# Supporting Information

## Ultrasound-responsive Gallium Protoporphyrin and Oxygen loaded Perfluoropentane Nanodroplets for Effective Sonodynamic Therapy of Implant Infections

Kaili Yang,[a,b] Xiaolong Chen,[a] Jianguang Li,[a] Weijun Xiu,[a] Lihui Yuwen,*[a] Jingyang Shan,[a] Heng Dong,[c] Shao Su*[a], Lianhui Wang*[a]

[a]State Key Laboratory of Organic Electronics and Information Displays & Jiangsu Key Laboratory for Biosensors, Institute of Advanced Materials (IAM), Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing University of Posts and Telecommunications, Nanjing 210023, China

[b]Department of Clinical Laboratory Medicine, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, 210008, Nanjing, China.

[c]Nanjing Stomatological Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing 210008, China

*Corresponding author

E-mail: iamlhyuwen@njupt.edu.cn (L. Yuwen), iamssu@njupt.edu.cn (S. Su) iamlhwang@njupt.edu.cn (L. Wang)

## Experimental Section

### Materials

Gallium protoporphyrin IX (GaPPIX, 95%) was provided by Frontier Scientific. 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-3-trimethylammonium-propane, chloride (DOTAP), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG), and 9,10-anthracenediyl-bis (methylene) dimalonic acid (ABDA, 90%) were provided by Sigma-Aldrich. Perfluoro-n-pentane (PFP, 98%) and tris (4,7-diphenyl-1,10-phenanthroline) ruthenium (II) dichloride ($Ru(dpp)_3Cl_2$, 99%) were obtained from Aladdin. Lactate dehydrogenase (LDH) cytotoxicity assay kit was purchased from Thermo Fisher. 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA, 97%), Calcein acetoxymethyl ester (Calcein-AM, 4 mM in DMSO), and propidium iodide (PI, 16 mM in DMSO) were provided by KeyGEN BioTECH.

### Characterization

The morphology of nanodroplets was observed by transmission electron microscopy (TEM, Hitachi-HT7700, Japan). The size of nanodroplets was counted by laser particle size analyzer (ZetaPALS, Brookhaven, USA). The composition of nanodroplets was analyzed by X-ray photoelectron spectroscopy (XPS, Ulvac-Phi, Japan). UV-3600 spectrophotometer (Shimadzu, Japan) was used to detect the ultraviolet-visible-near infrared (UV-vis-NIR) absorption spectra. The absorbance of solutions was measured by microplate spectrophotometer (PowerWave XS2, BioTek, USA). Electron spin resonance spectrometer (ESR, EMX-10/12, Bruker, Germany) was used for the qualitative analysis of free radicals in solution. Fluorescence images of biofilm was captured on a confocal laser scanning microscope (CLSM, FV1000MPE, Olympus, Japan).

### Toxicity of lipid-shelled PFP nanodroplets loaded with GaPPIX and $O_2$ (LPGO NDs)

Mouse blood was washed with physiological saline, and red blood cells (RBC) were collected. LPGO NDs dispersions (GaPPIX = 0, 10, 20, 40, 80, 160 μg/mL; 1 mL) were mixed with the physiological saline dispersion of RBC (0.1 mL) and shaken in a shaker at 37°C for 3 h. Meanwhile, physiological saline and 0.1% Triton was used as positive and negative controls, respectively. The supernatant of the mixture was collected by centrifugation and the absorbance at 540 nm was measured.

LPGO NDs dispersions (GaPPIX = 0, 10, 20, 40, 80, and 160 μg/mL, 100 μL) were added to the 96-well plate with mouse embryonic fibroblast (3T3) cells and incubated at 37°C for 24 h. The cytotoxicity of CaGaPP NSs was assessed by LDH cell toxicity assay.

Female Balb/c mice (18-20 g, Qinglongshan Company) were injected with physiological saline (200 μL) or LPGO NDs dispersions (200 μL, GaPPIX = 200 μg/mL) via tail vein and sacrificed on the 14th day. Major organs (heart, liver, spleen, lung, and kidney) of the mice were collected for hematoxylin and eosin (H&E) and Masson staining analysis.

**Antibiofilm Experiment in Vitro**

MRSA was cultured in Luria-Bertani (LB) medium for 12 h at 37℃. The MRSA suspension ($10^7$ CFU/mL) was diluted with LB medium containing 1% glucose and placed in a 96-well plate to form biofilm at 37℃ for 48 h.

Saline, LPO NDs, LG NPs, LPG NDs, and LPGO NDs (100 μL, GaPPIX = 20 μg/mL) were added into the 96-well plate containing MRSA biofilms. The MRSA biofilm was sonicated for 5 min (1 W/$cm^2$, 1 MHz, 50% duty cycle) and incubated for 6 h at 37℃. Firstly, the treated MRSA biofilm was stained with calcein acetoxymethyl ester (Calcein-AM) for 30 min and fluorescent images were captured using CLSM. Then, the treated MRSA biofilm was dispersed in physiological saline and quantified using the plate counting method. Finally, the treated MRSA biofilm was fixed with paraformaldehyde solution for 30 min, then stained with 0.02% crystal violet solution for 60 min. The excess crystal violet solution was gently washed away with physiological saline, and the MRSA biofilm was imaged using an inverted microscope. The crystal violet in the biofilm was dissolved in ethanol and the absorbance of solution at 590 nm was measured by a microplate spectrophotometer.

## Supporting Figures

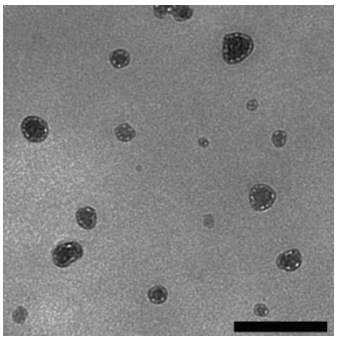

Figure S1 TEM image of lipid-shelled PFP/$O_2$ nanodroplets (LPO NDs). Scale bar is 200 nm.

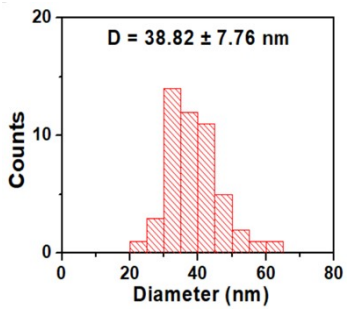


Figure S2 Size distribution histogram of LPO NDs.

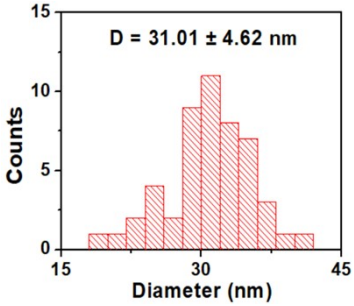


Figure S3 Size distribution histogram of LPGO NDs.

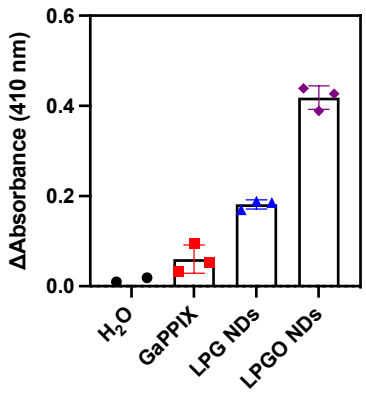


Figure S4 Absorbance at 410 nm of different samples containing DPBF under hypoxic conditions after US treatment.

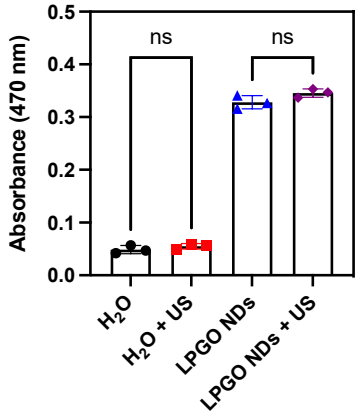


Figure S5 Absorbance at 470 nm of different samples containing XTT after US treatment.

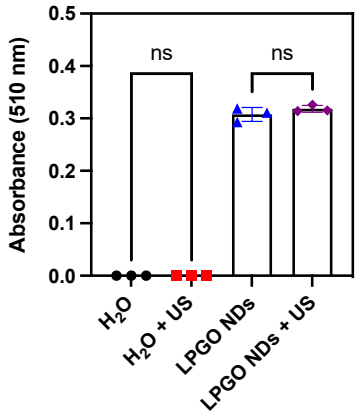


Figure S6 Absorbance at 510 nm of different samples containing SA after US treatment.

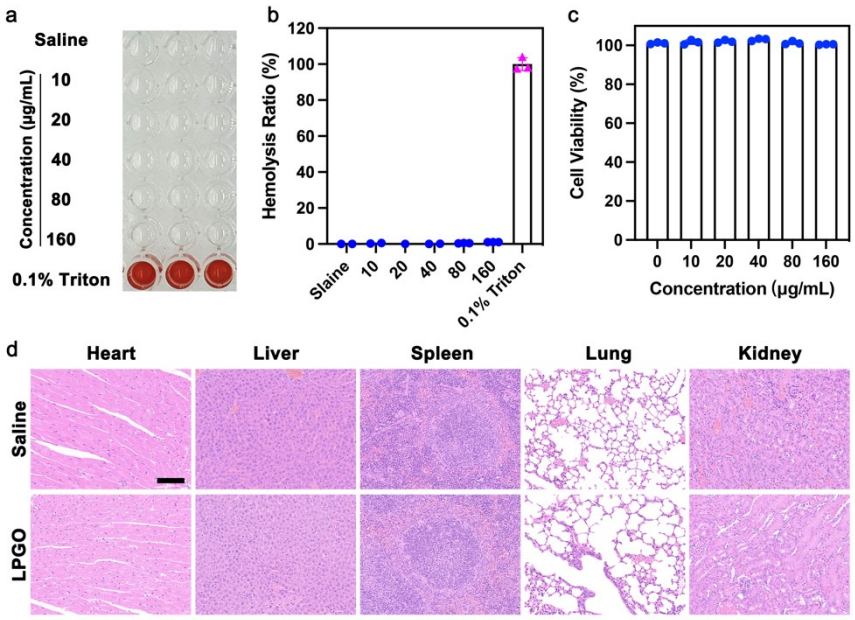


Figure S7 (a-b) Hemolysis ratio of RBC after exposure to saline, LPGO NDs dispersion (10, 20, 40, 80, 160 μg/mL), and 0.1% Triton for 3 hours. (c) Cell viability of 3T3 cells after incubation with LPGO NDs for 1 day. (d) Microscopy photographs of major organs in mice after treated with LPGO NDs and stained with H&E. Scale bar is 100 μm.

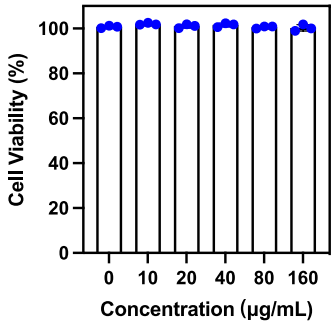


Figure S8 Cell viability of L-O2 cells after incubation with LPGO NDs for 24 h.

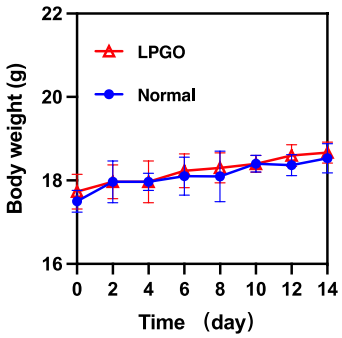


Figure S9 Weight of mice after intravenous injection of LPGO NDs.