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PII: S0003-2670(19)30672-5

DOI: https://doi.org/10.1016/j.aca.2019.05.059

Reference: ACA 236823

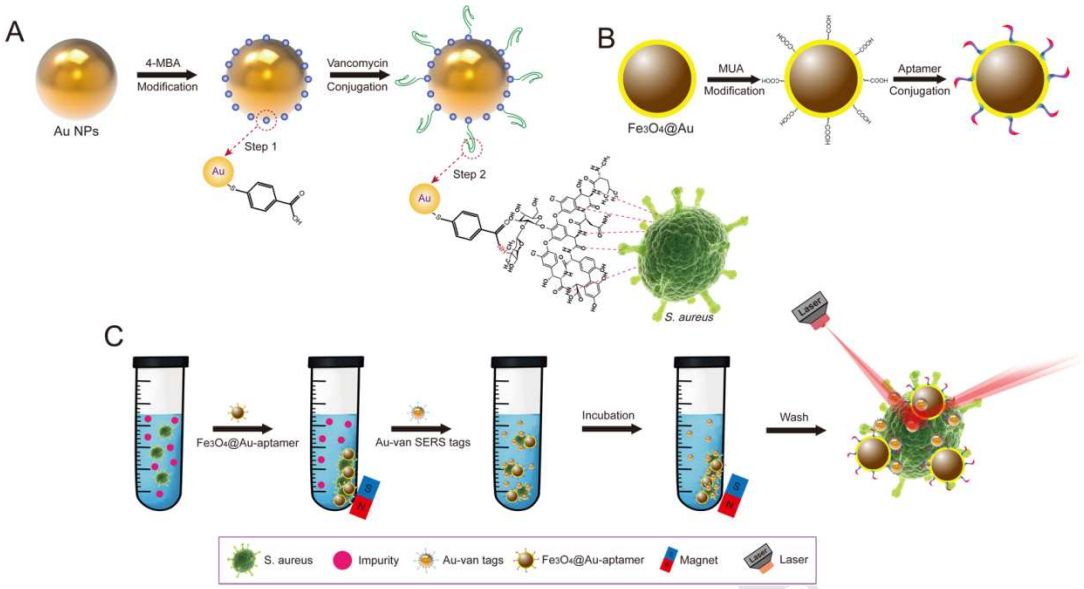
To appear in: *Analytica Chimica Acta*

Received Date: 12 March 2019

Revised Date: 21 May 2019

Accepted Date: 24 May 2019

Please cite this article as: Y. Pang, N. Wan, L. Shi, C. Wang, Z. Sun, R. Xiao, S. Wang, Dual-recognition surface-enhanced Raman scattering(SERS)biosensor for pathogenic bacteria detection by using vancomycin-SERS tags and aptamer-Fe_3O_4@Au, *Analytica Chimica Acta*, https://doi.org/10.1016/j.aca.2019.05.059.



Dual-recognition surface-enhanced Raman scattering（SERS）biosensor for pathogenic bacteria detection by using vancomycin-SERS tags and aptamer-Fe_3O_4@Au

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ABSTRACT

Rapid and reliable detection of pathogenic bacteria is vital to prevent and control bacterial diseases. In this study, we present a magnetically assisted surface-enhanced Raman scattering (SERS) biosensor based on the dual-recognition of bacterial cell by aptamer and antibiotic molecules. Aptamer-Fe_3O_4@Au magnetic nanoparticles (AuMNPs) were synthesized as magnetic and SERS activated substrate for specific bacteria enrichment, vancomycin-SERS tags (Au@MBA) were prepared for the sensitive quantification of pathogenic bacteria. Due to the Au-shell based dual-SERS enhancement and aptamer/vancomycin based dual-recognition ability, a detection limit of 3 cells/mL with a wide dynamic linear range from 10 to 10^7 cells/mL can be achieved within 50 min without other non-target bacteria interference. When applied in real samples, the approach shows recoveries from 95.0% to 106.4% with relative standard derivation (RSD) less than 5.3%. The SERS strategy could be used to detect a broad range of bacteria by using different aptamers, moreover, the simple operation and precise quantification ability empower this assay great potential in the application of food safety and infectious disease point-of-care diagnosis.

Keywords: Surface-enhanced Raman scattering, Bacteria detection, Dual-recognition, Vancomycin-SERS tag, Aptamer

1. Introduction

Infectious diseases, especially caused by pathogenic bacterial infections, have posed a great threat to public health worldwide [1]. Once delay in the treatment after the onset of symptoms, rapid eterioration of patients' health will be caused. Hence, real-time monitoring of pathogenic bacteria is essential for disease control and public health. Traditional methods for detecting pathogens include the standard plate colony counting, polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA) [2]. Colony counting is the standard method for detection of bacterial pathogens but labor-intensive and time-consuming with 24-72 h requirement. ELISA and PCR can perform high sensitivity and reduce the assay time to several hours, however, both of them requires multiple pretreatment steps, precision instruments, professional operation, therefore, their widespread applications in point-of-care (POC)diagnosis are limited [3,4]. To overcome such deficiencies, the development of POC biosensors with much shorter analysis time, higher sensitivity and specificity is very important.

Surface-enhanced Raman spectroscopy (SERS) has been used in various pathogenic bacteria detection because of its high sensitivity, specificity and high speed. Compared with fluorescence and electrochemistry based methods, SERS can provide steady and sharp fingerprint spectra of the bacteria or label molecule in complex sample environment with fewer background interference [5, 6]. The typical SERS detection for bacteria was usually composed by two strategies: label based and label-free based method [7, 8], both of them have their relative merits [9]. Here, our research will focus on the label based method. The typical label method combined the SERS tags labelling with a magnetic separation, where the magnetic nanoparticle was applied to capture and isolate bacteria from a complex matrix volume, giving rise to an increased SERS signal [10, 11]. For example, Zhang has reported aptamer-magnetic gold nanoparticles and aptamer-Au-Raman reporter as the capture and label probes respectively for pathogenic bacteria detection [12], Wang has respectively reported aptamer and antibody modified Au-coated magnetic nanoparticles and SERS tags for bacteria detection [13, 14]. Drake has reported a single domain antibody coated Au nanoparticles (NPs) and iron-oxide NPs for Staphylococcus aureus detection [15]. Although these methods performed well with high sensitivity and speed, both the capture and labelling steps depended on antibody or aptamer recognition. Nevertheless, antibodies are produced in immunized animals, which are expensive and subject to poor reproducibility [16]. The screening process of aptamers with high combining capacity is very difficult and the binding time of the aptamer with target bacteria was nearly 1 hour [17]. Moreover, as a pair of antibody or aptamer is required for one target bacterium, the multiple binding steps have to be optimized; the competition between of the same pairs on the limit binding site of the bacteria is also inevitable.

Theses years, antibiotics with small size, low price, and broad availability have been used as recognition molecules for bacteria detection as they can interact with bacteria via hydrogen bonds between the carbonyl and amine groups and the peptidoglycan of the cell wall[18]. For the SERS strategy, the antibiotic of

vancomycin (Van) was usually modified on Au, Ag nanoparticles or on magnetic nanoparticle (MNP) substrate for bacteria capturing in complex environments. For example, Zhang has reported Van-modified MNP nanoparticles for clinical bacteria capture and detection [19], Wang has applied the Van-modified Ag-coated magnetic nanoparticles for rapid SERS detection of bacteria [20]. Liu has designed a Van-coated Ag SERS substrates to differentiate different bacterial in human blood [21].Wu has reported a Van -functionalized Ag nanorod array as the SERS substrate to detect six foodborne pathogens in mung bean sprout samples[22]. However, one limit for Van-based methods was that the vancomycin is not specific for one kind bacteria but positive to conjunct with all Gram-positive bacteria. In our previous research, we have reported an aptamer/vancomycin system for SERS bacteria detection by combining the specificity of aptamer and the low price of vancomycin [19]. However, there were still some drawbacks. For example, due to the non-specificity of vancomycin-MNP, both target and non-target bacteria can be captured on the surface of the MNP. After SERS tags were added to label the target bacteria, Raman laser was used to stimulate the Raman signal. However, some SERS tags can be blocked by the non-target bacteria due to the incommensurable size difference (bacteria ~1000 nm, SERS tags ~50 nm), which can decrease the SERS intensity. Therefore, the detection limit (20-50 cell/mL) was not so good compared with the aptamer or antibody pairs SERS biosensors mentioned above (~10 cell/mL). Here, in order to improve the sensitivity, we designed a dual-recognition SERS biosensor with specific capture and universal labelling process. Vancomycin molecule was combined with the Au@MBA through chemical bonding to form Au@MBA@vancomycin as universal van-SERS tag.

There were two steps for our strategy. Firstly, aptamer-Fe_3O_4@Au MNP were applied for aptamer-specific bacteria capture; secondly, after washing away the non-target bacteria, the SERS tags were incubated with the Fe_3O_4@Au/target bacteria for labelling and detection step. There are three main characters for our system. First, the Fe_3O_4@Au capture substrate and Au NP SERS tags substrate can provide dual-SERS enhancement of the Ramen molecule (MBA) to induce intense Raman signals; Second, vancomycin with low price and aptamer with specificity were conjunct to improve the application in point-of-care setting. Third, the Van-SERS tags can be used as universal tag for common bacteria labelling which were selected captured by aptamer-Fe_3O_4@Au within 15 min. By using *staphylococcus aureus* (*S. aureus*) as an example, a detect limit of 3 cells/mL with a liner range from 10 to 10^7 cells/mL can be achieved. Moreover, the combination of aptamer and antibiotic can overcome the individual limitations of antibodies' cross-reactivity and aptamers' slow binding kinetics. The total detection process can be completed within 50 min, which effectively shortens the time needed for traditional bacterial identification. To our best knowledge, no published procedure for preparation and application of Van-SERS tag has been reported before. This method further exhibited high specificity and selectivity for real samples and thus is a promising tool for food safety and infectious disease point-of-care diagnosis.

2. Experimental

2.1 Materials and apparatus

Ferric chloride ($FeCl_3 \cdot 6H_2O$), chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$), Vancomycin(Van), trisodium citrate solution, polyethyleneimine (PEI), 4-mercaptobenzoic acid (4-MBA),ethanolamine,11-mercapto-undecanoic acid (MUA), 2-(N-morpholino) ethanesulfonic acid (MES) and N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) were obtained from Sigma-Aldrich (USA). The *S. aureus* aptamer (SA) was synthesized by Sangon Biotech (Shanghai) and sequence was shown in Table S1. *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Staphylococcus epidermidis* (*S. epidermidis*), *Listeria monocytogenes* (*L. monocytogenes*) and *Salmonella typhimurium* (*S.typhimurium*) were provided by the Institute for Disease Control and Prevention, Academy of Military Medical Sciences. UV-vis spectra were detected on a Shimadzu 2600 spectrometer. The size distribution of all nanoparticles was measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (NANO-ZS 90, Malvern, UK). The surface morphology was detected by scanning electron microscopy (FE-SEM) (JEOL JSM-7001F, Japan). Energy-dispersive X-ray (EDX) spectra and high-resolution transmission electron microscopy (HRTEM) images were detected on a JEOL JEM-2010F microscope (200 kV). X-ray photoelectron spectroscopy (XPS) was performed by a Thermo ESCALAB 250XI XPS spectrometer using the monochromatic Al Ka line at 1486.6 eV. The ζ-potential measurements were completed on a Zetasizer Nano ZS ZEN3600 system (Malvern Instruments, United Kingdom). The magnetic properties of the prepared MNPs were studied via a superconducting quantum interference device magnetometer (SQUID, MPMSXL-7) at 300 K. All Raman spectra were measured by Raman system B&W Tek, i-Raman Plus BWS465-785H spectrometer. Each sample was excited by a 785 nm laser with a power of 25 mW and a total acquisition time of 20 s.

2.2 Synthesis of vancomycin-SERS tags and aptamer-Fe_3O_4@Au MNP

The synthetic process of vancomycin-SERS tags (Van-SERS tag) is illustrated in Scheme 1A. Firstly, Au@MBA was prepared according to our previous report [19]. Briefly, Au NPs were synthesized by hydrothermal method using 1 mL $HAuCl_4$ (1% ,w/v) and 1 mL of trisodium citrate solution (1%) at 100 ^oC for 15 min. Secondly, the yielded Au NPs were incubated with 10 mL of MBA (1 mM) at room temperature for 4 h to form Au@MBA. Finally, Au@MBA was reacted with 20 mL of EDC (5 mg mL^{-1}) for 20 min to activate the COOH of the MBA, then, 5 mg of vancomycin was added to link the MBA through COOH-NH_2 condensation. After sonicated for 2 h at room temperature, 100 mL of ethanolamine was added to deactivate the unreacted carboxyl groups. The final Au@MBA@vancomycin SERS tags were purified through two rounds of centrifugation (5800 rpm, 7 min) and then resuspended in 1 mL of PB buffer (10 mM, pH 7.4) at 4 ^oC until use.

The synthetic process of aptamer-Fe_3O_4@Au MNPs is illustrated in Scheme 1B. Firstly, Fe_3O_4@Au MNPs were synthesized by a PEI-mediated seed growth method as reported in our previous research [23]. Secondly, the Fe_3O_4@Au MNPs (10 mg) were sonicated in a MUA ethanol solution (20 mM) for 2 h to get surface

carboxylation. Then, COOH-Fe_3O_4@Au MNPs were dispersed in 10 mL of MES buffer (10 mM, pH 6.0) with 10 mg of EDC to activate the carboxyl. Finally, 20 mL of the NH_2-aptamer SA (10 μM) was added to link the active Fe_3O_4@Au through of amino-carboxyl condensation. After shaken vigorously for 2 h, unreacted COOH groups on the MNPs were blocked with 5% ethanolamine for 1 h. Then, the final products were magnetic separated, washed three times with deionized water and stored in PBS buffer.

2.3 Bacterial sample preparation and capture efficiency experiment

The strains of S. aureus was cultured with Luria−Bertani (LB) broth at 37 °C overnight and determined by the plate dilution method. Briefly, 0.1 mL of the bacterial culture was diluted 1×10^5 times with medium and coated on agar plates at 37 ^oC overnight. After that the colony forming units (CFUs) was measured. Followed by centrifugation at 4500g for 10 min at 4 ^oC, the remaining bacteria were washed twice with PBS and the final bacterial cell numbers were diluted to 1×10^7 cfu /mL with PBS. The capture efficiency experiment was performed as follow: Fe_3O_4@Au-aptamer (20 mg/mL) were incubated with *S. aureus* (10^4 cells/mL) spiked in health human blood samples for 30 min. After magnetically separation of the Fe_3O_4@Au-aptamer/S. aureus complex, the residual supernatant was cultivated in blood agar at 37 ^oC overnight to count the remaining bacteria. The capture efficiency could be calculated by the subtraction of the original amount and remained amount in the supernatant. The same process was carried out by using Fe_3O_4@Au MNPs as a control.

2.4 Detection of *S. aureus* by using the dual-recognition SERS strategy

Specifically, 500 μL of various concentrations (0-10^7 cell/mL, respectively) of *S. aureus* were incubated with 4 μL of aptamer-Fe_3O_4@Au MNPs (20 mg/mL) for 30 min at room temperature with shaking. Then, the Fe_3O_4@Au/bacterial complexes were quickly separated using an external magnet and then washed twice with PBS to remove non-captured bacteria. Then, the precipitate was incubated with 50 μL of vancomycin-SERS tags (50 mg/mL) and 150 μL of binding buffer (10 mM PBS, 1 mM Mg^{2+}, pH 7.4) for 15 min. Finally, the Fe_3O_4@Au/bacteria/SERS tag complexes were washed three times with PBS and detected by Raman signals. For validation of the result, each sample was measured three times independently to calculate the average and standard deviation. The Raman intensity at 1074 cm^{-1}(signal peak for MBA) was used for sensitivity calculation. The selectivity was performed by detection of *S. aureus* (10^4 cells/mL) and other four interfering bacteria (10^7 cells/mL of each kind) in the same experiment conditions as previous description.

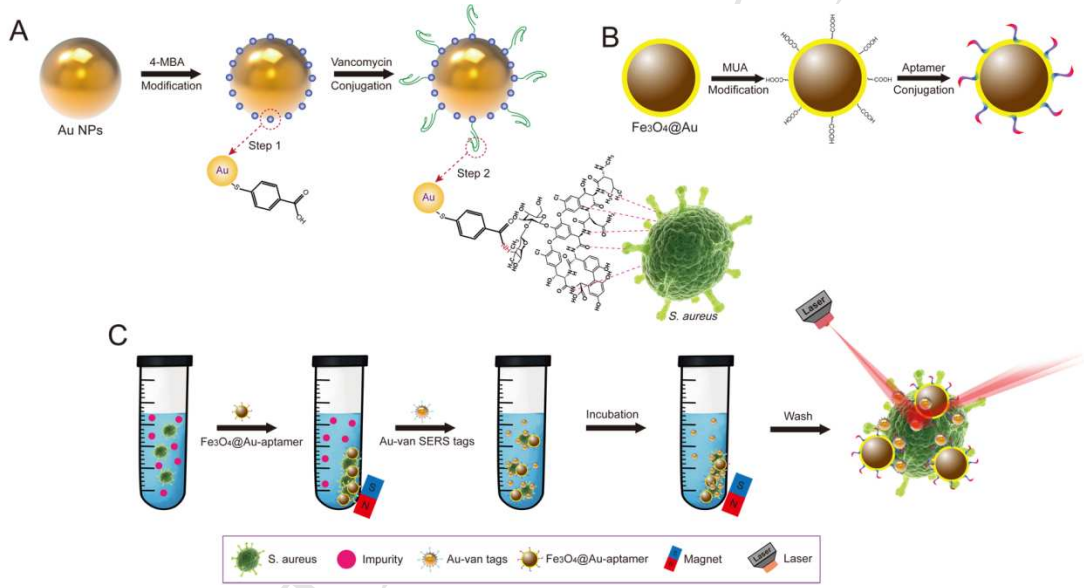
2.5 Assay of *S. aureus* in real samples

The real samples of qualified fresh milk, orange juice and human blood were obtained from a local supermarket and healthy volunteers respectively. Target bacteria with known concentration were added at into the real samples. Then, the SERS biosensor was applied to evaluate the feasibility according to the general procedure. The accuracy of real samples detection was estimated by calculating the recoveries.

3. Results and discussion

3.1 Synthesis and operating process of the dual-recognition SERS biosensor

Scheme 1A and B illustrated the synthesis process of Van-SERS tags and aptamer-Fe_3O_4@Au MNPs respectively. Scheme 1C showed the operating procedure for *S. aureus* detection by this strategy. When a sample containing one or more species of bacteria was mixed with aptamer-Fe_3O_4@Au MNPs, only the target bacteria can be captured via aptamer recognition. After magnetic separation, the captured bacteria can be labeled by Van-SERS tags. Finally, the Fe_3O_4@Au/bacteria/SERS tag magnetic sandwich complexes were separated by a magnet and the Raman signals for each sample were measured and analyzed. Due to the dual-SERS enhancement of the Fe_3O_4@Au and the Au NP of SERS tag, plus the magnetic concentration induced high density of hot spots, the Raman intensity can be enhanced largely which was responsible for higher sensitivity.



Scheme 1. Schematic illustration of (a) the synthesis of Au-Van SERS tags, (b) the synthesis of aptamer-modified Fe_3O_4@Au MNPs, and (c) the operating procedure for *S. aureus* detection via the dual-recognition SERS biosensor.

3.2 Characterization of the Van-SERS tags and Fe_3O_4@Au MNPs

The character of the Van-SERS tags (Au@MBA@vancomycin) was expressed in Fig.1. As shown in the TEM image (Fig.1A), the Van-SERS tags are monodisperse with estimated diameter of 52 ± 3 nm. Dynamic light scattering (DLS) and UV-vis spectra were also detected to confirm the size distribution of Au NPs and Van-SERS tags. As shown in Fig. 1B, the average diameter of Au NPs increased from 46 to 52 nm after vancomycin conjugations according to the DLS data. UV-vis spectra (Fig. 1C) also demonstrated that the absorption peak of Au NPS slightly shifted from 530 to 532 nm upon vancomycin conjugation, which indicated that the modification of vancomycin made no effect on the surface plasmon band of SERS tags [24].

The vancomycin molecules binding on the surface of SERS tag can be confirmed by the XPS spectrum where the presence of N 1s (398.4 eV) was derived from vancomycin (Fig. 1D). To confirm the SERS ability, the SERS spectra of the Au NPs,

Au@MBA and Au@MBA@vancomycin were detected respectively. As shown in Fig.1E, both Au@MBA and Au@MBA@vancomycin can induced strong SERS intensity of the Raman molecule MBA. Here, SERS intensity at 1074 cm^{-1} was chosen for the quantitative calculation. Finally, Van-SERS tags and S. aureus were incubated for 15 min, after centrifugation and washing, the TEM morphology of the precipitate was shown in Fig.1F. It can be seen that the Van-SERS tags effectively combined on the surface of S. aureus. To further confirm the stability of the Van-SERS tags, the SERS signal of the Van-SERS tags with time was detected in the PBS buffer (PH 7.4,10 mM). Fig. S1 showed the SERS intensity change at 1074 cm^{-1} of the Van-SERS tags after storage at 4 °C for 90 days. It can be seen that the assemblies can provide steady SERS signal with time lapses. Therefore, this SERS biosensor was stable enough for POC diagnostic application.

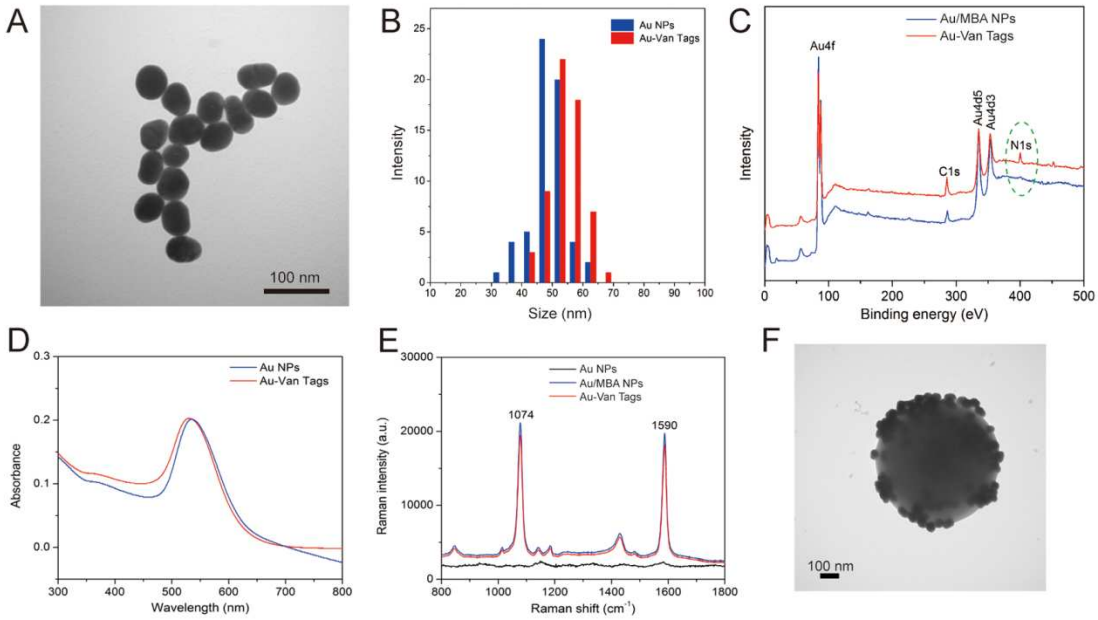
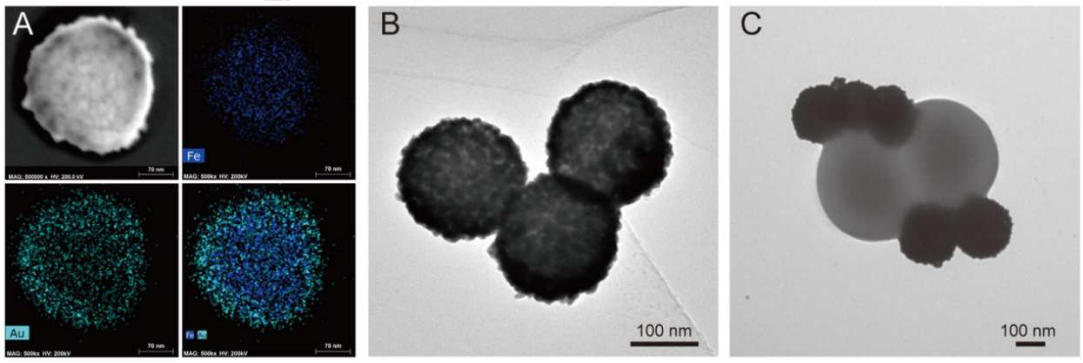


Figure 1. (A) TEM image of Van-SERS tags, (B) DLS distributions and (C) XPS spectra of Au NPs and Au-Van SERS tags. (D) UV-vis spectra and (E) SERS intensity of Au NPs and Au-Van SERS tags. (F) TEM image of *S. aureus* after incubation with Au-Van SERS tags.

The morphology character of Fe_3O_4@Au MNPs was shown in Fig.S2. It can be seen that uniform and monodisperse Fe_3O_4 and Fe_3O_4@Au magnetic nanoparticles can be synthesized with diameter of approximate average 150±4 and 200±6 nm, respectively, thence; the thickness of the Au shell is approximately 25±5 nm. The bright field TEM image of an individual Fe_3O_4@Au MNP and the associated EDX elemental mappings was shown in Fig.2A. It indicates that Fe atoms (blue) are mainly located in the core area and Au atoms (green) are homogeneously distributed around the Fe core to form the core-shell structure. Fig.2B showed the HRTEM image of Fe_3O_4@Au-aptamer MNPs with monodispersity. To confirm conjunction of the S. aureus aptamers with Fe_3O_4@Au, the negative shift zeta potential variation of the

Fe_3O_4@Au MNPs before and after incubated with S. aureus aptamer was detected. As shown in Fig.S3, obvious negative shift ζ-potential value of the aptamer-Fe_3O_4@Au(Fig.S3B) can be found compared with the Fe_3O_4@Au(Fig.S3A), which demonstrated the successful preparation of aptamer-Fe_3O_4@Au. The saturation magnetization (MS) values of the Fe_3O_4, Fe_3O_4@Au and Fe_3O_4@Au-aptamer MNPs are 81.3, 45.5 and 41.4 emu/g, respectively (Fig.S4). The MS decreases about 8.8% after aptamer coating, therefore, the Fe_3O_4@Au-aptamer MNPs still have strong magnetic responsiveness for the rapid separation of bacteria from solution [25]. To investigate the stability of the Fe_3O_4@Au nanoparticles, we detected the hydrodynamic sizes of the Fe_3O_4@Au after storage at 4 ^oC for 90 days. As shown in Fig.S5, the hydrodynamic sizes are steady with time lapses. Therefore, this SERS biosensor was stable enough for clinical diagnostic application.

The binding ability of aptamer-Fe_3O_4@Au nanoparticles was displayed via TEM measurement [26]. After incubation of aptamer-Fe_3O_4@Au and S. aureus in the binding buffer, the product was washed 3 times and separated by magnet for TEM detected. As shown in Fig.2C, several Fe_3O_4@Au nanoparticles were tightly conjugated around the *S.aureus* cells, suggesting that the aptamer-Fe_3O_4@Au has strong binding affinity to capture S. aureus from the solution. The bacterial capture efficiency of aptamer-Fe_3O_4@Au in blood sample was further investigated by using pure Fe_3O_4@Au as a control. Fe_3O_4@Au and Fe_3O_4@Au-aptamer were incubated with S. aureus (10^4 cells/mL) in health human blood samples for 30 min respectively. After magnetically separation, the supernatant from each group was cultivated in blood agar at 37 ^oC overnight to count the remaining bacteria. The capture efficiency could be calculated by the subtraction of the original amount and remained amount in the supernatant. As shown in Fig.2D, photographs of the agar plates demonstrated that over 80% of S. aureus in culture medium were captured by aptamer-Fe_3O_4@Au while few bacteria were absorbed by pure Fe_3O_4@Au. This result was consistent with the previous report [27] and demonstrated that our aptamer-Fe_3O_4@Au can recognize and bind to target bacteria with high specificity and strong affinity.



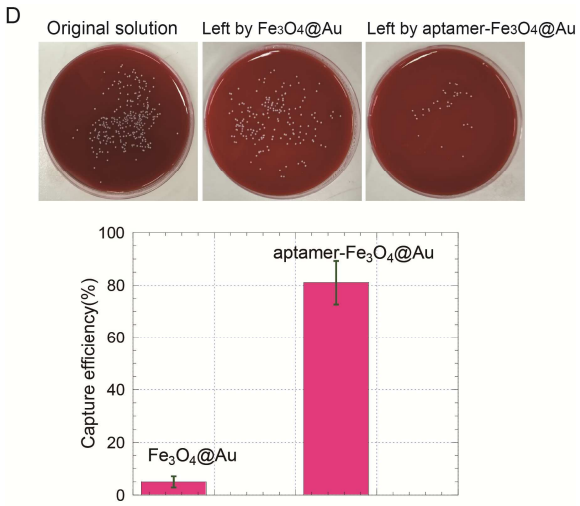


Figure 2. (A) Elemental mapping images of Fe_3O_4@Au MNPs, (B) HRTEM image of Fe_3O_4@Au-aptamer MNPs, and (C) TEM image of *S. aureus* captured by Fe_3O_4@Au-aptamer MNPs. (D) Capture efficiency of pure Fe_3O_4@Au and aptamer-Fe_3O_4@Au.

3.3 Optimization of incubation time and aptamer-Fe_3O_4@Au dosage

To form the Fe_3O_4@Au/*S. aureus*/Van-SERS tags sandwich complexes and obtain the best SERS intensity, the incubation times of the Van-SERS tags with *S. aureus*(labelling step) and the Fe_3O_4@Au-aptamer dosage for S. aureus capture(capture step) were two important factors. To obtain the optimized dosage of aptamer-Fe_3O_4@Au for bacterial capture, different amounts of aptamer-Fe_3O_4@Au MNPs (20 mg/mL)were used to capture the same *S. aureus* samples (500 μL, 10^4 /mL).Here, the dosage of SERS tags was excessed for effective labelling with 50 mg /mL, 50 μL). It can be seen in Fig.3B, the SERS intensity increased with the amount of aptamer-Fe_3O_4@Au increasing and come to a peak when the dosage was 4 μL, then, the SERS intensity decreased with more aptamer-Fe_3O_4@Au added. That was because the excessive aptamer-Fe_3O_4@Au in the solution can block the followed Van-SERS tag labeling and Raman laser stimulation, which resulted in the SERS signal decreasing. Under the optimized aptamer-Fe_3O_4@Au dosage (4 μL), the incubation times with Van-SERS tags was studied. The SERS intensity turn to maximum when the incubation time was 15 min, which indicated that the absorption of Van-SERS tags on the surface of *S. aureus* has been saturated. Therefore, the labelling process can be finished within 15 min. The TEM images of the formed Fe_3O_4@Au/*S. aureus*/Van-SERS tags sandwich complexes with different incubation times was also shown in Fig.3A. The increasing amount of Van-SERS tags on the surface of S. aureus with time can be seen clearly.

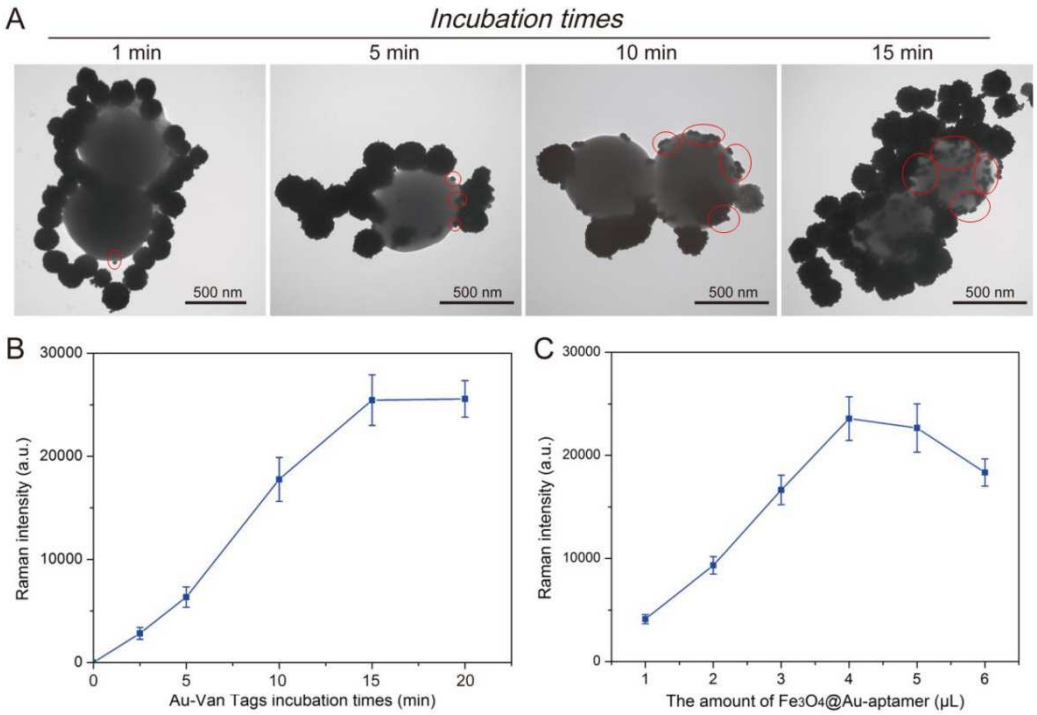


Figure 3. (A) TEM images of the formed Fe_3O_4@Au/*S. aureus*/Van-SERS tags sandwich complexes with different SERS tags incubation times. The SERS tags were marked by red circles. (B) SERS intensity at 1074 cm^{-1} versus the amount of Fe3O4@Au-aptamer (20 mg/mL) using for *S. aureus*. (B) And versus the time of Au-Van tags incubation(C). Error bars are the standard deviation of three repetitive experiments.

3.4 SERS detection of bacteria

Firstly, to study the influence of pH, the SERS signals of our Fe_3O_4@Au-aptamer MNPs and Van-SERS tags system for bacteria detection (10^4 cells/mL) were detected in the same conditions but with different pH values from 3 to 10. As shown in Fig. S6, no obvious effects of pH on the SERS signals were observed. The result indicates that our SERS system can be used as efficient tools for bacterial detection in a wide pH range.

To confirm the reproducibility of our method, we assessed five SERS biosensors for detection of bacteria (10^4 cells/mL) in the same manner. For each biosensor, SERS signals were measured 3 times to get the standard deviation. As shown in Fig.S7, the distribution of the Raman intensity (at 1074 cm^{-1}) revealed a low RSD of 4.03 %. This result indicates that SERS intensity variation of five sensors is very low. Based on this experimental data, the SERS sensors are suitable for quantitative detection of bacteria with consistent reproducibility. I have added the content in the manuscript.

To conform the SERS ability of Fe_3O_4@Au, the comparison of the two systems: Fe_3O_4 (20 mg/mL) and SERS tag (50 mg/mL);Fe_3O_4@Au (20 mg/mL) and SERS tag (50 mg/mL), was performed for the *S.aureus*(10^4 cells/mL)detection in the same other

conditions. As shown in Fig.S8, The SERS intensity of Fe_3O_4@Au system increased 2-fold compared with Fe_3O_4 system for the same concentration of *S. aureus*.

To verify the sensitivity, the S. aureus was spiked in a PBS solution from 10 to 10^7 cells/mL for the SERS intensity detection. Fig. 4A shows the SERS spectra for different concentrations of *S. aureus*. The corresponding calibration curves are displayed in Fig. 4B. It can be seen that the LOD for S. aureus was 3 cells/mL with a linearity range of 10-10^7 cells/mL (R^2 = 0.968). The detection limit is based on the calculation formula LOD=3N/S (N is the standard deviation of blank sample signal, S is the slope of standard curve). All the experiments were performed three times and the error bar was the standard deviations from five SERS scans. The LOD was lower than our previous method (20-50 cells/mL) and comparable with other pair aptamer or antibody modified MNP and SERS tag strategy (~10 cells/mL), but with less time consuming (within 50 min) compared with those systems (at least 120 min) [12-14]. In addition, a brief comparison of recent SERS-based strategies for detection of *S. aureus* is performed (Table S2). Compared with others; our SERS strategy for *S. aureus* is preferable due to its high specificity and sensitivity, short time and cost-effective consumption in POC settings. Fig. S9 shows the SERS spectra of different concentrations of *S. aureus*, obtained using the Fe_3O_4@Au MNPs as SERS substartes. The intensity of the SERS signals of the bacteria obviously increased with the bacterial concentration (1×10^5 to 1×10^8 cells/mL). The Raman peaks of *S.aureus* barely observable when the concentration of the bacteria solution at 10^5 cells/mL. In current study, we reported highly sensitive SERS platform by combining aptamer-modified Fe_3O_4@Au MNPs and vancomycin-modified SERS tags for the detection of *S. aureus*. This platform is a typical label-based SERS method, and the SERS spectra for *S. aureus* was dominated by the characteristic Raman peaks at 1074 cm^{-1} attributable to MBA. The LOD of the proposed platform for *S. aureus* was as low as 3 cells/mL. Moreover, the Raman reporters modified SERS tags have very strong and characteristic Raman signal, and will conceal the intrinsic Raman spectra of bacterial cell. Therefore, we could not see any Raman spectra correspond to bacterial cell upon increasing the concentration in this study.

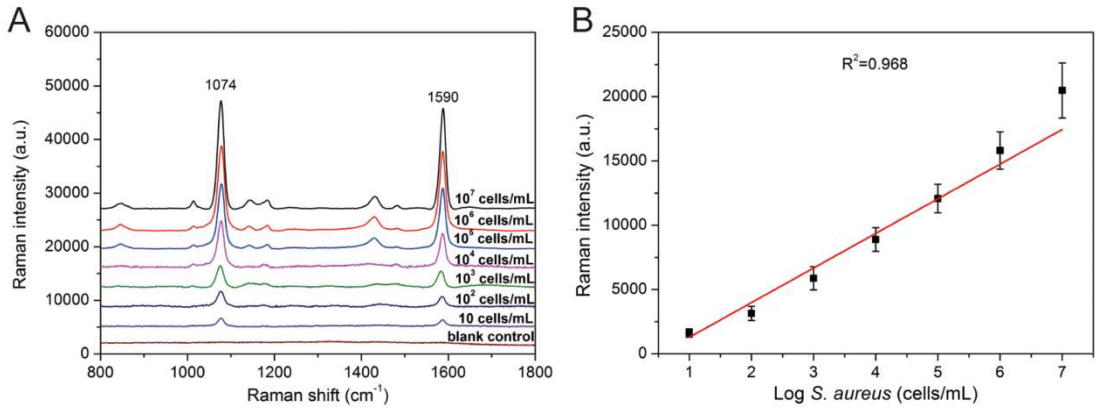


Figure 4. (A) SERS spectra taken from the Fe_3O_4@Au/*S. aureus*/Au-Van tags sandwich complexes with various concentrations of *S. aureus* (10^7-10 cells/mL) and blank control (0 cell/mL). (B) The liner relationship of the Raman intensity (at 1074 cm^{-1}) vs the logarithm value of the *S. aureus*. The error bars represent the standard deviations from five measurements.

3.5 Specificity and S. aureus detection in complex matrixes.

The selectivity of the SERS biosensor for S. aureus (10^4 cells/mL) was tested by comparing the SERS intensity at 1074 cm^{-1} with other interfering bacteria (10^7 cells/mL) in the same other conditions. Here, 4 most common clinic pathogens of *E. coli*, *S. epidermidis*, *L. monocytogenes* and *S.typhimurium* were selected as the unspecific bacteria. Experimental result was shown in Fig. 5A. It can be seen that the signal intensities of the other bacteria are almost the same with the blank control (0 cell/mL) while the *S. aureus* showed strong SERS signal because of the high affinity between the aptamer and its target. This result indicated that our SERS method possesses good specificity for the target bacteria detection in multiple pathogens environment.

In order to identify the practical applicability of SERS dual-recognition strategy for *S. aureus* detection in real samples, qualified milk, orange juice from a local supermarket and blood samples from healthy volunteers spiked with *S.aureus*(10^4 cells/mL) were compared with that in the PBS buffer. As shown in Fig.5B, the SERS intensity of the same *S.aureus* changed little (RSD of 3.1%) when detected in different matrixes. The recoveries of the *S. aureus* with known spiked amounts in different matrixes were also detected and the result was summarized in Table 1. The recoveries were between 95.0% and 106.4% with a relative standard derivation (RSD) of 5.3%, indicating that the SERS assay could be applied for effective quantification of S. aureus in real samples.

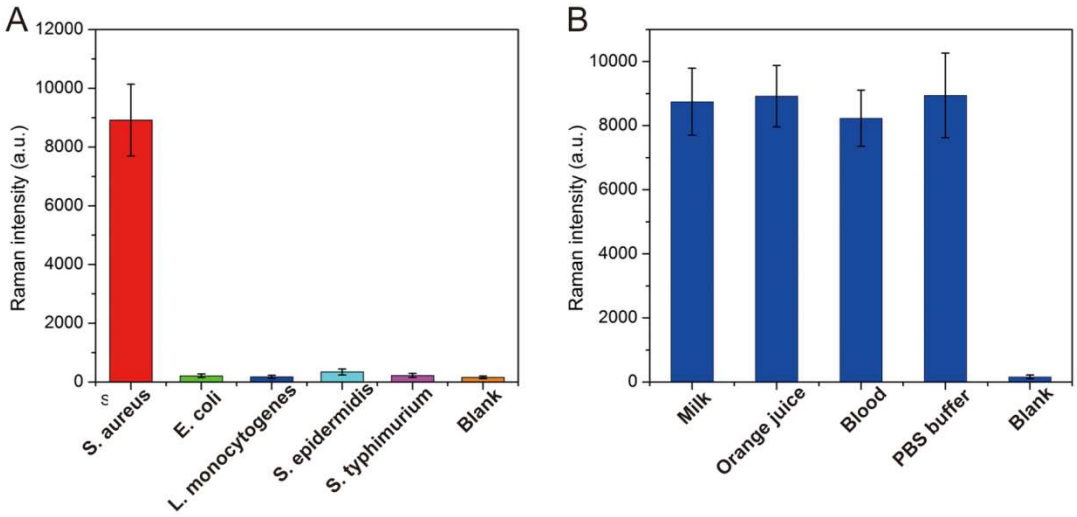


Figure 5. (A) Specificity of the dual-recognition SERS biosensor. The concentrations of the five interfering bacteria are all at 10^7 cells/mL, and the concentration of *S. aureas* group is at 10^4 cells/mL. (B) Detection results of complex solution samples spiked with *S. aureas* (10^4 cells/mL). Error bars are standard deviation of three repetitive experiments.

samples	Spiked(cell/mL)	measured(cell/mL)	Recovery(%)	RSD(%,N=3)
milk	10	9.6±0.3	96.0	3.3
	100	97.1±4.2	97.1	2.7
	500	513.6±12.1	102.6	5.1
Orange juice	20	21.1±1.5	105.5	4.2

	200	196.8±5.5	98.4	2.5
	1000	969.4±79.3	96.9	3.6
blood	10	9.5±0.3	95.0	5.3
	50	53.2±2.4	106.4	4.4
	200	197.5±5.1	98.7	2.9

Table 1. Recovery efficiency of *S. aureus* detected in fresh milk, orange juice and human blood samples based on the SERS method.

Conclusion

In this paper we demonstrated that the dual-recognition SERS biosensor can serve as a POC platform for pathogenic bacteria selective identification and sensitive quantification. The aptamer-Fe_3O_4@Au MNPs provided a magnetic SERS substrate for target bacteria capture via aptamers recognition, the Van-SERS tags was applied to label the bacteria which was selected captured. With the dual-SERS enhancement and dual-recognition, a LOD of 3 cells/mL for *S. aureus* with high specificity can be achieved within 50 min, which was much shorter than the common methods. Moreover, the SERS strategy can also be applied to other bacterial targets with the transformation of the corresponding aptamers. Therefore, the dual-recognition SERS biosensor provides an effective avenue for simple, fast, sensitive and selective assays of a variety of pathogenic bacteria not only in the laboratory but also in a POC setting.

Acknowledgements

This work was supported by the financial support through the National Natural Science Foundation of China (81702106) and the Infectious Diseases Major Project (2018ZX10712001-010).

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Dual-recognition surface-enhanced Raman scattering（SERS）biosensor for pathogenic bacteria detection by using vancomycin-SERS tags and aptamer-Fe_3O_4@Au

Highlight:

1. Dual-recognition SERS biosensor for pathogenic bacteria detection has been designed.
2. Vancomycin-Au@MBA was designed as SERS tag and aptamer-Fe_3O_4@Au was designed as specific magnetic concentration and dual-SERS substrate.
3. Vancomycin with low price and aptamer with specific and relative low cost were conjunct to improve the selectivity and efficiency for application in point-of-care setting.
4. Due to the dual-SERS enhancement and dual-recognition, a detection limit of 3 cells/mL with a wide dynamic linear range from 10 to 10^7 cells/mL can be achieved within 50 min
5. The approach shows recoveries from 95.0% to 106.4% with relative standard derivation (RSD) less than 5.3% when applying in real samples.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: