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Fluorescent biosensor based on MB-APT combined with Pt NPs for the detection of infectious bacteria in mouse and human wounds

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ABSTRACT

Bacterial infection of wounds is one of great concern to patients, and rapid and correct detection of bacterial infection is crucial to ensure accurate diagnosis and early intervention. Based on the principle that glucose can only be consumed by live bacteria, a fluorescent biosensor was constructed to detect four kinds of common infectious bacteria (Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus) in skin wounds, taking advantage of magnetic bead-aptamer for recognizing, sorting and enrichment, platinum nanoparticles for signal amplification. The linear detection range of AB, EC, PA and SA were 27– 8×10^6 CFU/mL, 10– 2.5×10^7 CFU/mL, 34– 2.5×10^7 CFU/mL and 10– 1.0×10^7 CFU/mL, respectively, and the limits of detection were 27 CFU/mL, 10 CFU/mL, 34 CFU/mL and 2 CFU/mL. Furthermore, all target bacteria in samples containing 8×10^8 CFU/mL of other interfering bacteria have been successfully identified and quantified. The proposed method was also successfully applied to the detection of bacterial infection in skin wounds of mouse and human, including the detection of separate bacterial infection as well as a coinfection. The recovery of this method was in the range of 90.161– 109.961%. Thus, this proposed method can be a promising candidate for rapid and convenient evaluation of infectious bacteria in point-of-care settings.

1. Introduction

More than 6.5 million people are suffered from Chronic wound disease worldwide due to the lack of adequate medical care [1]. Among numerous wound diseases, wound infection is of great concern to the public. The skin is the outermost layer of the human body, and it is the first line of defense against a variety of external pathogens [2]. Owing to the support of frequent contact with the external environment, the skin is vulnerable to being attacked by external factors such as machinery, heat, and chemicals, which eventually resulted in skin tissue damage and wound formation. Subsequently, the wound is easy to be colonized by bacteria and cause infection. Bacterial infection of the wound is a major clinical problem, and severe infections are the inducement of high incidence rate and high mortality. And it affects about 8.2 million people around the world [3], and the annual cost of wound treatment is estimated to exceed 32 billion US dollars, which leads to huge global financial expenditure every year [4].

Many studies have shown that the most common infectious bacteria which has pathogenicity in the wound are *Staphylococcus aureus* (SA, 39.28%), *Pseudomonas aeruginosa* (PA, 19.64%), *Acinetobacter baumannii* (AB, 10.71%) and *Escherichia coli* (EC, 30.35%) [5]. The types of infectious bacteria are complex and bacterial content is low in an early wound, so it is difficult to detect these harmful bacteria in a short time. It may lead to delaying the treatment time and will cause serious local and systemic complications, such as cellulitis, osteomyelitis, and sepsis. Therefore, it is urgent to develop new bacterial detection techniques for infectious samples in wounds.

At present, three conventional quantitative methods are used for the

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determination of infectious bacteria, including plate culture counting, molecular biological detection (e.g. polymerase chain reaction and loop-mediated isothermal amplification), and immunological detection (e.g. enzyme linked immunosorbent assay and chemiluminescence analysis) [6]. Among them, the plate culture counting method based on bacterial culture is the "gold standard" for infection diagnosis, but it takes a long time to get diagnostic results [7]. Although the method of DNA/RNA analysis based molecular technology and antibody analysis based immunology technology can improve sensitivity and shorten the detection time, they are mostly on the level of low efficiency, high cost and single target, especially in quantitation of live bacteria due to the presence of dead cells and extracellular DNA [8].

Bacterial biosensors have emerged as advanced detection tools in many research fields, including environmental monitoring, detection of airborne pathogens, and real-time detection of human blood components and pathogens [9]. Compared with antibodies that are often used as recognition elements in biosensors, aptamers have been used as new recognition elements. Aptamers are specific nucleic acid sequences that can fold into stable structures and specifically bind to target substances, such as whole cells, proteins, peptides, small molecules and other target substances [10]. And they have the characteristics of easy synthesis and modification, high stability, good reproducibility, low immunogenicity level, and low production cost. Consequently, aptamers combined with various signal transduction strategies of optics-based and electrochemistry-based analysis, such as surface plasmon resonance (SPR) [11], surface-enhanced Raman scattering (SERS)[12], colorimetry [13], fluorescence [14], electrochemistry [15] and chemiluminescence [16], which are widely used to construct sensors to detect bacteria samples from milk, apple juice, urine, blood [17–19].

Glucose, a standard carbon and energy source for bacteria According to the principle of adding glucose oxidase to consume glucose and produce hydrogen peroxide, it has been used for micro detection of glucose [20,21], and sensors with fluorescence [22], colorimetric [23], and electrochemical methods can be used to quantify bacteria based on the principle of glucose consumption by bacteria [24–26]. Furthermore, we try to achieve the classification and quantitative detection of wound infection bacteria by combining suitable ligands and hydrogen peroxide color reaction. However, the application requirements of biosensor based on aptamers merit further concerned. Potential interfering substances (such as particles, organic or inorganic pollutants and other biomolecules) interfere with the signals, reduce the sensitivity of the sensor and limit the service life of the sensor. So, it is worthy of research task how to detect the live bacteria accurately in the infectious wound by aptamers biosensors.

In this study, the bacterial wound infection model based on mouse skin is used to simulate clinical wound infection. The fluorescent biosensor we constructed consists of two parts: one is the Magnetic beads-aptamer (MB-APT) that is used to separate and enrich target bacteria; another is platinum nanoparticles (Pt NPs) that are used to catalyze O-phenylenediamine (OPD) to form 2,3-diaminophenazine (OPDox) with bright yellow fluorescence, and there is a positive correlation between the fluorescence intensity (FI) and targeted bacterial concentration. This study provides an experimental basis for the development of a simple, rapid, sensitive and specific method for detecting the live bacteria in wound infection. In addition, this method can be well applied to the actual detection of mouse and human wound samples, and it may provide the practical reference value for the accurate diagnosis of the wound infection.

2. Materials and methods

2.1. Materials and reagents

Standard bacterial strains of AB (ATCC 19606), EC (ATCC 25922), PA (ATCC 27853) and SA (ATCC 33591) were purchased from Beina Chuanglian Biotechnology Co., Ltd. *Aeromonas* (N5), *Psychrophilic bacillus* (N12), *Pseudomonas* (C6), *Shewanella* (TB-1) and *Vibrio* (VR-5) were from the strain preservation library of our laboratory.

OPD and HEPES buffer were purchased from McLean Biochemical Technology Co., Ltd (Shanghai, China); Chloroplatinic acid hexahydrate was purchased from Aneji Chemical Co., Ltd; Glucose oxidase (Gox) and Sodium borohydride were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd; Glucose (Glu), Trisodium citrate, KCl, $MgCl_2$, $CaCl_2$ and NaCl were purchased from Sinopharm Chemical Reagent Co., Ltd; Streptavidin modified magnetic beads (MBs, 0.1 μm) and biotin modified aptamer (biotin-APT) were purchased from Sangong Bioengineering (Shanghai, China) Co., Ltd. The synthetic sequence of APT_{AB}[27], APT_{EC}[28], APT_{PA}[29] and APT_{SA}[30] were shown in Table S1.

2.2. Bacterial culture and plate colony counting

AB, EC, PA and SA were inoculated in Luria-Bertani (LB) liquid medium and cultured overnight at 180 rpm at 37 ℃. The absorbance value of bacterial fluid at OD_{600} was measured by the microplate reader (SYNERGY-H1, BIOTEK, America), and the concentrations of AB, EC, PA, and SA were calculated by plate colony counting at the corresponding absorbance.

2.3. Synthesis of Pt NPs

Pt NPs were prepared according to the previously reported method [31]. 1 mL $H_2PtCl_6{\cdot}6\,H_2O$ aqueous solution (16 mM) and 1 mL trisodium citrate solution (40 mM) were added into a round bottom flask filled with 38 mL deionized water, stirred in the dark at room temperature for 30 min, then added 200 μL sodium borohydride solution (50 mM) reacted at room temperature for 1 h. the brownish-yellow Pt NPs solution was obtained and stored at 4 ℃.

2.4. Characterization of Pt NPs

The particle size and shape of Pt NPs were measured by Transmission Electron Microscopy (TEM, HT 7700, HITACHI, Japan) and Marvin Particle Sizer (Mastersizer, MALVERN, Britain).

2.5. Incubation of MBs with APT

500 μL 1 mg/mL streptavidin-MBs solution were incubated with 70 μL biotin-APT_{AB}, biotin-APT_{EC}, biotin-APT_{PA}, and biotin-APT_{SA} (10 μM) and reacted at 37 °C for 1 h, respectively. The connection effect was verified by Zeta potential analyzer (Nano-ZS, MALVERN, Britain).

2.6. Incubation of MB-APT with target bacteria

10 μL MB-APT solution was added to different concentrations of target bacterial solutions respectively (AB added MB-APT_{AB}, EC added MB-APT_{EC}, PA added MB-APT_{PA}, SA added MB-APT_{SA}), which mixed with 400 μL binding buffer (40 mM pH 8.0 HEPES buffer, 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 150 mM NaCl) [32] for 30 min at 37 ℃, and MB-APT-bacteria was dispersed in 100 μL PBS(0.01 M) buffer. Then, TEM and Scanning Electron Microscope (SEM, Quanta250, FEI, America) were used for characterization.

2.7. Detection of one type of bacteria in PBS buffer

100 μL Glu solution (2 M) was mixed with different concentrations of MB-APT-bacteria, cultured at 37 ℃ for 30 min, and then MB-APT-bacteria was removed by magnetic separation within 1 min. The remaining Glu solution was incubated with 100 μL Gox solution (20 U/mL, 30 ℃) for 30 min 200 μL Pt NPs were added to the above solution and reacted at room temperature for 10 min, and OPD solution (100 μL, 0.4 mM, 37 ℃) was added for 30 min, then the FI was measured by a

high sensitivity fluorescence spectrometer (FluoroMax-4, HORIBA, France). ΔFI = FI (tested sample) − FI_0 (negative control sample), was defined as differences in measured fluorescence values between positive tested groups (infectious bacteria) and negative control group were considered as net fluorescence values. All experiments were conducted in three replicates, and results were presented as mean ± standard deviation.

2.8. Detection of multiple bacteria in PBS buffer

The mixed bacterial solution (8 $\times 10^8$ CFU/mL) was prepared by EC, PA, SA, C6, N5, N12, TB-1 and VR-5 solution (the concentration of each group was 1 $\times 10^8$ CFU/mL), in which AB solution with different concentrations (19, 190, 1900, 19,000, 190,000, 1900,000 CFU/mL) was added, respectively. According to 2.6 and 2.7, the detection of EC, PA and SA in a mixed bacterial solution except for the target bacteria, and the subsequent steps were the same as above.

2.9. Detection of one type of bacteria in wound sample from infected mouse and human

The wounds were made on the mouse epidermis used by a puncher under aseptic conditions. Then AB, EC, PA, and SA were added to different mouse wounds, respectively. Wound samples were obtained by a sterile cotton swab, other operations were the same as 2.7. The study was approved by the Ethics Committee of Fuzhou University (No. 2020-SG-001), and all experimental procedures were carried out under the regulations on the management of experimental animals. Besides, the collection of clinical wound samples followed the relevant national legal requirements with consent and approval from the patients, and also approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University (No. 2022KY056). The obtained samples were

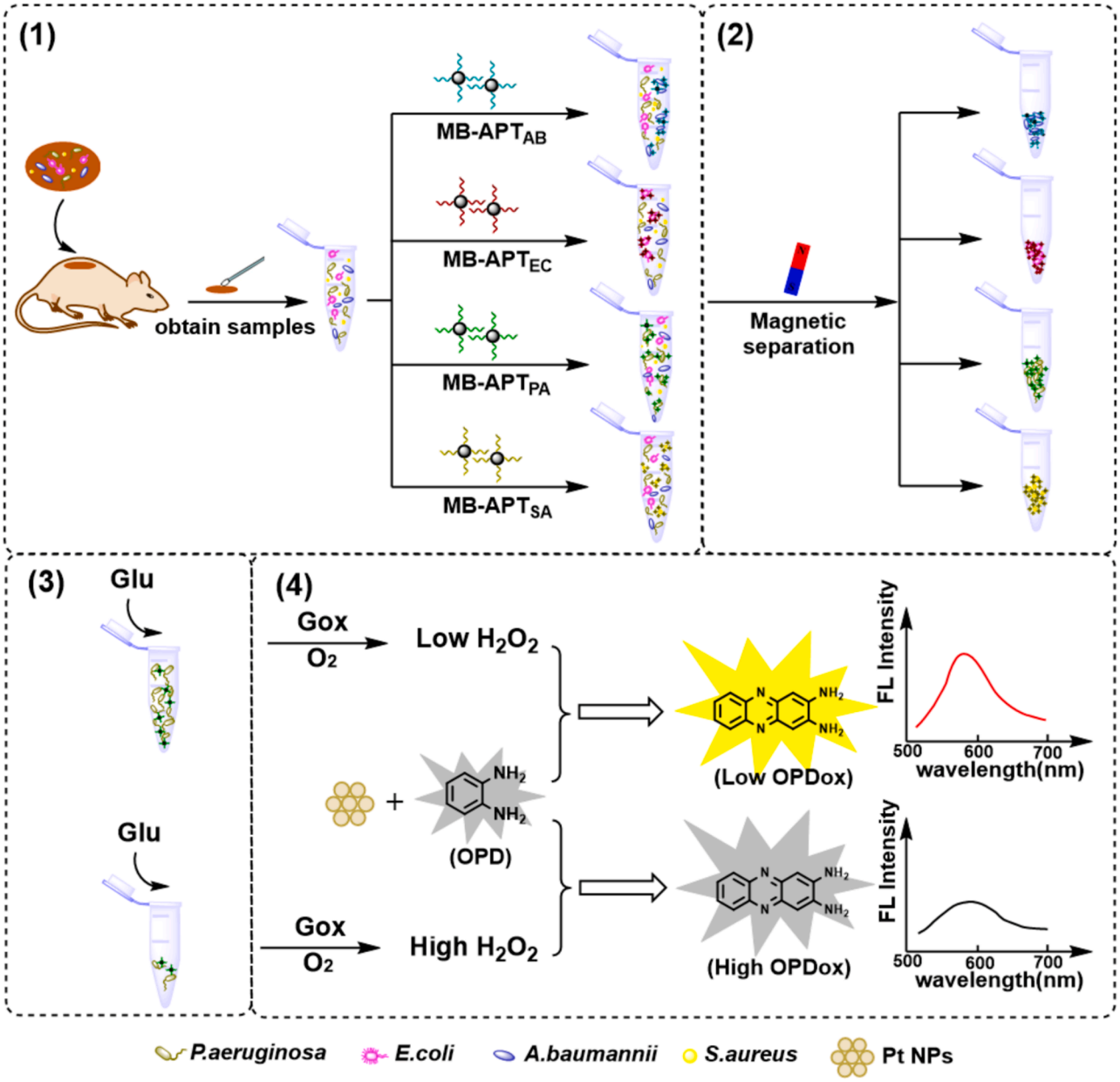

Fig. 1. Schematic diagram of fluorescent biosensor for detecting infectious bacteria on wounds (1) Selective capture of functionalized MB-APT; (2) Isolation and enrichment of target bacteria; (3) Glu consumed by live bacteria and (4) Response mechanism of FI to bacterial concentration. Note: High OPDox content leads to self-aggregation, the fluorescence is inhibited subsequently and decreases.

sterilized by filter and added the target bacteria as the tested samples of one type of bacterial infection.

2.10. Detection of multiple bacteria in wound sample from infected mouse and human

The mixed bacterial solution of AB, EC, PA, SA, TB-1, VR-5, N12, C6 and N5 were inoculated on mouse wounds, other operations were the same as 2.7 and 2.9. Clinical wound samples from complex bacterial infections were provided by the First Affiliated Hospital of Fujian Medical University.

3. Results and discussions

3.1. Principle and design of biosensors for bacteria detection

Fig. 1 shows the sensing mechanism of the fluorescent biosensor for detecting live AB, EC, PA, and SA. Firstly, wound samples from infected mouse are collected and prepared (Figs. 1–1). Then MB-APT could be used to capture, isolate and enrich the target bacteria in wound samples (AB was captured by MB-APT_{AB}, EC was captured by MB-APT_{EC}, PA was captured by MB-APT_{PA}, and SA was captured by MB-APT_{SA}), and form compound of MB-APT-bacteria separated by MBs (Figs. 1–2). Next, the response mechanism of biosensor for the qualitative and quantitative analysis of bacteria is divided into two parts (Figs. 1–3 and 1–4): we use the principle that Glu is consumed by live bacteria in their metabolic process, and the residual Glu after MB-APT-bacteria consumption is catalyzed by Gox to produce oxygen-dependent H_2O_2. As a hydrogen peroxide mimic enzyme, Pt NPs catalyze H_2O_2 to generate hydroxyl radical, and the latter further oxidizes OPD to produce OPDox with bright yellow fluorescence. With the increase of H_2O_2 concentration, increased OPDox content leads to self-aggregation raise, the fluorescence of the latter is inhibited subsequently and the FI decreases. Thus, the more bacteria in a wound sample, the more Glu consumption, which results in lower content of OPDox produced by Pt NPs oxidation, and the strong fluorescence signal are finally detected. On the contrary, if few bacteria are in the wound, the detected FI is low. Therefore, the FI of OPDox depends on the concentration of bacteria, and the concentration of bacteria in wound samples can be determined by measuring the FI.

3.2. Characterization of Pt NPs

It is reported that Pt NPs with a diameter of less than 6 nm can have the oxidation catalytic function of the H_2O_2 enzyme [33]. The DLS results of Pt NPs with an average particle size about 4.6 nm (Fig. S1B) were consistent with the literature [34]. TEM images further verified that Pt NPs with a diameter of 5 nm were evenly dispersed in Fig. S1A. And the fluorescence results showed that the synthesized Pt NPs had peroxidase mimic activity (Fig. S3).

3.3. Characterization of MBs-APT

The streptavidin-MBs were used to connect with biotin-APT. As shown in Fig. S2, compared with the streptavidin-MBs, the zeta potentials of MB-APT_{AB}, MB-APT_{EC}, MB-APT_{PA} and MB-APT_{SA} changed from -17.0 ± 0.6 to -21.7 ± 0.3, -21.70 ± 0.2, -20.4 ± 1.7 and -20.8 ± 0.7 mV, respectively. The reason for these results was the aptamer has net negative charge at neutral pH, the further decline in the zeta potentials of MB-APT represented that the aptamer was successfully bound to the MBs.

3.4. Feasibility analysis of fluorescent biosensor for detecting bacteria

Firstly, TEM and SEM images showed the morphology and size of AB, EC, PA, and SA in Fig. 2A. We could clearly observe that MBs (particle size was about 0.1 μm) with uniform size and regular shape gathered around the target bacteria. It indicated that the target bacteria were captured by MB-APT because of the specific adsorption provided by aptamers with a high affinity to target bacteria.

Secondly, the fluorescence response of fluorescent biosensor composed of Pt NPs, OPD and H_2O_2 was explored. As shown in Fig. S3, no emission peak of Pt NPs was observed at 580 nm under 478 nm excitation light. While Pt NPs, OPD, and H_2O_2 were simultaneously added to the reaction system, the maximum fluorescence signal was found at 580 nm, and there was a dose-response relationship between H_2O_2 concentrations and FI (the FI decreases with the increase of H_2O_2 concentration). So, the H_2O_2 in the fluorescent biosensor based on Pt NPs played a key role in the fluorescence quenching effect.

Furthermore, based on the principle that Gox catalyzed the oxidation of Glu by oxygen to produce H_2O_2, the same fluorescence quenching effect mentioned above was also observed in Fig. 2B. When Pt NPs, OPD, Glu and Gox existed simultaneously in the reaction system, there was a maximum emission peak at 580 nm. And FI decreased with the increase of Glu concentration. Subsequently, we verified the response of the fluorescent biosensor to different Glu concentrations. In Fig. 2D and E, the FI showed a significant linear dose-response relationship with Glu concentration in the range of 10 μM–2 mM, and $R^2 = 0.998$. These results showed that the biosensor based on Pt NPs has a good linear fluorescence response to Glu concentration.

Finally, we further validated the feasibility of the fluorescent biosensor for detecting live bacteria. As shown in Fig. 2C, compared with group b (PBS), the FI significantly increased in the group a (SA), c (H_2O_2 + Glu) and d (H_2O_2 + Gox), and the results indicated that Glu catalyzed by Gox to produce a large amount of H_2O_2 could decrease the FI; Moreover, the increased FI in the group a indicated bacterial consumption, resulting in the decrease of Glu concentration significantly enhanced the FI. Consequently, similar results also appeared in the relationship between the FI and SA concentration (47–47 $\times 10^6$ CFU/mL) in Fig. 2F and G, and there was a good positive correlation (R^2 =0.995). Hence, it is feasible to use such a fluorescent biosensor based on Glu consumed by bacteria to detect live bacteria. On this basis, we optimized the concentration of GOX, as shown in Fig. S4. The fluorescence reached a plateau when the concentration was 20 U/mL.This data was used for subsequent experiments. Moreover, we also used the fluorescent biosensor for the detection of AB, EC, and PA. The FI showed a good linear relationship with the concentrations of AB, EC and PA in Fig. S5, and R^2 were 0.993, 0.995 and 0.994 respectively, indicating that the biosensor has the ability to quantify AB, EC and PA accurately.

3.5. Detection of one type of bacteria in PBS buffer

Given excellent capture ability of MB-APT, the fluorescent biosensor was used to detect the concentration of four species of infectious bacteria in PBS with one type of bacteria. The target bacterial concentration corresponding to the FI was determined by the plate colony counting method in Fig. S6. As shown in Fig. 3A, C, E and G, there were good linear correlations between the FI and the concentration of AB, EC, PA, and SA, and R^2 were 0.994, 0.990, 0.999, and 0.999, respectively. The linear detection ranges of the four infectious bacteria were different. We attribute such results to the various metabolic capacities possessed by different bacteria, which resulted in diverse abilities to consume Glu and finally assorted responses to our sensor. By calculation, the detection ranges of AB, EC, PA, and SA were as follows: 27– 8×10^6 CFU/mL,10– 2.5×10^7 CFU/mL,34– 2.5×10^7 CFU/mL, and 10– 1.0×10^7 CFU/mL, and the theoretical limits of detection (LODs) were 27 CFU/mL, 10 CFU/mL, 34 CFU/mL and 2 CFU/mL, respectively (Table S2). The results demonstrated that MB-APT could effectively enrich the target bacteria, and the biosensor for the detection of infectious bacteria based on MB-APT and Pt NPs had some advantages such as wide detection range, low detection limit, high accuracy and sensitivity.

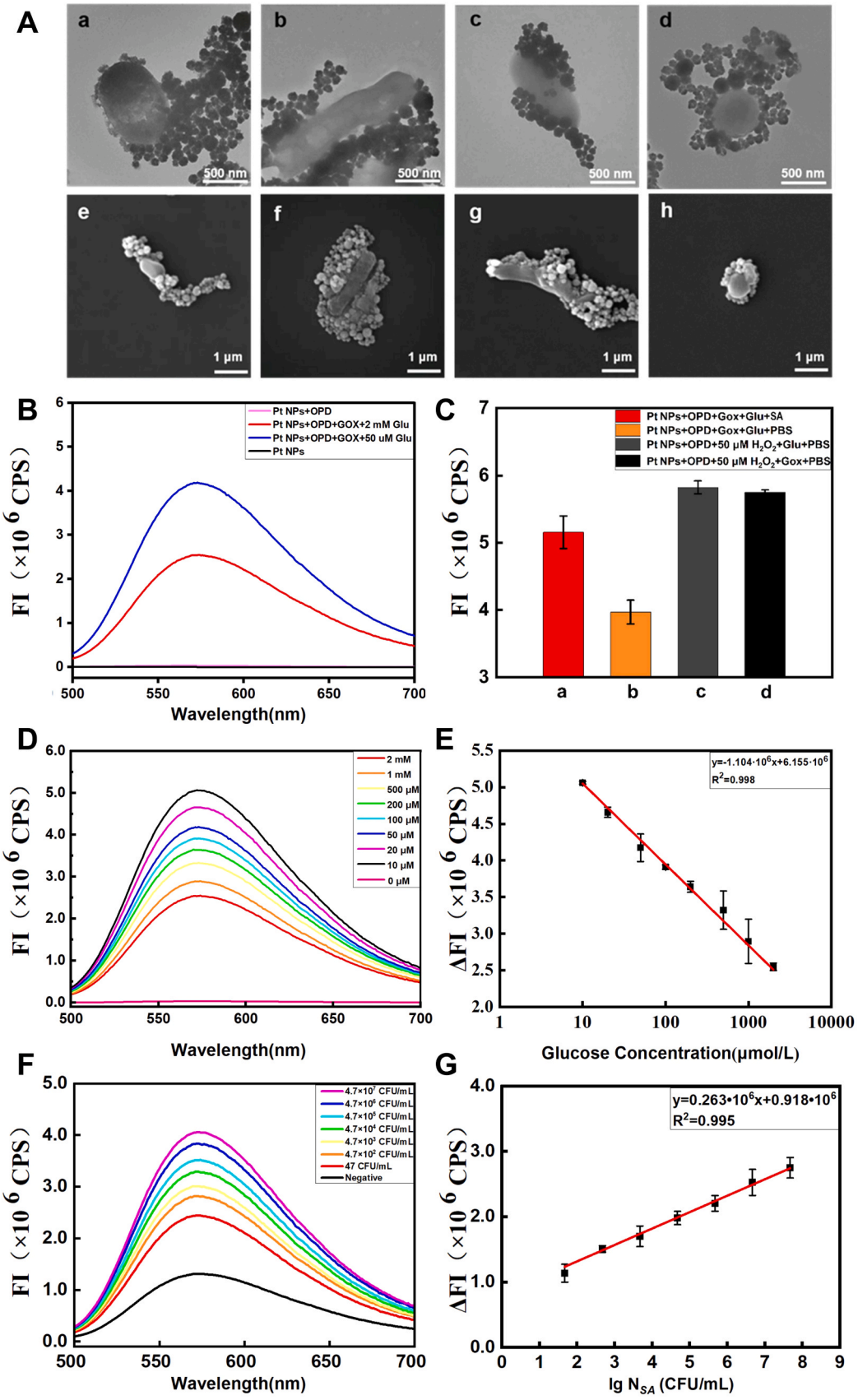

Fig. 2. Feasibility analysis of fluorescent biosensor. (A) TEM (a-d) and SEM (e-h) images of target bacteria after incubated with MB-APT. a and e: AB captured by MB-APT_{AB}; b and f: EC captured by MB-APT_{EC}; c and g: PA captured by MB-APT_{PA}; d and h: SA captured by MB-APT_{SA}. (B) Response of fluorescent biosensor based on Pt NPs to Glu; (C) Feasibility analysis of fluorescent biosensor based on Pt NPs for detecting live bacteria; (D) Fluorescence spectra of different Glu concentrations; (E) Relationship between Glu concentration and FI; (F) Fluorescence spectra of different SA concentrations; (G) Relationship between SA concentration and FI.

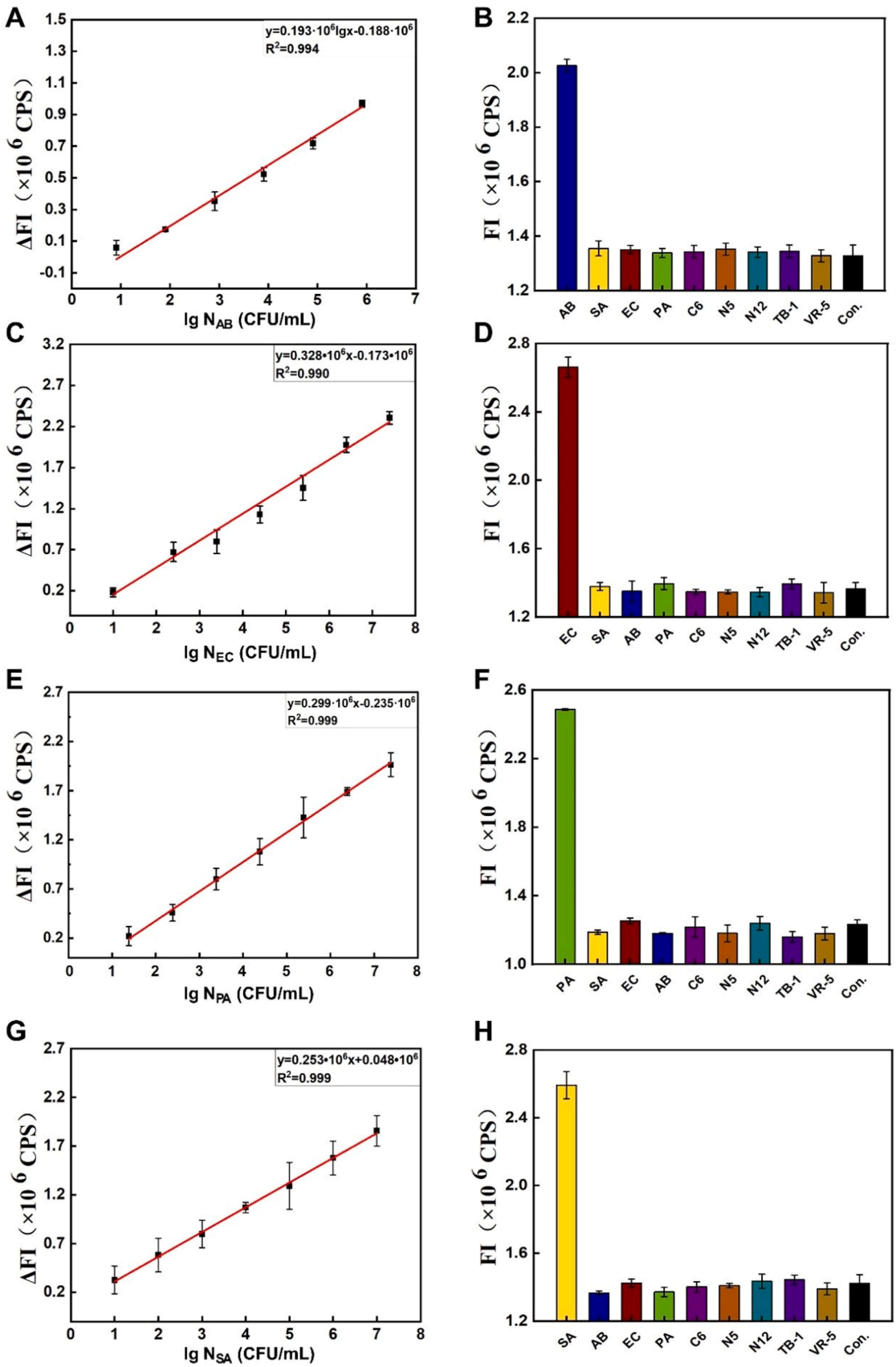

Fig. 3. Linearity (A, C, E, G) and selectivity (B, D, F, H) of fluorescent biosensor for detecting AB, EC, Pa and SA. N_{AB}, N_{EC}, N_{PA} and N_{SA} represent the number of bacteria after separating the magnetic bead adapter and bacteria, and the unit is CFU/mL.

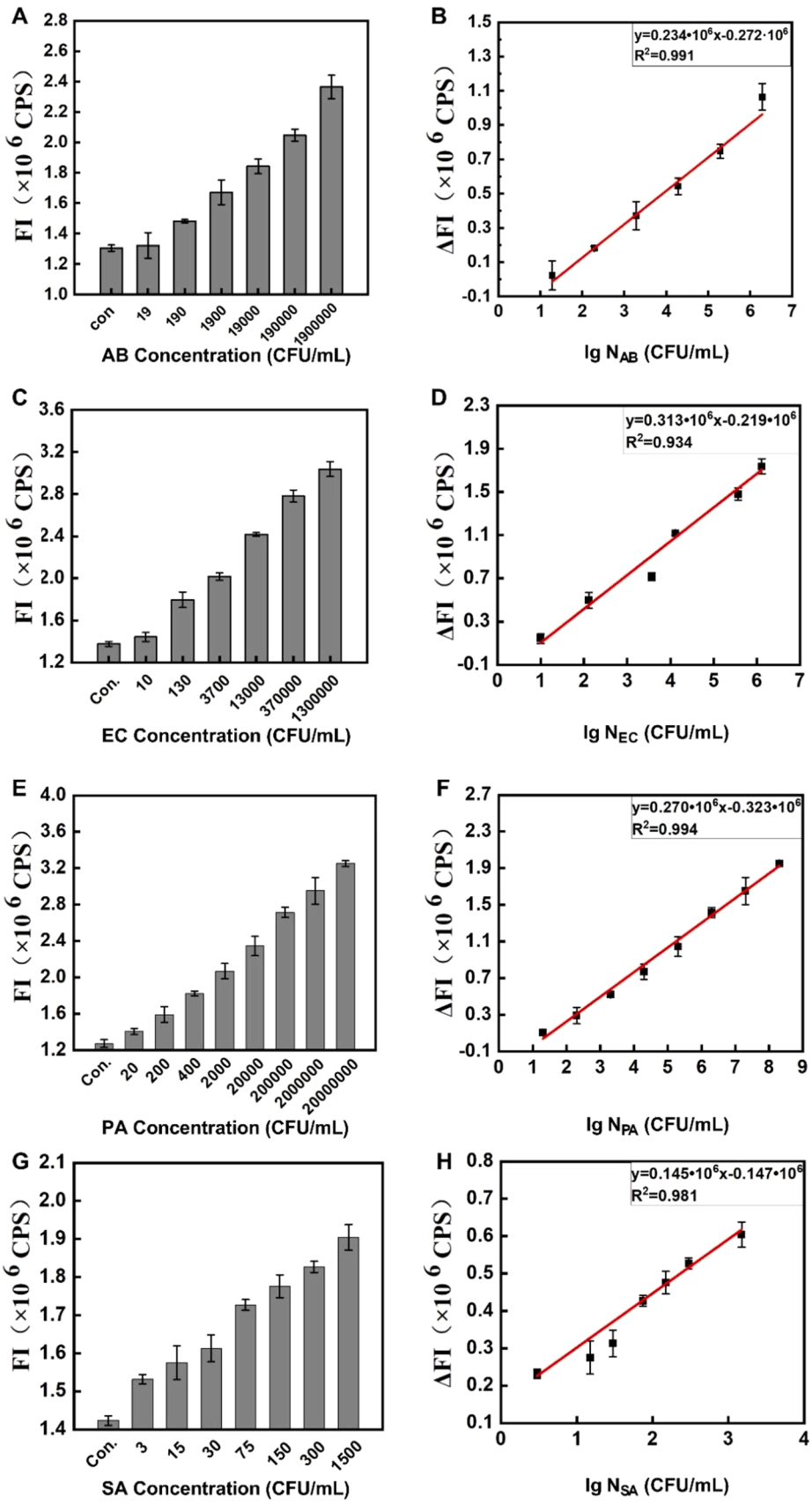

Fig. 4. Detection effect of four infectious bacteria in mixed bacterial solution after separating the magnetic bead adapter and bacteria. FI of AB (A), EC (C), PA (E) and SA (G); Relationship between FI and AB (B), EC (D), PA (F) and SA (H) concentrations.

3.6. Selectivity analysis of fluorescent biosensor

Since wound infection is often caused by many different types of bacteria, it is necessary to confirm the selectivity of fluorescent biosensors for detecting AB, EC, SA, and PA. Besides the four target bacteria, the other five species of interfering bacteria (including C6, N5, N12, TB-1, VR-5) and a control group (PBS buffer) were set for detection, and the bacterial concentration of each group was about 1×10^5 CFU/mL (no bacteria in the control group). As shown in Fig. 3, the group AB in Fig. 3B, group EC in Fig. 3D, group PA in Fig. 3F, and group SA in Fig. 3H have significantly higher levels of the FI compared with every interfering bacteria group and control group, and there were negligible differences between interfering bacteria groups and control group. The experimental data fully proved that the only target bacteria specifically was attracted by MB-APT, so our designed fluorescent biosensor has excellent selectivity for the detection of AB, EC, PA, and SA.

3.7. Detection of multiple bacteria in PBS buffer

To further explore whether the fluorescent biosensor possesses high sensitivity to detection of the AB, EC, PA, and SA in complex samples, a mixed bacterial solution with gradient concentration was prepared with PBS buffer. The target bacterial concentration corresponding to the FI was determined by the plate colony counting method in Fig. S7. Even in the coinfection system containing multiple bacteria, there is also a very good linear relationship between the FI and the concentration of AB, EC, PA, and SA (Fig. 4), R^2 was 0.991, 0.934, 0.994 and 0.981, and the LODs of AB, EC, PA and SA were 19 CFU/mL, 10 CFU/mL, 20 CFU/mL and 3 CFU/mL respectively. In particular, the changed fluorescence signal of 3 CFU/mL SA mixed in 8×10^8 CFU/mL interfering bacteria could still be detected in Fig. 4G and H. Thus, the results of the quantitative assessment also demonstrated good performance of our fluorescent biosensor to identify and quantify AB, EC, PA, and SA in mixed samples, and suggested that it might have a promising application prospect.

3.8. Detection of one type of bacteria in wound samples from infected mouse and human

Infectious wound in mouse (Fig. S8) and human (Fig. S9) were obtained and further demonstrated the practicability of biosensor for the detection of common infectious bacteria (AB, EC, PA and SA). The detection results were shown in Table S3 (added concentration of target bacteria was determined by the plate colony counting method in Fig. S10). The recoveries of AB, EC, PA and SA in mouse were were 99.8-102.9%, 96.8- 108.4%, 93.1- 101.1% and 98.3- 101.0% (human wound samples: 92.3- 107.4%, 90.1-101.9%, 93.1- 101.60%, 102.802%-109.890%), and the RSD were 1.916- 5.643%, 1.267-3.389%, 0.362-2.899% and 0.327- 2.608% (human wound samples: 0.275- 1.978%, 1.211- 2.402%, 2.381- 5.729%, 3.776- 7.697%) respectively. The results indicated that our method could be well applied to the qualitative and quantitative analysis of AB, EC, PA and SA in mouse and patients with infected wounds.

3.9. Detection of multiple bacteria in wound samples from infected mouse

In addition, mouse (Fig. S11) and human infected by multiple bacteria was used for verifying the detection effect of the fluorescent biosensor. Fig. S12 showed that added concentration of target bacteria was determined by the plate colony counting method. And the fluorescent biosensor in mixed bacteria from mouse wounds had the similar results as excellent as one type of bacteria-infected wound, as exhibited in Table 1, the recoveries of AB, EC, PA, and SA were 92.2- 109.8%, 102.1- 106.1%, 94.9- 100.9%, and 98.5- 109.9% (human wound samples: 103.8- 105.2%, 93.7- 97.9%, 103.9- 107.6%, 94.8- 106.0%), and the RSD were 0.646- 4.437%, 0.537- 2.462%, 1.690- 4.498%, and 1.724-4.294% (human wound samples: 1.347- 1.905%, 1.932- 2.290%, 0.772-4.294%, 1.197- 3.197%), respectively. Taken together with the results described above, our method was illustrated to be good capability and reliability, and it could accurately detect a broad range of bacteria.

Table 1
Recovery efficiency of target bacteria in mouse and human wound samples infected by multiple bacteria.

Type of bacteria	Sample	Added (lg CFU/mL)	Found (lg CFU/mL)	Recovery (%)	RSD (%, n = 3)
AB	Mouse 1	5.906	6.406	92.206	0.646
	Mouse 2	4.906	4.843	101.310	1.662
	Mouse 3	3.906	3.556	109.843	4.437
	Human 1	5.000	4.817	103.789	1.347
	Human 2	4.301	4.143	103.820	1.350
	Human 3	4.000	3.800	105.257	1.905
EC	Mouse 1	5.405	5.175	104.446	1.175
	Mouse 2	3.405	3.209	106.105	0.537
	Mouse 3	2.405	2.357	102.055	2.462
	Human 1	5.699	5.821	97.899	1.932
	Human 2	4.699	4.935	95.217	2.036
	Human 3	3.699	3.946	93.730	2.290
PA	Mouse 1	4.342	4.559	95.245	1.724
	Mouse 2	3.342	3.312	100.892	4.498
	Mouse 3	2.342	2.466	94.960	1.690
	Human 1	5.301	5.100	103.945	0.772
	Human 2	4.301	4.135	104.016	1.804
	Human 3	3.301	3.067	107.629	4.294
SA	Mouse 1	4.405	4.468	98.582	4.294
	Mouse 2	3.405	3.360	101.340	3.384
	Mouse 3	2.405	2.056	109.961	1.724
	Human 1	5.000	4.717	106.010	1.197
	Human 2	4.000	3.828	104.501	2.328
	Human 3	3.000	3.164	94.827	3.197

3.10. Comparison between this method and other reported methods

Compared with the reported methods in Table 2, this method in this paper has a wider linear-detecting range, can to detect a variety of infectious bacteria, and has a better detection capacity, even in skin wounds of mouse and human. Moreover, the LOD of this method was lower than some traditional colorimetric methods, but is consistent with the electrochemical biosensors reported by previous studies. However, it should be noted that most of the previous electrochemical biosensors were used for the detection of foods such as water and milk, but were not tested in skin wounds. Furthermore, due to the relatively facile preparation and low cost of Pt NPs, and the shorter testing time, our biosensor could be a powerful tool for reliable identification and quantitation of infectious bacteria.

4. Conclusions

In this study, a new fluorescent biosensor based on MB-APT with sorting and enriching bacteria combined with Pt NPs with catalytic enzyme activity was successfully constructed to identify and quantify live AB, EC, PA, and SA in wounds. The proposed fluorescent biosensor had the characteristics of fast speed, high sensitivity, and good accuracy for the qualitative and quantitative analysis of four kinds of infectious bacteria. The LODs of AB, EC, PA, and SA were 27 CFU/mL, 10 CFU/mL,

Table 2
Comparisons between this work and other reported bacterial detection methods.

Analytical methods	Detection target	Linear range	Limit of detection	Sample	Ref.
SPR	EC SA	10^5-10^8 CFU/mL	10^5 CFU/mL 10^6 CFU/mL	—	[11]
SERS	SA	10–10^7 CFU/mL	3 CFU/mL	Orange juice human blood	[12]
Colorimetric	SA	100–10^7 CFU/mL	81 CFU/mL	milk sample	[13]
Fluorescent	SA	80–8 × 10^6 CFU/mL	39 CFU/mL	tap water lake water	[14]
Electrochemical	EC SA	2.5 × 10^3-2.5 × 10^8CFU/mL 4.1 × 10^3-4.1 × 10^8CFU/mL	2.3 × 10^4 CFU/mL 4.0 × 10^3 CFU/mL	tap water	[15]
Pulse voltammetry	EC	100–10 × 10^7 CFU/mL	32 CFU/mL	tap water juices milk	[35]
This work	AB EC PA SA	27–8 × 10^6 CFU/mL 10–25 × 10^6 CFU/mL 34–24 × 10^6 CFU/mL 10–10 × 106 CFU/mL	27 CFU/mL 10 CFU/mL 34 CFU/mL 2 CFU/mL	skin wound in mouse	—

Note: SERS (Surface-enhanced Raman scattering); LSPR (Local Surface Plasmon Resonance).

34 CFU/mL, and 2 CFU/mL respectively, and it was far lower than some reported traditional colorimetric methods. Additionally, in the detection of mixed bacterial with a concentration of 8 × 10^8 CFU/mL, the test results reflected that this method could avoid the influence of interfering bacteria, and showed the high selectivity and sensitivity to the target bacteria. More importantly, as for the detection of the target bacteria in infection wounds from mouse and patients, whether a single strain of bacteria infection or multiple strains of bacteria infection, both showed good recovery rates. Therefore, the constructed fluorescent biosensor in this study might have great application potential in the rapid clinical diagnosis of live bacteria in wound infection.

CRediT authorship contribution statement

Lanmei Gao, Houbing Zheng, Fang Zhang, Zhenyu Lin, Meishui Wang, Zuquan Weng: Concept and design. **Lanmei Gao, Houbing Zheng, Yi Zhong, Linhai Jiang, Yuanzi Wu, Fen Yan, Da Huang, Jianhua Li, Fang Zhang, Zuquan Weng**: Procedures. **Lanmei Gao, Houbing Zheng, Yuanlong Hu, Fang Zhang, Meishui Wang, Zuquan Weng**: Writing of article. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

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Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2023.134318.

References

[1] T. Criscitelli, The future of wound care, AORN J. 107 (2018) 427–429.
[2] M.L. Clausen, T. Agner, Antimicrobial peptides, infections and the skin barrier, Curr. Probl. Dermatol. 49 (2016) 38–46.
[3] C.K. Sen, Human Wound and Its Burden: Updated 2020 Compendium of Estimates, Adv. Wound Care (N. Rochelle) 10 (2021) 281–292.
[4] S.R. Nussbaum, M.J. Carter, C.E. Fife, J. DaVanzo, R. Haught, M. Nusgart, et al., An economic evaluation of the impact, cost, and medicare policy implications of chronic nonhealing wounds, Value Health 21 (2018) 27–32.
[5] A.F. Cardona, S.E. Wilson, Skin and soft-tissue infections: a critical review and the role of telavancin in their treatment, Clin. Infect. Dis. 61 (Suppl 2) (2015) S69–S78.
[6] D. Li, L. Liu, Q. Huang, T. Tong, Y. Zhou, Z. Li, et al., Recent advances on aptamer-based biosensors for detection of pathogenic bacteria, World J. Microbiol Biotechnol. 37 (2021) 45.
[7] K.S. Park, Nucleic acid aptamer-based methods for diagnosis of infections, Biosens. Bioelectron. 102 (2018) 179–188.
[8] C. Acquah, D. Agyei, E.M. Obeng, S. Pan, K.X. Tan, M.K. Danquah, Aptamers: an emerging class of bioaffinity ligands in bioactive peptide applications, Crit. Rev. Food Sci. Nutr. 60 (2020) 1195–1206.
[9] Y. Zhang, B.S. Lai, M. Juhas, Recent Advances in Aptamer Discovery and Applications, Molecules 24 (2019).
[10] K.L. Hong, L.J. Sooter, Single-Stranded DNA Aptamers against Pathogens and Toxins: Identification and Biosensing Applications, Biomed. Res Int 2015 (2015), 419318.
[11] W.W. Wang, X. Han, L.Q. Chu, Polyadenine-mediated Immobilization of Aptamers on a Gold Substrate for the Direct Detection of Bacterial Pathogens, Anal. Sci. 35 (2019) 967–972.
[12] Y. Pang, N. Wan, L. Shi, C. Wang, Z. Sun, R. Xiao, et al., Dual-recognition surface-enhanced Raman scattering(SERS)biosensor for pathogenic bacteria detection by using vancomycin-SERS tags and aptamer-Fe3O4@Au, Anal. Chim. Acta 1077 (2019) 288–296.
[13] T. Yu, H. Xu, Y. Zhao, Y. Han, Y. Zhang, J. Zhang, et al., Aptamer based high throughput colorimetric biosensor for detection of staphylococcus aureus, Sci. Rep. 10 (2020) 9190.
[14] R. Cai, F. Yin, Z. Zhang, Y. Tian, N. Zhou, Functional chimera aptamer and molecular beacon based fluorescent detection of Staphylococcus aureus with strand displacement-target recycling amplification, Anal. Chim. Acta 1075 (2019) 128–136.
[15] X. Zhang, X. Wang, Q. Yang, X. Jiang, Y. Li, J. Zhao, et al., Conductometric sensor for viable Escherichia coli and Staphylococcus aureus based on magnetic analyte separation via aptamer, Mikrochim Acta 187 (2019) 43.
[16] X. Dong, Z. Shi, C. Xu, C. Yang, F. Chen, M. Lei, et al., CdS quantum dots/Au nanoparticles/ZnO nanowire array for self-powered photoelectrochemical detection of Escherichia coli O157:H7, Biosens. Bioelectron. 149 (2020), 111843.
[17] X. Wei, W. Zhou, S.T. Sanjay, J. Zhang, Q. Jin, F. Xu, et al., Multiplexed Instrument-Free Bar-Chart SpinChip Integrated with Nanoparticle-Mediated Magnetic Aptasensors for Visual Quantitative Detection of Multiple Pathogens, Anal. Chem. 90 (2018) 9888–9896.
[18] X. Zhang, G. Xie, D. Gou, P. Luo, Y. Yao, H. Chen, A novel enzyme-free electrochemical biosensor for rapid detection of Pseudomonas aeruginosa based on high catalytic Cu-ZrMOF and conductive Super P, Biosens. Bioelectron. 142 (2019), 111486.

[19] X. Shi, J. Zhang, F. He, A new aptamer/polyadenylated DNA interdigitated gold electrode piezoelectric sensor for rapid detection of Pseudomonas aeruginosa, Biosens. Bioelectron. 132 (2019) 224–229.
[20] Q. Zhang, X. Wang, Y. Kang, et al., Natural products self-assembled nanozyme for cascade detection of glucose and bacterial viability in food[J]. Foods 10 (11) (2021) 2596.
[21] P. Qiu, P. Yuan, Z. Deng, et al., One-pot facile synthesis of enzyme-encapsulated Zn/Co-infinite coordination polymer nanospheres as a biocatalytic cascade platform for colorimetric monitoring of bacteria viability[J], Microchim. Acta 188 (2021) 1–10.
[22] S.J. Kwon, D. Kim, I. Lee, et al., Sensitive multiplex detection of whole bacteria using self-assembled cell binding domain complexes[J], Anal. Chim. Acta 1030 (2018) 156–165.
[23] J. Sun, J. Huang, Y. Li, et al., A simple and rapid colorimetric bacteria detection method based on bacterial inhibition of glucose oxidase-catalyzed reaction[J], Talanta 197 (2019) 304–309.
[24] M. Xu, R. Wang, Y. Li, An electrochemical biosensor for rapid detection of E. coli O157: H7 with highly efficient bi-functional glucose oxidase-polydopamine nanocomposites and Prussian blue modified screen-printed interdigitated electrodes[J], Analyst 141 (18) (2016) 5441–5449.
[25] F. Huang, L. Xue, W. Qi, et al., An ultrasensitive impedance biosensor for Salmonella detection based on rotating high gradient magnetic separation and cascade reaction signal amplification[J], Biosens. Bioelectron. 176 (2021), 112921.
[26] L. Xue, R. Guo, N. Jin, et al., Rapid and automatic Salmonella typhimurium detection integrating continuous-flow magnetic separation and dynamic impedance measurement[J], Food Control 143 (2023), 109316.
[27] S. Rasoulinejad, S.L.M. Gargari, Aptamer-nanobody based ELASA for specific detection of Acinetobacter baumannii isolates, J. Biotechnol. 231 (2016) 46–54.
[28] M. Nosrati, J. Amani, In silico screening of ssDNA aptamer against Escherichia coli O157:H7: A machine learning and the Pseudo K-tuple nucleotide composition based approach, Comput. Biol. Chem. 95 (2021), 107568.
[29] R. Das, A. Dhiman, A. Kapil, V. Bansal, T.K. Sharma, Aptamer-mediated colorimetric and electrochemical detection of Pseudomonas aeruginosa utilizing peroxidase-mimic activity of gold NanoZyme, Anal. Bioanal. Chem. 411 (2019) 1229–1238.
[30] 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.
[31] J. Chen, Y. Lu, F. Yan, Y. Wu, D. Huang, Z. Weng, A fluorescent biosensor based on catalytic activity of platinum nanoparticles for freshness evaluation of aquatic products, Food Chem. 310 (2020), 125922.
[32] D. Cheng, M. Yu, F. Fu, W. Han, G. Li, J. Xie, et al., Dual Recognition Strategy for Specific and Sensitive Detection of Bacteria Using Aptamer-Coated Magnetic Beads and Antibiotic-Capped Gold Nanoclusters, Anal. Chem. 88 (2016) 820–825.
[33] C.T. Campbell, The energetics of supported metal nanoparticles: relationships to sintering rates and catalytic activity, Acc. Chem. Res 46 (2013) 1712–1719.
[34] H. Deng, S. He, X. Lin, L. Yang, Z. Lin, R. Chen, et al., Target-triggered inhibiting oxidase-mimicking activity of platinum nanoparticles for ultrasensitive colorimetric detection of silver ion, Chin. Chem. Lett. 30 (2019) 1659–1662.
[35] S. Bu, K. Wang, Z. Li, C. Wang, Z. Hao, W. Liu, et al., An electrochemical biosensor based on methylene blue-loaded nanocomposites as signal-amplifying tags to detect pathogenic bacteria, Analyst 145 (2020) 4328–4334.

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