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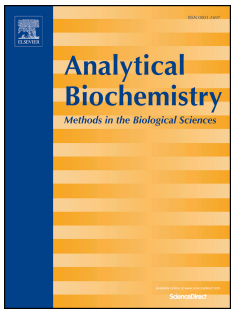

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An aptasensor for *staphylococcus aureus* based on nicking enzyme amplification reaction and rolling circle amplification

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Highlights

- A chemiluminescence aptasensor for *S. aureus* detection based on aptamer recognition and DNA amplifying cycle was established.
- The LoD was as low as 5 CFU/mL with a good linear correlation at 5-10^4 CFU/mL.
- The aptasensor can selectively distinguish living *S. aureus* against dead ones inactivated by HTHP method.

Abstract

An ultra-sensitive aptamer-based biosensor for the detection of *staphylococcus aureus* was established by adopting the nicking enzyme amplification reaction (NEAR) and the rolling circle amplification (RCA) technologies. Aptamer-probe (AP), containing an aptamer and a probe sequence, was developed to act as the recognition unit of the biosensor, which was specifically bound to *S. aureus*. The probe was released from AP and initiated into the subsequent DNA amplification reactions where *S. aureus* was present, converting the detection of *S. aureus* to the investigation of probe oligonucleotide. The RCA amplification products contained a G-quadruplex motif and formed a three dimensional structure in presence of hemin. The G4/hemin complex showed horseradish peroxidase (HRP)-mimic activity and catalyzed the chemiluminescence reaction of luminol mediated by H_2O_2. The results showed that the established biosensor could detect *S. aureus* specifically with a good linear correlation at 5-10^4 CFU/mL. The signal values based on NEAR-RCA two-step cycle were boosted acutely, much higher than that relied on one-cycle magnification. The limit of detection (LoD) was determined to be as low as 5 CFU/mL. The established aptasensor exhibited a good discrimination of living against dead

S. aureus, and can be applied to detect *S. aureus* in the food industry.

Keywords: aptamer, NEAR- RCA, G-quadruplex, *S. aureus* detection

1. Introduction

Staphylococcus aureus is one of the life-threatening pathogens occurring in a variety of foods. Cases of food poisoning incidents caused by microbes rank high and have been documented to occur up to 3,181 times accounting for 53.7% of the total cases according to National Health and Family Planning Commission of China, 2015 [1]. The emergence of *S. aureus* with methicillin-resistant (MRSA) poses a serious challenge to the prevention and control of pathogenic infections. Studies show that about one in three people carry *S. aureus* in their nose, usually without any illness and that two in 100 people carry MRSA [2]. Traditional methods of detecting *S. aureus* were based on growth culture and enzyme-linked immunosorbent assays (ELISA) which were time consuming, had low sensitivity and involved complex sample preparation [3, 4]. Therefore, a sensitive and rapid method is urgently needed to detect *S. aureus* effectively.

In the recent years, DNA nanobiosensors have been widely explored for the inspection of pathogens [5, 6]. Compared to traditional methods such as colony counting, ELISA, PCR etc., DNA nano-sensors perform faster, more efficient and more accurate owing to the advantages of DNA i.e. high stability, spatial structure variability, easy synthesis and modification [7]. The working mechanism of biosensors mainly consists of target recognition and signal conversion. The frequently used recognition elements are based on antigen-antibody, base pairing, particular chemical bond interactions (such as amino to carboxyl groups and sulfur bond to gold atom), intermolecular interactions (π-π stacking) and embedment (nitrocellulose membrane) technologies [8, 9] and all these strategies can identify their target. In particular, biosensors using aptamer as the recognition unit have rapidly developed and are capable of detecting small molecular substances, proteins, toxins, bacteria and cells [10, 11]. An aptamer is a kind of nucleic acid, DNA or RNA, with special properties of selective recognition and binding to its target in a similar manner as antigen-antibody reactions [12]. The aptamer's binding ability can be largely enhanced through meticulous modification, and a target owns several aptamers combines with it [13, 14]. These advantages facilitate the performance of aptamer-based sensor and widen its application fields.

There are various tactics for signal conversion when transforming the input target into output signals. The commonly used transducers are based on fluorescence produced by chimeric dyes or

fluorescence resonant energy transfer (FRET) technology [15, 16]. Intercalated dyes for DNA bases are convenient and universal but lack selectivity. The FRET, as a common and promising tactic, has been applied in numerous sensors yet obstructed by the limited number of fluorescent molecules. Sensors based on electrochemical character [17] and interfacial property [18, 19] have also been developed. And biosensors which rely on enzyme catalytic ability possess various forms of signal output such as electricity, thermal and various kinds of spectroscopy [20]. Noticeably, DNAzyme and RNAzyme are fairly stable in complex constitution samples compared to proteineous enzymes, further strengthening the stability of biosensors [21, 22].

G-quadruplex (G4) is a kind of DNAzyme with a spatial configuration which is formed in the presence of K^+ [23]. This structure usually occurs in some G-rich sequences and consists of several planar G-tetrad formations [24]. G4 is an aptamer for compounds containing a porphyrin structure, especially bound with hemin to form the G4/hemin complex [25]. Studies have shown that the oxidation activity of the complex to H_2O_2 increases sharply and much higher than that of hemin alone [26, 27]. Nowadays, G4 DNA architecture is widely applied in biosensor signal conversion systems [28] due to its broad range of affinity [29, 30] and the horseradish peroxidase (HRP)-like activity [31-34].

Currently biosensors for *S. aureus* detection are usually established on indirectly monitoring a particular bacterial DNA sequence or characteristic biotoxin produced by strains [35]. However, it is hard to distinguish dead bacteria from living ones and sometimes be hysteretic of detection. The aptamer-based biosensor can detect pathogens in samples directly and on time. In this study, an aptamer-based biosensor was established for selective recognition and quantitative analysis of *S. aureus*. The established biosensor possess various characteristics which include easy-operation, quick response and strong signal output hence providing a reference for detection of *S. aureus* and other pathogens in food industry.

2. Materials and Methods

2.1 Reagents

Sodium chloride (NaCl), magnesium chloride ($MgCl_2$), potassium chloride (KCl), potassium hydroxide (KOH), potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), dimethyl sulfoxide (DMSO) and 30% H_2O_2 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hydroxyethyl piperazine ethanesulfonic acid (HEPES), Triton X-100 and hemin were purchased from Sangon Biological Engineering Technology & Co. Ltd.

(Shanghai, China) whereas 1× HEPES buffer with 80 mM K^+ and 1× PBS buffer were prepared in the laboratory. Hemin was prepared using DMSO to make stock solution (10 mM), and subsequently diluted with 1× HEPES buffer to 2 mM hemin for experimental use. 30% H_2O_2 were diluted to afford a final concentration of 0.3%. Luminol (Solarbio, Beijing) stock solution (10mM) was dissolved in 0.1 M NaOH and diluted to 0.5 mM before use. Bst DNA polymerase (800 U) and 10 mM dNTPs were obtained from Novoprotein Scientific Inc. (Shanghai, China). The Nb.BbvCI (1000U) nicking enzyme and NEB buffer was purchased from New England bioLabs Inc. (NEB).

Reagents used in this study were of analytical grade without any purification done before use. Ultra-pure water (18.2 MΩ • cm) was directly applied to solution preparations. The double distilled water (ddH2O) used for DNA amplification and *S. aureus* detection was sterilized under high temperature and high pressure (HTHP).

2.2 Bacteria strains and culture

A variety of bacterial strains such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus warneri*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Clostridium perfringens* were used. The accession numbers and sources of all bacteria are outlined in Table S1. These bacteria were cultured in commonly used LB agar medium over a period of 1-2 days by plate culture method [36] but as for culturing in liquid media, different strains have different culturing periods according to the optical density (OD) value.

2.3 Sequences design

An aptamer that selectively binds with *S. aureus* was acquired, according to previous studies [14]. The aptamer has a length of 45 nt with a dissociation constant (K_d) of 210.70 ± 135.91 nM [14]. The probe sequence that was to act as the primer for subsequent DNA amplifications was designed so as to be paired to the 5' end of the aptamer strand, forming a partial complementary region of AP. The linear template (LT) for NEAR replication and the circular template (CT) for RCA were designed and listed in Table S2. The recognition site of Nb.BbvCI was employed in both the LT and CT templates. The construction procedure of CT referred a studied protocol and the concrete operations are not outlined in this paper [37]. The CT was designed to contain C-rich fragment in order to enrich the G-rich sequences. The base compositions of the G-rich sequence were AAGGGTAGGGCGGGTTGGGA, which formed an intra-molecular G4 spatial conformation.

2.4 Detection of *S. aureus*

The ratio of aptamer to probe is a very important factor for the biosensor's performance. Initially, optimization for constituent proportion of the AP was conducted with different ratios (aptamer to probe, 1:1, 5:4, 5:3, 5:2, 5:1, 5:0). The AP detection probe was then prepared by adding 50 µL of 5 µM aptamer with optimized concentration of the probe. The mixture was initially melted at 94□ for 3 min, gradually reducing the temperature to 37□ whereby the mixture was incubated at this temperature for 30 min.

The operations for the biosensor were described as follows; 5 µL of prepared AP solution was mixed with 5 µL of tested homogeneous sample and incubated at 37□ for 30 min, then added into 1 µL of LT, 1 µL of CT, 2 µL NEB buffer, 2 µL dNTPs, 0.5 µL Bst polymerase and 0.5 µL Nb.BbvCI nickase, up to a final volume of 20 µL with ddH_2O. The amplification reactions were conducted at 50□ for 50 min and inactivated at 80□ for 10 min. Subsequently, DNA products were immediately placed on ice and incubated with 30 µL of hemin solution at 37□ for 1 h. Finally, the above mixtures were transferred into a 96-well plate and blended with 100 µL luminol solution and 5 µL diluted H_2O_2 for immediate chemiluminescence detection.

2.5 Detection of chemiluminescence of luminol (CL)

Chemiluminescence of luminol signals were measured on the Varioskan® Flash machine (Thermo Fisher Scientific, USA) at wavelengths ranging from 350 nm to 500 nm, with a scanning step size of 2 nm. From the scanning spectrogram, a maximum absorption wavelength of CL was determined at 426 nm where single-wavelength measurements for samples were carried out. In order to exclude background interference, the chemiluminescence signals of blank control and sample detections were respectively measured and the responding values of samples were represented as $(L- L_0) / L_0$, namely $\Delta L/ L_0$, in which L represents signals of samples and L_0 blank control.

2.6 Gel electrophoresis analysis

DNA amplified products of NEAR-RCA cycles were validated by gel electrophoresis. 6 µL products were mixed with 1 µL loading buffer then placed in a constant voltage of 110V for 35 min in 2% agarose gel. Images of the results were taken via a gel imaging and analysis systems (Syngene G:BOX F3,USA) machine.

2.7 Colony counting

A serial dilution method was employed to estimate the approximate number of *S. aureus* colonies in the sample and the resulting solutions were separately plated on the LB media and cultured in 37□

for 1d. The colonies on plates were counted and the total number of colonies in testing samples calculated.

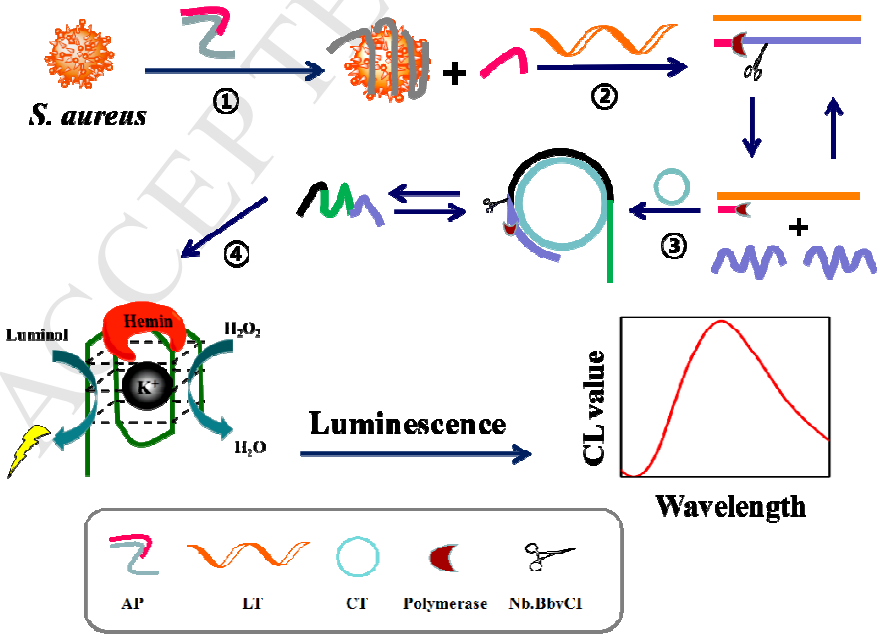
2.8 Data analysis and determination of limit of detection (LoD)

The acquired data were analyzed using Microsoft Excel. All response values were represented as ± standard error (SE, *n*=*3*). The LoD for this biosensor was determined as the concentration that of the average value of blank control adding 3 times of its standard deviation (SD, *n*=7).

3. Results and discussion

3.1 Scheme of aptasensor for *S. aureus* detection

The scheme of the biosensor is shown in Scheme 1. An aptamer-probe (AP) was designed to act as the recognition unit of the biosensor converting the input, *S. aureus* strain, into a probe sequence signal. The concentration of released probe sequence was directly proportional to the count of input i.e. *S. aureus* colony. Subsequently, the resultant free probe acted as a primer and initiated the nicking enzyme amplification reaction (NEAR) and rolling circle amplification (RCA) reactions to further magnify the detection signals. The G-riched fragments were enriched by amplifying circular template (CT) of RCA and forming the G4 architecture in the presence of K^+. Finally, G4 and hemin formed the G4/hemin complex that catalyzed the H_2O_2-mediated luminol oxidation thus triggering the chemiluminescence signals.



Scheme 1: The chemiluminescent aptasensor for *S. aureus* detection that was conceived in this study.

Aptamer has been widely employed as the biosensor recognized unit because of its low-price,

convenient synthesis, easy modification and strong binding ability. For example, a dual-aptamer based sandwich biosensor for *S. aureus* was established by utilizing aptamers conjugated with silver nanoparticles [38] and an aptamer-graphene-based piezoelectric sensor for the detection of *S. aureus* [39] was also developed. These sensors all showed great accuracy and good selectivity of their target. Recently, Guo et al. [40] applied RCA technology to magnify the responding signal of the target in aptamer based biosensor and identified *E.coli* with a LoD of 8 CFU/mL. In the current investigation, an ultra-sensitive aptasensor for *S. aureus* was designed combining the advantages of aptamer technology and the magnifying effect of isothermal amplification. To our knowledge, this is the first time to adopt a double-step amplifying tactic into an aptamer sensor which improved the LoD of *S. aureus* detection remarkably.

3.2 Optimization of this sensor

To develop an ideal diagnostic biosensor, the key step was selecting a suitable recognition element that selectively discriminates against its target from other substances. In this study, AP acted as the recognition element hence aptamer specifically recognized the target in the presence of *S. aureus* whereas the probe was released from AP to initiate the amplification process. Therefore, the constitution of AP has a significant influence on the performance of the sensor. The released probe influenced the enhancement of background signal in the absence of *S. aureus*, which seriously affected the sensitivity of the sensor. Firstly, optimization of the ratio between aptamer and probe was carried out as shown in Fig. 1. The signal leakage was rather serious when aptamer and probe were in equal proportion. This was probably because the probe was not completely hybridized with aptamer hence leaving some parts of the probe free in solution. The background interference was however stronger at the ratio of 5: 4. At lower probe composition ratios such as 5: 3, 5: 2 and 5: 1, the interference almost disappeared as compared to the absence of probe sequence demonstrating that background noise can be dwindled when the proportion of probe in AP is lowered. However, considering that the signal values of samples were closely related to the amount of probe, the ratio of 5:3 was adopted in this experiment.

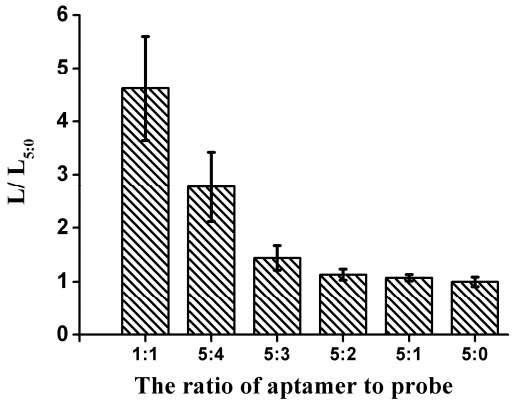


Fig. 1: Optimization of the composition of AP recognition probe. L represents the chemiluminescence under various ratios between aptamer and probe, $L_{5:0}$ represents the signal without probe sequence.

The conditions of NEAR-RCA cycle were also crucial in the establishment of a biosensor. In previous reports, isothermal amplification reactions usually required DNA polymerase and nicking enzyme [41]. DNA polymerase with strand displacement activity, such as Bst large fragment, phi 29 DNA polymerase, klenow fragment polymerase, etc., were mostly used and nick enzyme-like Nb.BbvCI was required to induce incision in double strands which reinforced the chain displacement reactions thereby releasing a large number of target fragments [42]. However, different enzymes have different optimum reaction temperatures. In this study, Bst polymerase with a relative high enzyme activity was chosen and NEAR with RCA reactions occurred simultaneously in an isothermal condition. Thus, it is necessary to select a suitable temperature in order to maintain the activity of the stronger enzyme at a relative condition. According to the manufacture's guide (see Table S3), 50□ was selected as the optimum reaction temperature for the NEAR-RCA magnifying cycle owing to the self-meltdown of AP (T_{mAP} = 75.3□).

3.3 Feasibility analysis of the biosensor

Firstly, the recognition function of the AP to *S. aureus* was analyzed. The melting temperature (T_m) of the partial complementary structure of AP was critical to the following amplification step. The T_m value was predicted as 75.3□ through the online software (Fig. S1) [43]. After that, the binding affinity of AP to *S. aureus* was validated using gel electrophoresis analysis as shown in Fig. 2A. AP detection probe was successfully hybridized as it showed a distinct band with a larger molecular weight (lane 3) than aptamer (lane 1) or probe sequence (lane 2) alone. Moreover, aptamer band was observed as almost disappeared in the lane 4 indicating that the aptamer can specifically be recognized and bound

by *S. aureus*. Consequently, aptamer sequence in AP can be taken away by its target leaving the free probe sequence in the gel (lane 5). Therefore, the AP detection probe can be applied as the recognition element in the biosensor.

Subsequent NEAR-RCA cycle was further verified as shown in Fig. 2B revealing only a single band on the gel imaging through the NEAR amplification. The massive fragments released from NEAR reaction could be used as primers of RCA. Ultimately, consecutive bands were observed after the combined NEAR-RCA cycle suggesting that the magnification of probe signal can take place at the chosen temperature.

The fragments generated by RCA were G-rich sequences and they induced the signal outputs. It exhibited the scanning chromatograms of chemiluminescence signals of samples as shown in Fig. 2C. The sample signal exceed 3 times than that of control which was one-step magnification. It is worth to note that the response values of samples were remarkably enhanced, exhibiting values 5 times than that of control one and 1.5 times that of one-step magnification, when the NEAR-RCA two-step strategy was applied. Hence, the developed *S. aureus* biosensor realized a high ratio of signal to noise which will elevate the detection sensitivity for *S. aureus*.

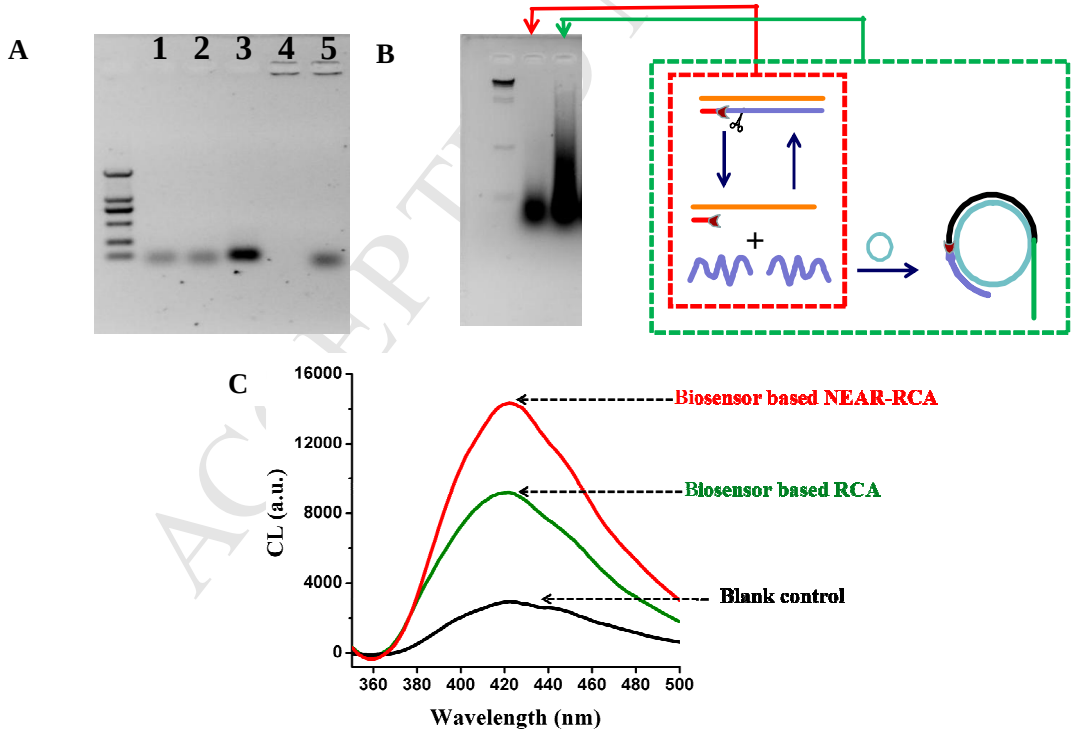


Fig. 2: Feasibility of the aptamer-based sensor. A) Validation of the AP detection probe, in which lane 1: aptamer; lane 2: probe sequence; lane 3: AP; lane 4: aptamer and *S. aureus*; lane 5: AP and *S. aureus*. B) Integrated NEAR-RCA amplification cycle, in which NEAR was framed up by red and NEAR-induced

RCA in green. C) The scanning chromatogram of chemiluminescencethis of luminol.

3.4 Performance of this biosensor

The sensitivity of the biosensor was verified using a series of concentration gradients of *S. aureus*. The scatter diagram of the luminol chemiluminescence values and corresponding colony numbers of *S. aureus* as shown in Fig. 3A demonstrates that the signal intensity was closely related to the count of *S. aureus*. Within the range of 5-10^4 CFU/mL, a linear regression equation was obtained by regression analysis, with a correlation coefficient of R^2=0.922 as shown in Fig. 3B. The LoD of the biosensor was determined as 5 CFU/mL. Therefore, the established aptasensor showed a good analytical performance and can thus achieve the quantitative detection of *S. aureus*.

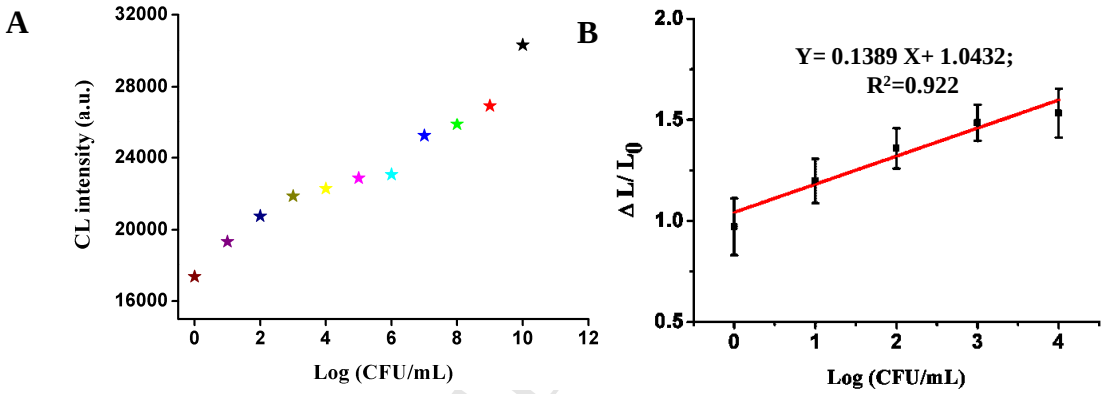


Fig. 3: Quantitative analysis of the sensor. A) The scatter diagram of the CL intensity and corresponding numbers of colony. B) Linear correlation between numbers of colony and response values.

The universality of the biosensor was validated using five strains of *S. aureus*. The results showed the sensor can detect these *S. aureus* strains specifically as they exhibited bands of RCA products on the gel imaging (Fig. 4A). The specificity of this aptasensor was verified with several different pathogens and the results showed that the sensor can discriminate *S. aureus* against the other marked strains (Fig. 4B). The selectivity of the sensor mostly relies on the features of the aptamer. *S. epidermidis* and *S. warneri* can't combine with the aptamer though they belong to the genus of *Staphylococcus*, likewise for other pathogens such as *P. aeruginosa* and *E. coli* can't fuse with the aptamer either (Fig. S2).

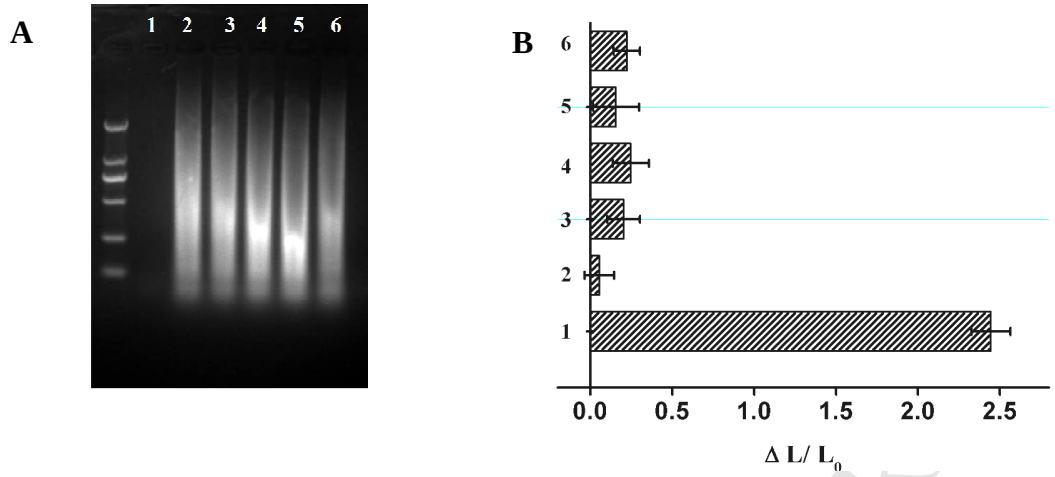


Fig. 4: Universality and selectivity analysis. A) Gel analysis of amplified products, lane 1: negative control; lane 2-6 represented five strains of *S. aureus*. B) Selectivity of the aptasensor, lane 1: *S. aureus*; lane2: *S. epidermidis*; lane 3: *S. warneri*; lane 4: *P. aeruginosa*; lane 5: *E. coli*; lane 6: *C. perfringens.*

Many biosensors have been developed for *S. aureus* based on nucleic acid probe with good sensitivity and selectivity. For example, the LoD of the aptamer sensor with fluorescent label and silica nanoparticles was 93 CFU/mL [44]. The fluorescence aptesensor using up-converting fluorescent materials can achieve the LoD of 8 CFU/mL with a linear range of 10-10^5 CFU/mL [45]. Recently, a colorimetric assay that combined phagomagnetic separation with immunoassay has been developed. By using double site recognition of bacteriophage and mammal IgG, the LoD for the assay was 2.47×10^3 CFU/mL of *S. aureus* in PBS [46]. And a paper-based colorimetric biosensor was also established, which utilized the proteolytic activity of *S. aureus* proteases on a specific peptide substrate. The colour change can be detected by the naked eye and the results showed the detection limits as low as 7 CFU/mL for *S. aureus* in pure broth culture [47]. The biosensor established in this study had an ultra-sensitivity with the LoD at as low as 5 CFU/mL which could meet the requirements for *S. aureus* detection in food industry. A comparison of the biosensor with others was described in detail in Supplementary Table S4.

3.5 Discrimination of *S. aureus* inactivated by HTHP

An effective method for the detection of pathogens which could distinguish dead from live bacteria is really important. So far, most biosensors don't have such a character because they target bacterial DNA or biotoxin which exists in both live and dead cells while aptamer can recognize and bind the whole cell directly. It is presumed that a specific aptamer suitable for the live cells can be

developed. Interestingly, an unexpected result emerged in this study when testing the *S. aureus* that was inactivated by HTHP method. The chemiluminescence values for inactivated target were relatively lower than those of living cells, and the dead cells of *S. aureus* couldn't bind with aptamer leaving a distinct band on the gel image (Fig. 5). This may be attributed to the SELEX process (systematic evolution of ligand by exponential enrichment) used to screen the aptamer of *S. aureus* [14]. The targets for *S. aureus* according to previous studies were active cells and the screened aptamer, as reported, may bind with the living cells or just a small substance on its surface. The cells were inactivated by HTHP which destroyed the bacterial surface to some extent thus the inactivated cells could not bind to the aptamer. Therefore, the aptasensor could selectively distinguish living *S. aureus* from HTHP inactivated ones hence this method could be applied to evaluate the residue of *S. aureus* in food industry.

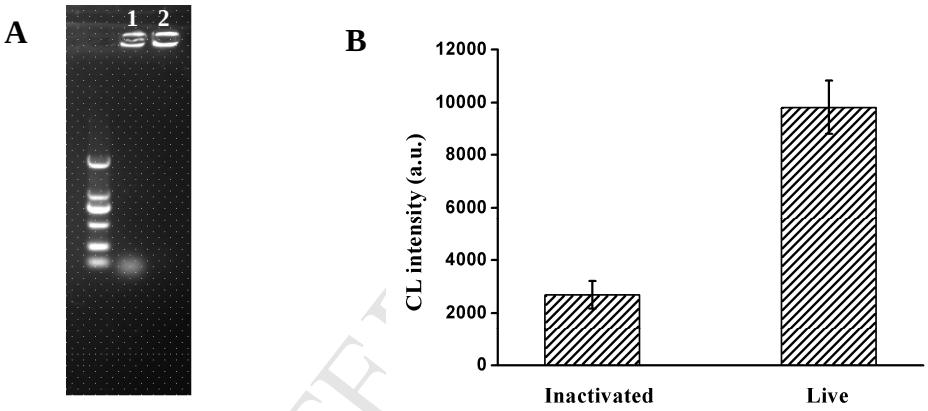


Fig. 5: Discrimination of *S. aureus* inactivated by HTHP. A) Gel electrophoresis analysis, lane 1: inactivated *S. aureus* with aptamer; lane 2: live *S. aureus* with aptamer. B) Responding signals of chemiluminescence of luminol.

4. Conclusion

In conclusion, a chemiluminescence aptasensor for *S. aureus* detection has successfully been established based on aptamer recognition and NEAR-RCA magnifying cycle. Results from the present study showed that the biosensor exhibited a good linear correlation at a concentration of 5-10^4 CFU/mL. The sensitivity of this sensor improved significantly to the 5 CFU/mL by NEAR-RCA two-step amplifying cycle. The biosensor showed notable specificity of *S. aureus* individually and eliminated the interference from other pathogens. In addition, the established aptasensor exhibited a good discrimination of living against dead *S. aureus*. Therefore, the biosensor can detect *S. aureus*

specifically and sensitively thus avoiding the complicated DNA extraction procedure, which has potential application in the detection of *S. aureus* and other pathogens in food industry.

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Appendix A. Supplementary data

Conflicts of interests

The authors declare having no conflict of interest.

References

[1] National Health and Family Planning Commission of China (2015), http://www.nhfpc.gov.cn/yjb/s7859/201604/8d34e4c442c54d33909319954c43311c.shtml, (January 1, 2018).

[2] General Information: About MRSA in the Community, Centers for Disease Control and Prevention, https://www.cdc.gov/mrsa/community/index.html#how2.

[3] O. Lazcka, F.J. Del Campo, F.X. Munoz, Pathogen detection: a perspective of traditional methods and biosensors, Biosens Bioelectron, 22 (2007) 1205-1217. http://dx.doi.org/10.1016/j.bios.2006.06.036.

[4] P.D. Skottrup, M. Nicolaisen, A.F. Justesen, Towards on-site pathogen detection using antibody-based sensors, Biosens Bioelectron, 24 (2008) 339-348. http://dx.doi.org/10.1016/j.bios.2008.06.045.

[5] T.N.T. Dao, E.Y. Lee, B. Koo, C.E. Jin, T.Y. Lee, Y. Shin, A microfluidic enrichment platform with a recombinase polymerase amplification sensor for pathogen diagnosis, Analytical biochemistry, 544 (2017) 87-92. http://dx.doi.org/10.1016/j.ab.2017.12.030.

[6] W. Wang, T. Bao, X. Zeng, H. Xiong, W. Wen, X. Zhang, S. Wang, Ultrasensitive electrochemical DNA biosensor based on functionalized gold clusters/graphene nanohybrids coupling with exonuclease III-aided cascade target recycling, Biosens Bioelectron, 91 (2017) 183-189. http://dx.doi.org/10.1016/j.bios.2016.12.017.

[7] V. Perumal, U. Hashim, Advances in biosensors: Principle, architecture and applications, J Appl Biomed, 12 (2014) 1-15. http://dx.doi.org/10.1016/j.jab.2013.02.001.

[8] J. Zhu, L. Wang, X. Xu, H. Wei, W. Jiang, Modular Nuclease-Responsive DNA Three-Way Junction-Based Dynamic Assembly of a DNA Device and Its Sensing Application, Anal Chem, 88 (2016) 3817-3825. http://dx.doi.org/10.1021/acs.analchem.5b04889.

[9] J. Liu, Z. Cao, Y. Lu, Functional nucleic acid sensors, Chem Rev, 109 (2009) 1948-1998. http://dx.doi.org/10.1021/cr030183i.

[10] N.R. Ha, I.P. Jung, I.J. La, H.S. Jung, M.Y. Yoon, Ultra-sensitive detection of kanamycin for food safety using a reduced graphene oxide-based fluorescent aptasensor, Sci Rep-Uk, 7 (2017).

http://dx.doi.org/ 10.1038/srep40305.
[11] C.Y. Lu, J.J. Xu, Z.H. Wang, H.Y. Chen, A novel signal-amplified electrochemical aptasensor based on supersandwich G-quadruplex DNAzyme for highly sensitive cancer cell detection, Electrochem Commun, 52 (2015) 49-52. http://dx.doi.org/10.1016/j.elecom.2015.01.015.
[12] J.G. Bruno, Predicting the Uncertain Future of Aptamer-Based Diagnostics and Therapeutics, Molecules, 20 (2015) 6866-6887. http://dx.doi.org/10.3390/molecules20046866.
[13] M. Darmostuk, S. Rimpelova, H. Gbelcova, T. Ruml, Current approaches in SELEX: An update to aptamer selection technology, Biotechnol Adv, 33 (2015) 1141-1161. http://dx.doi.org/10.1016/j.biotechadv.2015.02.008.
[14] X.X. Cao, S.H. Li, L.C. Chen, H.M. Ding, H. Xu, Y.P. Huang, J. Li, N.L. Liu, W.H. Cao, Y.J. Zhu, B.F. Shen, N.S. Shao, Combining use of a panel of ssDNA aptamers in the detection of Staphylococcus aureus, Nucleic acids research, 37 (2009) 4621-4628. http://dx.doi.org/10.1093/nar/gkp489.
[15] K. Quan, J. Huang, X. Yang, Y. Yang, L. Ying, H. Wang, N. Xie, M. Ou, K. Wang, Powerful Amplification Cascades of FRET-Based Two-Layer Nonenzymatic Nucleic Acid Circuits, Anal Chem, 88 (2016) 5857-5864. http://dx.doi.org/10.1021/acs.analchem.6b00609.
[16] E.J. Jo, H. Mun, S.J. Kim, W.B. Shim, M.G. Kim, Detection of ochratoxin A (OTA) in coffee using chemiluminescence resonance energy transfer (CRET) aptasensor, Food Chemistry, 194 (2016) 1102-1107. http://dx.doi.org/10.1016/j.foodchem.2015.07.152.
[17] E. Xiong, X. Yan, X. Zhang, Y. Liu, J. Zhou, J. Chen, Exonuclease III-assisted cascade signal amplification strategy for label-free and ultrasensitive electrochemical detection of nucleic acids, Biosens Bioelectron, 87 (2017) 732-736. http://dx.doi.org/10.1016/j.bios.2016.09.036.
[18] J. Dong, Y. Li, M.Y. Zhang, Z. Li, T.Y. Yan, W.P. Qian, Ultrasensitive surface-enhanced Raman scattering detection of alkaline phosphatase, Anal Methods-Uk, 6 (2014) 9168-9172. http://dx.doi.org/10.1039/c4ay01885k.
[19] C. Xiong, L.S. Ling, Localized Surface Plasmon Resonance Light-Scattering Sensor for Mercury(II) Ion with Label-Free Gold Nanoparticles, J Nanosci Nanotechno, 13 (2013) 1406-1410. http://dx.doi.org/10.1166/jnn.2013.6101.
[20] R. Miranda-Castro, M.J. Lobo-Castanon, A.J. Miranda-Ordieres, P. Tunon-Blanco, Comparative Study of HRP, a Peroxidase-Mimicking DNAzyme, and ALP as Enzyme Labels in Developing Electrochemical Genosensors for Pathogenic Bacteria, Electroanal, 22 (2010) 1297-1305. http://dx.doi.org/10.1002/elan.200900608.
[21] M. Wang, H. Zhang, W. Zhang, Y. Zhao, A. Yasmeen, L. Zhou, X. Yu, Z. Tang, In vitro selection of DNA-cleaving deoxyribozyme with site-specific thymidine excision activity, Nucleic acids research, 42 (2014) 9262-9269. http://dx.doi.org/10.1093/nar/gku592.
[22] Y.C. Su, S.F. Hickey, S.G.L. Keyser, M.C. Hammond, In Vitro and In Vivo Enzyme Activity Screening via RNA-Based Fluorescent Biosensors for S-Adenosyl-L-homocysteine (SAH), J Am Chem Soc, 138 (2016) 7040-7047. http://dx.doi.org/10.1021/jacs.6b01621.
[23] I. Russo Krauss, V. Spiridonova, A. Pica, V. Napolitano, F. Sica, Different duplex/quadruplex junctions determine the properties of anti-thrombin aptamers with mixed folding, Nucleic acids research, 44 (2016) 983-991. http://dx.doi.org/10.1093/nar/gkv1384.
[24] S. Burge, G.N. Parkinson, P. Hazel, A.K. Todd, S. Neidle, Quadruplex DNA: sequence, topology and structure, Nucleic acids research, 34 (2006) 5402-5415. http://dx.doi.org/10.1093/nar/gkl655.
[25] J. Kosman, B. Juskowiak, Bioanalytical Application of Peroxidase-Mimicking DNAzymes: Status

and Challenges, Adv Biochem Eng Biotechnol, (2017). http://dx.doi.org/10.1007/10_2017_7.

[26] L.C.H. Poon, S.P. Methot, W. Morabi-Pazooki, F. Pio, A.J. Bennet, D. Sen, Guanine-Rich RNAs and DNAs That Bind Heme Robustly Catalyze Oxygen Transfer Reactions, J Am Chem Soc, 133 (2011) 1877-1884. http://dx.doi.org/10.1021/ja108571a.

[27] P. Travascio, Y.F. Li, D. Sen, DNA-enhanced peroxidase activity of a DNA aptamer-hemin complex, Chem Biol, 5 (1998) 505-517. http://dx.doi.org/Doi 10.1016/S1074-5521(98)90006-0.

[28] H.Z. He, D.S. Chan, C.H. Leung, D.L. Ma, G-quadruplexes for luminescent sensing and logic gates, Nucleic acids research, 41 (2013) 4345-4359. http://dx.doi.org/10.1093/nar/gkt108.

[29] P. Zhang, H. Liu, S. Ma, S. Men, Q. Li, X. Yang, H. Wang, A. Zhang, A label-free ultrasensitive fluorescence detection of viable Salmonella enteritidis using enzyme-induced cascade two-stage toehold strand-displacement-driven assembly of G-quadruplex DNA, Biosens Bioelectron, 80 (2016) 538. http://dx.doi.org/10.1016/j.bios.2016.02.031.

[30] H. Tan, G. Tang, C. Ma, Q. Li, Luminescence detection of cysteine based on Ag(+)-mediated conformational change of terbium ion-promoted G-quadruplex, Anal Chim Acta, 908 (2016) 161-167. http://dx.doi.org/10.1016/j.aca.2015.12.035.

[31] H.Q. Wang, W.Y. Liu, Z. Wu, L.J. Tang, X.M. Xu, R.Q. Yu, J.H. Jiang, Homogeneous label-free genotyping of single nucleotide polymorphism using ligation-mediated strand displacement amplification with DNAzyme-based chemiluminescence detection, Anal Chem, 83 (2011) 1883-1889. http://dx.doi.org/10.1021/ac200138v.

[32] F. Gao, T. Fan, J. Wu, S. Liu, Y. Du, Y. Yao, F. Zhou, Y. Zhang, X. Liao, D. Geng, Proximity hybridization triggered hemin/G-quadruplex formation for construction a label-free and signal-on electrochemical DNA sensor, Biosens Bioelectron, 96 (2017) 62-67. http://dx.doi.org/10.1016/j.bios.2017.04.024.

[33] J. Li, Q.H. Yao, H.E. Fu, X.L. Zhang, H.H. Yang, High sensitive and label-free colorimetric DNA detection based on nicking endonuclease-assisted activation of DNAzymes, Talanta, 85 (2011) 91-96. http://dx.doi.org/10.1016/j.talanta.2011.03.042.

[34] Z. Zhou, Y. Du, L. Zhang, S. Dong, A label-free, G-quadruplex DNAzyme-based fluorescent probe for signal-amplified DNA detection and turn-on assay of endonuclease, Biosens Bioelectron, 34 (2012) 100-105. http://dx.doi.org/10.1016/j.bios.2012.01.024.

[35] H. He, Y. Wu, N. He, Y. Deng, The Latest Progress of On-Site Pathogens Detection Techniques and Instruments Based on Nucleic Acid, J Nanosci Nanotechnol, 15 (2015) 6342-6356. http://www.ncbi.nlm.nih.gov/pubmed/26716189.

[36] J. Guo, A. Wang, K. Yang, H. Ding, Y. Hu, Y. Yang, S. Huang, J. Xu, T. Liu, H. Yang, Z. Xin, Isolation, characterization and antimicrobial activities of polyacetylene glycosides from Coreopsis tinctoria Nutt, Phytochemistry, 136 (2017) 65-69. http://dx.doi.org/10.1016/j.phytochem.2016.12.023.

[37] H. Xu, D. Wu, Y. Jiang, R. Zhang, Q. Wu, Y. Liu, F. Li, Z.S. Wu, Loopback rolling circle amplification for ultrasensitive detection of Kras gene, Talanta, 164 (2017) 511-517. http://dx.doi.org/10.1016/j.talanta.2016.12.017.

[38] A. Abbaspour, F. Norouz-Sarvestani, A. Noori, N. Soltani, Aptamer-conjugated silver nanoparticles for electrochemical dual-aptamer-based sandwich detection of staphylococcus aureus, Biosens Bioelectron, 68 (2015) 149-155. http://dx.doi.org/10.1016/j.bios.2014.12.040.

[39] Y. Lian, F. He, H. Wang, F. Tong, A new aptamer/graphene interdigitated gold electrode piezoelectric sensor for rapid and specific detection of Staphylococcus aureus, Biosens Bioelectron, 65 (2015) 314-319. http://dx.doi.org/10.1016/j.bios.2014.10.017.

[40] Y. Guo, Y. Wang, S. Liu, J. Yu, H. Wang, Y. Wang, J. Huang, Label-free and highly sensitive electrochemical detection of E. coli based on rolling circle amplifications coupled peroxidase-mimicking DNAzyme amplification, Biosens Bioelectron, 75 (2016) 315-319. http://dx.doi.org/10.1016/j.bios.2015.08.031.

[41] L. Yan, J. Zhou, Y. Zheng, A.S. Gamson, B.T. Roembke, S. Nakayama, H.O. Sintim, Isothermal amplified detection of DNA and RNA, Molecular bioSystems, 10 (2014) 970-1003. http://dx.doi.org/10.1039/c3mb70304e.

[42] P. Menova, M. Hocek, Preparation of short cytosine-modified oligonucleotides by nicking enzyme amplification reaction, Chem Commun (Camb), 48 (2012) 6921-6923. http://dx.doi.org/10.1039/c2cc32930a.

[43] The DINAMelt Web Server, http://unafold.rna.albany.edu/?q=DINAMelt/Two-state-melting#opennewwindow.

[44] J. Shangguan, Y. Li, D. He, X. He, K. Wang, Z. Zou, H. Shi, A combination of positive dielectrophoresis driven on-line enrichment and aptamer-fluorescent silica nanoparticle label for rapid and sensitive detection of Staphylococcus aureus, Analyst, 140 (2015) 4489-4497. http://dx.doi.org/10.1039/c5an00535c.

[45] J. Yuan, S. Wu, N. Duan, X. Ma, Y. Xia, J. Chen, Z. Ding, Z. Wang, A sensitive gold nanoparticle-based colorimetric aptasensor for Staphylococcus aureus, Talanta, 127 (2014) 163-168. http://dx.doi.org/10.1016/j.talanta.2014.04.013.

[46] C.H. Yan, Y. Zhang, H. Yang, J.P. Yu, H.P. Wei, Combining phagomagnetic separation with immunoassay for specific, fast and sensitive detection of Staphylococcus aureus, Talanta, 170 (2017) 291-297. http://dx.doi.org/10.1016/j.talanta.2017.04.007.

[47] G.A.R.Y. Suaifan, S. Alhogail, M. Zourob, Rapid and low-cost biosensor for the detection of *Staphylococcus aureus*, Biosensors and Bioelectronics, 90 (2017) 230-237. http://www.ncbi.nlm.nih.gov/pubmed/27914366.