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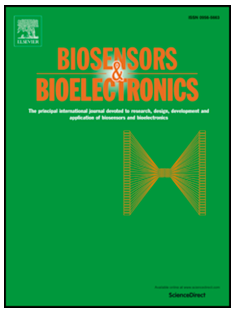

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Afang Zhu: Methodology, Investigation, Writing-original draft. **Shujat Ali:** Visualization, Data curation, Writing-review & editing. **Yi Xu:** Software, Formal analysis. **Qin Ouyang**: Conceptualization, Investigation, Validation, Data curation. **Quansheng Chen**: Conceptualization, Supervision.

A SERS Aptasensor Based on AuNPs Functionalized PDMS Film for Selective and Sensitive Detection of *Staphylococcus aureus*

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Abstract

In this study, a sensitive biosensor was developed based on aptamer functionalized polydimethylsiloxane (PDMS) film for the detection of *Staphylococcus aureus* (*S. aureus*) using surface-enhanced Raman scattering (SERS) technology. Initially, the surface of PDMS film was chemically modified by piranha and 3-Aminopropyltriethoxysilane (APTES), and then AuNPs-PDMS film was prepared by coating gold nanoparticles (AuNPs) through electrostatic interaction. Next, the aptamers were immobilized on the AuNPs-PDMS membrane via gold-sulfur bond to form the capture substrate. Meanwhile, gold-silver core-shell nanoflowers (Au@Ag NFs) modified with mercaptobenzoic acid (4-MBA) and aptamers were applied as a signal probe. In the presence of the target, the signal molecular probe and the capturing substrate specifically combined with the target and resulted in a sandwich structure "capture substrate-target-signal molecular probe". Under the optimized experimental condition, the signal of 4-MBA at 1085 cm^{-1} was linearly related to the *S. aureus* concentration in the range of 4.3×10 $cfu \cdot mL^{-1}$-4.3×10^{7} $cfu \cdot mL^{-1}$ ($y=326.91x-117.62$，$R^2=0.9932$) with a detection limit of 13 $cfu \cdot mL^{-1}$. The method was successfully applied to spiked actual samples and a 92.5-110% recovery rate was achieved.

Keywords: Surface-enhanced Raman scattering; Gold-silver core-shell nanoflowers; AuNPs-PDMS membrane; Aptasensor; *Staphylococcus aureus*; Fish

1. Introduction

Staphylococcus aureus (*S. aureus*) is a foodborne pathogenic bacteria which cause a lot of infectious diseases and other health problems. Food samples contaminated by *S. aureus* result in rapid decay and production of toxins (enterotoxins), and cause food poisoning in human beings (Rajkovic et al. 2020). Hence, a reliable and rapid method is urgently needed for the detection of *S. aureus* in food samples.

Previously, numerous analytical approaches have been reported for the determination of *S. aureus* in food samples. For instance, conventional bacterial culture method can be used for qualitative and quantitative detection of *S. aureus* in food. Despite the simplicity and economy of this method, it is time-consuming (requires 3-5 days) (Hameed et al. 2018), thus, is not suitable for emergency public health and food poisoning incidents. Besides, advanced microbiological detection methods have been used, including enzyme-linked immunosensor assay (ELISA) (Reddy et al. 2015), polymerase chain reaction (PCR) (Liu et al. 2019b; Qin et al. 2020) and immunomagnetic beads separation techniques (IMS) (Xu et al. 2019a), etc. These techniques are highly sensitive and specific but having the disadvantages of being complex and expensive, need trained personnel, require multiple pre-treatment steps and the contamination by bacteria during long-term assay make their implementation challenging (Byrnes et al. 2018; Farooq et al. 2018; Tokel et al. 2015). Consequently, it is crucial to develop a reliable, precise and economic method for the detection of *S. aureus*.

Surface-enhanced Raman spectroscopy (SERS) is a fast, sensitive, separation-free

and label-free technology for the detection of analytes in food. The application of SERS in biomedicine (Li et al. 2018), environmental monitoring (Pinzaru et al. 2018), material chemistry (Zhu et al. 2020) and food safety (Xu et al. 2019b) has attracted the attention of researchers and has become a popular and effective tool for testing pathogenic microorganisms. Zhu et al. have applied SERS technique for the quantitative analysis of *Escherichia coli* (detection limit was 10 cfu·mL^{-1}) by using a coated silica shell on the surface of AuNPs (Zhu et al. 2017). Wu et al. have used magnetic nanoparticles connected with aptamers as a capture probe and AuNPs linked with horseradish peroxidase/aptamers as a signal probe. A sandwich structure was formed by the specific binding of the target and aptamer, and the limit of detection was 12 cfu·mL^{-1} (Wu et al. 2015). Regarding the enhancement mechanism of SERS, two types have been recognized, physical enhancement (represented by local electromagnetic enhancement caused by surface plasmon resonance) (Liu et al. 2019a) and chemical enhancement (represented by charge transfer between metal nanomaterials and sample molecules) (Qi et al. 2016). In short, SERS is an optical enhancement phenomenon based on raw or irregular metal surfaces or Raman-active nanostructures. Metal nanomaterials have been the most widely used SERS substrates due to their small size, large specific surface area, unique optical, electrical, and physicochemical properties (Chen et al. 2018).

Generally, the existing SERS sensing methods have been applied in liquid systems, have many detection steps and are costly. Thus, greatly limits its application in practical detection. Therefore, it is of great significance to design a suitable, stable

and economic solid support substrate for reliable and rapid detection. Solid substrates have good stability and can be divided into rigid substrates and flexible substrates (Li et al. 2020). Traditional rigid substrates usually rely on metal electrodes, glass sheets, mica sheets, quartz sheets, silicon wafers, and other materials. Meanwhile, flexible SERS active substrates with support membranes gaining the attention of researchers. Compared with rigid substrates, flexible substrates have strong flexibility and mechanical resistance, can be cut into any shape and size, and can be adsorbed on any complex surface for in-situ detection (Parmeggiani et al. 2019; Zhao et al. 2019).

PDMS is a widely used solid material with transparency, good heat resistance and stability (Fortuni et al. 2017b). Previously, PDMS in combination with nanomaterials have been applied as a solid-state SERS substrate. Shiohara et al. have immobilized a monolayer of AuNPs on PDMS and used as an active SERS substrate for the detection of pesticides in peel. The target molecules were detected by covering the fruit surface with a layer of the flexible substrate (Shiohara et al. 2014). Fortuni et al. have applied PDMS film (coated with silver nanoparticles and gold atoms) as an active SERS substrate by using mercaptobenzoic acid (4-MBA) as the Raman molecule (Fortuni et al. 2017a). Due to the good optical transparency of PDMS, the laser can easily trigger nanoparticles loaded on PDMS to produce a localized surface plasmon resonance (LSPR) and result in the enhancement of the electromagnetic field (Yu et al. 2020). Hence, PDMS can not only retain the properties of nanomaterials but also improve the performance of composite materials (Xu et al. 2018).

Aptamer is a kind of synthetic oligonucleotides discriminated by systematic

evolution of exponential enrichment technology (SELEX) in vitro screening, which can bind to certain targets (small molecules, proteins, or even entire cells) with extremely high specificity (Yang et al. 2017). Due to the simple preparation, easy modification and good stability, aptamer has been widely used to construct biosensor for the detection of pathogenic microorganisms.

Herein, a sensitive biosensor was developed for specific detection of *S. aureus* in food matrix by using SERS technology. The aptasensor platform was constructed based on gold nanoparticles (AuNPs) functionalized PDMS film with the aid of the specificity between *S. aureus* and the aptamers. Firstly, the surface of PDMS membrane was chemically modified by piranha and 3-Aminopropyltriethoxysilane (APTES), and AuNPs-PDMS film was prepared by conjugation with AuNPs. Then, the thiol-terminal aptamer was immobilized on AuNPs-PDMS film by the Au-sulfur bond to form a solid-state capturing substrate. At the same time, hollow gold-silver core-shell nanoflowers (Au@Ag NFs) were synthesized and modified with 4-MBA and amino-terminal aptamers as a signal probe. In the presence of the target, the signal molecular probe and the capturing substrate specifically combined with the target and resulted in a sandwich structure "capture substrate-target-signal molecular probe". The detection of *S. aureus* was achieved by measuring the change of Raman intensity.

2. Materials and methods

2.1 Regents and Materials

Analytical grade chloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$), trisodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, ≥99.0%), sodium hydroxide (NaOH, ≥96.0%), hydroxylamine (NH_2OH), silver nitrate ($AgNO_3$, ≥99.0%), 3-Aminopropyltriethoxysilane (APTES), ethanol (C_2H_6O, 99%), mercaptobenzoic acid (4-MBA, 99%), PBS buffer solution (pH=7), sulfuric acid (H_2SO_4, 95~98%), hydrogen peroxide (H_2O_2), carbodiimide (EDC), and ethanolamine were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sylgard 184 (including PDMS monomer and curing agent) was obtained from Dow Corning (Michigan, USA). *S. aureus* (ATCC 29213), *Escherichia coli* (ATCC 12435) and *Salmonella* (ATCC 14028) strains were acquired from the American Type Culture Collection (ATCC). *Bacillus subtilis* (BNCC 251376) and *Pseudomonas* (BNCC 125486) strains were obtained from BeNa Culture Collection (BNCC). The aptamer sequence of *S. aureus* was synthesized by Shanghai Biotechnology Co., Ltd. The *S. aureus* aptamer sequence was 5'-NH_2-GCAATGGTACGGTACTTCCTCGGCACGTTCTCAGTAGCGCTCGCTG GTCATCCCACAGCTACGTCAAAAGTGCACGCTACTTTGCTAA-3' and 5'-SH-GCAATGGTACGGTACTTCCTCGGCACGTTCTCAGTAGCGCTCGCTGG TCATCCCACAGCTACGTCAAAAGTGCACGCTACTTTGCTAA-3' (Li et al. 2019). The fish used in the experiment was collected from Auchan Supermarket (Zhenjiang, Jiangsu Province, China), transported quickly to the laboratory and stored

in a refrigerator at -18 °C. Ultrapure water (18.2 MΩ cm) was produced using the Millipore water purification system.

2.2 Instrumentation

The energy dispersive spectroscopy (EDS) spectrum of the Au@Ag NFs was acquired through EDS instrument (S-3400N; Hitachi Ltd., Japan). Ultraviolet-Visible (UV-Vis) absorption spectra were measured by an 8453 UV-Vis spectrometer (Agilent Technologies Inc., USA). JEM-2100HR transmission electron microscopy (TEM, JEOL Ltd., Japan) was used to detect the size and morphology of the nanoparticles. The morphology of AuNPs modified PDMS film substrate was observed by field emission-scanning electron microscope (JSM-7800F; SEM, JEOL Ltd., Japan). Zeta potential measurement was performed by a Zetasizer nano instrument (model ZEN 3600, Malvern Instruments Ltd., UK) and Raman spectra were collected by SPLD-RAMAN-785-Q spectrometer (Hangzhou SPL photonics Co Ltd., China) under 785 nm wavelength incident laser light.

2.3 Preparation of bacterial strain samples

Firstly, the purchased lyophilized powder of *S. aureus* (ATCC 29213) was dissolved in sterile saline and then cultured with Lysogeny Broth (LB) medium (continuous activation two generations). Secondly, the activated *S. aureus* strain was cultured in LB liquid medium at 37 °C for 12 h and then 20 mL of *S. aureus* solution was centrifuged at 6000 rpm for 10 min. The supernatant was removed and dispersed in sterile saline. Finally, tenfold ratio of the obtained bacterial solution was used for gradient dilution, and a total of 8 gradients were obtained as experimental samples.

158 Meanwhile, the conventional plate counting method was used to determine the
159 number of bacteria, and the plate with the colony number between 30 and 300 was
160 selected to count. Three parallel repeats were set for each concentration gradient.

161 **2.4 Preparation of aptamer functionalized PDMS membrane**

162 All glassware used in the experiment were washed with aqua regia (HNO_3/HCl, 3:1,
163 *v/v*), rinsed thoroughly with ultrapure water, and dried before use. PDMS membrane
164 was prepared by mixing the main agent (polydimethylsiloxane prepolymer) and
165 auxiliary agent (curing agent) in a ratio of 10:1 followed by the evacuation at room
166 temperature for 1 h until the air bubbles in the colloid were disappeared. The mixture
167 was then solidified in an oven at 70 °C overnight. Samples (0.5 cm×0.5 cm) were cut
168 from the cured polymer (Zhang et al. 2008). The prepared PDMS membrane was
169 immersed in piranha solution (H_2SO_4: H_2O_2 = 3: 1, *v*/*v*) for 60 s at 40 °C, rinsed with
170 ultrapure water for three times immediately and then dried with nitrogen.
171 Subsequently, the hydroxylated PDMS membrane was moved into a centrifuge tube.
172 APTES ethanol solution (5%, *v/v*) was added and the reaction was carried out at
173 70 °C for 3 h. The small pieces of the prepared sample were moved out, washed with
174 plenty of ultrapure water and then dried with nitrogen gas to obtain
175 amine-functionalized PDMS film (Zhan et al. 2016). The aminated PDMS were
176 immersed in concentrated AuNPs solution and incubated overnight to form
177 AuNPs-PDMS film. The AuNPs were synthesized by a reported method with slight
178 modifications (Ziegler and Eychmüller 2011). Briefly, 0.25 mL (0.5 M)
179 $HAuCl_4 \cdot 3H_2O$ solution and 100 mL of ultrapure water were heated to boiling point

and continuously stirred for 10 min. Then 1.5 mL 1% trisodium citrate was quickly added to the above mixture and boiled for 15 min as its color turned into wine red indicated the formation of AuNPs. The prepared AuNPs-PDMS film was incubated with thiol-terminal *S. aureus* aptamers solution (70 nM) for 24 h at 37 °C. The AuNPs-PDMS film attached with aptamers was washed with anhydrous ethanol and ultrapure water for several times and stored for further use.

2.5 Preparation of signal probe

Au@Ag NFs were prepared according to the previously reported method (Garcia-Leis et al. 2015). In detail, 0.5 mL of NH_2OH (6×10^{-2} M) and 0.5 mL of NaOH (0.05 M) were mixed at room temperature for 5 min under vigorous stirring. Under dark conditions, 9 mL of $AgNO_3$ solution was added to the mixture and left to react for 5 min. Subsequently, 5 mL of $HAuCl_4\cdot3H_2O$ solution (8×10^{-4} M) and 100 µL of $C_6H_5Na_3O_7\cdot2H_2O$ solution (1%, *w/v*) were added simultaneously. The reaction mixture was stirred for 14 min at 100 °C to obtain Au@Ag NFs material. The solution was then centrifuged (6000 rpm, 10 min) and dispersed in 5 mL ultrapure water. Then, 30 µL (10 µM) 4-MBA ethanol solution was added to the above reaction mixture, stirred at room temperature for 3 h and centrifuged to remove the unconnected 4-MBA. The prepared Au@Ag NFs@MBA were dispersed in 5 mL ultrapure water. Then, 700 µL Au@Ag NFs@MBA solution was added with 20 µL of freshly prepared EDC (7 mg/mL) and stirred at 850 rpm for 15 min at 25 °C. It was then followed by the addition of 25 µL aminated *S. aureus* aptamers (10 µM) and 500 µL PBS buffer solution, and mixed at room temperature for 1 h. To close the free sites

(not connected with the aptamers), ethanolamine (1%, *v/v*) was added and shaken for 1 h. Finally, Au@Ag NFs@MBA connected with aptamers were centrifuged at 7500 rpm for 6 min, dispersed in 1 mL PBS buffer solution and stored in a refrigerator at 4 °C (Chen et al. 2018).

2.6 Detection of *S. aureus*

The prepared aptamer functionalized AuNPs-PDMS film was taken in a centrifuge tube. *S. aureus* (500 μL) solution with a known concentration (4.3×10 $cfu \cdot mL^{-1}$-4.3×10^7 $cfu \cdot mL^{-1}$) was added and incubated at 37 °C for 1 h. The PDMS film was washed twice with PBS buffer to remove the *S. aureus* physically adsorbed on the film. Then, the PDMS films were incubated with the prepared 500 μL signal probe solution for 1 h at 37 °C. Finally, the SERS detection was performed after rinsing with PBS buffer solution to determine the linear range and detection limit. All measurements were repeated three times in parallel.

2.7 Specificity experiment

To verify the specificity of this method, four pathogenic bacteria (*Escherichia coli*, *Salmonella*, *Bacillus subtilis* and *Pseudomonas*) were selected and tested under the optimal conditions simultaneously with *S. aureus*. The bacterial solution concentration was around 10^5 $cfu \cdot mL^{-1}$.

2.8 Blank spike recovery of *S. aureus* in fish samples

To study the reliability of the method, we evaluated the developed SERS biosensor using the plate counting method for spiked fish samples. Fresh fish was purchased from a local supermarket and shipped to the laboratory in ice packs. Then, 25 g of the

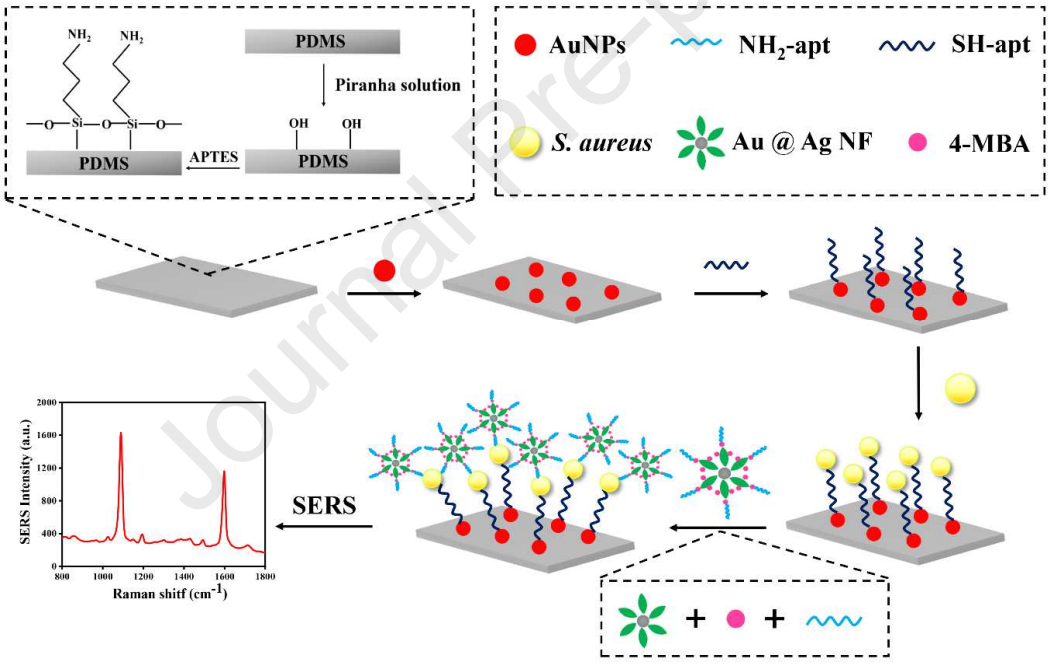
fish meat was weighted and irradiated with ultraviolet disinfection light for 30 min in a super clean bench to eliminate the interference of foodborne pathogens. Thereafter, *S. aureus* was added to the fish samples with concentrations of 1×10^3, 1×10^4, 1×10^5 and 1×10^6 cfu·mL^{-1} and incubated for 30 min. Then, the spiked samples were transferred to conical flasks (250 mL), mixed with 100 mL sterile saline and stirred for 3 min. Finally, the established SERS aptasensor platform was used to detect the prepared samples. The obtained results were compared with the traditional plate counting method and the recovery rate was calculated.

3. Results and discussion

3.1 Detection principle of SERS aptamer sensor platform based on AuNPs-PDMS film

A SERS aptamer for the quantitative detection of *S. aureus* was fabricated by utilizing AuNPs-PDMS film as a SERS active substrate. The schematic representation of the developed SERS aptasensor platform is shown in Scheme 1. Thiol-terminal aptamers conjugated with AuNPs-PDMS were assembled through two steps and used as a solid-state capture substrate. In the first step, negatively charged colloidal gold and positively charged amino group on the PDMS film modified by APTES were combined through electrostatic interaction. In the second step, the thiol-terminal aptamers were fixed on the film surface through a gold-sulfur bond. Then, the amino-terminal aptamers were assembled on the surface of Au@Ag NFs@4-MBA by amide condensation. The prepared aminated aptamer functionalized Au@Ag NFs@4-MBA was used as a SERS signal probe to express the Raman signal of *S.*

aureus. In the presence of the target bacteria, the aptamers on the capture nanoprobe and the signal nanoprobe were specifically bound to *S. aureus* simultaneously and a sandwich structure "capture nanoprobe-target-signal molecular nanoprobe" was formed. A dramatically strong Raman signal was generated due to the signal nanoprobes associated with the active substrate and the Raman intensity increased with the concentration of target bacteria. The proposed SERS aptamers sensor can provide a platform for the detection of other analyte molecules by using specific aptamers.



Scheme 1 Schematic representation of the developed SERS aptasensor.

3.2 Characterization of AuNPs modified PDMS film

The AuNPs were characterized by transmission electron microscopy (TEM) and UV-Vis absorption spectroscopy. The UV-Vis spectrum is shown in Fig. S1A. The maximum absorption peak of the AuNPs was at 520 nm. The TEM results are shown in Fig. S1B. The AuNPs were uniformly distributed spherical shape, with a size

around 15 nm. In addition, their surface was negatively charged (Fig. S1C). The prepared bare PDMS film was transparent. After being loaded with AuNPs, the PDMS film turned to wine red, which was the color of the AuNPs. The AuNPs-PDMS membrane was characterized by UV-Vis absorption spectroscopy, scanning electron microscope and Raman spectrometer. Bare PDMS exhibited no UV-Vis absorption spectrum, while AuNPs-PDMS showed a UV-Vis absorption peak at 520 nm, which was consistent with the UV-Vis absorption peak of the AuNPs (Fig. 1A). The scanning electron micrograph revealed that the AuNPs were evenly distributed on the surface of the PDMS film (Fig. 1B). The results of Raman spectroscopy are shown in Fig. S1D. The Raman spectrum of the 4-MBA solution and bare PDMS film are shown as horizontal lines, respectively. The Raman spectrum of the PDMS membrane incubated with 4-MBA also exhibited a horizontal line and the Raman spectrum of 4-MBA on the AuNPs-PDMS film showed a strong Raman characteristic peak due to the SERS enhancement effect of the AuNPs. The characteristic peaks at 1085 cm^{-1} and 1592 cm^{-1} were attributed to the stretching vibration of C-C and benzene ring. The results demonstrated that the AuNPs were successfully modified on the surface of the PDMS membrane, and were used in the subsequent experimental operations.

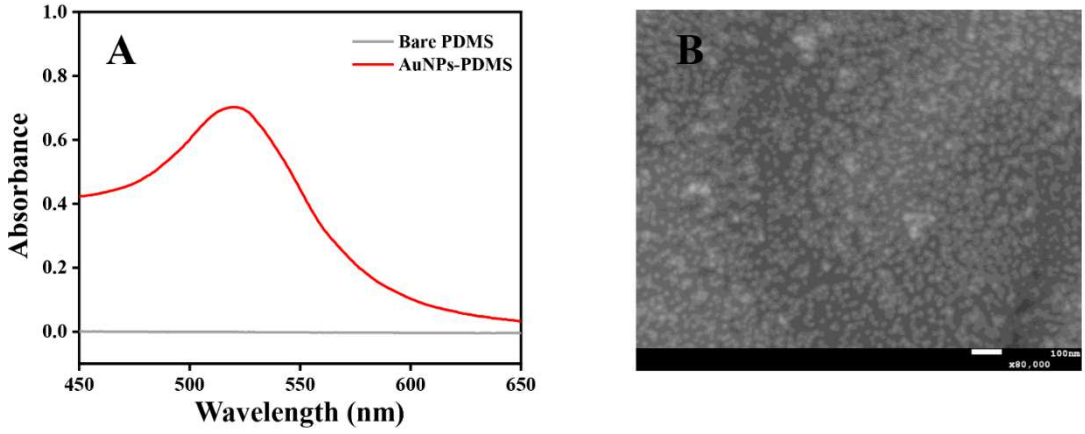


Fig. 1 UV-vis absorption spectra of PDMS film and AuNPs-PDMS film (A) and SEM image of the AuNPs-PDMS film (B).

3.3 Characterization of aptamer functionalized AuNPs-PDMS membrane

Thiol-terminal aptamers of *S. aureus* were adsorbed on the surface of the AuNPs-PDMS membrane through gold-sulfur bond. UV–Vis absorption spectroscopy was used to characterize whether the aptamers of *S. aureus* were successfully immobilized on the AuNPs-PDMS film. After incubation with the AuNPs-PDMS film, the absorbance value of the aptamers at 260 nm was significantly reduced compared to the initial aptamer solution (Fig. S2). Hence, confirmed that most of the aptamers in the reaction mixture were connected to the surface of the AuNPs-PDMS membrane.

3.4 Characterization of signal probes of *S. aureus*

Au@Ag NFs were obtained by galvanic replacement using silver nanoparticles (AgNPs) as a template. The materials were prepared through two-step reduction process and one-step replacement reaction: Hydroxylamine (HA) and trisodium citrate (CIT) reduction of silver nitrate, and the replacement of AgNPs. The prepared Au@Ag NFs substrate exhibited a distinct flower-like structure (Fig. 2A). The EDS

297 spectrum (Fig. 2B) showed that the synthesized substrate was mainly composed of
298 gold and silver elements. The Raman reporter molecule 4-MBA was conjugated to the
299 silver shell of the substrate through the sulfhydryl group. Raman spectrometer was
300 used to characterize whether 4-MBA was connected to the Au@Ag NFs material. The
301 Raman spectrum of 4-MBA and Au@Ag NFs were almost horizontal lines,
302 respectively. When the labeling molecule 4-MBA was added to the substrate surface,
303 obvious characteristic peaks were appeared at 1085 cm^{-1} and 1592 cm^{-1} (Fig. 2C).
304 Amino-terminal aptamers were linked to the surface of Au@Ag NFs@4-MBA
305 through amide condensation reaction. UV-Vis spectroscopy was used to characterize
306 the attachment of the aptamer with the Au@Ag NFs@4-MBA material. A UV-Vis
307 absorption peak at 260 nm was appeared when the Au@Ag NFs@4-MBA were
308 connected to the aptamers (Fig. 2D). Hence, indicated that the aptamers were
309 successfully attached to the surface of Au@Ag NFs @4-MBA.

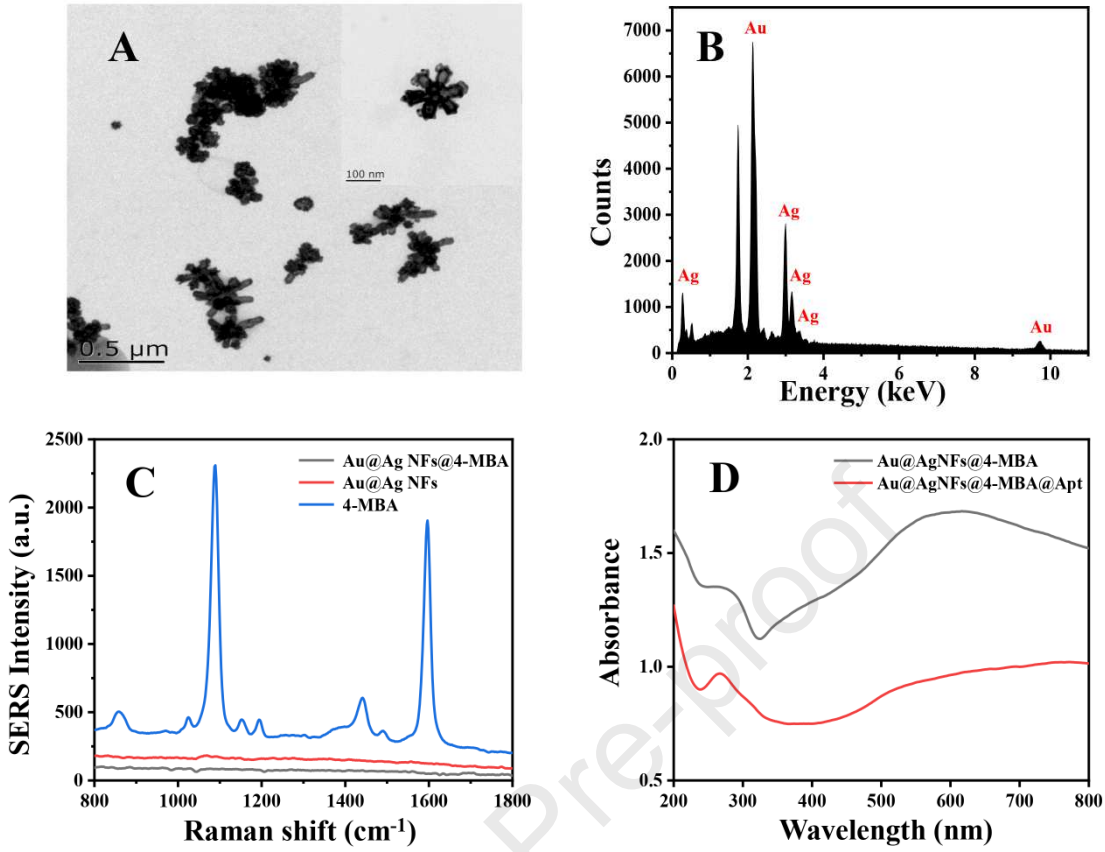


Fig. 2 Transmission electron micrograph (A) and EDS spectrum (B) of Au@Ag NFs. Comparison of SERS intensity obtained from 4-MBA, Au@Ag NFs and Au@Ag NFs@4-MBA (C). UV-Vis absorption spectra of Au@Ag NFs@MBA before and after attachment with the aptamers (D).

3.5 Optimization of experimental conditions

The concentration of *S. aureus* was quantified by measuring the SERS intensity of 4-MBA. The 4-MBA molecules were attached to the silver shell through Ag-S bonds. Less amount of 4-MBA resulted in insufficient signal molecules connected to Au@Ag NFS while an excessive amount of 4-MBA affected the stability of the SERS active substrate and the SERS signal intensity. Hence, the amount of 4-MBA was optimized for Au@Ag NFs@4-MBA formation (Fig. 3A). The aptamers linked to Au@Ag NFs@4-MBA were optimized by incubating different volumes of the

323 nanomaterials and fixed concentrations of the aptamers (Fig. 3B). The optimum
324 amount of the Au@Ag NFs was found to be 700 μL.

325 In addition, to obtain the optimal analytical performance of *S. aureus* SERS
326 aptasensor, the final concentration of the aptamer functionalized on the
327 AuNPs-PDMS film and incubation time between the aptamer and AuNPs-PDMS film
328 were investigated. First, the concentration of the aptamer solution incubated with
329 AuNPs-PDMS film was optimized. The optimization results are shown in Fig. 3C.
330 The difference value (ΔA260) of the absorbance intensity at 260 nm of the aptamers
331 before and after incubation with AuNPs-PDMS membrane was recorded by UV-Vis
332 absorption spectroscopy and used as the ordinate. The net increase in the absorbance
333 intensity was obtained by subtracting the absorbance value of the supernatant of the
334 aptamer functionalized AuNPs-PDMS membrane from that of the initial aptamer
335 solution. The higher the value of ΔA260, the greater the aptamers loading on the
336 AuNPs-PDMS. When the concentration of the aptamer was increased from 40 nM to
337 100 nM, the ΔA260 gradually increased and tended to be stable. The difference value
338 reached to the maximum at 70 nM aptamer concentration. This may be due to the
339 saturated state of the aptamers attached to the surface of the AuNPs-PDMS membrane.
340 Therefore, 70 nM was selected as the optimal aptamer concentration and used in the
341 subsequent experiments. Second, the incubation time of the aptamers and
342 AuNPs-PDMS membrane was optimized, the results are shown in Fig. 3D. UV-Vis
343 absorption spectroscopy was used to record the ΔA260 of aptamers before and after
344 incubation with the AuNPs-PDMS membrane. Up to 24 hours, the ΔA260 increased

gradually with incubation time and when incubation time exceeded 24 hours, the difference value decreased slowly with incubation time. This may be due to the fact that in the short incubation time, the aptamer did not connect with the AuNPs-PDMS membrane completely, while the long incubation time resulted in their disconnection. Hence, resulted in a low ΔA260 value. When the incubation time was reached to 24 h, the difference value reached to the maximum. Therefore, the optimal incubation time of the AuNPs-PDMS membrane was selected as 24 h.

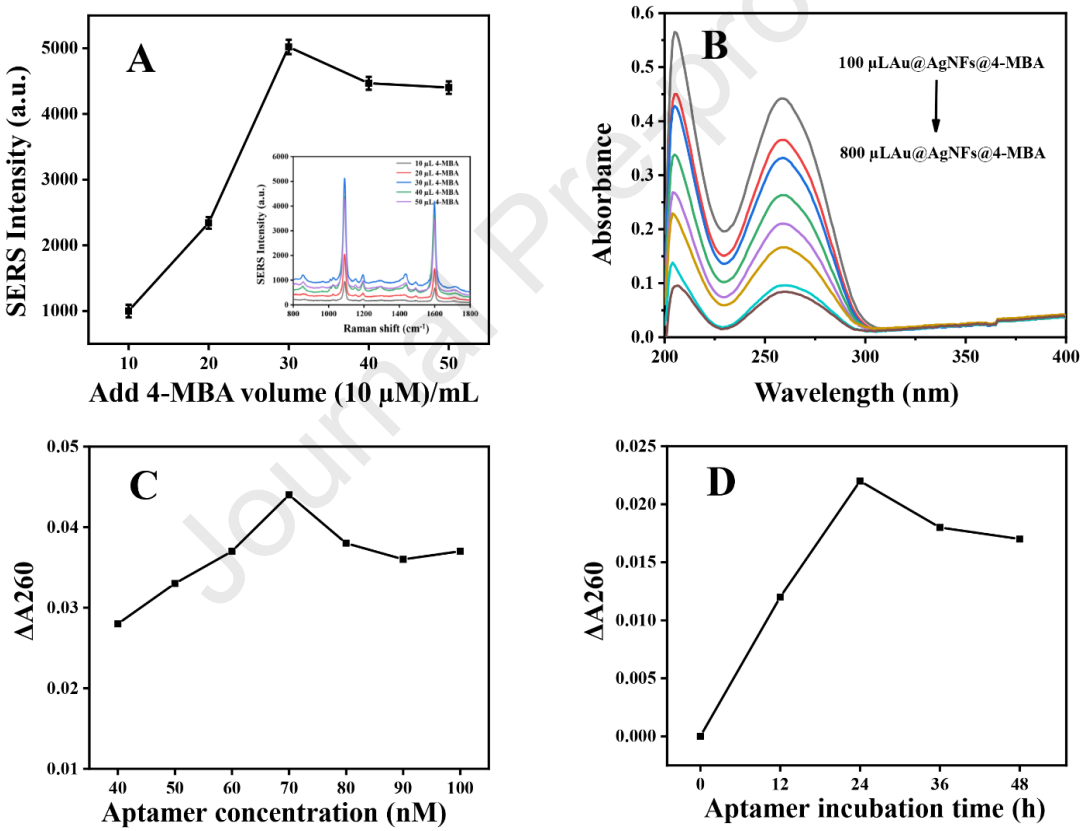


Fig. 3 Line chart of the optimized signal molecule 4-MBA and the insert is the photo of its corresponding SERS spectra (A). UV-vis spectra of different volumes of Au@AgNFs @4-MBA incubated with the fixed concentrations of the aptamers (B). Optimization of the concentration of the aptamers modified AuNPs-PDMS film (C) and time of the aptamers modified AuNPs-PDMS film (D). The error bars were

derived from the standard deviation of three measurements. Error bar=SD (n=3).

3.6 Linear range and detection limit

According to the designed experimental principle, when *S. aureus* was present in the system, the capture substrate and the signal molecule probe specifically bound to the target to form a "capture substrate-target-signal molecule probe" sandwich structure. Raman signal of the 4-MBA was generated at 1085 cm^{-1}. As the concentration of the *S. aureus* was increased, more aptamers were loaded on the solid substrate to capture the bacteria, so that more signal molecular probes were fixed to the substrate and caused the increase in the Raman intensity. Under the optimal experimental conditions, SERS detection of *S. aureus* with different concentrations was performed. The results of the Raman spectroscopy are shown in Fig. 4A. When the concentration of *S. aureus* was increased, the Raman intensity of 4-MBA at 1085 cm^{-1} was gradually increased. The linear results are shown in Fig. 4B. The logarithmic value of the number of *S. aureus* colonies was in the range of 4.3×10 $cfu \cdot mL^{-1}$-4.3×10^{7} $cfu \cdot mL^{-1}$, represented a good linear relationship with the Raman intensity of the signal molecular probe at 1085 cm^{-1} ($y=326.91x-117.62$, $R^2=0.9932$). The limit of detection was computed by the following formula: LOD=3S/M. Where S is the standard deviation of the blank sample (without *S. aureus*) and M is the slope of the standard curve. The limit of detection was 13 $cfu \cdot mL^{-1}$.

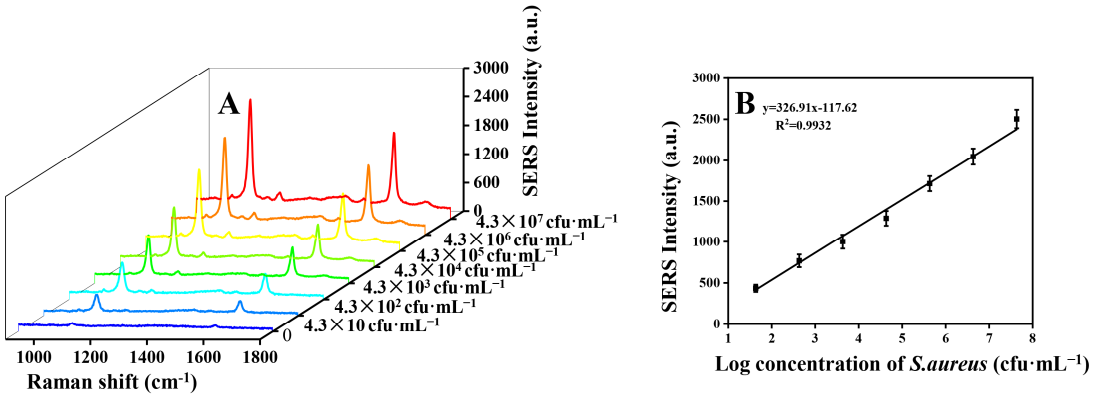


Fig. 4 SERS spectra in the presence of different concentrations of *S. aureus* (A) and the correlation between Raman intensity and concentrations of *S. aureus* (B). The error bars were derived from the standard deviation of three measurements. Error bar=SD (n=3).

3.7 Specificity experiment

To evaluate the specificity of the proposed SERS method, four pathogenic bacteria (*Escherichia coli*, *Salmonella*, *Bacillus subtilis* and *Pseudomonas*) were selected. For the best experimental results, a bacterial concentration of 10^5 cfu·mL^{-1} was selected. The results of the specificity analysis are shown in Fig. S3. Compared with *Escherichia coli*, *Salmonella*, *Bacillus subtilis* and *Pseudomonas*, *S. aureus* obtained a dramatically strong Raman signal at 1085 cm^{-1}, indicated that this method has good specificity and could be specifically applied for the detection of *S. aureus*.

3.8 Blank spike recovery of *S. aureus* in fish samples

The "sandwich" structure based on aptamer functionalized PDMS film for *S. aureus* detection was used for the blank spike recovery test of fish samples. *S. aureus* with different concentrations from 1×10^{3} to 1×10^{6} cfu·mL^{-1} was detected by the proposed method and traditional plate counting method respectively. Three replicates were set up for each concentration of fish samples and the obtained results were compared to calculate the recovery rate. The results are shown in Table 1. The detection result of this method possessed good accuracy for the quantification of *S. aureus* (the recovery rate was between 92.5% and 110%) and can be applied for the detection of *S. aureus* in actual samples. In addition, the experimental results were compared with the existing detection methods of *S. aureus*. Considering the detection range, detection limit and simplicity of the experimental operation. The proposed method showed certain advantages and could be used for quantitative detection of foodborne pathogens in fish samples (Table S1)

Table 1. Comparison of the results obtained through this method with the plate counting method on the spike recovery of fish samples.

Samples	Spiked ($cfu \cdot mL^{-1}$)	Plate counting method ± SD ($cfu \cdot mL^{-1}$)	This method ± SD ($cfu \cdot mL^{-1}$)	RSD%	Recovery%	P
1	1×10^3	$(0.919 \pm 0.04) \times 10^3$	$(0.925 \pm 0.05) \times 10^3$	5.41	92.5	P=0.306
2	1×10^4	$(0.973 \pm 0.02) \times 10^4$	$(1.01 \pm 0.03) \times 10^4$	2.97	101	
3	1×10^5	$(1.02 \pm 0.04) \times 10^5$	$(1.10 \pm 0.05) \times 10^5$	4.55	110	
4	1×10^6	$(0.983 \pm 0.03) \times 10^6$	$(0.962 \pm 0.03) \times 10^6$	3.12	96.2	

SD: standard deviation, n=3
P>0.05, indicate: no significant difference.

4. Conclusion

In this work, a highly sensitive and specific SERS biosensor based on aptamer functionalized composite PDMS film was constructed and successfully applied for the detection of *S. aureus* in fish samples. *S. aureus* was quantified using the developed aptasensor with high sensitivity. A good linear relationship (y=326.91x-117.62, R^2=0.9932) and LOD of 13 $cfu \cdot mL^{-1}$ were achieved. The spiked recovery test of fish samples showed no significant difference as compared to the plate count method and the spiked recovery rate was 92.5%-110% which indicated that the method can be applied for the detection of actual samples. The "sandwich" structure based on aptamer modified PDMS film provided a new idea for the rapid detection of foodborne pathogens in food production, processing and safety supervision. However, the stability of batch samples of PDMS film may affect the actual rapid detection of foodborne pathogens. Hence, new approaches to prepare functional PDMS membranes and to explore stable and rapid methods for the in situ detection of foodborne pathogens are the key development direction in the future.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

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Highlights

1. A SERS aptasensor was developed for selective and sensitive detection of *Staphylococcus aureus.*
2. Hollow Au@Ag NFs were synthesized and modified with 4-MBA and aptamers as signal probe.
3. A sandwich structure of "capture substrate-target-signal molecular probe" was obtained.
4. Under the optimized condition, a 13 cfu·mL^{-1} LOD was achieved.

Declaration of Interests Statement

I would like to declare on behalf of all the co-authors that the work described here is original research, that it is not published previously, that it is not under consideration for publication elsewhere in whole or in parts. No conflict of interest exists in the submission of this manuscript and supporting information. All the authors listed have approved the enclosed manuscript.

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