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A universal SERS-label immunoassay for pathogen bacteria detection based on Fe_3O_4@Au-aptamer separation and antibody-protein A orientation recognition

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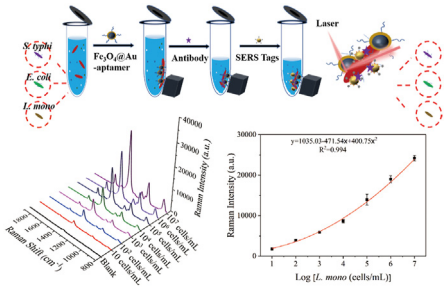
HIGHLIGHTS

- A SERS biosensor for sensitive and universal detection of bacteria was proposed.
- Fe_3O_4@Au-aptamer was used to specifically capture bacteria.
- PA modified-SERS tags and free antibody labeling were combined to achieve orientation binding.
- The LODs for three common pathogens were as low as 10–25 cells/mL.

GRAPHICAL ABSTRACT



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ABSTRACT

Rapid, reliable and sensitive detection methods for pathogenic bacteria are strongly demanded. Herein, we proposed a magnetically assisted surface enhanced Raman scattering (SERS)-label immunoassay for the sensitive detection of bacteria by using a universal approach based on free antibody labelling and staphylococcus proteins A (PA)-SERS tags orientation recognition. The SERS biosensor consists of two functional nanomaterials: aptamer-conjugated Fe_3O_4@Au magnetic nanoparticles (MNPs) as magnetic SERS platform for pathogen enrichment and PA modified-SERS tags (Au@DTNB@PA) as a universal probe for target bacteria quantitative detection. After target bacteria enriched, free antibody was used to specific marking target bacteria and provided numerous Fc fragment, which can guide the PA-SERS tags orientation-dependent binding. With this strategy, Fe_3O_4@Au/bacteria/SERS tags sandwich immunocomplexes for most bacteria (expect several species of *Staphylococcus*) were easy constructed. The limits of detection (LODs) of the proposed assay were found to be 10, 10, and 25 cells/mL for three common pathogens *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. mono*), and *Salmonella typhimurium* (*S. typhi*), respectively, in real food samples. The universal method also exhibits the advantages of rapid,

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robust, and easy to operate, suggesting its great potential for food safety monitoring and infectious diseases diagnosis.

1. Introduction

Bacterial infections or contaminations are always one of the most significant threats to public health and food safety [1]. For example, common foodborne pathogens, such as *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. mono*), *Shigella flexneri* (*S. flexneri*), and *Salmonella typhimurium (S. typhi)*, are blamed for more than 600 million foodborne and waterborne illnesses and 900,000 deaths each year [2–4]. The main methods for bacteria identification are plate culture, polymerase chain reaction (PCR), DNA sequencing, and mass spectrometry [5–7]. However, these methods require long detection times (2–16 h), large laboratory instruments, and skilled personnel and are inapplicable to point-of-care testing. Some biosensors based on specific immunoreactions, such as lateral flow immunoassay, chemiluminescence assay, immunofluorescence assay, and enzyme-linked immunosorbent assay, have been widely developed for rapid detection of pathogens [8–11]. However, the detection performance (e.g., sensitivity, throughput) of these strategies are far from satisfactory because they are restricted by lack of quantitative ability or sufficient sensitivity and presence of signal interference in complex samples.

In recent years, surface-enhanced Raman scattering (SERS) has been considered a promising approach for rapid detection of bacteria due to its advantages of single molecular sensitivity, nondestructive analysis, and high speed [12–15]. Two types of SERS technology, namely, label-free detection and label-based detection, have been proposed for detecting microorganisms [16]. The former strategy is direct analysis of the whole-organism fingerprint of bacteria by bringing the bacterial cell in close proximity to SERS-active substrates; as such, this method is easily subjected to interference in complex samples and usually lacks selectivity. Compared with label-free strategy, label detection is an indirect approach that uses SERS tags as quantitative reporters for bacteria detection. SERS tags are essentially small Au/Ag nanomaterials that are co-functionalized with Raman dye molecules and biorecognition molecules (e.g., antibodies, aptamers, lectin, antibiotic, etc.) to generate feature SERS signals and specifically bind to target bacteria [17–20].

More interestingly, recent studies have shown that the combination of separation tools, such as magnetic particles, microarrays, microfluidics, and SERS tags, can improve the sensitivity of SERS label-based biosensors and fulfill the real-time detection of target bacteria in complex samples [21–23]. For example, Rodríguez-Lorenzo reported the integration of SERS tags in a microfluidic system to rapidly and specifically detect *L. mono* in complex biological samples [24]. Our group developed a magnetically assisted SERS-label platform to simultaneously detect two pathogens in authentic urine samples with high sensitivity (10–25 cells/mL) [23]. One of the key but difficult challenge in these methods is to improve the binding efficiency of SERS tags to target bacteria. Hence, SERS tags with high specificity and stability are crucial for SERS label-based bacteria detection.

At present, two bottlenecks need to be addressed for developing a stable and universal bacteria detection system via SERS label-based technology. First, substrate/bacteria/SERS tags sandwich immunocomplex is most frequently introduced in SERS label-based detection, in which a pair of antibodies or aptamers (one for capture and one for detection) is usually required to identify different antigenic epitopes on the bacterial surface [25]. However, screening paired biorecognition molecules is labor and time consuming, especially for a multiplex detection system, which usually needs several pairs of different antibodies/aptamers. Second, labeling of biorecognition molecules for SERS tags is achieved by physical adsorption or chemical coupling [26,27]. However, these strategies easily bring the adsorption or coupling of the biorecognition molecules in a random orientation, leading to low binding activity and specificity. Thus, stable, active, and universal SERS tags for different target bacteria are strongly demanded.

Staphylococcus protein A (PA), a surface protein isolated from the cell wall of most *Staphylococcus aureus* (*S. aureus*), possesses four Fc binding domains that can specifically attach to the Fc region of various antibodies and leave the antigen-binding site (Fab region) free [28,29]. In this study, we propose a universal SERS-label detection system for bacteria by integrating magnetic capture, free antibody labeling, and PA-SERS tag binding. The proposed method consists of three steps performed simply in microtubes. First, the target bacteria were separated from the samples via aptamer conjugated Fe_3O_4@Au MNPs. Second, specific anti-bacteria antibody was incubated with Fe_3O_4@Au MNPs/bacteria for rapid labeling to provide abundant Fc region sites. Finally, PA-SERS tags were used to quantify the captured bacteria, which can bind efficiently and specifically to the Fc domains of Fe_3O_4@Au MNPs/bacteria/antibodies. The combined use of free antibody and PA-SERS tags not only simplifies the surface antibody modification of SERS tags but also reduces the inactivation of modified antibodies. The method also reduces the steric hindrance and increases the possibility of immune recognition reaction between SERS tags and bacteria, thereby improving the specificity and sensitivity of SERS label detection. In theory, the proposed universal SERS detection system should be applicable to most bacteria (except *S. aureus*). The feasibility of our method was verified by detection of three common pathogenic bacteria, namely, *E. coli*, L. mono, and S. typhi, with detection limits of 10, 10, and 25 cells/mL, respectively. Moreover, our method demonstrated high specificity and selectivity for real food samples (extracts of lettuce and beef) and human biological samples (urine and saliva). We believe the proposed method could serve as a universal sensing platform for monitoring food safety and diagnosis of bacterial infections.

2. Experimental section

2.1. Materials and apparatus

Polyethyleneimine (PEI), Protein A from *Staphylococcus aureus* (Catalog #P6031), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), polyvinylpyrrolidone (PVP, 40 K), *N*-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 11-mercaptoundecanoic acid (MUA), MES monohydrate, and Tween-20 were purchased from Sigma-Aldrich (USA). Phosphate buffer saline (PBS, 10 mM, pH 7.4) was obtained from Thermo Fisher (USA). Chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$), hydroxylamine hydrochloride, ethanolamine and trisodium citrate (TSC) were purchased from Sinopharm Chemical Reagent Co (Shanghai, China). Anti-*L. mono* antibody (Catalog #ab35132) was obtained

from Abcam, Monoclonal antibody (mAB) against *Salmonella* and *E. coli* O157 were bought from ACTHTEAM (Changzhou, China). The aptamers of *S. typhi*, *E. coli*, and *L. mono* were synthesized by Shenggong Biotech (Shanghai, China), and their sequences are shown in Table S1. *E. coli* O157:H7 (Catalog #ATCC 35150) was obtained from BeiNa Biotechnology Co (Shanghai, China). *S. typhi*, *S. aureus*, *Pseudomonas aeruginosa* (*P. aeruginosa*), and *L. mono* were provided by Xiao's group. Three kinds of target bacteria (*S. typhi*, *E. coli*, and *L. mono*) were verified by PCR (Fig. S1). The primers sequences and their target gene used in this study were provided in Table S2.

2.2. Apparatus

The morphologies of Fe_3O_4@Au and Au-PA nanotags were characterized by a Philips Tecnai G2 F20 transmission electron microscope (TEM). Zeta potential and size distribution of the prepared nanoparticles were measured by a Nano-ZS90 ZetaSizer. UV−vis spectra were recorded on a Shimadzu 2600 spectrometer. All Raman spectra were recorded using a portable Raman system (B&W Tek, i-Raman Plus BWS465-785H spectrometer) with a 785 nm excitation laser. The samples were excited with fixed laser power (10 mW) and acquisition time (10 s) for each SERS spectrum.

2.3. Preparation of Fe_3O_4@Au-Aptamer MNPs

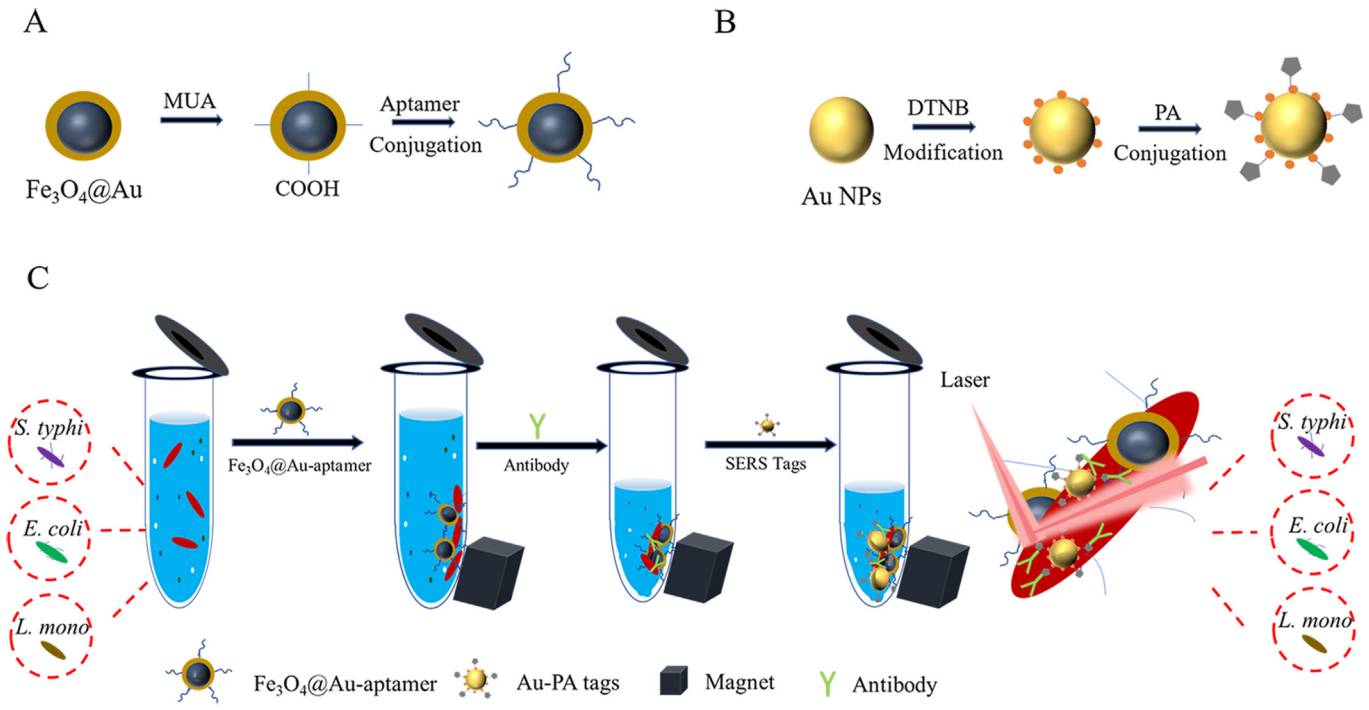
The synthesis route of Fe_3O_4@Au-aptamer MNPs is demonstrated in Scheme 1A. First, Fe_3O_4@Au core-shell MNPs were synthesized using our previously proposed PEI-mediated seed growth strategy [30]. In brief, Fe_3O_4 particle (160 nm) was selected as the superparamagnetic core to fabricate Fe_3O_4@Au MNPs because of its strong magnetic responsiveness and good stability. Fe_3O_4−Au seed MNPs were synthesized by self-assembly of positively charged PEI layer and negatively charged 3 nm Au NPs successively on the surface of Fe_3O_4 MNPs. Finally, uniform Au nanoshell surrounding the Fe_3O_4 core with high roughness was quickly formed by the in situ reduction of Au^{3+} on the dense 3 nm Au seeds.

Second, carboxyl-functionalized Fe_3O_4@Au MNPs were prepared by dispersing 10 mg of Fe_3O_4@Au MNPs in MUA ethanol solution (30 μM) under sonication for 60 min.

Third, MUA-modified Fe_3O_4@Au MNPs were magnetically separated and dispersed in 500 μL of MES buffer (0.1 M, pH 5.5) with 50 μL of EDC (10 mM) to activate the carboxyl groups. After reaction for 15 min, 25 μL of NH_2-aptamer (10 μM) was then added. The mixture was shaken with an oscillator at 37 °C for 3 h. The aptamer-modified Fe_3O_4@Au MNPs were incubated with 5 μL of ethanolamine for additional 1 h to block the unreacted carboxyl groups on the Au shell. The Fe_3O_4@Au-aptamer was magnetically separated, washed twice with PBS solution, and stored in 0.05% PBST buffer (10 mM, pH 7.4).

2.4. Preparation of Au-PA SERS tags

The synthesis process of Au-PA SERS tags is illustrated in Scheme 1B. Au@DTNB (~50 nm) was fabricated using our previously published method [31]. In brief, 1 mL of $HAuCl_4$ (1%, w/v) was added to 100 mL of deionized water, and the solution was heated to boiling. The solution was added quickly with 1 mL of TSC (1%, w/v) under vigorous stirring, and the reaction was kept at boiling point for 15 min. The solution was cooled, and the obtained 50 nm Au NPs were incubated with 200 μL of DTNB (10 mM) at room temperature for 4 h. The formed Au@DTNB NPs were purified by centrifugation at 4200 rpm for 6 min to remove excess Raman molecules. Finally, 1 mL of Au@DTNB NPs were reacted with 5 μL of EDC (10 mM) and 10 μL of NHS (0.1 M) for 15 min to activate the carboxyl group of DTNB. The activated Au@DTNB NPs were separated by centrifugation at 4200 rpm for 6 min and resuspended in 500 μL phosphate buffer (PB, 2 mM, pH 7.4) with 15 μg of PA. After incubation for 2 h, the mixture was added with 5 μL of ethanolamine to block the unreacted carboxyl sites. The final Au@PA SERS tags were purified



Scheme 1. Schematic of (A) synthesis of aptamer-modified Fe_3O_4@Au MNPs, (B) synthesis of Au-PA SERS tags, and (C) operating procedure for detection of bacteria (e.g., *S. typhi*, *E. coli*, and *L. mono*) via the proposed SERS immunoassay.

through two rounds of centrifugation (3800 rpm, 6 min) and resuspended in 500 μL of PB (2 mM, pH 7.4) at 4 °C until use.

2.5. Preparation of bacterial samples

The standard bacterial strains (*S. typhi*, *E. coli*, and *L. mono*) were cultivated in 5 mL of Luria–Bertani (LB) broth at 37 °C for 5 h. The bacterial concentration was verified via classical plate count method. In brief, 100 μL of the original bacterial solution was obtained and diluted 10^5, 10^6, and 10^7 times in 1 mL of LB medium. The diluted sample (100 μL) was spread on blood plates and cultured at 37 °C overnight. The formed bacterial colonies were counted, and the original bacterial solution was adjusted to 1×10^8 cells/mL.

2.6. Detection of bacteria by using the proposed method

The developed universal SERS platform for sensitive detection of bacteria is based on a sandwich-type SERS immunoassay with detection principle illustrated in Scheme 1. Our strategy involves three key steps. First, aptamer-modified Fe_3O_4@Au MNPs were used for specific pathogen capture and magnetic enrichment. Second, specific antibody was applied to mark the target bacteria and leave the Fc regions of antibody molecules free. Third, the PA-conjugated SERS tags were incubated with Fe_3O_4@Au/bacteria/antibody for SERS labeling and quantitative detection via the high affinity between antibody and protein A.

As shown in Scheme 1C, the operating procedure of the SERS biosensor was carried out in microtubes. First, 1 mL of different concentrations (0-10^7 cells/mL) of bacteria (*S. typhi*, *E. coli*, and *L. mono*) were incubated with 2 μL of Fe_3O_4@Au-aptamer MNPs and 5 μL of 1 M Mg^{2+}. The reaction was shaken (800 rpm) at room temperature for 45 min. After the bacteria were captured, the supernatant was discarded using an external magnet. Then, the Fe_3O_4@Au-bacteria complex was added with 100 μL of 0.05% PBST (10 mM, pH 7.4) and 0.8 μg of anti-bacteria monoclonal antibody and incubated for 20 min. The Fe_3O_4@Au/bacteria/antibody immunocomplexes were magnetically separated, and the precipitate was washed once with deionized water containing 0.05% Tween 20 to remove excess antibodies. The precipitate was dispersed by adding 40 μL of Au-PA SERS tags and 60 μL of PB (2 mM, pH 8), and the solution was shaken at room temperature for 20 min. Finally, the Fe_3O_4@Au/bacteria/SERS tags complex was washed once with water containing 0.05% Tween 20, redispersed in 2 μL of deionized water, and transferred to a silicon chip to facilitate the detection of its Raman signal.

2.7. Analysis of actual food samples

Bacterial samples (*S. typhi*, *E. coli*, and *L. mono*) were spiked in milk, orange juice, and tap water at different concentrations (10^6-10^2 cells/mL) to test the practicability of the proposed method in real food samples. Detection was performed according to the developed protocol in section 2.6. Each sample was measured at 10 random points on the silicon chip to ensure detection accuracy, and each SERS spectrum was the average of 10 independently collected spectra.

3. Results and discussion

3.1. Characterization of Fe_3O_4@Au-aptamer MNPs and Au@PA SERS tags

Monodisperse Fe_3O_4@Au core–shell MNPs with strong magnetic responsiveness and high SERS activity were fabricated by our previously proposed seed growth method [30,32] and used as magnetic capture tool and SERS substrate. Fig. 1A–C displays the TEM images of Fe_3O_4, Fe_3O_4–Au seed, and Fe_3O_4@Au MNPs, respectively. The prepared Fe_3O_4 MNPs were spherical and had an average diameter of 160 nm. After coating of the Au shell, the particle diameter of Fe_3O_4@Au MNPs increased to approximately 200 nm. The formation of Fe_3O_4@Au MNPs was further confirmed by UV–vis spectra. As shown in Fig. S2, an obvious absorption peak at 563 nm was observed for the Au coated Fe_3O_4 MNPs, which is consistent with previous report [25]. Elemental mapping results showed the distribution of Fe and Au in a single Fe_3O_4@Au MNP and its typical core-shell nanostructure. Aptamer functionalized-Fe_3O_4@Au MNPs were fabricated through a MUA-based coupling strategy (Scheme 1A). The MUA molecule was coated on the surface of the Au shell as the linker for aptamer modification to provide a terminal thiol group for forming a stable Au–S bond and a terminal carboxyl group [33]. The amino aptamer was then conjugated on the MUA coated surface via carbodiimide chemistry. As shown in Fig. S3, the zeta potential of Fe_3O_4@Au-MUA declined after aptamer conjugation, indicating the successful preparation of Fe_3O_4@Au-aptamer MNPs.

Scheme 1Bdemonstrates the sequential process for preparing Au-PA SERS tags. The DTNB molecules were modified onto the surface of Au NPs to generate characteristic SERS signal and provide terminal carboxyl groups for PA conjugation. Fig. 1E displays the TEM image of Au-PA SERS tags. The uniform-sized PA-modified SERS tags were monodispersed in the aqueous solution. The UV–vis spectral results indicated that the surface plasmon band of Au NPs was nearly unchanged upon DTNB modification and PA conjugation, thereby confirming that the conjugation of the PA protein did not affect the stability of SERS tags (Fig. 1F). The DLS data in Fig. 1G show that the average diameter of Au NPs increased from 45 nm to 53 nm after coating with the PA protein, suggesting the successful preparation of Au-PA SERS tags. Fig. S4 displays the SERS spectra of Au-DTNB and Au-PA SERS tags. The Au-PA SERS tags exhibited the same SERS ability as Au-DTNB NPs, and their main peak at 1331 cm^{-1} was selected for quantitative evaluation of bacteria in complex samples.

The binding abilities of Fe_3O_4@Au-aptamer MNPs and Au-PA SERS tags to target bacteria were next investigated. Three kinds of Fe_3O_4@Au-aptamer MNPs were incubated with the target bacteria (*S. typhi, E. coli*, and *L. mono*) for 40 min. After magnetic separation, the TEM morphologies of Fe_3O_4@Au–*S. typhi*, Fe_3O_4@Au-*E. coli*, and Fe_3O_4@Au-*L. mono* complexes are displayed in Fig. 2A, D, and 2G, respectively. All three bacteria were effectively captured by their corresponding Fe_3O_4@Au-aptamer MNPs from the solution. The capture efficiencies of Fe_3O_4@Au-aptamer MNPs for *S. typhi*, *E. coli*, and *L. mono* were 75.7%, 86.9%, and 77.8%, respectively, which were calculated using a colony counting method (Fig. S5).

Anti-*S. typhi*, anti-*E. coli*, and anti-*L. mono* antibodies were incubated with their target bacteria to verify the universality of Au-PA tags. The bacteria were then separated by centrifugation, washed with PBS, and incubated with Au-PA tags. As shown in Fig. 2B, E, and 2H, dense Au-PA tags effectively bound to the surface of the antibody modified-bacteria. No Au-PA tags combined with the bare bacterial cell (Fig. S6). These results indicated that the proposed Au-PA tags recognized and bound to antibody modified-bacteria with high specificity and affinity. After capturing by Fe_3O_4@Au-aptamer and modifying with specific antibodies, the obtained Fe_3O_4@Au/bacteria/antibody complexes exhibited strong binding affinity for Au-PA tags, thus easily forming Fe_3O_4@Au/bacteria/Au-PA tags sandwich complexes (Fig. 2C, F, I). The SERS signal of the sandwich complexes was proportional to the number of bacteria and their surface attached with Au-PA tags. This finding provided a foundation for quantitative analysis of bacteria.

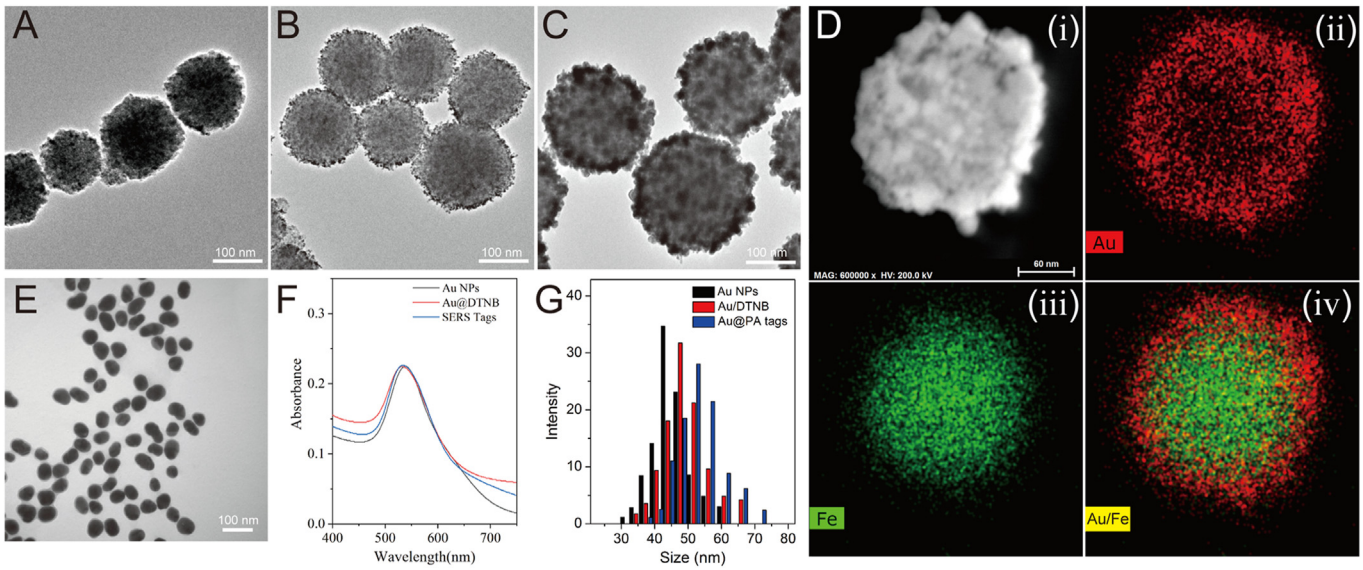


Fig. 1. TEM images of (A) Fe_3O_4, (B) Fe_3O_4—Au seed, and (C) Fe_3O_4@Au MNPs. (D) Elemental mapping images of a single Fe_3O_4@Au MNP. TEM image (E), UV—vis spectra (F), and DLS distribution of Au@PA SERS tags (G).

3.2. Optimization of assay conditions

The effects of antibody usage, antibody incubation time, and Au-PA tag reaction time were optimized to attach more Au-PA tags on the surface of Fe_3O_4@Au/bacteria complexes and achieve the best sensitivity of the assay. Different amounts of the detection antibody (0, 0.2, 0.4, 0.6, 0.8, and 1.0 μg) were used to incubate Fe_3O_4@Au/bacteria complexes to determine the influence of antibody concentration. As revealed in Fig. 3A, the SERS intensity of Fe_3O_4@Au/bacteria/Au-PA tags sandwich complexes increased with increasing antibody dosage (0—0.8 μg). When the antibody amount reached 0.8 μg, the SERS signal of the three Fe_3O_4@Au/bacteria complexes achieved the highest level, indicating that the surface of these bacterial cells was saturated with antibodies. Thus, 0.8 μg of the antibody was used for the assay. The optimal incubation time for antibody binding to the bacterial surface was 20 min, which was used to ensure the highest SERS signal (Fig. 3B). Excess Au-PA SERS tags (100 μL) were used to ensure sufficient immune binding between Fe_3O_4@Au/bacteria-antibody and Au-PA tags. The immunoreaction dynamics revealed that 20 min of immunoreaction was necessary for full binding of Au-PA tags (Fig. 3C).

3.3. Bacteria quantification via the proposed assay

Detection performance including sensitivity, quantitative ability, and detection range of the proposed SERS system for target bacteria in real food samples was assessed under the optimal conditions. Three groups of bacterial samples containing different concentrations (10^7—0 cell/mL) of *S. typhi*, *E. coli*, and *L. mono* were prepared by serial dilution using milk as solution. The assays were carried out for each group of bacteria according to the proposed detection procedure including capture of Fe_3O_4@Au-aptamer based bacteria, specific labeling of antibodies, and oriented binding of Au-PA SERS tags. The SERS intensities of 10 spectra for each test were averaged to obtain a reproducible signal. Three separate tests were performed for each sample, and standard deviation was calculated. Fig. 4A, B, and C display the SERS patterns for *E. coli*, *L. mono*, and *S. typhi*, respectively. The SERS intensity of Fe_3O_4@Au/bacteria/Au-PA tags complexes increased gradually with increasing concentrations of *E. coli*, *L. mono*, and *S. typhi*. The corresponding intensity-concentration calibration curves are shown in Fig. 4D, E, and F. These curves were constructed using the polynomial function of the target bacteria concentration and SERS intensity at 1331 cm^{-1} from the Au-PA tags. The limits of detection (LODs) for *E. coli*, *L. mono*, and *S. typhi* were approximately 10, 10, and 25 cells/mL, respectively, which were determined using the calculation protocol $LOD = y_{blank} + 3\ N$ (y_{blank} and N is the average SERS signal and standard deviation of the blank sample, respectively). Traditional plate counting, which is the gold standard of clinical test for bacterial detection, was used for confirming the bacteria concentration in the three test groups. As displayed in Fig. S7, the results of bacterial colony growth were consistent with the SERS detection results, demonstrating the excellent accuracy of the universal SERS platform for bacteria detection. Moreover, to compare the sensitivity of our proposed universal SERS-label immunoassay with that of the commonly used bacteria detection method, colloidal gold-based lateral flow immunoassays (AuNP-based LFA) were conduct to detect *S. typhi* and *E. coli* (Supporting information S1). Figs. 8a and 8b show the photographs of AuNP-based LFA tested at different concentrations of *S. typhi* and *E. coli*, respectively. The visualization limit of the red test lines for *S. typhi* (Fig. S8A) and *E. coli* (Fig. S8B) was 10^4 and 10^3 cells/mL, respectively. By comparison, the detection sensitivity of the proposed method for *S. typhi* and *E. coli* were about 400 and 100 times higher, respectively, than those of the AuNP-based LFA methods.

The specificity of the proposed SERS platform highly depends on the capture of the Fe_3O_4@Au-aptamer and binding of specific antibodies to target bacteria, both of which have high specificity and affinity to their target. In addition, aptamers are widely used to modify magnetic particles to capture bacteria from a complex solution due to their higher tolerance and chemical stability than antibodies. Specificity tests were conducted using the developed protocol to detect different bacterial samples containing 10^6 cells/mL of common pathogens (*S. aureus*, *P. aeruginosa*, *E. coli*, *L. mono*, and *S. typhi*) by using the system of Fe_3O_4@Au-aptamer$_{S.\ typhi}$/anti-*S. typhi* antibody, Fe_3O_4@Au-aptamer$_{E.\ coli}$/anti-*E. coli* antibody, and Fe_3O_4@Au-aptamer$_{L.\ mono}$/anti-*L. mono* antibody. As shown in Fig. 5, only the tube containing target bacteria generated distinct SERS signal at 1331 cm^{-1}, whereas no obvious SERS intensity of DTNB was observed in each sample of non-target bacteria. These results

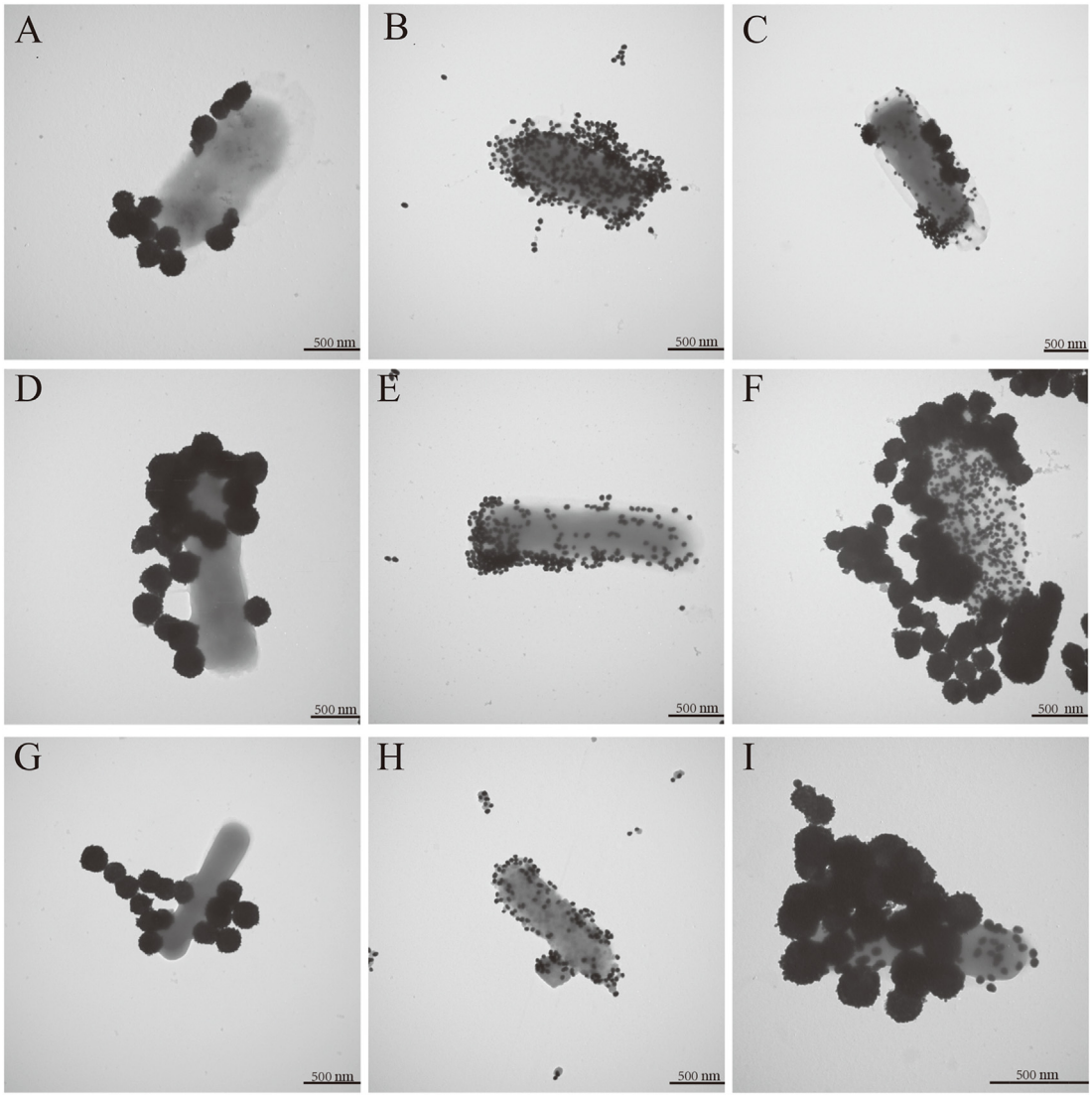


Fig. 2. TEM images of *S. typhi* (A), *E. coli* (D), and *L. mono* (G) captured by Fe_3O_4@Au-aptamer MNPs. TEM images of antibody modified *S. typhi* (B), *E. coli* (E), and *L. mono* (H) after incubation with Au-PA SERS tags. TEM images of the formed Fe_3O_4@Au/*S. typhi*/PA-SERS tags (C), Fe_3O_4@Au/*E. coli*/PA-SERS tags (F), and Fe_3O_4@Au/*L. mono*/PA-SERS tags sandwich complexes (I).

confirmed that the proposed SERS platform had good specificity for the detection of target bacteria.

The stability of the platform in complex samples is an important advantage of the proposed method. Target bacteria can be easily separated from complex samples to a clean PBS solution by using the aptamer-modified Fe_3O_4@Au to avoid the interference of environmental factors (e.g., pH value, salt ion strength) and ensure the stability of the proposed SERS platform. As revealed in Fig. S9, the sensitivity of the SERS platform for juice samples was almost equal to that for milk samples. Different concentrations of *E. coli* (10^6, 10^4, 10^2 cells/mL) were spiked in more food samples (extracts of lettuce and beef) and biological samples (human urine and saliva) to further verify the practical applicability of the SERS platform. Fig. S10 displays that all *E. coli*-spiked samples generated distinct and stable SERS signals, indicating the wide application potential of the proposed method. The values of recovery and coefficients of variability (CV) are summarized in Table 1. The recovery varied from 84.0% to 110.2% and the CV ranged from 1.9% to 13.9%, demonstrating the accuracy of our proposed method.

PA is a surface protein that is widely distributed on the cell wall of *S. aureus*. The strategy of free antibody labeling is unsuitable for *S. aureus* because the Fc region of the antibody binds preferentially to the surface PA of *S. aureus*; thus, no free Fc region could be exposed to the bacterial surface for Au-PA SERS tag binding. As shown in Fig. S11, the Au-PA SERS tags did not bind to Fe_3O_4@Au/*S. aureus*/antibody complexes; as such, no corresponding SERS signals were detected. The proposed method can be easily extended to most bacteria, except for *S. aureus*, by changing specific aptamer and antibody. Using one specific aptamer and one antibody with the help of universal PA-Au SERS tags can realize sensitive and quantitative detection of target pathogens with high specificity. This strategy is easier than developing a pair of specific antibodies or aptamers. Table S3 reveals the detection performance of our proposed universal SERS-label method and its comparison with the recently published foodborne pathogens detection biosensors. It can be concluded that the sensitivity and versatility of our method are superior or equivalent to these works.

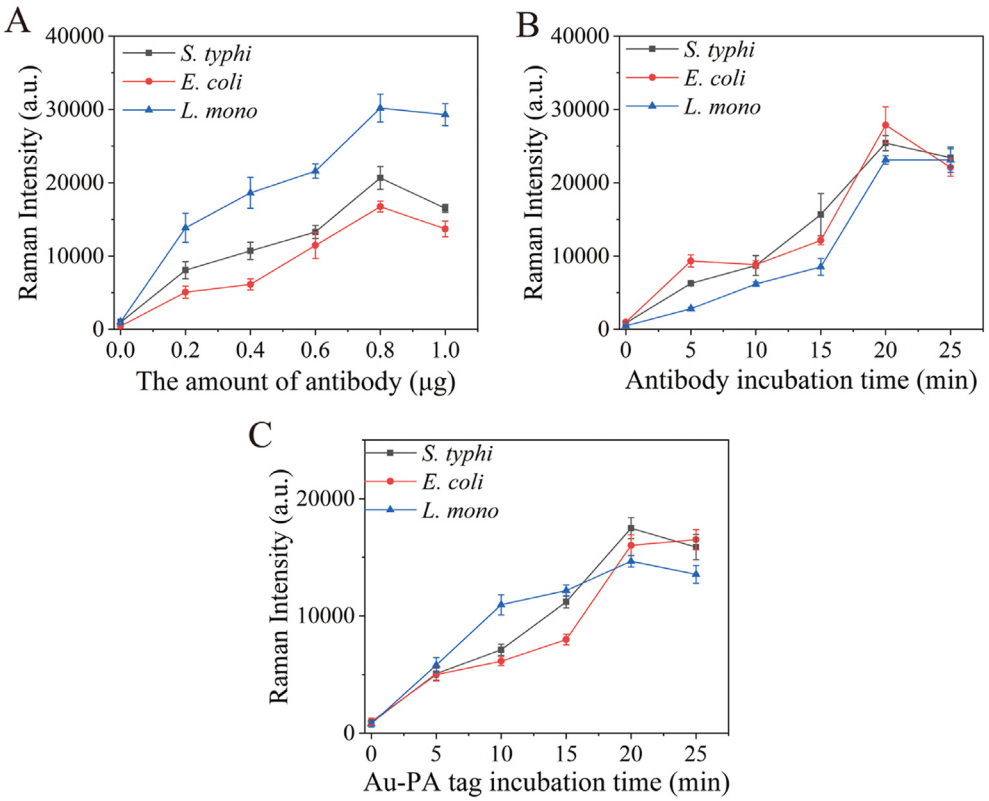


Fig. 3. (A) Optimization of the amount of antibody used for *S. typhi*, *E. coli*, and *L. mono*. (B) Optimization of antibody incubation time (B) and Au-PA SERS tags incubation time (C).

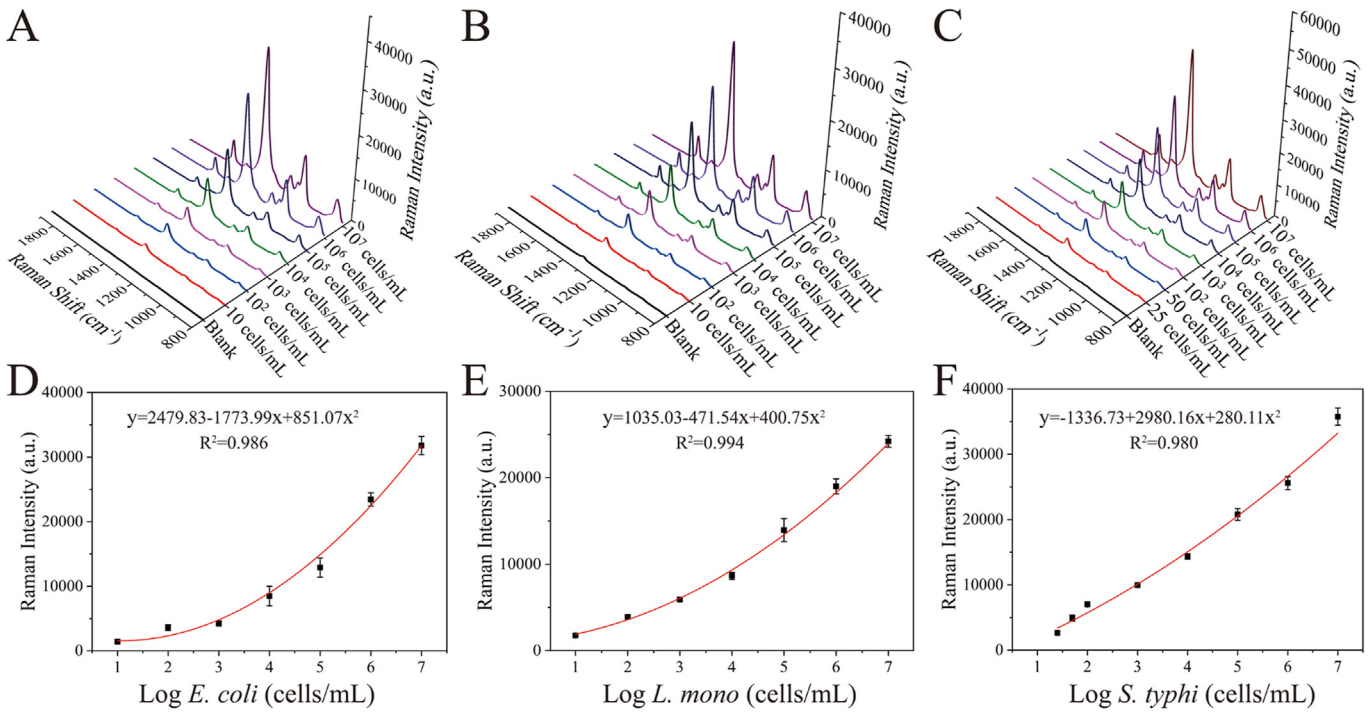


Fig. 4. (A) Detection performance of the universal SERS platform. Average SERS spectra recorded from the Fe_3O_4@Au/bacteria/PA-SERS tags with different concentrations (10^7–10 cells/mL) of (A) *E. coli*, (B) *L. mono*, and (C) *S. typhi*. The standard curves of the proposed SERS platform for (D) *E. coli*, (E) *L. mono*, and (F) *S. typhi*. Error bars represent the standard deviations from three independent assays.

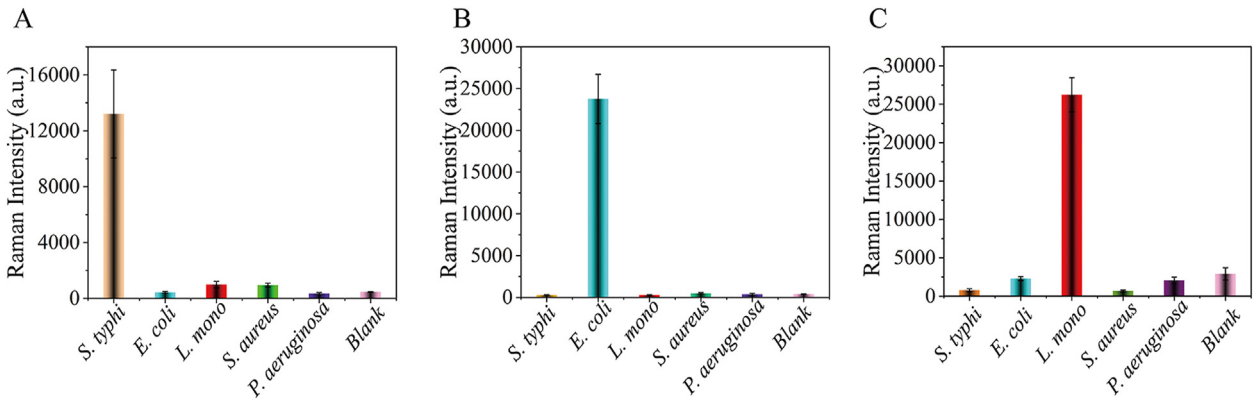


Fig. 5. Specificity of the universal SERS platform for bacteria detection. Using a combination of PA-Au SERS tags and (A) Fe_3O_4@Au-aptamer$_{S.\ typhi}$/anti-*S. typhi* antibody, (B) Fe_3O_4@Au-aptamer$_{E.\ coli}$/anti-*E. coli* antibody, and (C) Fe_3O_4@Au-aptamer$_{L.\ mono}$/anti-*L. mono* antibody. Error bars represent the standard deviation of three repetitive experiments.

Table 1
Recovery efficiency of *E. coli* detected in fresh milk, extract of lettuce and human urine samples based on the proposed SERS method.

Samples	Spiked (cells/mL)	Measured (cells/mL)	Recovery (%)	RSD (%)
Milk	5000	4872 ± 94	97.4	1.9
	500	433 ± 36	86.6	8.3
	50	42 ± 4	84.0	9.5
Extracts of lettuce	5000	5038 ± 102	100.8	2.0
	500	551 ± 19	110.2	3.4
	50	51 ± 4	102.0	7.4
Urine	5000	4952 ± 123	99.0	2.5
	500	467 ± 46	93.4	9.8
	50	43 ± 6	86.0	13.9

4. Conclusion

In this work, we developed a universal SERS-label based biosensor by using aptamer-functionalized Fe_3O_4@Au MNPs for separation of bacteria from the complex sample followed by specifically labelling the bacteria by using free antibodies and orientation binding of PA-SERS tags. Compared with conventional SERS tags, universal PA-SERS tags have obvious advantages including lower cost, better stability, easy preparation, lower steric hindrance, and higher binding efficiency to the target bacteria. The proposed SERS label biosensor can be applied to highly sensitive detection of most bacteria (expect several species of *Staphylococcus*) due to the stable and effective orientation binding between specific antibody and PA-SERS tags. The feasibility of the universal method was verified by detection of three important pathogenic bacteria, namely, *E. coli*, *L. mono*, *and S. typhi*, with LOD values of 10, 10, and 25 cells/mL, respectively. The excellent stability and specificity of the method in real food samples (milk, juice, extracts of lettuce, beef) and biological samples (human urine and saliva) were also demonstrated. To our best knowledge, this work is the first to propose the combined use of free antibody labelling and PA-SERS tag orientation binding to fabricate high-performance SERS label biosensors for bacteria detection. We believe the proposed universal SERS biosensor is an effective tool for detection of various pathogenic bacteria with high speed, specificity, and sensitivity.

CRediT authorship contribution statement

Zihui Zhou: Methodology, Writing – original draft. **Rui Xiao:** Methodology, Writing – original draft. **Siyun Cheng:** Methodology, Data curation. **Shu Wang:** Methodology. **Luoluo Shi:** Methodology. **Chongwen Wang:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision. **Kezong Qi:** Supervision, Funding acquisition. **Shengqi Wang:** Funding acquisition, Writing – review & editing, Supervision.

Declaration of competing interest

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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Appendix A. Supplementary data

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