Yin et al., 2015) and those assays which performed target binding and isothermal DNA amplification on the sensor surface (Table S2). In other words, the proposed biosensor was able to respond to 240 molecules of the synthetic target DNA in a 10 μL reaction mixture. The ultrahigh sensitivity was attributed to the high efficiency of homogeneous target binding, remarkable signal amplification performance and low background signal of DTITA.

To estimate the reproducibility of the developed electrochemical biosensor, the intra-assay imprecision of three different sensors at one assay and inter-assay imprecision at three different assays for detection of 1 pM target DNA were examined, respectively. The intra-assay coefficient of variation (CV) was 1.65% and the inter-assay CV was 7.56%, showing remarkable precision and reproducibility due to high conformational stability and homogenicity of the designed defective T junction.

3.6. Real sample analysis

In order to evaluate the practical applicability in real sample analysis, the established biosensor was employed to directly detect target gene from genomic DNA of serially diluted GBS without PCR. As shown in Fig. 6A, the DPV responses were proportional with the concentrations of genomic DNA in the range of 10^4–10^7 CFU mL^{-1}. According to the detection protocol, the detection limit was calculated to be about 400 copies of GBS genomic DNA, which was lower than that of PCR coupling with gel electrophoresis for pathogenic bacteria detection (Lei et al., 2015). The result also confirmed no loss of analytical performance of the biosensor for detection of enormous and complicated genomic DNA in real sample.

The proposed strategy was further applied to direct screening GBS from clinical vaginal/anal samples of pregnant women at 35–37 weeks of gestation without pre-PCR amplification. Among the 23 vaginal/anal lysates, 12 were identified as positive and 11 identified as negative by the proposed strategy with a positive threshold of 0.266 μA which was defined as three times of the standard deviation of 5 blank samples. Then these samples were identified with a commercial RT-PCR assay, which was approved by China Food and Drug Administration (CFDA). The proposed method agreed with the RT-PCR results completely, showing 100% of both positive predictive value and negative predictive value. When comparing the results of two assays for the 12 GBS positive samples using regression analysis, the plots of the cycle thresholds (*Ct*) obtained with the RT-PCR assay vs that of DPV peak currents obtained with the established biosensor gave a R^2 value of 0.961, still further confirming complete agreement of two methods (Fig. 6B). Furthermore, the proposed method could accurately identify four low bacterial load samples which displayed higher *Ct* in the range from 20 to 26. These results demonstrated that the proposed method held comparable sensitivity and specificity with CFDA-proposed RT-PCR method for GBS screening in real clinical samples. More importantly, these significant analytical performance was implemented without the need of sophisticated instrument for temperature cycling, complicated probes and procedures for cascade signal amplification and interfacial nano-fabrication, exhibiting great application potential towards point-of-care testing.

4. Conclusion

This work has demonstrated an ultrasensitive and specific electrochemical biosensor for convenient detection of target DNA by integrating a homogeneous target-dependent DTITA and the interface biosensing. The DTITA strategy demonstrates remarkable amplification performance by transition of efficient target binding to a lot of RNA transcription products. The designed method shows an ultra-high sensitivity, acceptable reproducibility and is applicable for handy screening of target DNA in real clinical samples without requirement of sophisticated thermal cycling and complicate detection system. This proposed strategy presents a facile and pragmatic platform toward ultrasensitive nucleic acids detection and would become a potential tool for general application in point-of-care nucleic acids assay.

Acknowledgments

This work was funded by the National Natural Science Foundation of PR China (81101638, 81371904 and 81572080), the Natural Science Foundation Project of Chongqing, PR China (CSTC2013jjB10019 and cstc2014kjrc-qnrc10001).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.09.009.

References

Chen, Y., Jiang, B., Xiang, Y., Chai, Y., Yuan, R., 2011. Chem. Commun. 47, 12798–12800.
Cheng, W., Zhang, W., Yan, Y.R., Shen, B., Zhu, D., Lei, P.H., Ding, S.J., 2014. Biosens. Bioelectron. 62, 274–279.
Debouck, C., Goodfellow, P.N., 1999. Nat. Genet. 21, 48–50.
Deng, R., Tang, L., Tian, Q., Wang, Y., Lin, L., Li, J., 2014. Angew. Chem. Int. Ed. 53, 2389–2393.

de-Paz, H.D., Brotons, P., Munoz-Almagro, C., 2014. Expert Rev. Mol. Diagn. 14, 827–843.
Divne, A.M., Allen, M., 2005. Forensic Sci. Int. 154, 111–121.
Dungchai, W., Chailapakul, O., Henry, C.S., 2009. Anal. Chem. 81, 5821–5826.
Gao, F.L., Lei, J.P., Ju, H.X., 2013. Anal. Chem. 85, 11788–11793.
Gao, Z.F., Ling, Y., Lu, L., Chen, N.Y., Luo, H.Q., Li, N.B., 2014. Anal. Chem. 86, 2543–2548.
Jiang, D.N., Liu, F., Liu, C., Liu, L.L., Li, Y., Pu, X.Y., 2014. Anal. Methods 6, 1558–1562.
Johri, A.K., Paoletti, L.C., Glaser, P., Dua, M., Sharma, P.K., Grandi, G., Rappuoli, R., 2006. Nat. Rev. Microbiol. 4, 932–942.
Kattah, M.G., Coller, J., Cheung, R.K., Oshidary, N., Utz, P.J., 2008. Nat. Med. 14, 1284–1289.
Lei, P.H., Tang, H., Ding, S.J., Ding, X.J., Zhu, D., Shen, B., Chen, Q., Yan, Y.R., 2015. Microchim. Acta. 182, 289–296.
Leontis, N.B., Kwok, W., Newman, J.S., 1991. Nucleic Acids Res. 19, 759–766.
Li, X., Wang, Y., Wang, L., Wei, Q., 2014. Chem. Commun. 50, 5049–5052.
Lu, J., Ge, S., Ge, L., Yan, M., Yu, J., 2012. Electrochim. Acta 80, 334–341.
Ma, F., Yang, Y., Zhang, C., 2014. Anal. Chem. 86, 6006–6011.
Miranda-Castro, R., Marchal, D., Limoges., B., Mavre, F., 2012. Chem. Commun. 48, 8772–8774.
Niemz, A., Ferguson, T.M., Boyle, D.S., 2011. Trends Biotechnol. 29, 240–250.
Palchetti, I., Mascini, M., 2008. Analyst 133, 846–854.
Park, J.S., Cho, D.H., Yang, J.H., Kim, M.Y., Shin, S.M., Kim, E.C., Park, S.S., Seong, M.W., 2013. Ann. Lab. Med. 33, 39–44.
Sendroiu, I.E., Gifford, L.K., Lupták, A., Corn, R.M., 2011. J. Am. Chem. Soc. 133, 4271–4273.
Tanaka, K., Iwashita, M., Matsushima, M., Wachi, Y., Izawa, T., Sakai, K., Kobayashi, Y., 2015. J. Matern-Fetal Neonat. Med. 0, 1–5.
Varma-Basil, M., El-Hajj, H., Marras, S.A., Hazbón, M.H., Mann, J.M., Connell, N.D., Kramer, F.R., Alland, D., 2004. Clin. Chem. 50, 1060–1062.
Verani, J.R., McGee, L., Schrag, S.J., 2010. Prevention of perinatal group B streptococcal disease, revised guidelines from CDC, 2010. MMWR Recomm. Rep. 59, 1–36.
Vijayendran, R.A., Leckband, D.E., 2001. Anal. Chem. 73, 471–480.
Wang, J., 2002. Anal. Chim. Acta. 469, 63–71.
Wharam, S.D., Marsh, P., Lloyd, J.S., Ray, T.D., Mock, G.A., Assenberg, R., Mcphee, J.E., Brown, P., Weston, A., Cardy, D.L., 2001. Nucleic Acids Res. 29, e54.
Yin, B.C., Wu, S., Ma, J.L., Ye, B.C., 2015. Biosens. Bioelectron. 68, 365–370.
Yu, C.Y., Yin, B.C., Wang, S., Xu, Z., Ye, B.C., 2014. Anal. Chem. 86, 7214–7218.
Zhang, H., Cheng, X., Richter, M., Greene, M.I., 2006. Nat. Med. 12, 473–477.
Zhang, H.T., Kacharmina, J.E., Miyashiro, K., Greene, M.I., Eberwine, J., 2001. Proc. Natl. Acad. Sci. USA 98, 5497–5502.
Zhang, Q., Chen, F., Xu, F., Zhao, Y., Fan, C., 2014. Anal. Chem. 86, 8098–8105.
Zhao, J., Zhang, L., Chen, C., Jiang, J., Yu, R., 2012. Anal. Chim. Acta. 745, 106–111.
Zhu, J., Zhang, L., Zhou, Z., Dong, S., Wang, E., 2013. Anal. Chem. 86, 312–316.