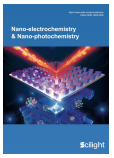
Scilight

Nano-electrochemistry & Nano-photochemistry

https://www.sciltp.com/journals/nenp



Supplementary Materials

Surface-Enhanced Raman Scattering Platform Based on ZnO@ZIF-8 Composite for the Detection of Periodontal Pathogens

Xinyi Zhang [1], Zhenhuan Liu [1], Qing Liu [1], Xuan Xu [2] and Tingting Zheng [1,*]

[1] Shanghai Key Laboratory of Green Chemistry and Chemical Processes, Department of Chemistry, School of Chemistry and Molecular Engineering, East China Normal University, Dongchuan Road 500, Shanghai 200241, China
[2] State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Xianlin Road 163, Nanjing 210093, China
* Correspondence: ttzheng@chem.ecnu.edu.cn

1. Apparatus and Instruments

Scanning electron microscopy (SEM) measurements were performed on scanning electron microscope (JSM-1788F PRIME) from Hitachi, Japan. X-Ray diffraction (XRD) patterns were performed on a D/max2550VB3+/PC X-ray diffractometer with Cu (40 kV, 100 mA) manufactured in Shanghai Jinpan Biotechnology Co. Ltd. Renishaw. XPS data were performed on Thermo ESCALAB 250XI from Thermo Fisher Scientific (USA). The UV-Vis absorption spectrum was obtained with an UV-4900 from Hitachi, Japan. Raman enhanced scanning was measured on a Renishaw Raman Microscope. Fourier transform infrared (FT-IR) spectra were examined with Fourier transform infrared spectrometer (Nicolet iS10, Thermo Fisher Scientific, USA). The magnetic stirrer made by Sh-sile (China, Shanghai) conducted all the stirring work.

2. Chemicals and Materials

Zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 6H_2O$) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); Diethylene glycol (DEG) and 4-cyanobenzoic acid were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China); N,N-dimethylformamide (DMF) was purchased from Anhui Senrise Technology Co., Ltd. (Hefei, China); 2-methylimidazole was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China); 3-Aminopropyltriethoxysilane (APTES) was purchased from Damas-beta (Shanghai, China); Chlorobenzene (CB) and Ethenylbenzene (EB) were purchased from TCI (Shanghai) Development Co., Ltd. (Shanghai, China); DNA aptamer (COOH-5′-TATGCCAGCATTTCGCCAACGGTGGTCATACAGTGTGAA-3′) and cDNA (5′-TTCACACTGTATGACCACCGTTGG-3′-NH_2) were purchased from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China); Artificial saliva was purchased from Merck KGaA (Darmstadt, Germany).

3. Synthesis of ZnO Nanoparticles

ZnO particles were synthesized using a two-step method reported previously [1]. In brief, 0.01 mol of $Zn(CH_3COO)_2 \cdot 2H_2O$ was first added to 100 mL of DEG in a glass flask. The mixture was then heated to 160 °C slowly and kept at 160 °C under magnetic stirring for 1 h for the formation of a cloudy colloidal suspension. The colloidal suspension was then cooled down to room temperature and centrifuged at 12,000 rpm for 5 min. The sediment was discarded, and the supernatant was used as a seed solution for further reactions. In a separate glass flask, another 0.01 mol of $Zn(CH_3COO)_2 \cdot 2H_2O$ was dissolved in 100 mL of DEG. The temperature of the mixture solution was slowly raised to 130 °C, followed immediately by the addition of 0.3 mL of seed solution. After that, the temperature was promptly raised to 160 °C. The mixture was kept at this temperature under magnetic stirring for 1 h for the formation of spherical ZnO nanoparticles. ZnO nanoparticles were then collected by centrifugation, followed by washing with distilled water and anhydrous ethanol several times. After that, ZnO nanoparticles were dried at 60 °C for 12 h in a vacuum drier before use.

4. Synthesis of ZnO@ZIF-8 Nanoparticles

0.0204 g of as-synthesized ZnO nanoparticles were first added to 16 mL of DMF/H2O (10:1 in volume ratio) solution dissolved with 0.11 g of 2-methylimidazole. The mixture was ultrasonicated for 10 min to disperse ZnO nanoparticles. After that, the mixture was transferred to an oil bath which is preheated to 70 °C. ZnO@ZIF-8 nanoparticles were formed after reacting at 70 °C for 3 h. They were collected by centrifugation and washed with DMF and ethanol several times. After that, ZnO@ZIF-8 nanoparticles were dried at 60 °C for 12 h in a vacuum drier before use.

5. Synthesis of ZIF-8 Nanoparticles

ZIF-8 was prepared using a modified method reported previously [2]. In brief, solution A was prepared by dissolving 9 mmol of $Zn(CH_3COO)_2 \cdot 2H_2O$ in 80 mL of methanol. Solution B was prepared by dissolving 36 mmol of 2-methylimidazole in 100 mL of methanol. After that, solution A was poured into solution B under intense magnetic stirring. The mixture was then heated at 50 °C under magnetic stirring in an oil bath for 2 h for the formation of ZIF-8 nanoparticles. As-prepared ZIF-8 nanoparticles were collected by centrifugation and washed with methanol several times before use.

6. P-NTP Detection (Liquid Phase) on ZnO@ZIF-8 Nanoparticles

0.5 mg of ZnO@ZIF-8 nanoparticles were dispersed in 5 mL of pNTP solution (ethanol) of a certain concentration. The mixture was incubated for 2 h. ZnO@ZIF-8 nanoparticles were then collected by centrifuge and transferred onto the stage of the Raman microscope for direct SERS measurement.

7. Synthesis of Amino-Modified ZnO@ZIF-8

Add an appropriate amount of ZnO@ZIF-8 to 5 mL of ethanol solution containing 2 mL of APTES and gently shake for 6 h. After the reaction is completed, centrifuge with anhydrous ethanol, wash 2–3 times, and finally freeze-dry for 12 h.

8. SERS Measurements

A 532 nm laser was used for all the measurements. In the vitro experiment, a 50× (NA 0.5) microscope objective with a working distance of 0.38 mm and spot-focused laser was used. The laser power and acquisition time were 10 mW and 5 s, respectively.

9. Construction and Detection of the SERS Platform for *P. gingivalis*

First, dissolve 5 mg of amino-functionalized ZnO@ZIF-8 in 3 mL of ultrapure water, and while stirring, add 30 μL of the aptamer. Stir for 1 h to obtain solution A. Meanwhile, dissolve 10 mg of 4-cyano-benzoic acid in 3 mL of ultrapure water, and while stirring, add 30 μL of cDNA. Stir for 1 h to obtain solution B. Mix solution A with solution B, and stir for 2 h to obtain solution C. The SERS platform is successfully established. Add a sample of *P. gingivalis*. The cell concentration of the *P. gingivalis* culture was evaluated by measuring the optical density at 600 nm (OD_{600}), and the measured value was 0.403. Based on the reported concentration conversion standard for *P. gingivalis* (i.e., an OD_{600} of 0.1 corresponds to approximately 1×10^8 CFU/mL), the viable cell concentration of the culture was calculated to be 4×10^8 CFU/mL. To establish a series of concentration gradients, the aforementioned bacterial suspension was subjected to serial gradient dilution, resulting in 6 groups of bacterial suspensions with 6 different concentrations (from 4×10^3 CFU/mL to 4×10^8 CFU/mL) in decreasing order of concentration. All diluted bacterial suspensions were placed in a constant-temperature stirring device and continuously stirred for 2 h.

10. Detection of the SERS Platform for *P. gingivalis* (in Artificial Saliva Solution)

3 mL of solution C from the aforementioned concentration gradient system was pipetted and mixed thoroughly with 3 mL of artificial saliva. Add a sample of *P. gingivalis*. After uniform oscillation of the mixed system, the optical density at 600 nm (OD_{600}) was measured using a UV-visible spectrophotometer, yielding a result of 0.367. To further establish a multi-concentration analysis model, the mixed solution was subjected to systematic gradient dilution, sequentially preparing 6 groups of *P. gingivalis* suspensions with different concentrations (from 4×10^3 CFU/mL to 4×10^8 CFU/mL), decreasing in order. All diluted bacterial suspensions were placed in a constant-temperature stirring device and continuously stirred for 2 h.

11. Construction of SERS Platform

As shown in Figure S1, amino groups were first modified on the surface of ZnO@ZIF-8. In the Fourier transform infrared spectroscopy (FT-IR) (Figure S1), a new strong absorption peak at 3320 cm^{-1} appeared in the aminated ZnO@ZIF-8, which was different from ZnO@ZIF-8 and corresponded to the stretching vibration of the N-H bond, proving the successful modification of amino groups on the surface of ZnO@ZIF-8 and obtaining the aminated modified ZnO@ZIF-8. Then, the DNA aptamer of *P. gingivalis* with carboxyl groups modified at the 5′ end was added. Through the chemical reaction of amino and carboxyl groups forming amide bonds, the DNA aptamer was stably fixed on the substrate. On the other hand, 4-cyanobenzoic acid with carboxyl groups was selected as the signal molecule. The molecule structure included a cyano group, and its characteristic fingerprint peak was located in the "Raman biological silent zone" (1800–2800 cm^{-1}). It was combined with the cDNA chain modified with amino groups at the 3′ end through amide bonds. Next, the cDNA chain modified with the signal molecule was added to the ZnO@ZIF-8 substrate connected with the aptamer. DNA and cDNA were connected into a double helix structure through base complementary pairing, and the signal molecule 4-cyanobenzoic acid appeared in the electromagnetic enhancement area of ZnO@ZIF-8, and an obvious Raman signal enhancement could be observed. Finally, *P. gingivalis* was added, which specifically bound to the aptamer and occupied the position of the cDNA chain. The cDNA chain connected with the signal molecule 4-cyano-benzoic acid was released from the aptamer, and a significant decrease in Raman signal could be observed. In addition, the UV-Vis spectra are shown in Figures S2–S4. In Figure S2, two obvious absorption peaks at 211 nm and 228 nm were observed, which belonged to the aminated ZnO@ZIF-8. The complex, a composed of aminated ZnO@ZIF-8 and the DNA aptamer, also had two absorption peaks at 225 nm and 246 nm. Compared with the former, the two absorption peaks had the same height but were obviously shifted, confirming that the amino groups on the aminated ZnO@ZIF-8 and the carboxyl groups on the aptamer formed amide bonds through chemical reactions and stably fixed the DNA aptamer on the substrate. In Figure S3, from 312 nm, the peaks of the signal molecule 4-cyanobenzoic acid and the complex b composed of 4-cyano-benzoic acid and cDNA began to deviate, confirming that the cDNA and the signal molecule 4-cyano-benzoic acid were successfully connected through the chemical reaction of amino and carboxyl groups, forming amide bonds. In Figure S4, a significant absorption peak at 368 nm was observed, which belonged to the complex a composed of aminated ZnO@ZIF-8 and aptamer; while in the range of 210 nm-293 nm, the UV absorption peaks were consistent with those of the complex b composed of cDNA and the signal molecule 4-cyano-benzoic acid, indicating that a and b were successfully connected, confirming the successful construction of the SERS platform.

12. The Infrared Spectra of ZnO@ZIF-8 and Amino Modified ZnO@ZIF-8

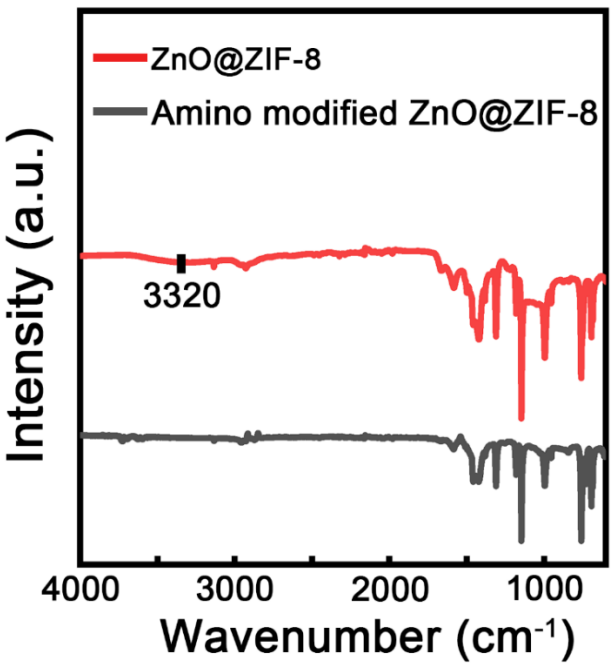


Figure S1. The Infrared spectra of ZnO@ZIF-8 and amino modified ZnO@ZIF-8.

13. The UV-Vis Absorption Spectra of Amino Modified ZnO@ZIF-8 Complex with DNA Aptamer and Amino Modified ZnO@ZIF-8

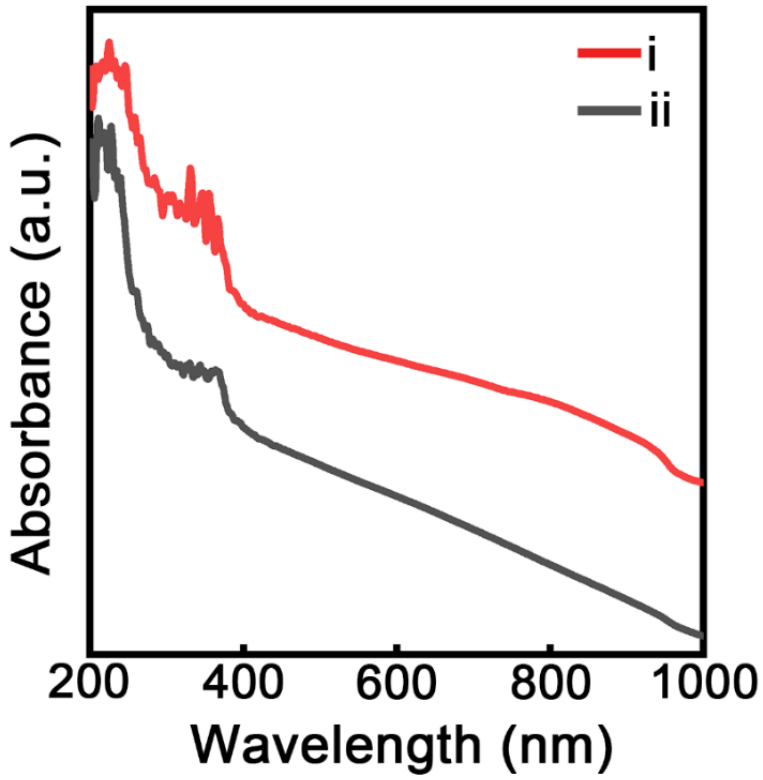


Figure S2. The UV-Vis absorption spectra of (**i**) amino modified ZnO@ZIF-8 complex with DNA aptamer and (**ii**) amino modified ZnO@ZIF-8.

14. The UV-Vis Absorption Spectra of 4-Cyanobenzoic Acid Complex with cDNA and 4-Cyanobenzoic Acid

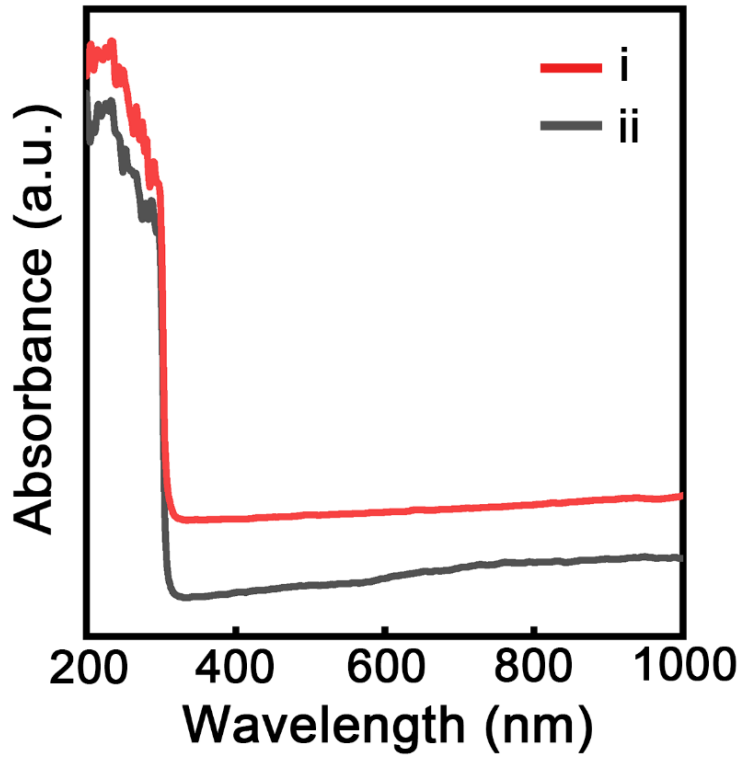


Figure S3. The UV-Vis absorption spectra of (**i**) 4-cyanobenzoic acid complex with cDNA and (**ii**) 4-cyanobenzoic acid.

15. The UV-Vis Absorption Spectra of Amino Modified ZnO@ZIF-8 Complex with DNA Aptamer, Complex with 4-Cyanobenzoic Acid and cDNA, and SERS Platform

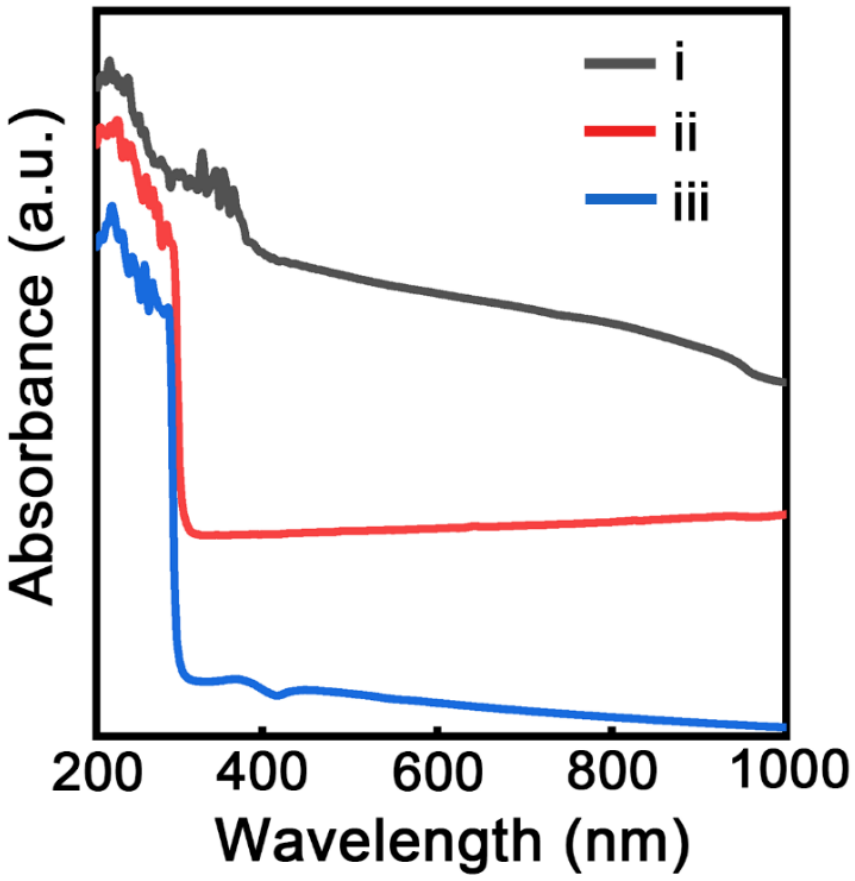


Figure S4. The UV-Vis absorption spectra of (**i**) amino functionalized ZnO@ZIF-8 complex with DNA aptamer; (**ii**) complex with 4-cyanobenzoic acid and cDNA; and (**iii**) SERS platform.

16. The SEM Image of ZnO

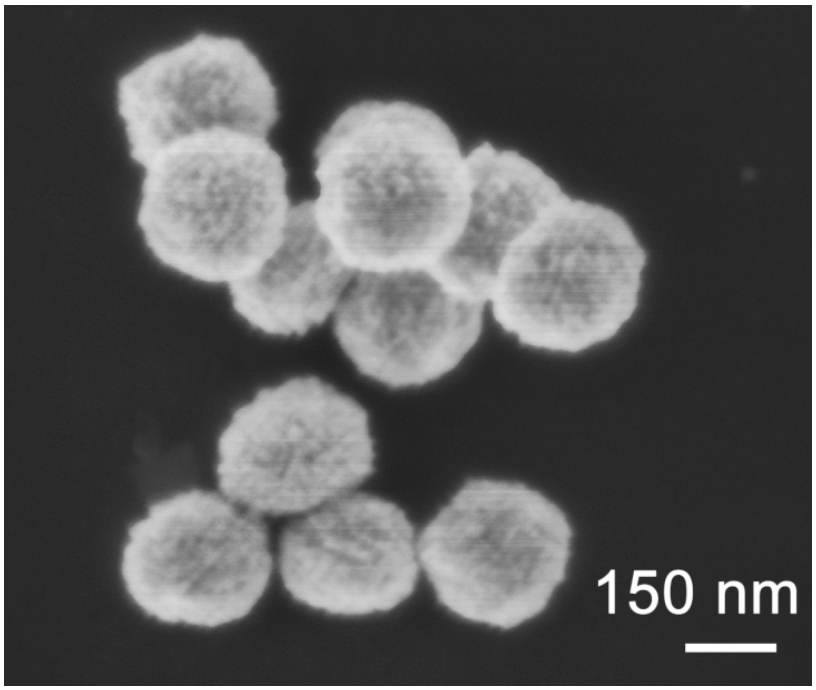


Figure S5. SEM image of ZnO.

17. The SEM Image of ZIF-8

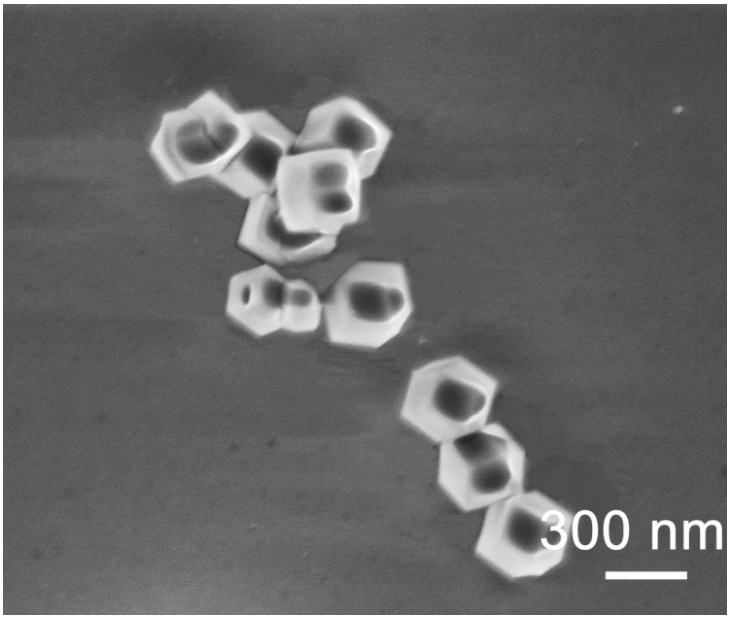


Figure S6. SEM image of ZIF-8.

18. Enhancement Factor Calculations for 4-Cyanobenzoic Acid on ZnO@ZIF-8

EF value was calculated based on the following formula:

$$EF = (\frac{I_{surf}}{N_{surf}})/(\frac{I_{bulk}}{N_{bulk}}) \quad \text{(S1)}$$

I_{surf} represents the Raman signal intensity of the measured molecule in the SERS region, I_{bulk} represents the Raman signal intensity of the measured molecule in the blank region, N_{surf} represents the number of molecules adsorbed on the SERS substrate surface during the SERS test, and N_{bulk} represents the number of signal molecules in the blank region. According to the formula, the spot diameter $d = 1.22\ \lambda/NA$, the spot area $A = \pi(d/2)^2$, and the molecule number $N = nN_AA/S$. Substituting the *EF* value calculation formula, the *EF* value was calculated to be approximately 5.3×10^4. The calculation results show that the ZnO@ZIF-8 substrate has a significant Raman signal enhancement effect on the 4-cyanobenzoic acid signal molecule, with an enhancement factor of up to 5.3×10^4, proving that this composite material has good SERS activity.

19. LOD Calculation

As shown in Figure 3C in manuscript, the linear relationship between SERS intensity ratio I_{2232}/I_{1455} and *P. gingivalis* concentration is $y = -0.9981x + 10.6781$.

The standard curve was given as Equation (2), where *A* and *B* were the variables obtained via least-square root linear regression for the signal concentration curve, and variable *Y* represented the normalized signal at *P.gingivalis* concentration of C. SD was the standard deviation, and Y_{blank} is the signal of the blank sample. The linear regression was shown in Equation (4)

$$Y = A + B \times \log C \quad \text{(S2)}$$

$$SD = \sqrt{\frac{1}{n-1} \times \sum_{i}^{n} (x_i - x_{average})^2} \quad \text{(S3)}$$

$$LOD = 10^{[(Y_{blank}+3SD)-A]/B} \quad \text{(S4)}$$

For the photothermal method, *Y* represents the normalized ratiometric SERS signal (I_{2232}/I_{1455}), and the linear regression was: $Y = 10.6781 - 0.9981 \times \log C$. $A = 10.6781$, $B = -0.9981$, $Y_{blank} = 7.18$, $SD = 0.64$. The *LOD* was calculated to be 37.98 CFU/mL ≈ 38 CFU/mL.

20. RSD Calculation for Stability and Repeatability for SERS Detection Platform

As shown in Figure 3F, selecting Raman peak at 2232 cm^{-1} as the internal standard peak, the RSD value of Raman peak intensity for stability evaluation was calculated to be 8.532% using the shown RSD calculation formula. Data are as follows: 8477.04492, 8261.03223, 8029.47315, 7507.19190, 7249.86231, 7010.59668, 6780.86084, 6886.96924, 6918.75147, 6889.88965, 6818.08545.

As shown in Figure 3G, the ratio of Raman peak intensity remained nearly stable, the RSD value for stability evaluation was calculated to be 1.476%. Data are as follows: 7.24667, 7.16333, 7.09333, 7.20333, 7.05333, 6.91333, 7.11333, 7.25333, 7.15, and 7.03.

$$RSD = \frac{S}{x} \times 100\% = \frac{1}{x_{average}} \times 100\% \times \sqrt{\frac{1}{n-1} \times \sum_{i}^{n} (x_i - x_{average})^2} \quad \text{(S5)}$$

21. The Influence of Environmental Temperature on SERS Detection Process

Add a sample of *P. gingivalis* in 15 mL of solution C (seen in manuscript 2.6). After uniform oscillation of the mixed system, the optical density at 600 nm (OD_{600}) was measured using a UV-visible spectrophotometer, yielding a result of 0.507. Dilute the sample to a concentration of around 10^5 CFU/mL. Divide the above samples evenly into 15 portions, with 3 samples in each group. By using refrigeration and a constant-temperature water bath for insulation, the temperatures of groups 1 to 5 were respectively controlled at 4 °C, 25 °C, 37 °C, 45 °C, and 60 °C respectively. Measure the Raman signal values of each group and obtain the signal ratio at I_{2232}/I_{1455}. The stability of the platform varies with temperature is illustrated as Figure S7.

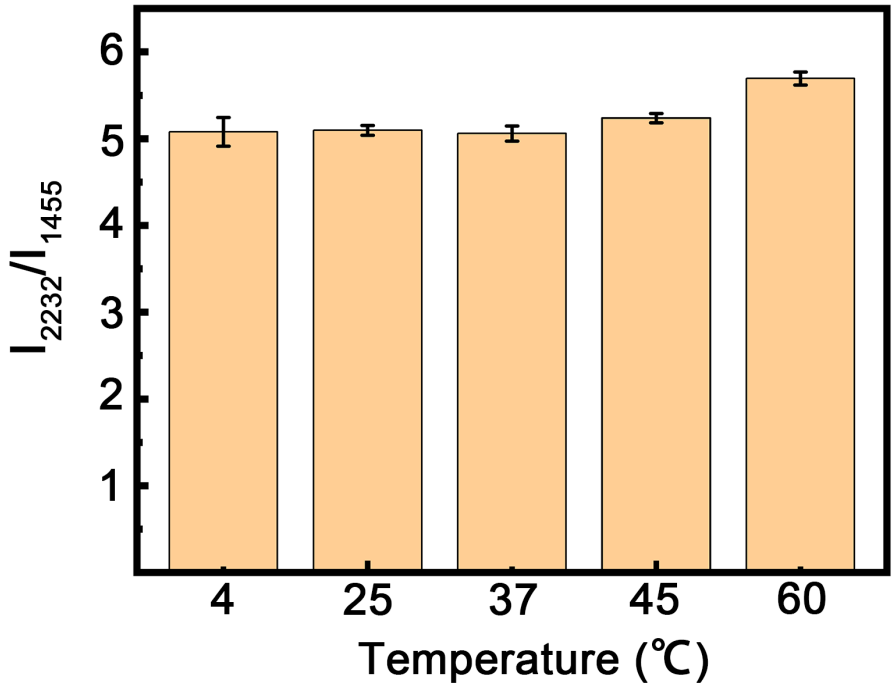


Figure S7. The stability of the platform varies with temperature.

22. The Influence of Environmental pH on SERS Detection Process

The sample preparation process and signal measurement process were the same as those in Section 21. The pH of the mixture was adjusted by the addition of 0.5 mol/L NaOH or 0.5 mol/L HCl, and was monitored in real time using a pH meter. The stability of the platform varies with pH is illustrated as Figure S8.

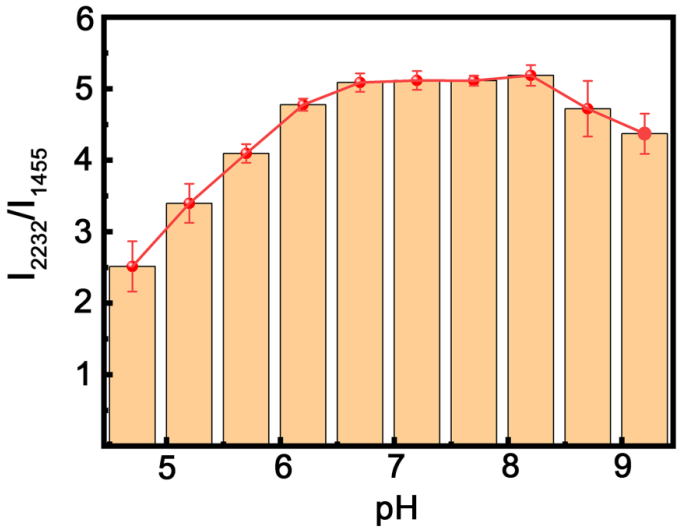


Figure S8. The stability of the platform varies with pH.

23. The Spike Recovery Experiment of *P. gingivalis*

Take 1 mL of the *P. gingivalis* solution with a known concentration of 4×10^5 CFU/mL, and mix it with 0.01 M PBS buffer solution at a volume ratio of 1:4. Then, the spiked sample concentration will be 8×10^4 CFU/mL. Next, prepare a solution of the same total volume using the same concentration of PBS buffer solution as the blank sample. Conduct the detection using the SERS platform that has been set up as described above. The concentration of *P. gingivalis* in the spiked group could be detected by the built SERS platform at 74,404, 82,148, 83,000 CFU/mL respectively while the concentration in the blank group accordingly were 158, 404, 275 CFU/mL. Recovery (%) = $[(C_1 - C_0)/C_a] \times 100\%$. The experiment was repeated three times, and the relative standard deviation (RSD) of the recovery was calculated. According to the calculation, the recovery of *P. gingivalis* using this method ranges from 92% to 103% with an RSD = 5.93% < 6%.

24. Comparison to the Common Detection Methods

Table S1. Comparison of the common methods for *P. gingivalis* detection.

Method	LOD	Linear range	Stability	Ref.
Culture	1000 CFU/mL	10^3–10^4 CFU/mL	/	[3]
PCR	300 CFU/mL	10^3–10^4 CFU/mL	/	[4]
Real-time PCR	1000 copies/mL	1×10^3–1×10^6 copies/mL	7 days	[5]
MB-LAMP	1×10^{-10} ng/μL	1×10^{-7}–1×10^{-1} ng/μL	/	[6]
This work	38 CFU/mL	4×10^3–4×10^8 CFU/mL	60 days	

25. Comparison of the SERS Substrates

Table S2. Comparison of the EF & LOD values for ZnO as different SERS substrates.

Method	EF Value	Substrates	LOD	Ref.
SERS	10^3	one-dimensional semiconducting ZnO nanostructures (nanowires and nanocones)	/	[7]
SERS	10^3	ZnO nanocrystals	/	[8]
SERS	10^4	ZnO films with {0001} and {10$\bar{1}$1}	1×10^{-6} M and 5×10^{-7} M	[9]
This work	5.3×10^4	Spherical ZnO nanoparticles	38 CFU/mL	

References

1. Ji, W.; Li, L.; Song, W.; et al. Enhanced Raman Scattering by ZnO Superstructures: Synergistic Effect of Charge Transfer and Mie Resonances. *Angew. Chem. Int. Ed.* **2019**, *58*, 14452–14456.
2. Wang, W.; Deng, C.; Xie, S et al. Photocatalytic C-C Coupling from Carbon Dioxide Reduction on Copper Oxide with Mixed-valence Copper(I)/Copper(II). *J. Am. Chem. Soc.* **2021**, *143*, 2984–2993.

3. Gomes, B.P.F.A.; Pinheiro, E.T.; Gadê-Neto, C.R.; et al. Microbiological Examination of Infected Dental Root Canals. *Mol. Oral Microbiol.* **2004**, *19*, 71–76.
4. Gu, B.L.; Qi, Y.J.; Kong, J.Y.; et al. An Evaluation of Direct PCR Assays for the Detection and Quantification of *P. gingivalis*. *Epidemiol. Infect.* **2020**, *148*, e107.
5. Ingalagi, P.; Bhat, K.G.; Kulkarni, R.D.; et al. Detection and Comparison of Prevalence of *Porphyromonas gingivalis* through Culture and Real Time-polymerase Chain Reaction in Subgingival Plaque Samples of Chronic Periodontitis and Healthy Individuals. *J. Oral Maxillofac. Pathol.* **2022**, *26*, 288.
6. Su, Y.X.; Huang, S.M.; Hong, L.; et al. Establishment of the Molecular Beacon-loop-mediated Isothermal Amplification Method for the Rapid Detection of *P. gingivalis*. *J. Microbiol. Methods* **2019**, *160*, 68–72.
7. Shin, H.Y.; Shim, E.L.; Choi, Y.J.; et al. Giant enhancement of the Raman response due to one-dimensional ZnO nanostructures. *Nanoscale* **2014**, *6*, 14622–14626.
8. Wang, Y.; Ruan, W.; Zhang, J.; et al. Direct observation of surface-enhanced Raman scattering in ZnO nanocrystals. *J. Raman Spectrosc.* **2009**, *40*, 1072–1077.
9. Luo, Y.W.; Niu, L.Y.; Wang, Y.F.; et al. Experimental and theoretical evaluation of crystal facet exposure on the charge transfer and SERS activity of ZnO films. *Nanoscale* **2022**, *14*, 16220–16232.