Biosensors and Bioelectronics 132 (2019) 224–229

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A new aptamer/polyadenylated DNA interdigitated gold electrode piezoelectric sensor for rapid detection of *Pseudomonas aeruginosa*

Xiaohong Shi[a,b], Jialin Zhang[a], Fengjiao He[a,*]

[a] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China
[b] Department of Chemistry, Taiyuan Normal University, Jinzhong 030619, China

ARTICLE INFO

Keywords:
P. aeruginosa
Aptamer
Poly(A)DNA
Au IDE-MSPQC sensor
Magnetic bead

ABSTRACT

Rapid detection of *Pseudomonas aeruginosa* (*P. aeruginosa*) is of great importance for accurate diagnosis and treatment of infected patients. In this study, a novel method was developed for the selective detection of *P. aeruginosa* by combing the sandwich type complex of magnetic bead/aptamer/polyadenylated-DNA with the sensitive detection platform of gold (Au) interdigital electrode connected to a multichannel series piezoelectric quartz crystal (Au IDE-MSPQC) system. Here, the magnetic bead (MB) was used as carrier for immobilization of the aptamer of *P. aeruginosa*. Polyadenylated DNA was bound to the aptamer through complementary strand pairing. When the *P. aeruginosa* was present in the sample solution, the polyadenylated DNA was replaced by the *P. aeruginosa* because of the specific interaction between *P. aeruginosa* and its aptamer. The released polyadenylated DNA strand in the detected solution could adsorb onto the surface of Au IDE by virtue of the strong interaction between adenine (A) and Au IDE, and result in sensitive frequency shift response of the MSPQC sensor. The limits of detection (LOD) of the method were as low as 9 CFU/mL in buffer and 52 CFU/mL in simulated blood sample. The proposed method was successfully applied to the selective detection of *P. aeruginosa* in blood samples. The constructed sensor is expected to find application for the rapid detection of *P. aeruginosa* in environment, food and clinical diagnosis.

1. Introduction

P. aeruginosa is an opportunistic pathogen that is widely distributed in soil, water, air, food, animals and humans (Lister et al., 2009). It is also one of the main pathogens of nosocomial infection (Choi et al., 2013). When the immune system is attacked by the *P. aeruginosa*, serious infection may occur, such as metabolic disease, cancer, etc (Wiehlmann et al., 2012; Zhang et al., 2012). Therefore, the rapid detection of *P. aeruginosa* is significantly important for making a timely and accurate clinical diagnosis, water and food safety control (Ardura et al., 2013; Tang et al., 2013).

Currently, the most commonly used methods for detecting *P. aeruginosa* are via culture-dependent means (Deschaght et al., 2011; Weiser et al., 2014), but these are time consuming. In addition, a subsequent biochemical identification is needed for further confirmation as to whether the infection is due to *P. aeruginosa* (Aghamollaei et al., 2015). Molecular-based biological methods have also been developed to shorten the detection time. For example, PCR-based assays are used to simultaneously detect and identify *P. aeruginosa* (Gall et al., 2013). These assays are specific and sensitive, but some limitations exist, such as the tedious process that is involved for the RNA or DNA extraction, the extremely strict operation and the high cost of the instrument and reagents. The loop-mediated isothermal amplification (LAMP) method is a new nucleic acid amplification technology that has been used for the identification of *P. aeruginosa* (Diribe et al., 2015). Although the cost of LAMP is less than PCR, it also requires DNA extraction and strict operating conditions. Wang et al. proposed the colloidal gold immunochromatography assay for the detection of *P. aeruginosa* using the specific interaction between the outer membrane protein (OprF) of *P. aeruginosa* and its antibody (Wang et al., 2011b). This strategy, based on the reaction between the antigen and the antibody, is simple, rapid and specific, but the cost of the antibody is high. As a new type of recognition molecule, aptamers have been used to detect a variety of analytes (ions, small molecules, proteins, cells, etc.) (Bahareh et al., 2018; Lu et al., 2010; So et al., 2005; Tan et al., 2013). An aptamer is a short single-stranded DNA molecule with a special three-dimensional structure that has a selective recognition to its target. The chain length of an aptamer is usually 20–80 bases (Sun and Zu, 2015). Compared with an antibody, an aptamer has a higher stability and is easier to synthesize and modify (Chen and Yang, 2015). A *P. aeruginosa* aptamer

* Corresponding author.
E-mail address: fengjiaohe@hnu.edu.cn (F. He).

https://doi.org/10.1016/j.bios.2019.02.053
Received 18 December 2018; Received in revised form 13 February 2019; Accepted 19 February 2019
Available online 04 March 2019
0956-5663/

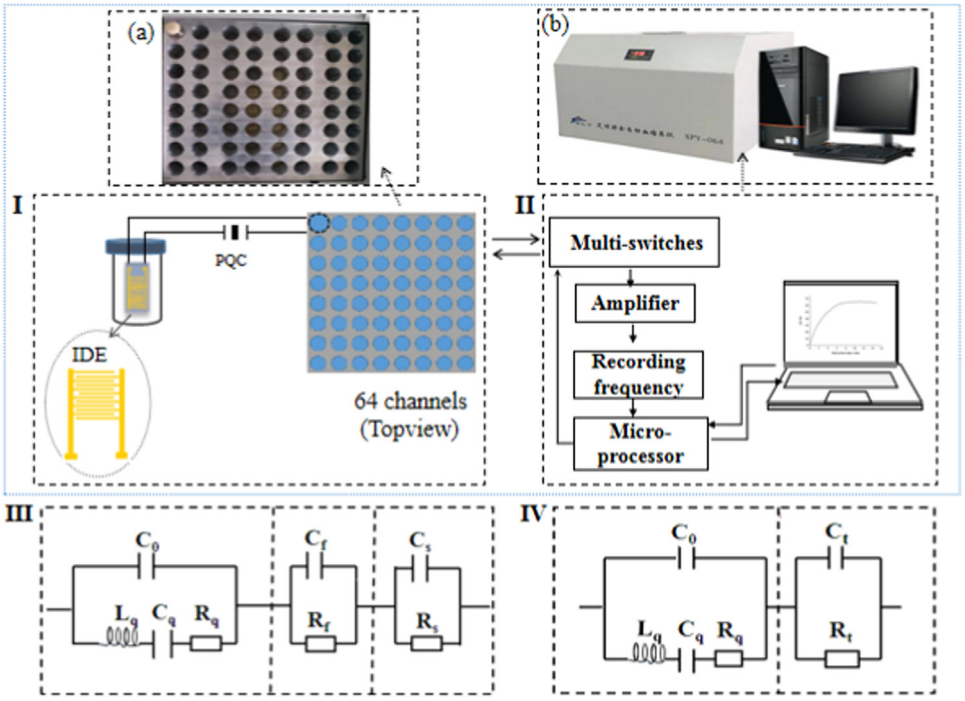

Fig. 1. Schematic representation of the IDE-MSPQC sensor: I, detection system; II, microprocessor & data output system; III, equivalent electric circuit model of the IDE-MSPC; IV, simplified equivalent electric circuit model of the system; (a) and (b), photographs of the sensor.

(F23) (Wang et al., 2011a) can be used to detect *P. aeruginosa* in water by a fluorescent labeling method (Kim et al., 2013). This method is fast, specific and sensitive, but it requires a fluorescent labeling marker. Besides, the fluorescence assay requires costly instrument, and is susceptible to background fluorescence interference. Therefore, it is necessary to develop new low-cost, simple, label-free, rapid and specific methods for detecting *P. aeruginosa* in complicated samples.

Piezoelectric quartz crystal (PQC) sensors have attracted a lot of attention due to their sensitive response, easy operation and low cost (Chen et al., 2018; Lakshmanan et al., 2014; Zhang et al., 2017a). A series piezoelectric quartz crystal (SPQC) sensor is constructed by connecting a piezoelectric quartz crystal (PQC) with a conductive electrode in series. The SPQC sensor shows a sensitive frequency response to changes in the electric parameters in a solution (Jang et al., 2009) and is more stable than common PQC (Shen et al., 1993). The development of a multi-channel SPQC (MSPQC) sensor allows for the parallel detection of multiple samples (Mi et al., 2012; Tong et al., 2014). An interdigital electrode (IDE) consists of a series of parallel microband electrodes. The micro-gap between the cathode and the anode allows the IDE to detect tiny changes on its surface. An IDE-MSPQC has a sensitive frequency response to electrical parameters of the electrode surface and has been used to construct sensors for the rapid detection of targets (He et al., 2016; Zhang et al., 2017b).

Poly adenines Poly(A) have strong affinity with Au (Pei et al., 2012). Compared with HS-DNA, Poly(A)-DNA has greater coverage density on electrode surface at the same concentration (Zhu et al., 2016). In this study, a novel strategy was developed, which utilized magnetic bead-aptamer-Poly(A) DNA complex coupled with IDE-MSPQC for the rapid and selective detection of *P. aeruginosa*. The magnetic bead-aptamer-Poly(A) DNA had a sandwich-type structure, and a magnetic bead (MB) was used to immobilize the *P. aeruginosa* aptamer. The Poly (A) extended DNA strand was partially complementary to the aptamer. To ensure that only polyA existed in the final test solution, it was necessary to separate the Poly(A)DNA and aptamer-MB for avoiding the non-specific adsorption of aptamer on the surface of the gold surface. The magnetic separation is a simple and effective way to get the detected Poly(A)DNA probe, so the MB is selected. The sensor was applied to selectively detect *P. aeruginosa*.

2. Experimental

2.1. Materials

Streptavidin-MB (Henan Huier Nano Technology Co., Ltd.); Sterile defibrinated sheep blood (Guangzhou Ruite Biotechnology Co., Ltd.); Blood agar (Guangdong Central Key Microbial Technology Co., Ltd.); Buffer A (2 × washing and binding buffer): 10 mM Tris-HCl, 1 mM EDTA, 2 M NaCl, pH 7.5; Buffer B (for blocking non-specific adsorption sites on MB): 0.1 M NaCl, 0.1 M PBS (pH 7.3), 0.05% Tween-20; Buffer C (for DNA hybridization and storage): 10 mM Tris-HCl, 5 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 150 mM NaCl, pH 7.5; nutrient broth(NB): beef extract 0.5 g, peptone 1 g, NaCl 0.5 g, ultrapure water 100 mL; pH 7.2.

P. aeruginosa aptamer (Biotin-target probe):

Biotin-$(CH_2)_6$CCCCCGTTGCTTTCGCTTTTCCTTTCGCTTTTGTTCGTTTCGTCC CTGCTTCCTTTCTTG;

Poly(A)-DNA (Poly(A)-detection probe):

AAAAAAAAAACAAGAAAGGAAGCAGGG

All of the above DNA sequences were synthesized and purified by Shanghai Sangon Biological Science and Technology Company Co., Ltd. (Shanghai, China).

2.2. Bacteria

Pseudomonas aeruginosa (*P. aeruginosa* ATCC 27853), Escherichia coli (*E. coli* ATCC 25922), *Staphylococcus aureus* (*S. aureus* ATCC 25923), *Klebsiella pneumoniae* (*K. pneumoniae* ATCC33495), *Streptococcus pyogenes* (*S. pyogenes* ATCC 19615), *E. cloacae* (*E. cloacae* clinical isolate strain) and *Enterococcus faecalis* (*E. faecalis* clinical isolate strain) were obtained from the Hunan Children's Hospital (China). Each strain in pure culture was transferred into a sterilized conical flask containing Nutritional broth (NB). After incubation in shaker for 24 h at 37 °C, the conical flask was removed from the shaker and preserved in a refrigerator at 4 °C for further use.

2.3. Apparatus

MSPQC (self-designed product in our lab, China), its schematic diagram is shown in Fig. 1a. Au IDE was ordered from the Dalian Institute of Chemical Physics (Chinese Academy of Science). It patterned on SiO_2 surface using an image reversal technique. The middle layer was Ti that its thickness was about 20 nm. The outermost layer was deposed with Au which thickness was about 200 nm. The whole electrode contained six interdigital couples. The dimension of each electrode was 3 mm in length and 0.1 mm in width with a distance between electrodes of 0.1 mm.

2.4. Preparation of the MB-aptamer-Poly(A)DNA

First, 20 μL of the 10 mg/mL streptavidin-labeled MB was washed with buffer A to remove the residual NaN_3 protection solution. Then, it was resuspended in 100 μL buffer A. Next, 100 μL of 1.0 μM biotinylated aptamer was added, and it was incubated with the streptavidin-labeled MB for 30 min to generate the aptamer-MB. This was further washed with 400 μL buffer B to remove the unbound aptamer, and the exposed reaction sites of the MB were blocked. After magnetic separation, the MB-aptamer was resuspended in 100 μL of buffer C. Finally, 100 μL of 1.0 μM Poly(A)DNA was added into the MB-aptamer suspension reaction, and it was incubated for 1 h to form the MB-aptamer-Poly(A)DNA. Finally, the MB-aptamer-Poly(A)DNA was washed using buffer C until no DNA was detected in the discarded solution.

2.5. Detection of P. aeruginosa in simulated blood samples using the proposed method

Sterile defibrinated sheep blood was spiked with different concentrations of *P. aeruginosa* and was used as the simulated positive control samples. A blank control was used as the negative sample. A total of 100 μL of the Poly(A)DNA-aptamer-MB was added to 500 μL of the sample solution. The obtained mixture was incubated at room temperature for 30 min followed by magnetic separation to obtain the test solution, which contained Poly(A)DNA. The test solution was added to the test cells in the Au IDE-MSPQC system, and the change of the IDE surface was monitored in real-time. The curve of the frequency shift (ΔF)-time (t) was automatically recorded and saved.

3. Results and discussion

3.1. The Au IDE-MSPQC system

The schematic diagram of the detection system and the corresponding equivalent circuit are shown in Fig. 1.

As shown in Fig. 1(III), C_0, L_q, C_q and R_q are static capacitance, motional inductance, motional capacitance and motional resistance of AT cut 9 MHz quartz crystal, respectively; C_s and R_s are equivalent capacitance and resistance of detection medium, respectively; C_f and R_f are equivalent capacitance and resistance of adsorbent layer of IDE, respectively. Because C_s and R_s of the solution changed little during the detection process, so Fig. 1(III) is simplified to Fig. 1(IV). The detailed explanation of the frequency shift response characteristics of the electric parameters was described in Supporting Information.

The change of resistance (i.e.R_t) and capacitance (i.e.C_t) of IDE and solution in detection process can result in the sensitive frequency shift response of Au IDE-MSPQC sensor.

3.2. Strategy of the P. aeruginosa sensor based on magnetic bead-aptamer-polyadenylated DNA and Au IDE-SPQC

The detection mechanism diagram of the proposed sensor is shown in Fig. 2. The aptamer was immobilized onto the MB through the interaction between the terminal biotin of the aptamer and the modified streptavidin on the MB. The modification of the Poly(A)DNA onto the MB-aptamer was achieved through the complementary base-pairing reaction of the Poly(A)DNA with the aptamer. When the *P. aeruginosa* was present in the sample, it bound to the MB and replaced the Poly(A) DNA, because the interaction between *P. aeruginosa* and its aptamer was stronger. The released Poly(A)DNA adsorbed on the gold surface of the IDE effectively because of the high affinity between the adenines of the Poly(A) sequences and gold. The adsorption triggered a sensitive signal shift in the frequency of the MSPQC sensor.

3.3. The typical response curve of the proposed sensor to P. aeruginosa

The typical response curve (ΔF versus t) of the Poly(A)DNA-Au IDE-MSPQC sensor to indirectly detect 8.1×10^4 CFU/mL of *P. aeruginosa* is shown in Fig. 3 (curve c).

Curve (a) and curve (b) represent the response curves of the Au IDE-MSPQC corresponding to blank control sample without *P. aeruginosa* and the sample without the ployA DNA modified MB-aptamer probe for the detection of the *P. aeruginosa* sample with 8.1×10^4 CFU/mL, respectively. There was no obvious frequency shift (ΔF) response in curve (a) and curve (b), which indicated that the resistance in the IDE surface did not change in the testing process. In contrast, the ΔF in curve (c) increased gradually over about the first 10 min, and then, it reached a maximum and plateaus over the remaining time. This phenomenon originated from the interaction between the Au IDE and the Poly(A) DNA, which was released from the MB into the test solution, due to the presence of *P. aeruginosa*. The frequency shift (ΔF) increased with the increase in the resistance of the electrode surface via the accumulation of the Poly(A) onto the Au IDE, and then, it reached a plateau. The obvious difference in the value of ΔF at the plateau in curve (a) and curve (b) indicated that the presence of Poly(A)DNA substituted by *P. aeruginosa* resulted in the ΔF response of the MSPQC.

3.4. Optimization of parameters in preparation of the proposed sensor

In this study, we tried to optimize the effective experimental parameters of the aptamer concentration and the reaction time of aptamer with *P. aeruginosa*. The detection results are shown in Fig. S1. As shown in Fig. S1 (a), the frequency shift increased with the increase of aptamer concentration from 200 nM to 1.0 μM. It suggested that sparse aptamer could not detect the target completely. When the concentration of aptamer increased up to 1.2 μM, the frequency shift decreased. It meant that the dense aptamer molecules might cause space obstacle and excessive accumulation which could evidently reduce the identification sites and influence the recognition ability of the target molecules. So, 1.0 μM aptamer was used to immobilize on MB for subsequent experiments. From Fig. S1 (b), it could be seen that the ΔF increased with the extension of the reaction time between aptamer and its target. When the reaction time over 30 min, the change of the signal intensity was quite slow and small. Thus, the 30 min was chosen as the reaction time.

3.5. Detection of different concentrations of P. aeruginosa by the proposed method

In the proposed method, different concentrations of *P. aeruginosa* were detected, and the results are shown in Fig. 4. The frequency shift (ΔF) increased with the concentration of *P. aeruginosa*. A linear relationship was obtained between ΔF and the logarithm concentration of *P. aeruginosa*, and it was in the range of 8.1×10^1 to 8.1×10^5 CFU/mL. The equation was $\Delta F = 23.9 \lg c - 5.36$, $R^2 = 0.98$. The relative standard deviation (RSD) of five parallel experiments was in the range of 1.2% to 4.5%, which indicated a good reproducibility. The detection limit was 9 CFU/mL, which was calculated as the ratio of three times the noise standard deviation of the blank over the sensitivity.

The classic plate count method was used to verify the accuracy of the proposed method for the detection of *P. aeruginosa*, and the results

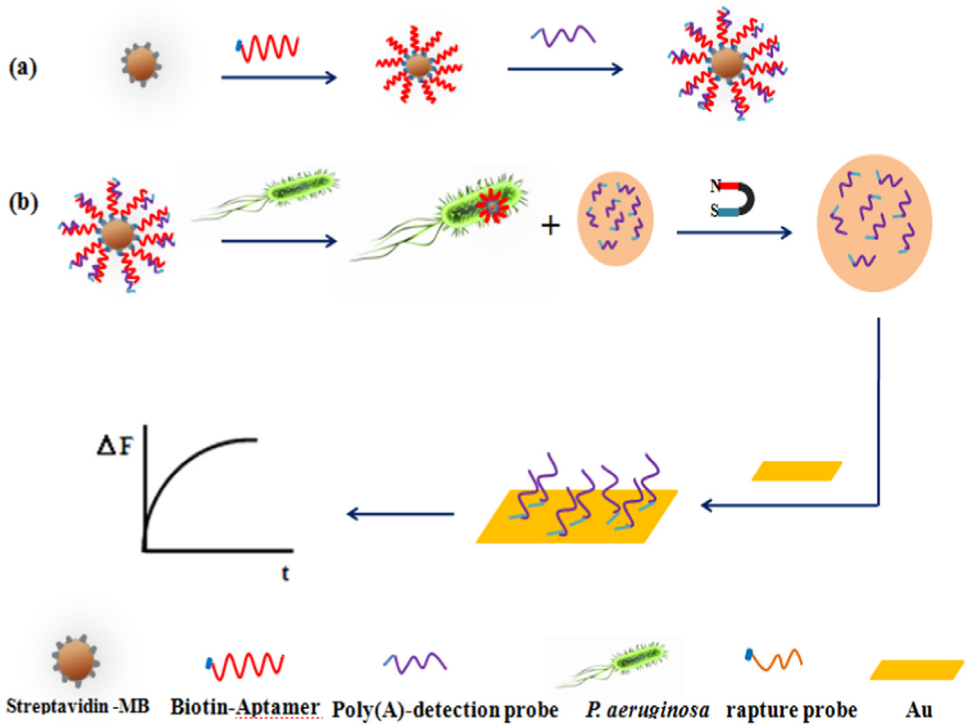

Fig. 2. Strategy of the MB-aptamer-Poly(A)DNA coupled with Au-IDE-MSPQC system to detect *P. aeruginosa*. (a) The preparation of MB-aptamer-Poly(A)DNA; (b) The detection of *P. aeruginosa* by the adsorption of released Poly(A)DNA onto the Au IDE surface.

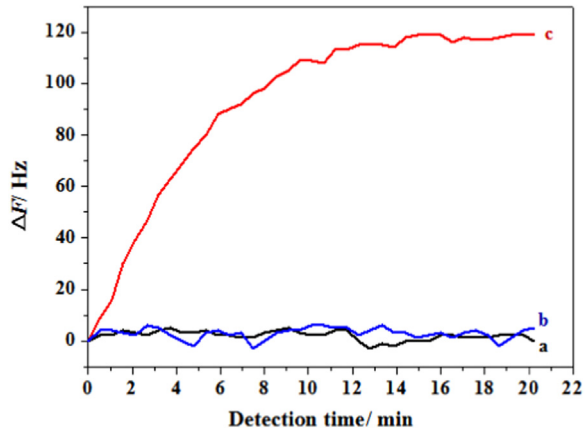

Fig. 3. The typical response curves (a) no *P. aeruginosa*; (b) no ployA DNA modified MB-aptamer; (c) 8.1×10^4 CFU/mL *P. aeruginosa* were detected by the proposed sensor.

are shown in Table S1. The results were analyzed by the T-test: $P = 0.899 > 0.05$, which indicated that there was no significant difference between the two methods. However, the detection time of the proposed method was shorter than the culture method. The comparision of the proposed method with previously reported one is shown in Table S2. The proposed method showed a high specificity and sensitivity for the detection of *P. aeruginosa*.

3.6. The specificity analysis of the sensor

To evaluate the selectivity of the proposed assay, six other pathogenic bacteria (*E. coli*, *S. aureus*, *E. faecalis*, *K. pneumonia*, *S. pyogenes* and *E. cloacae*) were detected using our method. The concentration of these strains was 1.0×10^3 CFU/mL. The obtained frequency shifts are shown in Fig. 5. Remarkably, there was no obvious signal response of these strains except for *P. aeruginosa*. This demonstrated that the sensor could selective detection of *P. aeruginosa*.

3.7. Detection of P. aeruginosa in a simulated blood sample

To test the potential application of the proposed method in practical samples such as blood samples, we further applied our method to detect *P. aeruginosa* in simulated blood samples. The sterile defibrinated sheep blood samples were spiked with five different concentrations of *P. aeruginosa* to obtain simulated positive samples, with three replicates for each sample. The calibration curve for the detection of *P. aeruginosa* in simulated blood samples is shown in Fig. 6. A linear relationship was obtained between ΔF and the logarithm concentration of *P. aeruginosa*, and it was in the range of 1.9×10^2 to 1.0×10^6 CFU/mL. The equation was $\Delta F = 24.70 \lg c - 7.38$, $R^2 = 0.99$. The sensor response obtained from negative blood sample was considered as blank signal in this case. Since the negative samples did not contain any bacteria, we attribute the response signal to the nonspecific adsorption of protein components of blood on the gold surface of the IDE. For the 20 simulated negative control samples, the average value of ΔF was 22 Hz and standard deviation (S_d) was 4 Hz. At the 99% confidence interval range ($\bar{x} \pm 3S_d$), frequency shifts of the negative samples were from 10 to 34 (Hz). The 34 Hz frequency shift detected by the proposed sensor was used as the criterion to differentiate a negative or positive result. If the detected frequency shift $\Delta F \leq 34$ Hz, the result was considered negative, and if $\Delta F > 34$ Hz, the result was considered positive. Therefore, according to the criteria for the positive/negative assessment, the LOD was 52 CFU/mL. The assay could be improved by selecting more suitable sensing electrode and better sample handling. To evaluate the specificity of the proposed assay in sterile defibrinated sheep, 1.0×10^4 CFU/mL bacteria that commonly appeared in the clinic (*E. coli*, *S. aureus*, *E. faecalis*, *K. pneumonia*, *S. pyogenes* and *E. cloacae*) were detected. The values of ΔF are 31, 21, 23, 28, 26 and 30 (Hz), respectively, these were the averages of three replicates, these values were below 34 Hz. These results indicate that the selected bacteria are not significant in causing interference with the determination of *P. aeruginosa* in sterile defibrinated sheep samples. The developed method exhibits

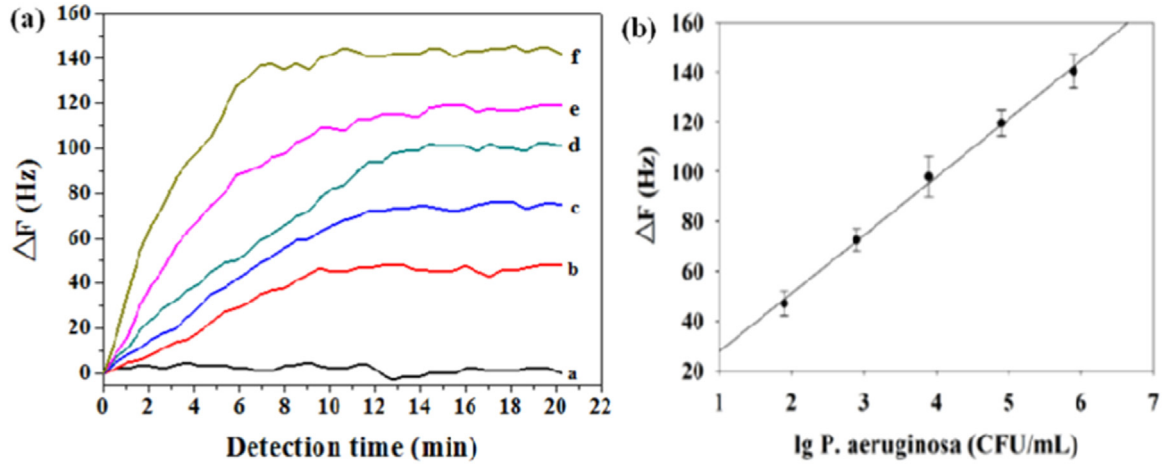

Fig. 4. (a) Response curves of different concentration *P. aeruginosa* (a, 0 CFU/mL; b, 8.1 $\times 10^1$ CFU/mL; c, 8.1 $\times 10^2$ CFU/mL; d, 8.1 $\times 10^3$ CFU/mL; e, 8.1 $\times 10^4$ CFU/mL; f, 8.1 $\times 10^5$ CFU/mL); (b) The calibration curve between the frequency change and concentration of *c*(logarithm). The measurements were repeated 3 times to obtain the standard deviation.

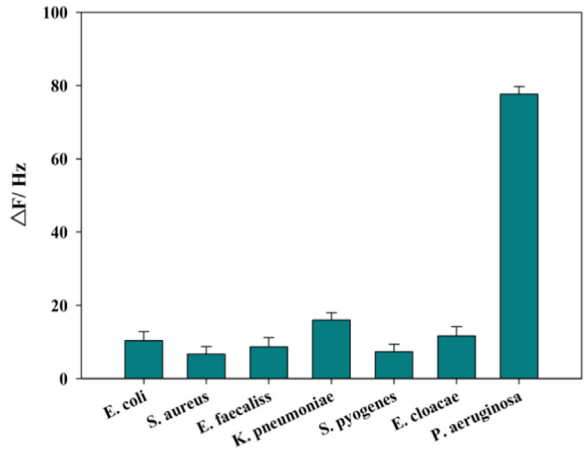

Fig. 5. Frequency shift response of different bacteria. The concentration for all detected strains was 1.0 × 10^3 CFU/mL. The error bars present the standard deviation of three replicate determinations.

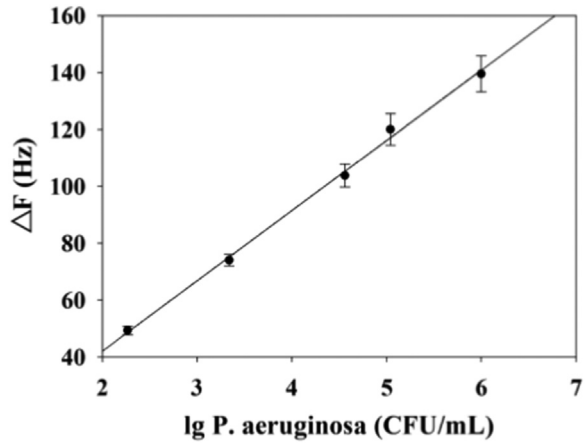

Fig. 6. Standard calibration for *P. aeruginosa* in simulated blood samples. Each data point represents the mean and standard deviation of three replicates.

good specificity for *P. aeruginosa*. The analysis of real samples (urine, blood, etc.) is our future work to further ensure the diagnosis sensitivity and specificity in the clinic.

4. Conclusion

In this study, a novel sensing method was developed to specifically detect *P. aeruginosa* by combining a magnetic bead-aptamer-poly(A) DNA with the sensitive Au IDE-MSPQC technology. The sensing electrode of the sensor did not any modifications, which was in constrast with the conventional electrochemical electrode. The proposed assay was simple, fast, specific and sensitive. Thus, this strategy provides a new path for the selective detection of *P. aeruginosa* in the clinic and in food and environmental sectors. In addition, alternative detection probes and capture probes can be used for detection of other targets. At this time, the real samples from patients were not detected, this analysis is being pursued in our future work.

CRediT authorship contribution statement

Xiaohong Shi: Conceptualization, Formal analysis, Methodology, Visualization, Writing - original draft. **Jialin Zhang:** Methodology, Data curation. **Fengjiao He:** Funding acquisition, Resources, Validation, Writing - review & editing.

Acknowledgements

This research work was supported by the National Natural Science Foundation of China (no. 21275042) and Hunan Province Science and Technology Plan Project (No. 2015JC3072).

Credit author statement

X.H. S. designed and carried out the experiments, and wrote the manuscript. J.L. Z. carried out the bacterial count and analyzed some experimental results. F.J. H. analyzed experimental results, supervised the work and revised the manuscript.

Declaration of interests

None.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2019.02.053.

References

Aghamollaei, H., Moghaddam, M.M., Kooshki, H., Heiat, M., Mirnejad, R., Barzi, N.S., 2015. J. Infect. Public. Heal. 8 (4), 314–322.

Ardura, A., Linde, A., Garcia-Vazquez, E., 2013. Int. J. Environ. Res. Public Health 10 (9), 3954–3966.

Bahareh, B., Abdollah, S., Hallaj, Rahman, H., 2018. Biosens. Bioelectron. 102, 328–335.

Chen, A.L., Yang, S.M., 2015. Biosens. Bioelectron. 71, 230–242.

Chen, J.Y., Penn, L.S., Xi, J., 2018. Biosens. Bioelectron. 99, 593–602.

Choi, H.J., Kim, M.H., Cho, M.S., Kim, B.K., Kim, J.Y., Kim, C.K., Park, D.S., 2013. Appl. Microbiol. Biot. 97, 3643–3651.

Deschaght, P., Van daele, S., De Baets, F., Vaneechoutte, M., 2011. J. Cyst. Fibros. 10 (5), 293–297.

Diribe, O., Fitzpatrick, N., Sawyer, J., Ragione, R.L., North, S., 2015. J. Equine Vet. Sci. 35 (11–12), 929–934.

Gall, F.L., Berre, R.L., Rosec, S., Hardy, J., Gouriou, S., Boisrame-Gastrin, S., Vallet, S., Rault, G., Payan, C., Hery-Arnaud, G., 2013. BMC Microbiol. 13 (143), 1–9.

He, F.J., Xiong, Y.C., Liu, J., Tong, F.F., Yan, D.Y., 2016. Biosens. Bioelectron. 77, 799–804.

Jang, H.D., Chang, K.S., Lee, Y.G., Lee, S.J., Hsu, C.L., 2009. Eur. Food Res. Technol. 229, 349–355.

Kim, L.H., Yu, H.W., Kim, Y.H., Kim, I.S., Jang, A., 2013. J. Korean Soc. Appl. Bi. 56 (2), 165–171.

Lakshmanan, R.S., Efemov, V., Cullen, S.M., Killard, A.J., 2014. Sens. Actuators B: Chem. 192, 23–28.

Lister, P.D., Wolter, D.J., Hanson, N.D., 2009. Clin. Microbiol. Rev. 22, 582–610.

Mi, X.W., He, F.J., Xiang, M.Y., Lian, Y., Yi, S.L., 2012. Anal. Chem. 84, 939–946.

Pei, H., Li, F., Wan, Y., Wei, M., Liu, H.J., Su, Y., Chen, N., Huang, Q., Fan, C.H., 2012. J. Am. Chem. Soc. 134 (29), 11876–11879.

Shen, D.Z., Zhu, W.H., Nie, L.H., Yao, S.Z., 1993. Anal. Chim. Acta 276, 87–97.

So, H.M., Won, K., Kim, Y.H., Kim, B.K., Ryu, B.H., Na, P.S., Kim, H., Lee, J.O., 2005. J. Am. Chem. Soc. 127, 11906–11907.

Sun, H.G., Zu, Y.L., 2015. Molecules 20 (7), 11959–11980.

Tan, W.H., Donovan, M.J., Jiang, J.H., 2013. Chem. Rev. 113 (4), 2842–2862.

Tang, Y.J., Zou, J., Ma, C., Ali, Z., Li, X.L., Ma, N.G., Mou, X.B., Deng, Y., Zhang, L.M., Li, K., Lu, G.M., Yang, H.W., He, N.Y., 2013. Theranostics 3 (2), 85–92.

Tong, F.F., Lian, Y., Zhou, H., Shi, X.H., He, F.J., 2014. Anal. Chem. 86, 10415–10421.

Wang, K.Y., Zeng, Y.L., Yang, X.Y., Li, W.B., Lan, X.P., 2011a. Eur. J. Clin. Microbiol. 30 (2), 273–278.

Wang, Y., Dou, H.L., Chen, K.X., Zhang, H., Hu, C.G., 2011b. Scand. J. Infect. Dis. 43 (5), 329–338.

Weiser, R., Donoghue, D., Weightman, A., Mahenthiralingam, E., 2014. J. Microbiol. Methods 99, 8–14.

Wiehlmann, L., Cramer, N., Ulrich, J., Hedtfeld, S., Weißbrodt, H., Tümmler, B., 2012. Int. J. Med. Microbiol. 302, 69–77.

Zhang, P., Guo, X.L., Wang, H.H., Sun, Y., Kang, Q., Shen, D.Z., 2017a. Sens. Actuators B: Chem. 248, 551–559.

Zhang, Q.L., Smith, J.C., Zhu, Q.J., Guo, Z.Q., MacDonald, N.E., 2012. Int. J. Infect. Dis. 16, 628–632.

Zhang, X.Q., Feng, Y., Yao, Q.Q., He, F.J., 2017b. Biosens. Bioelectron. 98, 261–266.

Zhu, D., Song, P., Shen, J.W., Su, S., Chao, J., Aldalbahi, A., Zhou, Z., Song, S.P., Fan, C.H., Zuo, X.L., Tian, Y., Wang, L.,H., Hao, P., 2016. Anal. Chem. 88 (9), 4949–4954.