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RESEARCH PAPER

A highly sensitive DNAzyme-based SERS biosensor for quantitative detection of lead ions in human serum

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Abstract

Lead ions (Pb^{2+}), one form of the toxic heavy metal, have drawn significant attention due to their harmful effects on human health and the environment. Although many analytical techniques have been developed over the past few decades, the development of a sensitive, selective, and rapid method to detect Pb^{2+} remains a challenge. In this work, we developed a sensitive surface-enhanced Raman scattering (SERS) biosensor for highly sensitive detection of Pb^{2+} by using DNAzyme-modified Fe_3O_4@Au@Ag nanoparticles (Fe_3O_4@Au@Ag NPs). Firstly, the thiolated 5'-Cy3 DNA probe was modified on the surface of Fe_3O_4@Au@Ag NPs, which hybridized with the Pb^{2+}-specific DNAzyme to form a SERS biosensor, and the Cy3 labels were used to detect Pb^{2+}. In the presence of Pb^{2+}, the DNAzyme cleaves the Cy3-labeled DNA probe, leading to the release of Cy3-labeled DNA probe from the Fe_3O_4@Au@Ag NPs. Therefore, the Raman intensity of the Cy3 labels decreases. The proposed biosensor exhibited excellent linearity in the range from 0.01 to 1.0 nM, with a limit of detection for Pb^{2+} of 5 pM. It features superior selectivity to Pb^{2+} over other interfering metal ions and good application in the determination of Pb^{2+} in tap water and human serum samples. The SERS biosensor provides a novel, simple and sensitive method for detection of Pb^{2+} and sheds new light on the design and synthesis of analogous SERS biosensors for the detection of other heavy metal ions.

Keywords SERS · DNAzyme · Fe_3O_4@Au@Ag NPs · Lead ions · Human serum

Introduction

Lead ions (Pb^{2+}) are categorized as a hazardous heavy metal ion in geochemical cycling and industrial waste water, wherein they pose serious danger to environmental and human health [1, 2]. Lead can enter the human body through air, drinking water, and food [3, 4]. Therefore, it is a serious threat to the major organs of the human body. The maximum allowable content of Pb^{2+} is 15 ppb (72 nM) in drinking water and 100 ppb (483 nM) in human blood as defined by the US Environmental Protection Agency (USEPA) [5, 6]. In addition, even trace amounts of lead ions may cause serious harm to human health due to their long-term accumulation in the human body. Therefore, it is necessary to develop a simple and sensitive methods for quantitative detection of Pb^{2+}.

Although many traditional analytical methods for the Pb^{2+} quantification have been developed, such as atomic absorption spectrometry (AAS) [7, 8], inductively coupled plasma–mass spectrometry (ICP-MS) [9, 10], and inductive coupled plasma–atomic emission spectrometry (ICP-AES) [11], the majority of them require sophisticated sample handling processes, professional operation, and complicated instruments. Surface-enhanced Raman scattering (SERS) has received extensive attention due to its significant advantages including extraordinary sensitivity, special spectroscopic fingerprint, and rapid detection. Therefore, taking advantage of these qualities, SERS is considered as a promising approach for sensitive detection of Pb^{2+} [12–14]. SERS is a plasmon

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phenomenon that mediates the collective oscillation of the conductive electrons of noble metal nanoparticles, and known as localized surface plasmon resonance (LSPR). According to previous reports, the intensity of SERS signal can reach up to the order of 10^6–10^{14} [15–17]. In addition, SERS has also been combined with DNAzyme for highly sensitive and specific detection of Pb^{2+} [18, 19].

In recent years, considerable efforts have focused on the development of DNAzyme-based Pb^{2+} detection methods. DNAzyme is a group of functional DNA molecules with enzymatic activity, which can cleave a substrate at its adenine ribonucleotide (rA) site [20, 21]. DNAzymes display high binding activity and excellent specificity via in vitro selection [22, 23]. "8–17" DNAzyme molecules have been developed to detect Pb^{2+}, including fluorescence [24, 25], electrochemical signals [26, 27], and colorimetry [28]. The Pb^{2+}-dependent DNAzyme sensors have high enzymatic activity for Pb^{2+} as their cofactor. Furthermore, DNAzymes provide excellent biocompatibility and low toxicity, making it possible to develop biosensors for determination of a variety of metal ions. For example, Niu et al. developed a "turn-on" fluorescence sensor based on the specific DNA technology for sensitive determination of Pb^{2+} [29]. Subsequently, Zhang et al. reported a photoelectrochemical DNAzyme sensor based on the DNAzyme–ZnO nanoflower for highly sensitive Pb^{2+} detection [30]. Yang et al. developed a sensing strategy based on a DNAzyme-based two-photon imaging probe for Zn^{2+} detection in living cells [31]. Although these methods can achieve the advantages of simple, sensitive for detection of heavy metals ions, a DNAzyme-based SERS biosensor for quantitative detection of Pb^{2+} has not been reported.

In this work, we propose a DNAzyme-based SERS biosensor to detect Pb^{2+} employing DNAzyme-modified Fe_3O_4@Au@Ag nanoparticles (Fe_3O_4@Au@Ag NPs) as SERS substrate. In this sensing platform, Cy3-labeled thiolated DNA substrates (SH-DS) are used as the SERS probe which were immobilized on the Fe_3O_4@Au@Ag NPs surface for quantitative detection of Pb^{2+}. In the presence of Pb^{2+}, the Cy3-labeled DS was cleaved by the Pb^{2+}-dependent DNAzyme, which caused a remarkable decrease of SERS intensity. In addition, the DNAzyme-based SERS biosensor demonstrated some unique advantages that were not accessible with traditional metal sensors. At the same time, this SERS biosensor was proved to be useful for determination of Pb^{2+} in human serum.

Experiment

Materials and regents

The DNAs purified by HPLC and used in the experiments were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The nucleotide sequences are shown below: DNAzyme sequence, 5′-TCATCTCTTCTCCG AGCCGGTCGAAAGTG-3′; Cy3-labeled DNA substrate (DS), 5′-Cy3-ACTrAGGAAGAGATGA-SH-3′ [32]. Gold chloride trihydrate ($HAuCl_4$), sodium borohydride ($NaBH_4$), polyethylene glycol-6000 (PEG-6000), branched polyetherimide (PEI, MW 25 kDa), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), ethylenediaminetetraacetic acid (EDTA), and *p*-aminothiophenol (p-ATP) were procured from Sigma-Aldrich (St. Louis, MO). Ethylene glycol (EG), ethanol, ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium acetate (NaAc), silver nitrate ($AgNO_3$), trisodium citrate (Na_3Ct), hexadecyltrimethylammonium bromide (CTAB), ascorbic acid (AA), and hydrochloric acid (HCl) were procured from Shanghai SinopharmChemical Reagent Co., Ltd. (Shanghai, China). All of these reagents were analytical grade. Ultrapure water (18.2 MΩ cm^{-1}) was used throughout the study.

Instruments

The morphologies of the Au@Ag NPs and Fe_3O_4 MNPs were characterized by transmission electron microscopy (TEM) and scanning electron microscopy (SEM, S-4800, Hitachi, Japan). Energy-dispersive X-ray (EDX) spectra and high-resolution transmission electron microscopy (HRTEM) images were obtained from a JEOL-2010 microscope (200 kV). The crystalline structure was collected on a Philips X-Pert Pro X-ray diffractometer (XRD) with Cu Ka radiation ($\lambda K\alpha 1$ = 1.5418 Å). Ultraviolet–visible (UV–vis) absorption spectra were collected on a Shimadzu 2550 spectrometer. SERS measurements were performed using a portable Raman system B&W Tek, i-Raman Plus BWS465-785H spectrometer at 785 nm and a laser power of 30 mW.

Synthesis of Fe_3O_4 MNPs

The Fe_3O_4 magnetic nanoparticles (Fe_3O_4 MNPs) were prepared through a modified solvothermal reaction as previously reported [33]. Typically, 1.35 g $FeCl_3{\cdot}6H_2O$ and 1 g PEG were added into 40 mL of ethylene glycol under stirring. Subsequently, 3.6 g NaAc was added into the mixture under stirring to form a homogeneous solution. Afterward, the prepared solution was transferred to a Teflon-lined stainless autoclave, heated to 200 °C for 10 h, and then cooled to room temperature. Finally, the final products were washed with water and ethanol several times and dried at 60 °C for 10 h.

Preparation of Au@Ag NPs

AuNPs of 30 nm size were synthesized according to a previous report with slight modifications [34]. Briefly, 100 mL of 0.01% $HAuCl_4$ solution was heated to boiling point under

vigorous stirring and 1 mL of 1.0% trisodium citrate solution was quickly added into the reaction mixture under stirring. The mixture was refluxed for about 30 min until the color became wine red and then cooled to room temperature. The Au@Ag NPs were synthesized as follows: 1.5 mL of 0.1 M ascorbic acid and 1 mM $AgNO_3$ solution (0.5, 1.5, 2.5 mL) were mixed into 10 mL Au NPs solution under vigorous stirring. After the solution was stirred for 30 min, the wine red color of the solution turned orange yellow, and the Au@Ag NPs were obtained. A series of Au@Ag core-shells with Ag shell thickness of 1, 3, and 5 nm were obtained.

Preparation of Fe_3O_4@Au@Ag NPs

The Fe_3O_4@Au@Ag NPs were synthesized by two simple steps. First, the Fe_3O_4@PEI was synthesized according to the previous report with minor modification [35]. Typically, 40 mg Fe_3O_4 MNPs was added into 40 mL PEI solution (10 mg/mL) under stirring for 2 h. The obtained Fe_3O_4@PEI were separated by a magnet and washed with deionized water to remove excess PEI. Second, 10 mg Fe_3O_4@PEI was added into 100 mL of Au@Ag NPs aqueous solution and stirred for 30 min to form Fe_3O_4@Au@Ag NPs. Finally, the products were magnetically collected from excess Au@Ag NPs colloid solution and washed with deionized water four times.

Preparation of DNAzyme-based SERS biosensor

The thiolated probe (DS) was immobilized on the surface of Fe_3O_4@Au@Ag NPs via Ag–S bonds. In detail, 90 μL DNAzyme (10 μM), 30 μL thiolated probe (10 μM), and 20 μL TCEP (5 mM) were added into 50 mM Tris-HCl (pH 7.0), and the mixture was incubated at room temperature for 1 h. Subsequently, this solution was added into 1.5 mL Fe_3O_4@Au@Ag NPs (1 mg/mL) solution and NaCl was added to make a final concentration of 0.3 M. After incubation at 4 °C for 24 h, the DS-Fe_3O_4@Au@Ag NPs were gathered by magnet and stored at 4 °C in Tris-HCl buffer.

Quantitative detection of Pb^{2+} ions

The as-prepared SERS biosensor was immersed into different Pb^{2+} concentrations at room temperature for 40 min. The solution was separated by a magnet to remove Cy3-DNA dissociated from the Fe_3O_4@Au@Ag NPs surface and redispersed into doubly distilled water. SERS measurements were performed using a portable Raman instrument (B&W Tek, USA) equipped with a 785-nm laser for a total acquisition of 15 s.

Results and discussion

Principle of SERS strategy for lead ion detection

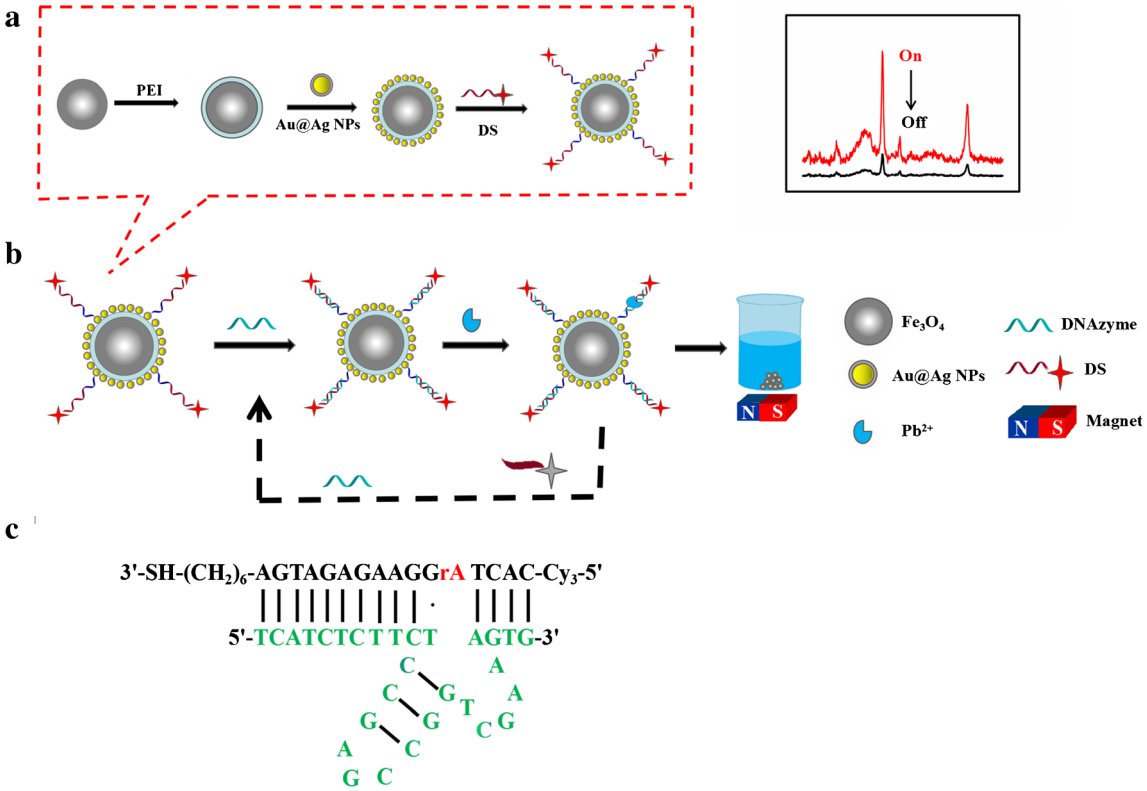
As illustrated in Scheme 1, the DNAzyme-based SERS biosensor consisted of three parts: Fe_3O_4@Au@Ag MNPs, DNAzyme strand, and the cleavage substrate (DS). Herein, the substrate strands of DS were labeled with Cy3 fluorophores at the 5′ end and thiol group (SH) at the 3′ end. Scheme 1A shows illustrates the preparation of a thiolated-DS embedded on Au@Ag NPs. The cleavage substrate (DS) was assembled on Fe_3O_4@Au@Ag MNPs through the Ag–S bonds, and a powerful Raman intensity was yielded by the approach even between Au@Ag NPs and Cy3. Scheme 1B demonstrates the principle of the DNAzyme-based SERS biosensor for detection of Pb^{2+}. This strategy is based on formation of double-stranded DNA (dsDNA) when the DNAzyme strands were added to the DS-Fe_3O_4@Au@Ag MNPs solution, and they will hybridize with DS. On the other hand, in the presence of the Pb^{2+} ions, the DNAzyme could cleave the DS at the adenine ribonucleotide (rA) site into two parts, causing the release of the Cy3-labled short fragment and resulting in decrease of SERS intensity. Scheme 1C shows the secondary structure of the 8–17 DNAzyme hybridized with the Cy3-labeled substrate strand (DS). Thus, the concentration of Pb^{2+} is investigated by monitoring the Cy3 Raman intensity.

Feasibility of DNAzyme-based SERS biosensor

In order to confirm the feasibility of the DNAzyme-based SERS biosensor for Pb^{2+} detection, the SERS signal response for Pb^{2+} was validated. As shown in Fig. 1, no SERS signal is detected when there is no Cy3-DNA probe on the surface of Fe_3O_4@Au@Ag NPs (curve a). Hence to assess the DNAzyme cleaving ability, the SERS intensity due to the Cy3-labeled DNA in close proximity to the surface of Fe_3O_4@Au@Ag NPs (curve b) was observed without Pb^{2+}. Typical Raman peaks assigned to Cy3 at 1078, 1180, and 1586 cm^{-1} were observed. Upon addition of 10 nM Pb^{2+} ions to the mixture, the Raman intensity at 1078 cm^{-1} (Cy3) increased after incubation for 60 min (curve c). These results demonstrate that the SERS biosensor using Cy3-DNA probe as a Raman reporter is feasible for quantitative detection of Pb^{2+}.

Characterization of Fe_3O_4 MNPs

Images of the as-synthesized products are shown in Fig. 2. As shown in the SEM image, Fe_3O_4 magnetic nanoparticles (Fe_3O_4 MNPs) with a diameter of approximately 400 nm were synthesized (Fig. 2A). It has been reported that hydrophilic PEI could quickly self-assemble on the surface of Fe_3O_4 particles to form a thin shell and improve the dispersity of Fe_3O_4



Scheme 1 Fabrication of DNAzyme-based SERS biosensor (**A**) and a SERS sensing protocol for Pb^{2+} (**B**). **C** Structure of Pb^{2+}-specific DNAzyme

MNPs [36]. Consistently, the diameter of Au NPs was 30 nm according to HRTEM (Fig. 2B). Further characterization of the Au@Ag NPs by HRTEM (Fig. 2C) showed that the thickness of the silver shell was about 5 nm. The HRTEM image of Fe_3O_4@Au@Ag NPs further indicated that Au@Ag NPs are uniformly adhered to the surface of the Fe_3O_4@PEI microspheres (Fig. 2D). In order to further evaluate the elemental distribution of the composite, the elemental mapping of Ag, Au, Fe, and O were collected by energy dispersive spectrometry (EDS) area scans shown in Fig. 2E.

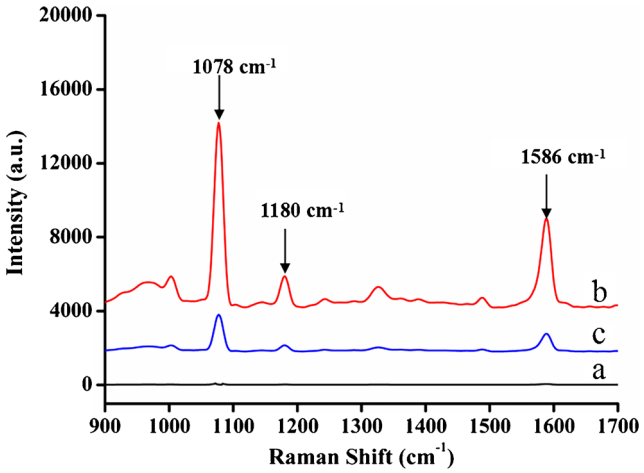


Fig. 1 **a** Raman spectra of Fe_3O_4@Au@Ag MNPs in solution. **b** Cy3-labeled DNAzyme–Fe_3O_4@Au@Ag MNPs in the absence of Pb^{2+}. **c** Raman spectra of DNAzyme-based SERS biosensor in the presence of Pb^{2+}

The TEM images showed that the average diameter of Au NPs was around 30 nm (see Electronic Supplementary Material (ESM) Fig. S1A). Subsequently, Au@Ag NPs were synthesized by the seed-mediated and surfactant-directed synthesis method. In this method, the thickness of silver was controlled by changing the amount of the $AgNO_3$ aqueous solution. The thickness of silver shells increased with the amount of $AgNO_3$, and the thickness was evaluated as 1, 3, and 5 nm in the range from 0.5 mM to 3.5 mM (ESM Fig. S1B–D). The UV–vis spectra indicated that the absorption peak of Au at 520 nm had disappeared, and the absorption peak of Ag was blue-shifted to 400 nm with the increasing of silver shell thickness, and the solution changed from red to yellow (ESM Fig. S1E). The crystal structure of as-prepared Fe_3O_4 MNPs was also determined by X-ray diffraction (XRD) (ESM Fig. S2). XRD analysis of synthesized Fe_3O_4 MNPs afforded six diffraction peaks at 2θ values of 30°, 35.4°, 43°, 53.5°, 56.9°, and 62.5° that refer to the (220), (311), (400), (422), (511), and (440) planes of the cubic inverse spinel Fe_3O_4, respectively, and all of which could be indexed to the cubic structure of Fe_3O_4 (JCPDS card no. 19–0629) [37]. The HRTEM image and EDS results confirmed the presence of gold and silver elements within the nanoparticles, proving that Au@Ag NPs were successfully synthesized (ESM Fig. S3).

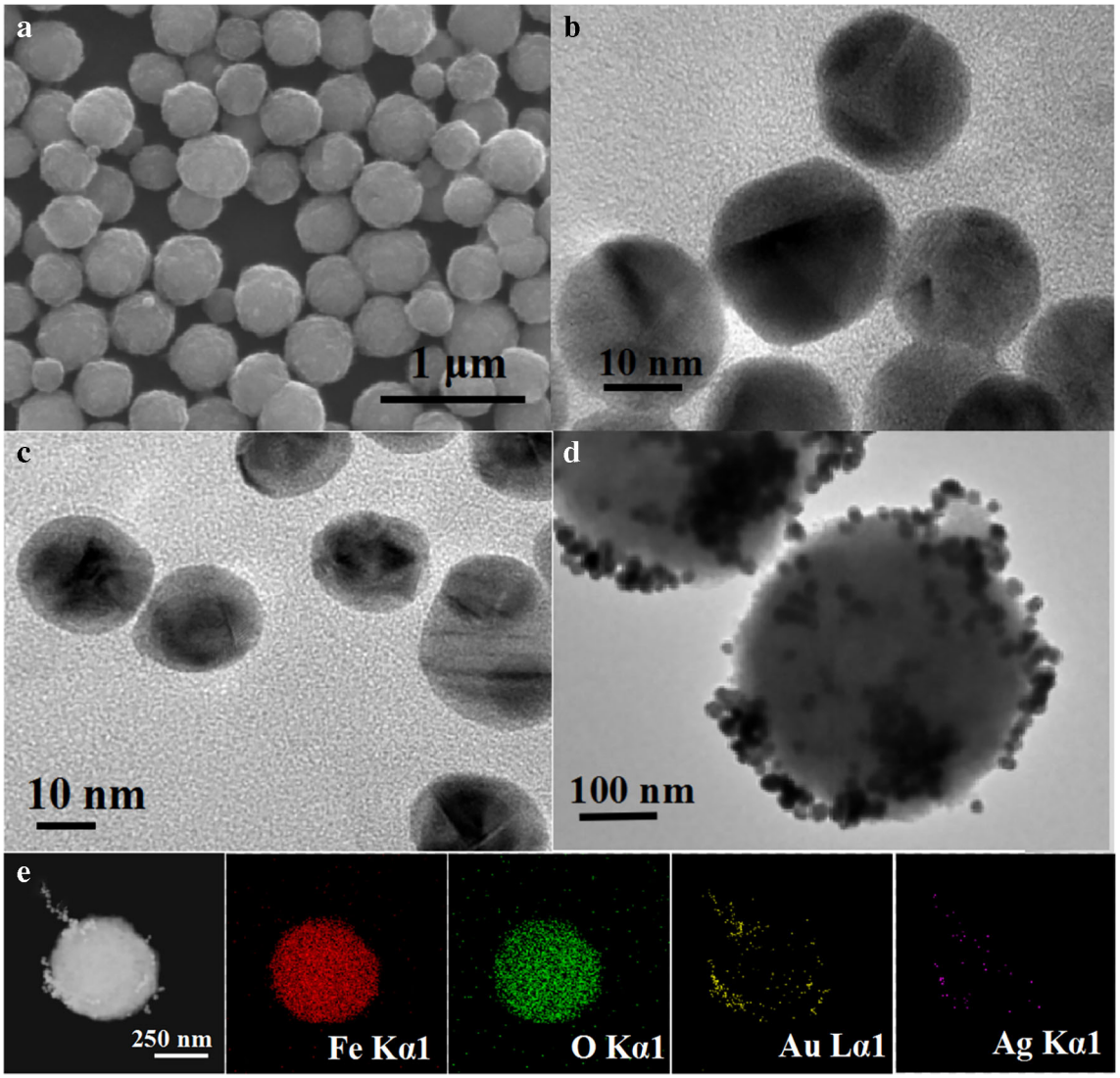


Fig. 2 **A** SEM of Fe_3O_4 NPs. **B** TEM of AuNPs, **C** Au@AgNPs, **D** Fe_3O_4@Au@Ag MNPs. **E** Elemental mapping images of Fe Kα1, O Kα1, Au Lα1, and Ag Kα1 according to **D**

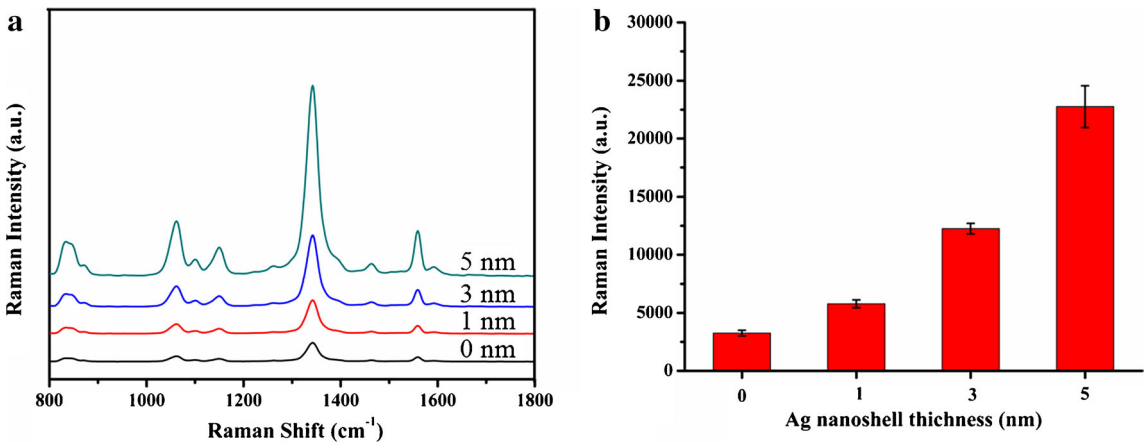


Fig. 3 **A** SERS spectra of DTNB with different silver shell thickness. **B** Corresponding Raman intensity with different silver shell thicknesses at 1338 cm^{-1} (DTNB concentration is 1×10^{-3} M in Fe_3O_4@Au@Ag MNPs colloid)

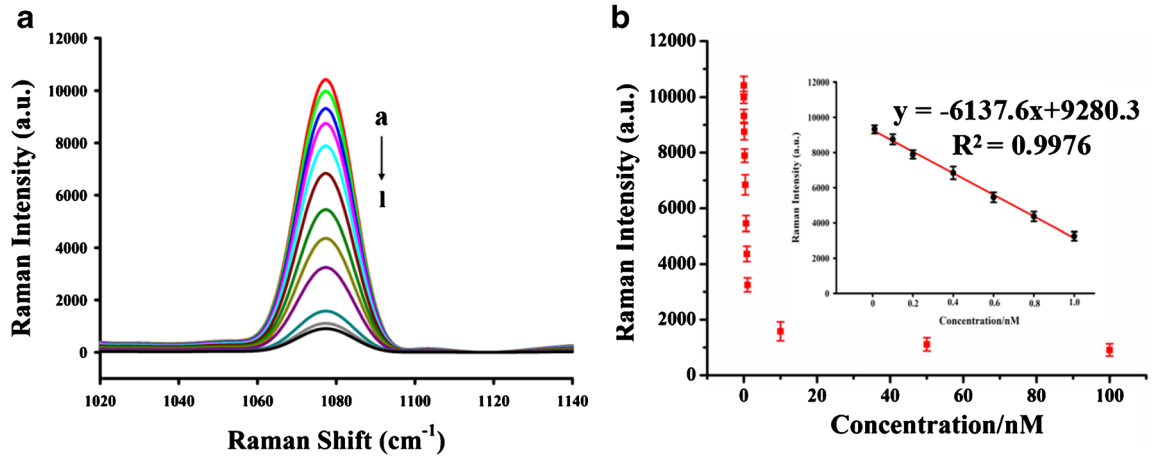


Fig. 4 **A** SERS spectra of Cy3 with a different concentration of Pb^{2+} ions: **a** 0 M, **b** 0.005 nM, **c** 0.01 nM, **d** 0.1 nM, **e** 0.2 nM, **f** 0.4 nM, **g** 0.6 nM, **h** 0.8 nM, **i** 1 nM, **j** 10 nM, **k** 50 nM, **l** 100 nM. **B** Correlation analysis of SERS intensity (at 1078 cm^{-1}) and the Pb^{2+} concentration from 0.01 to 100 nM. The inset shows a linear relationship between the value of SERS intensity (at 1078 cm^{-1}) and the Pb^{2+} concentration. Error bars represent the standard deviations of three independent assays

It is reported that Au NPs have some advantages such as long-term stability, good biocompatibility, and controllable size distribution. In contrast, Ag NPs have disadvantage of size instability but stronger Raman enhancement than that of gold. Interestingly, here we combined the advantages of both noble metals and chose Au@Ag NPs as SERS substrates to satisfy the size stability and Raman enhancement [38]. DTNB was chosen as a probe molecule to evaluate the SERS activity of Au@Ag NPs. Thus, 100 μL of DTNB (3×10^{-2} M) was added to 3 mL Au@Ag NPs colloid solution and the mixture was sonicated for 1 h to afford obvious enhancement of signals of Au@Ag NPs at the Ag shell thickness of 0 to 5 nm (Fig. 3A). Figure 3B demonstrates that the Raman intensity at 1338 cm^{-1} was chosen as the main peak; as the shell thickness of silver gradually increases, the SERS intensity became higher; and the SERS intensity was highest at a shell thickness of 5 nm. Therefore, we used the Au@Ag NPs with 5 nm Ag shell thickness as the SERS active substrates. For the detection of the SERS sensitivity of the as-prepared Fe_3O_4@Au@ Ag MNPs, a series of p-ATP alcoholic solutions were tested at different concentrations ranging from 10^{-6} to 10^{-12} M (ESM Fig. S4).

Optimization of SERS conditions

In order to achieve the best performance of the DNAzyme-based SERS biosensor, several crucial experiment parameters such as the cleavage time, the reaction temperature, and pH were investigated in this study with Pb^{2+}-specific DNAzyme. As shown in ESM Fig. S5, when 10 nM Pb^{2+} was analyzed, the Raman intensity at 1078 cm^{-1} (Cy3) decreased rapidly after the reaction time from 0 to 60 min, until it reached a steady state at 60 min; thus 60 min was chosen as the incubation time in the following experiments. It is noteworthy that the pH and temperature influence can also affect the sensing assay of biosensor systems. However, ESM Fig. S6 shows that the SERS signal only changed slightly under different pH values and temperatures. These results illustrated that the

Table 1 Comparison of the proposed SERS biosensor with other platforms for lead ion detection

Target	Method	Detection strategy	Detection limit	Reference
Lead ions	SERS	DNAzyme and DNA-modified Au NPs for signal amplification	20 nM	[39]
Lead ions/mercury ions	SERS	Oligonucleotide-functionalized and gold-coated polystyrene microspheres as signal probe	0.1 nM/1.0 nM	[40]
Lead ions	SERS	Rox-DNA functionalized AuNPs and SERS quadratic amplification	70 fM	[41]
Lead ions	SERS	Cy5-DNA and Au@Ag@Si as SERS substrate for Pb^{2+} detection	8.9 pM	[42]
Lead ions	SERS	H_2O_2–$HAuCl_4$–nanogold and rhodamine S as SERS probe	50 nM	[43]
Lead ions	SERS	DNAzyme-linked plasmonic nanomachine and substrate DNA as a spacer	1.0 nM	[44]
Lead ions	SERS	Magnetic substrate (Fe_3O_4@Au@Ag NPs) and DNAzyme as SERS signal amplification	5 pM	This work

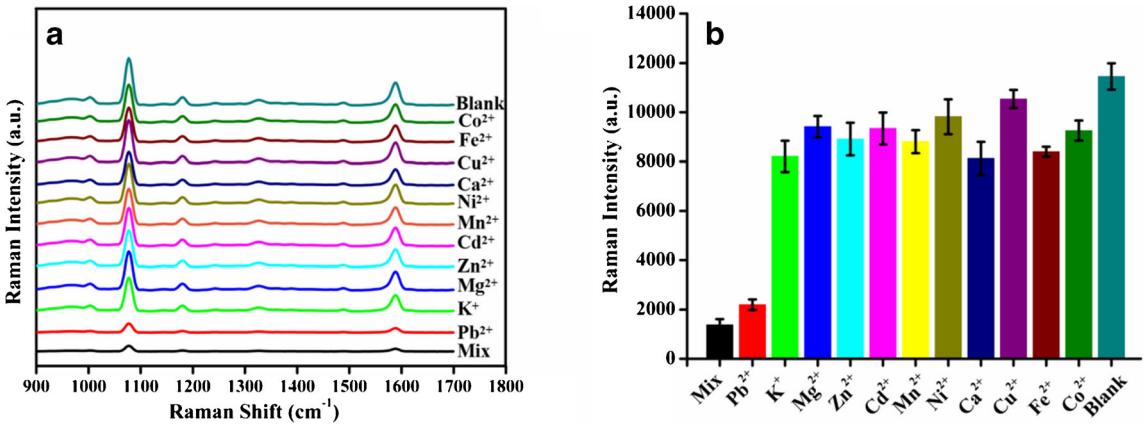


Fig. 5 Specificity of the SERS detection system for Pb^{2+}. **A** Raman spectra of DNAzyme-based SERS biosensor and **B** corresponding Raman intensity at 1078 cm^{-1} for various divalent metal ions (10 nM for Pb^{2+} and 200 nM for other metal ions) and the mixture of these metal ions (Mix). Error bars were from three repeat measurements

proposed SERS system can effectively remove the influence of the temperature and pH on the biosensor performance.

Sensitivity of DNAzyme-based SERS biosensor

To explore the DNAzyme-based SERS biosensor for quantitative SERS detection of Pb^{2+}, SERS signal was measured in aqueous buffer solution under optimized conditions. We observed a significant decrease in Raman intensity with Pb^{2+} concentrations in the range from 0.005 to 100 nM (Fig. 4A). As shown in Fig. 4B, there is a good linear relationship between Raman intensity and Pb^{2+} concentration in the range from 0.01 to 1.0 nM. The present limit of detection (LOD) for the DNAzyme-based SERS biosensor was estimated to be 5 pM (S/N = 3), which is much lower than the maximum contaminant level for Pb^{2+} in drinking water (72 nM) defined by USEPA. The linear equation is $y = -6137.6x + 9280.3$ with the correlation coefficient $R^2 = 0.9976$, where y is the normalized SERS intensity at 1078 cm^{-1} and x is the corresponding concentration of Pb^{2+} ions. These results demonstrated that the DNAzyme-based SERS biosensor assay provides a wider linear range of Pb^{2+} detection. To further explain the high performance of this platform, we compared it with existing biosensors for Pb^{2+} (Table 1). This revealed that the SERS sensing platform is efficient for quantitative and sensitive detection of Pb^{2+}.

Selectivity of DNAzyme-based SERS biosensor

To investigate the selectivity of this SERS strategy for Pb^{2+} detection, 11 metal ions (Pb^{2+}, K^+, Mg^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Ni^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}) and mixed samples containing Pb^{2+} were further tested (Fig. 5). The concentration was set as 10 nM Pb^{2+} and 200 nM for the other metal ions. As shown in Fig. 5A, Pb^{2+} and the Pb^{2+} mixture afford significant changes of SERS intensity and the other metal ions show no apparent changes of the SERS intensity. The excellent selectivity of this method is mainly due to coordination mechanisms between Pb^{2+} and DNAzyme. For direct quantitative comparison, the corresponding Raman intensities of the 1078 cm^{-1} peak of

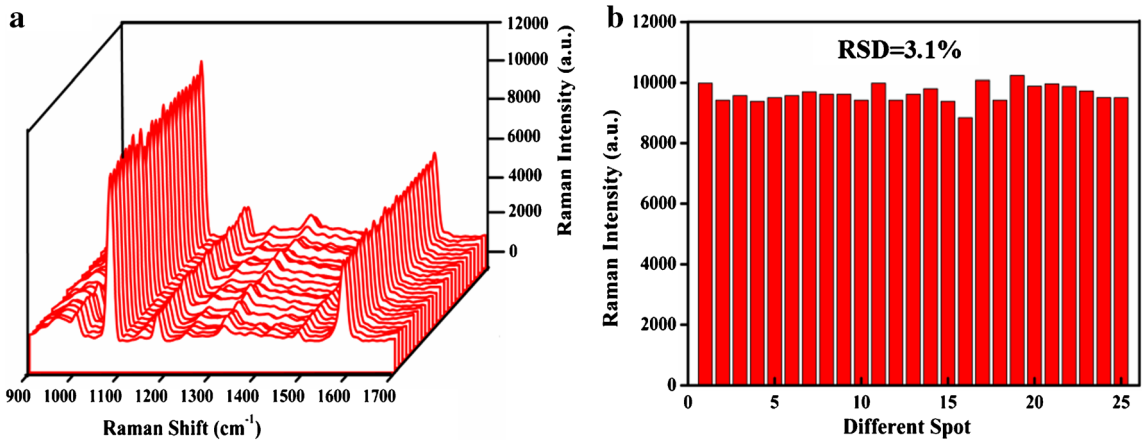


Fig. 6 **A** SERS spectrum measured from 25 different spots. **B** Raman intensity at 1078 cm^{-1} ("on" status)

Table 2 Analysis data of the DNAzyme-based SERS biosensor for Pb^{2+} determination in 5% tap water and 1% human serum ($n = 3$)

Samples	Added[a] (nM)	Mean ± SD[b] (nM)	Recovery (%)	RSD (%)
Tap water	10	9.73 ± 0.24	97.3	2.5
	50	49.47 ± 1.83	98.9	5.8
	100	101.31 ± 1.37	101.3	1.4
Human serum	10	9.24 ± 0.89	92.4	9.6
	50	48.85 ± 2.61	97.7	5.3
	100	102.61 ± 2.43	102.6	2.4

[a] Added amount of Pb^{2+} in the real samples

[b] Standard deviation

Cy3 in the presence of the aforentioned substances are shown as histograms in Fig. 5B. These results clearly demonstrated that the developed DNAzyme-based SERS biosensor exhibits a high specificity to detect Pb^{2+} over other metal ions.

Reproducibility and stability of proposed SERS strategy

It is well known that the reproducibility of SERS intensity is a critical property for DNAzyme-based SERS biosensor assays. The signal reproducibility was investigated with 25 replicate measurements of 100 pM Pb^{2+} concentration (Fig. 6A). To get a statistically significant result, the relative standard deviation (RSD) of the Raman intensity at 1078 cm^{-1} for Cy3 is estimated as 3.1%. In this assay, this DNAzyme-based SERS biosensor exhibits good reproducibility. Moreover, the developed DNAzyme-based SERS biosensor remains stable and available to use even after 30 days' storage at 4 °C. Thus, this DNAzyme-based SERS biosensor has excellent storage stability and can be used for SERS detection of Pb^{2+} (ESM Fig. S7).

Analysis of real samples

In order to investigate the analytical application and potential of the proposed biosensor for detection of Pb^{2+} in real samples, Pb^{2+} was added into 5% tap water and 1% human serum samples (collected from The Third Xiangya Hospital, Central South University, China) at a wide range of concentrations, and then incubated with the biosensor for the recovery experiments. The SERS biosensor was incubated with Pb^{2+} ion (1, 10, and 100 nM) spiked in tap water or human serum sample for 60 min. The detailed recovery results are shown in Table 2. The results displayed outstanding recoveries from 92.4% to 102.6%. Moreover, the RSD values were less than 9.6%. Therefore, these results demonstrated that the SERS strategy can be used to detect Pb^{2+} in real samples with excellent sensitivity and generally satisfactorily.

Conclusion

We developed a novel SERS biosensor for simple, reliable, selective determination of Pb^{2+} ions. The DS-Fe_3O_4@Au@Ag NPs were used as SERS magnetic substrate, which can detect target Pb^{2+} via specific recognition. In addition, the proposed SERS strategy allows the detection of Pb^{2+} with a LOD of 5 pM, which is lower than those of most other available methods. These results indicate that the DNAzyme-based SERS biosensor can be easily used for qualitative detection of Pb^{2+} in tap water and human serum samples. Therefore, the SERS strategy is a promising alternative method for highly sensitive and selective detection of heavy metal ions.

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Compliance with ethical standards

Written informed consent was obtained and this study was approved by the Ethics Committee of The Third Xiangya Hospital of Central South University (No. 2020-S233).

Conflict of interest All authors declare that they have no conflict of interest.

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