Supporting Information

Dual-Responsive Polydopamine-Embellished Zn-MOFs Enabling Synergistic Photothermal and Antibacterial Metal Ion Therapy for Oral Biofilm Eradication

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Table of Contents

Methods

S1 Characterization

To analyze the morphology and size distribution of nanoparticles, we employed transmission electron microscopy (TEM, Hitachi, Japan) and field scanning electron microscope (SEM, model XL-30 from the Netherlands) equipped with an energy-dispersive X-ray spectrometer (EDS). The crystalline structures of ZIF-8 NPs and ZIF-8@PDA NPs were examined using X-ray diffraction (XRD, model D8-ADVANCE from Germany) with Cu Kα radiation as the light source. The X-ray photoelectron spectroscopy (XPS) was conducted with a Shimadzu AMICUS XPS spectrometer (model smartlab) using monochromatized Al Kα excitation, and the binding energies were calibrated against contaminant carbon (C1s = 284.6 eV). The operating conditions for the XRD were set at 40 kV and 40 mA. The Bruker ALPHA FT-IR spectrometer was utilized to obtain the Fourier-transform infrared (FT-IR) spectra of the samples. UV-vis absorption spectra were recorded using a TU-1900 UV-Vis spectrophotometer (Persee Analytics, China). The confocal images of cells were captured using a confocal laser fluorescence scanning microscope (Nikon, Japan).

S2 Antibacterial activity *in vitro*

S. mutans was cultured in Brain Heart Infusion (BHI) broth at 37°C. The bacterial suspensions (1×10^6 CFU mL^{-1}) were subjected to various treatments, as shown in Table S1. Subsequently, all the samples were incubated at 37°C for 6 h. After treatment, bacterial suspensions were diluted 10^4 times with the culture medium. Then, 100 μL of each suspension was spread on BHI agar plates and incubated at 37°C for 24 h. The number of colony-forming units (CFUs) was then determined. To visualize microbial morphology, 100 μL of bacterial suspensions after various treatments were fixed with glutaraldehyde for 12 h, dehydrated using a gradient ethanol method, and observed with SEM on a silicon wafer.

Table S1. Abbreviations and brief descriptions of experimental groups.

Groups	Treatments	Concentration and irradiation
I	Control	The concentrations of PBS were 0.8 μg mL^{-1}
II	Zn^{2+}	The concentrations of $Zn(OAc)_2 \cdot 2H_2O$ were 20 μg mL^{-1}
III	ZIF-8 NPs	The concentrations of ZIF-8 NPs were 200 μg mL^{-1}
IV	PDA NPs +NIR	The concentrations of PDA NPs were 200 μg mL^{-1} with 808 nm NIR laser, 1.5 W cm^{-2}, 10 min
V	ZIF-8@PDA NPs	The concentrations of ZIF-8@PDA NPs were 200 μg mL^{-1}
IV	ZIF-8@PDA NPs +NIR	The concentrations of ZIF-8@PDA NPs were 200 μg mL^{-1} with 808 nm NIR laser, 1.5 W cm^{-2}, 10 min

To assess the variation in pH value over time during the bacterial growth process, *S. mutans* was cultured in the BHI broth supplemented with 1% sucrose. The bacterial suspensions were adjusted to a concentration of 1×10^7 CFU mL^{-1} and incubated in media with different pH levels ranging from 5.5 to 7.0. In the control group, the bacterial suspensions were treated with PBS solution. In the experimental group, the bacterial suspensions were treated with a medium containing ZIF-8@PDA NPs at 200 μg mL^{-1} and irradiated with an 808 nm NIR laser. After incubating the samples for 6 h, the optical density (OD) of the bacterial suspensions at 600 nm was measured. The difference in OD_{600nm} values between the control and experimental groups was calculated, and the change in pH of the culture over time was recorded.

S3 Eradication of oral biofilms *in vitro*

We used *S. mutans* to establish an *in vitro* oral biofilm model. *S. mutans* bacterial suspensions (1×10^6 CFU mL^{-1}) were mixed with 1% sucrose in a 24-well plate and incubated at 37 °C for 24 h to form mature biofilms. Then, the mature biofilms were washed 3 times by PBS and with various treatments, as shown in Table 1. The 24-well plate was then placed in a 37°C incubator for 6 hours. Subsequently, each well was

gently washed 3 times with PBS. Then, the staining of the biofilm was performed using a live/dead bacterial viability kit and observed on the biofilm using a confocal laser scanning microscope (CLSM). The captured biofilm has dimensions of 210 μm × 210 μm × 100 μm. Additionally, the treated biofilms were collected and diluted into PBS by intense eddy dissolution. The bacterial suspensions were spread on an agar plate and incubated at 37 °C for 24 h to count the number of CFUs. Simultaneously, the biofilm was fixed with methanol, stained with 0.1% crystal violet, and dissolved with acetic acid after staining. The biofilms were quantitatively calculated according to the optical density (OD) value at 550 nm measured by a UV-Vis spectrophotometer. The residual biofilms (%) were calculated using the formula (1):

$$\text{Residual biofilms (\%)} = N/Nc \times 100\% \quad (1)$$

Here, *N*c represents the optical density (OD) value at 550 nm ($OD_{550\ nm}$) in the control group, and *N* represents the $OD_{550\ nm}$ in the experimental group.

S4 Cell Biocompatibility assay

Mouse gingival fibroblast (L929) cells were cultured in DMEM medium supplemented with 1% and penicillin-streptomycin 10% FBS until they reached the logarithmic growth phase. The cells were then seeded in a 96-well plate and incubated for 24 h. The culture medium was then replaced with DMEM containing either PBS (control group) or ZIF-8@PDA NPs at a concentration of 200 μg mL^{-1}. In the ZIF-8@PDA NPs+NIR group, the samples were exposed to an 808 nm laser (1.5 W cm^{-2}, 10 min). All the samples were incubated at 37 °C for 24 h. Cell viability was assessed using the CCK-8 assay to evaluate the cytotoxicity of different treatments. The OD_{450nm} was measured, and the formula for calculating cell viability was calculated as follows:

$$\text{Cell viability (\%)} = OD_{test}/OD_{control} \times 100\% \quad (2)$$

In the cell staining assay for live and dead cells, the cells were incubated with a culture medium containing nanoparticles for 24 h. After that, they were stained with Calcein-AM/PI in the dark for 15 minutes. The stained cells were then observed using a CLSM.

For the cell cytoskeleton staining testing, the cells were incubated with a culture medium containing NPs for 24 h. Subsequently, they were fixed with 4% paraformaldehyde for 10 min. Then, the cells were permeabilized with 0.5% Triton X-100 for 5 min. Next, the cell nuclei and cytoskeleton were stained using RhB-phalloidin and DAPI by immersing the samples in the dark for 45 min. Finally, the stained cells were observed using a CLSM.

S5 Hemolysis assay

Fresh mouse blood (1mL) was obtained from Sprague-Dawley (SD) rats, centrifuged and resuspended in PBS. The samples were divided into four groups: (1) PBS (negative group), (2) ZIF-8@PDA NPs group, (3) ZIF-8@PDA NPs +NIR and (4) H_2O (positive group). In the ZIF-8@PDA NPs +NIR and ZIF-8@PDA NPs groups, cells were suspended in PBS containing ZIF-8@PDA NPs (200 μg mL^{-1}) and either irradiated with NIR or left untreated. The negative group consisted of cells mixed with PBS, while the positive group involved cells mixed with ultrapure water. The samples were cultured at 37 °C for 1 h and centrifuged at 2000 rpm for 10 min to obtain the supernatants. The OD_{540nm} was measured and the hemolysis rate (HR) was calculated according to the formula (3).

$$HR(\%) = \frac{OD_{experimental\ group} - OD_{negative\ control}}{OD_{positive\ component} - OD_{negative\ control}} \times 100\% \quad (3)$$

S6 Animal experiment

All animal experiments to the guidelines established by the Animal Care and Ethics Committee of the Changchun Institute of Applied Chemistry (CIAC), Chinese Academy of Sciences. Female SD rats were fed the caries-inducing Keyes 2000 diet (Syony Bioengineering Co., LTD, Jiangsu, China) and 5% sucrose solution. The rats were infected with *S. mutans* bacterial suspensions for five consecutive days. An additional 5 days were permitted for the infection to develop, and then the rats were randomly divided into three treatment groups (n = 3): (I) PBS group, (II) ZIF-

8NPs+NIR group, (III) PDA NPs+NIR group, (IV) ZIF-8@PDA NPs group, and (V) ZIF-8@PDA NPs+NIR group. In each group NPs (100 μL, 200 μg mL^{-1}) were applied to the rats' teeth using an applicator, and the rats' teeth were exposed to NIR for 10 min, repeated twice a day. In the ZIF-8@PDA NPs group, there were the rats' teeth that were not exposed to NIR. In the control group, the rats' teeth were treated with PBS. The treatment lasted 7 days, during which the rats' body weight and physical appearance were recorded daily. At the end of the experiment, oral microbial samples were collected using sterile swabs, inoculated on BHI plates, and incubated at 37 °C for 24 h. Animals were euthanized by asphyxiation before sterile dissection. The teeth of rats were collected and fixed in 4% formalin. The occurrence of lesions on rat teeth' smooth surface and fissure surface was measured using a stereomicroscope (NSZ-405, China). The hearts, livers, spleens, lungs, kidneys, tongue mucosa, lips, and gingival tissues of rats were collected for H&E staining.

S7 Statistical analysis

All data obtained are presented as mean ± standard deviation (SD). All results were repeated three times (n = 3), and the final result was taken as the average. Significant differences were evaluated using Student's t-test and denoted as * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and N.S. for no significant difference.

Figures mentioned in the main text

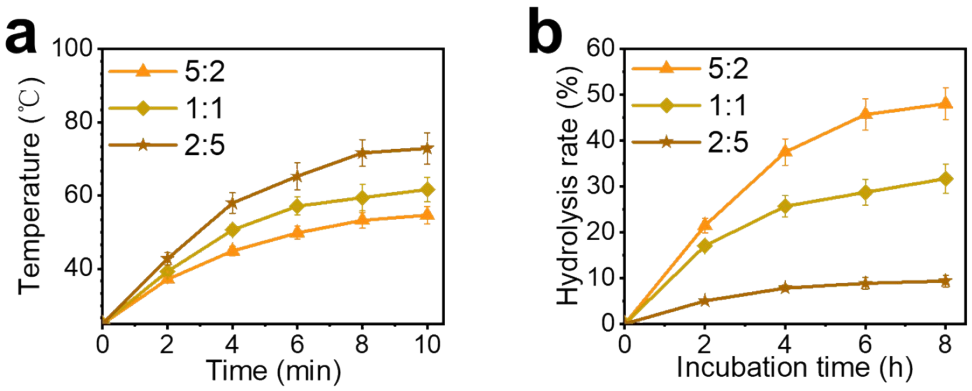

Fig. S1. (a) Temperature versus time curves for ZIF-8@PDA NPs under an 808 nm laser (1.5 W cm^{-2}, 10 min). The proportions between ZIF-8 and PDA were 5:2, 1:1 and 2:5, respectively. (b)Hydrolysis rate of ZIF-8@PDA NPs over time in a solution with pH 5.5. The proportions between ZIF-8 and PDA were 5:2, 1:1, and 2:5, respectively.

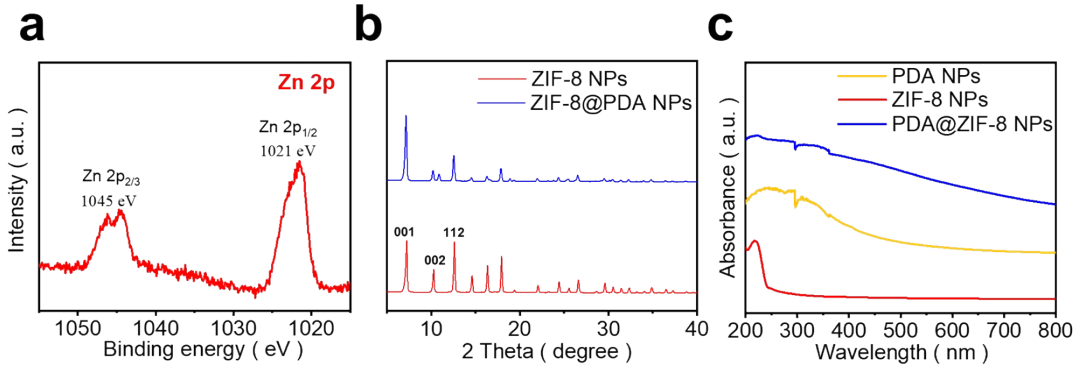

Fig. S2. (a) XPS of Zn2p spectrum of ZIF-8@PDA NPs. (b) XRD of ZIF-8 NPs and ZIF-8@PDA NPs. (c) UV-vis spectra of PDA NPs, ZIF-8 NPs and ZIF-8@PDA NPs.

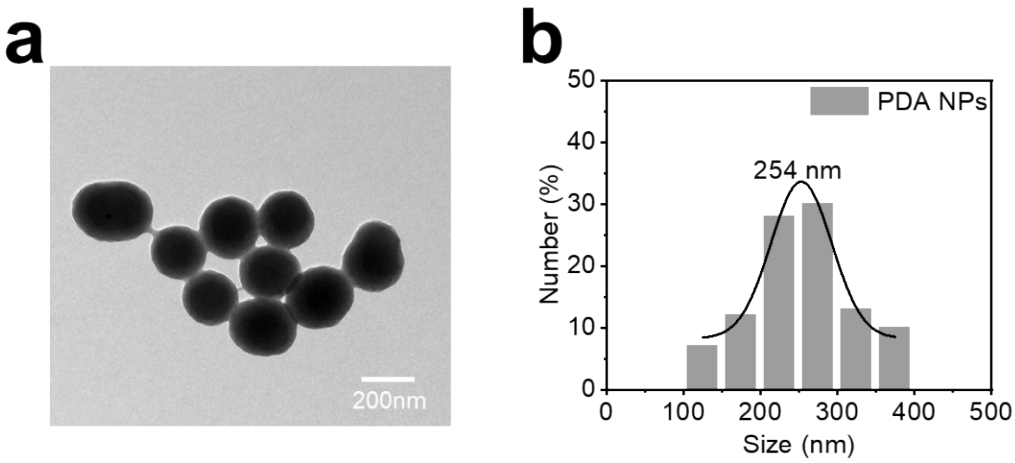

Fig. S3. (a) TEM images of PDA NPs. (b) particle size distribution of PDA NPs.

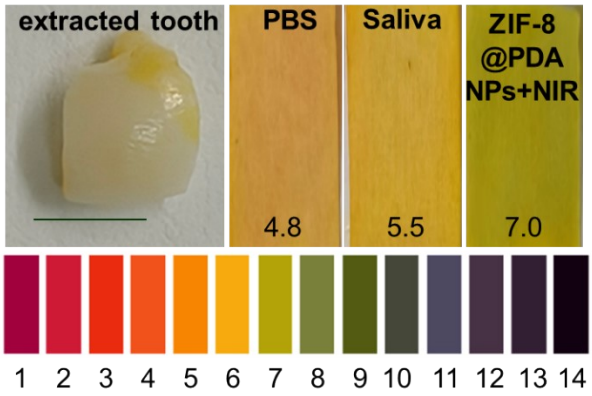

Fig. S4. pH value of the biofilm-formed tooth surface, after immersion in PBS solution, artificial saliva, and ZIF-8@PDA NPs solution for 30 minutes. (Scale bar = 5 mm).

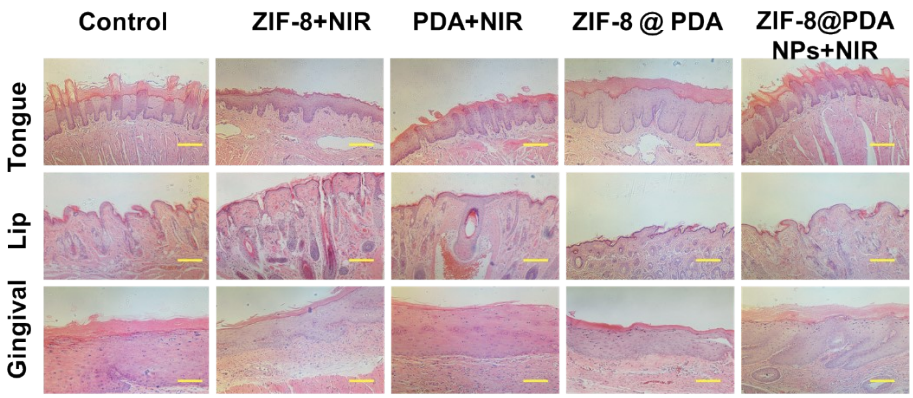

Fig. S5. H&E staining images of the rat tongue, lips, and gingival after 7 days treatment (Scale bar = 100 μm).

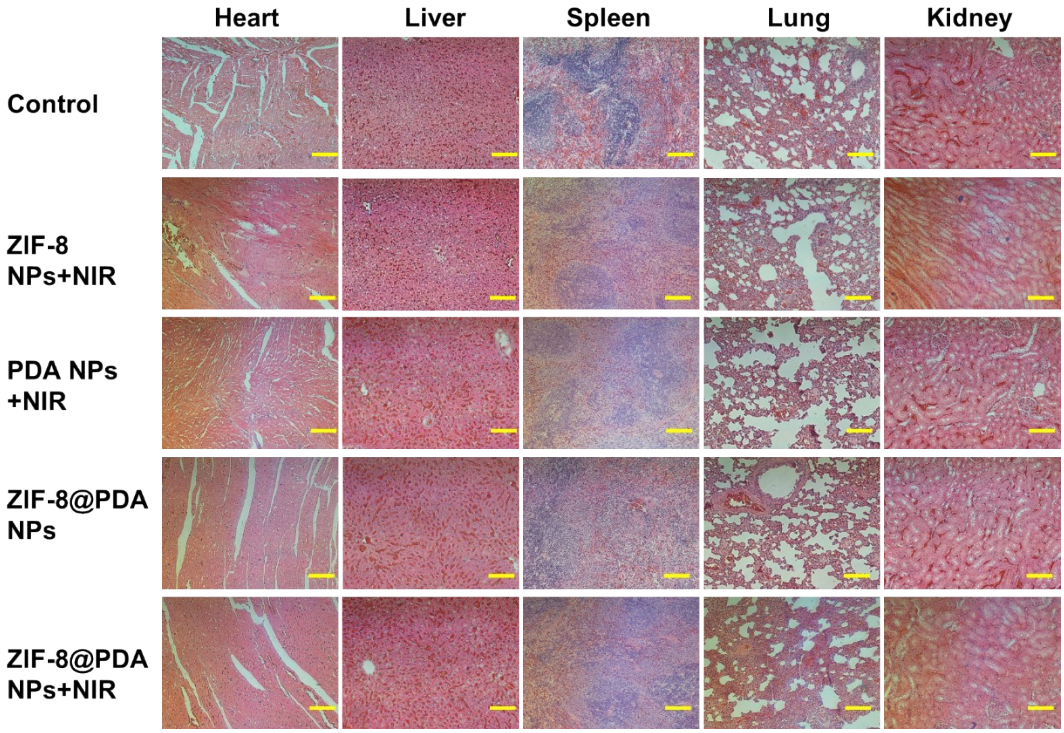

Fig. S6. H&E staining images of the rat vital organs (heart, liver, spleen, lung and kidney) by H&E staining (Scale bar = 100 μm).

Table S1. Comparative analysis of oral biofilm therapy methods

Treatment Method	**Effectiveness**	**Safety**	**Patient Comfort**	**Ref**
ZIF-8@PDA NPs+NIR Therapy	High (multiple mechanisms)	High (good biocompatibility)	High (non-invasive, painless)	This work
1% NaOCl	High (broad-spectrum antibacterial)	Low (strong irritation)	Low (irritative discomfort)	[1]
Photothermal Therapy	Medium (efficient antibacterial)	Medium (potential thermal damage)	Medium (localized heat sensation)	[2]
Photodynamic Therapy	Medium (selective antibacterial)	Medium (phototoxicity)	Medium (light sensitivity)	[3]
Zn-MOF	Medium (antibacterial activity)	Medium (potential toxicity)	High (non-invasive, painless)	[4]

References

1. P. Pladisai, R. S. Ampornaramveth and P. Chivatxaranukul, *Journal of Endodontics*, 2016, **42**, 460-464.
2. J. Wang, B. Hao, K. Xue, H. Fu, M. Xiao, Y. Zhang, L. Shi and C. Zhu, *Advanced Materials*, 2022, **34**, 2205653.
3. S. H. Ibbotson, T. H. Wong, C. A. Morton, N. J. Collier, A. Haylett, K. E. McKenna, R. Mallipeddi, H. Moseley, L. E. Rhodes, D. C. Seukeran, K. A. Ward, M. F. Mohd Mustapa and L. S. Exton, *British Journal of Dermatology*, 2019, **180**, 715-729.
4. C. Zhang, Z. Shu, H. Sun, L. Yan, C. Peng, Z. Dai, L. Yang, L. Fan and Y. Chu, *Applied Surface Science*, 2023, **611**, 155599.