Supplementary information

Photothermally-induced HClO releasing nanoplatform for imaging-guided tumor ablation and bacteria prevention

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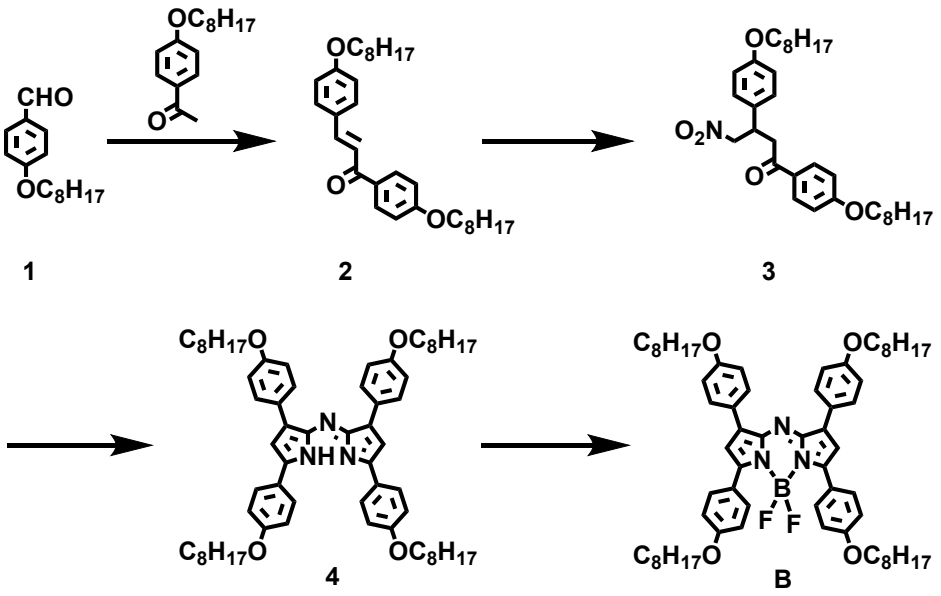
Contents

Part I. Experimental methods

Materials and characterization. All starting reagents and materials were purchased from commercial sources and used without further purification, unless otherwise specified. Deionized water was used to prepare all aqueous solutions. Cell culture reagents and fetal bovine serum (FBS) were purchased from Gibcco. The ^{1}H NMR spectra were recorded on a Bruker Ultra Shield Plus 400 MHz NMR instrument at 298 K using deuterated solvents. Chemical shifts are given in ppm, and are referenced against external Me_4Si (^{1}H, ^{13}C, ^{19}F). Mass spectra were obtained on a Bruker autoflex matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometer. The visible absorption spectra were obtained with a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer. Photoluminescence spectra were measured on an Edinburgh FL 920 spectrophotometer equipped with a temperature controller. The morphology of nanoparticles was measured by high resolution transmission electron microscopy (TEM, JEM-2100), and the accelerating voltage is 200 KV. Dynamic light scattering (DLS) was performed on a particle size analyzer (Brookhaven BI-90Plus). The methyl thiazolyl tetrazolium (MTT) assay was performed by a Power Wave XS/XS2 microplate spectrophotometer. Infrared thermal imaging was measured by FLIR E50 infrared camera. MW-GX-730/2000mW laser is used as a laser for photothermal and photodynamic experiments. The power density meter is VLP-2000 laser power meter. Confocal fluorescence imaging was measured with Olympus Fluo View FV1000 laser scanning confocal microscopy.

Synthesis.



Scheme S1. Synthesis routes of aza-BODIPY (B).

Synthesis and characterization of compound B. Compound B was synthesized according to our previous work.[1] ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.05 (d, *J* = 2.0, 4 H), 8.03 (d, *J* = 2.0 Hz, 4 H) 6.98 (d, *J* = 8.8 Hz, 8 H,), 6.92 (s, 2 H), 4.03 (q, *J* = 19.2 Hz, 8 H), 1.86-1.78 (m, 8 H), 1.51-1.44 (m, 8 H), 1.37-1.28 (m, 32 H), 1.11 (s, 12 H), 0.90 (t, *J* = 13.6 Hz 12 H,). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 161.35, 160.30, 131.46, 130.72, 114.62, 68.18, 38.16, 31.89, 31.25, 29.71, 29.30, 26.07, 22.68, 14.12. ^{19}F NMR (376.5 MHz, $CDCl_3$) δ (ppm): -131.62 (q, 2 F). MALDI-TOF-MS (m/z): calcd for $C_{64}H_{86}BF_2N_3O_4$, 1009.67; found, 1008.49.

Synthesis and characterization of compound BClO. The HClO probe BClO was synthesized according to the literature.[2] ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.41 (s, 1H), 6.08 (s, 2H), 5.66 (s, 1H), 3.28-3.17 (t, *J* = 16.0 Hz, 2H), 2.83 (t, *J* = 16.0 Hz, 2H), 2.55 (s, 6H), 2.40 (s, 6H), 2.20 (s, 3H), 2.03 (s, 3H).

Preparation of TCCA solution. Saturated cyanuric acid was prepared with deionized water and cyanuric acid. And the pH of the saturated cyanuric was adjusted to 4 with HCl. Finally, trichloroisocyanuric acid (10.0 mg) was added to the mixture (100 mL).

Synthesis and characterization of Lipo-B-TCCA, Lipo-B and Lipo-TCCA. The molar ratios of DPPC/DSPC/DSPE-PEG_{2000}/cholesterol was 71:14:5:10.[3] Then 7.4/3.5/2.8/38.7 mg of DPPC/DSPC/DSPE-PEG_{2000}/cholesterol were dissolved in 50 mL chloroform for preparing the phospholipids solution. And then B (1.0 mg) was added to 5 mL phospholipids solution. The mixture was dried *via* a rotary evaporator under a decreased pressure to form a lipid film. 10 mL saturated cyanuric acid containing 0.1 mg trichloroisocyanuric acid was added to the lipid film. And then the mixture was treated with the continuous sonication (360 W) at room temperature for 30 s. The suspension was centrifuged at 10000 rpm for 10 min, and washed 3 times with ultrapure water to obtain **Lipo-B-TCCA**. The drug loading content (DLC) and drug loading efficiency (DLE) of B in **Lipo-B-TCCA** was 78 μg/mg and 40.9%, respectively. Lipo-B and Lipo-TCCA was synthesized without adding TCCA or B. And they were stored in -20 ^{o}C.

Concentration detection of Lipo-B-TCCA. Standard concentration curve of B was obtained by UV-vis spectrophotometer at different concentrations. The AO7 was used as the TCCA probe. And its absorption at 486 nm would decrease when it reacted with trichloroisocyanuric acid and HClO. According to the absorption intensity of **Lipo-B-TCCA** and AO7 dissolved in dichloromethane, the concentration of B and TCCA in **Lipo-B-TCCA** could be confirmed.

Photothermal effect of **Lipo-B-TCCA, Lipo-B and Lipo-TCCA.** To evaluate the photothermal performance of **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA, different concentrations of them (260, 520 and 780 μg/mL respectively) were irradiated with laser (730

nm, 500 mW/cm^2). And their temperature changes were recorded by FLIR thermal camera for 6 min.

Photothermal conversion efficiency calculation of Lipo-B-TCCA. The photothermal conversion efficiency of **Lipo-B-TCCA** was calculated according to the following formula:

$$\eta = \frac{hS\Delta T_{\max} - Q_s}{I(1 - 10^{-A_{730}})}$$

$$\tau_s = \frac{m_D C_D}{hS}$$

Where h is the heat transfer coefficient, S is the surface area of the container, and the value of hS is obtained from the Figure 1f. The ΔT_{max} is the temperature change of **Lipo-B-TCCA** aqueous solution at the maximum steady-state temperature, I is the laser power, A_{730} is the absorbance of **Lipo-B-TCCA** at 730 nm, and Q_s expresses the heat associated with light absorption by the solvent. The variable τ_s is the sample-system time constant, and m_D and C_D are the mass and heat capacity (4.2 J/g) of the deionized water.

Photothermal stability of Lipo-B-TCCA. **Lipo-B-TCCA** solution was irradiated with 730 nm laser (500mW/cm^2) till its temperature stop increasing. Then the irradiation was turned off, and the solution was cooled to room temperature naturally. This process was repeated for 3 times.

Thermally-induced TCCA releasing from Lipo-B-TCCA. Firstly, the absorption spectrum of **Lipo-B-TCCA** with AO7 at 20 ^{o}C was recorded with UV-vis spectrophotometer. Then **Lipo-B-TCCA** was heated at 20, 37, 42, 50, 60 and 70 ^{o}C for 6 min with water bath. And their absorption was recorded.

Photothermally-induced TCCA releasing from Lipo-B-TCCA, Lipo-B and Lipo-TCCA. Firstly, the absorption spectra of **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA with AO7 at 20 ^{o}C were recorded with UV-vis spectrophotometer. And they were irradiated with 730 nm laser (500 mW/cm^2) for 6 min. Then, their absorption spectra were recorded.

HClO detection *in vitro*. BClO was used as the HClO probe. And **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA in methanol were irradiated with 730 nm laser (500 mW/cm^2) for 6 min. And then, the 2 μM BClO was added and excited at 480 nm. The fluorescence was recorded at 525 nm by luminescence spectrometer.

Cell culture. The Hela cell lines (human cervical cancer) were purchased from the Institute of Biochemistry and Cell Biology, SIBS, CAS (China). The cells were incubated in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum), 100 mg/mL streptomycin and 100 U/mL penicillin at 37 ^{o}C with 5% CO_2.

HClO detection in cells. Hela cells were seeded in cell culture dishes and incubated for 24 h at 37 ^{o}C. Then Hela cells were cultured with **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA for 24 h in dark. And they were irradiated with 730 nm laser (500 mW/cm^2) for 6 min. The cells were cultured with fresh medium with 2 μM BClO for 20 min. Then the medium was removed and the cells were washed with PBS for 3 times. Finally, the cells were observed with confocal laser scanning microscope.

MTT assays. To measure the dark toxicity of **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA, methyl thiazolyltetrazolium (MTT) assays was taken on Hela cells. 10^4/well in 96-well cell culture plate were cultured at 100% humidity 37 °C with 5% CO_2 for 24 h. Different concentrations of **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA (0, 130, 260, 520, 780, 1040, 1300 μg/mL in DMEM) were added into the wells, respectively. The cells were incubated in dark

for another 24 h. Finally, MTT (10 μL/well, 5 mg/mL) were added to each well and the cells were incubated for 4 h. After sucking out the medium, 150 μL DMSO was added to each well. Enzyme-linked immunosorbent assay (ELISA) reader was used to measure the OD570. The viability of cell growth was calculated by the formula:

Cell viability (%) = (mean of absorbance value of treatment group)/(mean of absorbance value of control group) ×100.

Phototoxicity assays *in vitro*. To measure the phototoxicity of **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA, methyl thiazolyltetrazolium (MTT) assays was taken on Hela cells. 10^4/well in 96-well cell culture plate were cultured at 100% humidity 37 °C with 5% CO_2 for 24 h. Different concentrations of **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA (0, 130, 260, 520, 780, 1040, 1300 μg/mL in DMEM) were added into the wells, respectively. And then the cells were incubated in dark for another 24 h. Then, they were irradiated with 730 nm laser (500 mW/cm^2) for 6 min. Finally, MTT (10 μL/well, 5 mg/mL) were added to each well and the cells were incubated for 4 h. After sucking out the medium, 150 μL DMSO was added to each well. Enzyme-linked immunosorbent assay (ELISA) reader was used to measure the OD570. The viability of cell growth was calculated by the formula:

Cell viability (%) = (mean of absorbance value of treatment group) / (mean of absorbance value of control group) × 100%.

AM/PI assays *in vitro*. Hela cells were seeded into a glass bottom petri dish and cultured at 100% humidity 37 °C with 5% CO_2 for 24 h. **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA (260 μg/mL in DMEM) were added into the wells, respectively. The cells were incubated for another 24 h. Finally, the media was removed and the cells were washed with PBS for 3 times. 5 μL PI and 10 μL AM were added to the cells in dark for 15 min. Then the cells were

irradiated with a 730 nm laser (500 mW/cm^2) for 6 min. After irradiation, the cells imaging was observed by the confocal microscope in green channel for AM (λ_{ex} = 488 nm, λ_{em} = 500-560 nm) and red channel for PI (λ_{ex} = 488 nm, λ_{em} = 600-680 nm).

Flow cytometry experiments. Annexin V-FITC/PI Apoptosis Detection Kit was used for detection of **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA mediated induced cell Apoptosis. Hela cells were seeded into a glass bottom petri dish and cultured at 100% humidity 37 °C with 5% CO_2 for 24 h. **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA (260 μg/mL in DMEM) were added into the wells, respectively. The cells were incubated for another 4 h. Finally, the media was removed and the cells were washed with PBS for 3 times. Then the cell was stained with 5 μL annexin V-FITC and 10 μL PI at room temperature for 10 min in dark. After that, the cells were treated with 730 nm NIR light excitation (500 mW/cm^2) for 6 min or not respectively. And then the viability of Hela cells was obtained with a flowcytometry (flow sight).

Analysis of mitochondrial membrane potential. Hela cells were seeded into a glass bottom petri dish and cultured at 100% humidity 37 °C with 5% CO_2 for 24 h. **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA (260 μg/mL in DMEM) were added into the wells, respectively. The cells were incubated for another 24 h. Then the cells were irradiated with a 730 nm laser (500 mW/cm^2) for 6 min. The cells were treated with JC-1 (5 mg/mL) for 20 min at 37 ^oC. The fluorescence intensity of JC-1 was observed by the confocal microscope in the red channel for J-aggregates (λ_{ex} = 515 nm, λ_{em} = 580-640 nm) and the green channel for JC-1 monomers (λ_{ex} = 515 nm, λ_{em} = 530-560 nm).

Bacterial culture. S. aureus (ATCC25923) in Luria-Bertani (LB) medium (5 mL, NaCl 10 g/L, yeast extract 5 g/L, and tryptone 10 g/L) was shaken with 220 rpm at 37 ^oC for 12 h. And then the bacteria were diluted to 2 × 10^6 CFU/mL with saline. The concentration of bacteria

was estimated by the optical density calue at the wavelength of 600 nm (OD_{600} of 0.1 corresponds to □ 10^8 CFU/mL).

***In vitro* antibacterial activity of Lipo-B-TCCA, Lipo-B and Lipo-TCCA**. **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA cultured Hela cells were irradiated with 730 nm laser (500 mW/cm^2) for 6 min. Then the medium was removed and the cells were washed with PBS for 3 times. And then the bacteria (100 μL, 2 × 10^6 CFU/mL) were mixed with the cells. The bacteria were cultured at 37 ^{o}C in dark. The concentration of bacteria was estimated by the optical density value at the wavelength of 600 nm at different time. Finally, the bacteria were dispersed in one hundred microliters and spread onto the LB agar plates for 6 h.

Animals. All animal experiments were performed in accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication no. 85-23 Rev. 1985) and approved by the Jiangsu Administration of Experimental Animals. All Hela tumor model was established from Jiangsu Keygen Biotech Co., Ltd. and used referring to the standard of the Laboratory Animal Center of Jiangsu Keygen Biotech Co., Ltd.

***In vivo* imaging**. The tumor bearing nude mice were injected with **Lipo-B-TCCA** (1300 μg/mL, 160 μL) through tail intravenous injection. At different time (0, 0.5, 2.5, 4.0, 6.0, 12.0, 24.0 h) after tail intravenous injection, the fluorescence imaging of the tumor bearing nude mice were observed by a small animal *in vivo* imaging system (λ_{ex} = 720 nm, λ_{em} = 780 nm).

***In vivo* photothermal effect detection**. The mice were injected with **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA (1300 μg/mL, 160 μL) through tail intravenous injection respectively. And 2.5 h later, the mice were irradiated with a 730 nm laser (500 mW/cm^2) for 6 min. The temperature change of them was recorded by a FLIR camera.

***In vivo* HClO detection**. Mice were injected with **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA (1.3 mg/mL, 160 μL) through tail intravenous injection. And 2.5 h later, the mice were irradiated with a 730 nm laser (500 mW/cm^2) for 6 min. Then, the mice were injected with BClO (20 μM, 100 μL) in the tumor region at different time after irradiation. After irradiation, 3 mice were executed 20 min later after the injection of BClO for collecting their tumors. And then the tumor sections were observed with confocal microscopy (λ_{ex} = 488 nm). At 4 and 8 h after irradiation, the mice which injected with **Lipo-B-TCCA** and Lipo-B were executed 20 min later after the injection of BClO for collecting their tumors. And then the tumor sections were observed with confocal microscope (λ_{ex} = 488 nm).

***In vivo* antitumor efficacy**. The mice were divided into 7 groups (n = 3) when the tumors reached ~ 200 mm^3: **Lipo-B-TCCA** + Laser, **Lipo-B-TCCA** + no Laser, Lipo-B + Laser, Lipo-B + no Laser, Lipo-TCCA + Laser, Lipo-TCCA + no Laser and PBS + Laser (control). The mice were injected with **Lipo-B-TCCA**, Lipo-B, Lipo-TCCA and PBS respectively. The mice were irradiated with 730 nm laser (500 mW/cm^2) or not 2.5 h after injection every 2 d for 3 times. And their weights and tumor sizes were measured and recorded every 2 d for 20 d. After treatment, all mice were sacrificed and their tumors and main organs (heart, liver, spleen, lung and kidney) were collected.

***In vivo* bacteria inhibition**. The mice were injected with **Lipo-B-TCCA** and Lipo-B, and irradiated with 730 nm laser (500 mW/cm^2) every 2 d for 3 times. And then, the mice were injected with S. aureus (100 μL, 1 × 10^8 CFU/mL) at the tumor region every time after irradiation for 3 times. 20 d later, the mice were sacrificed for collecting the wound tissues.

Histological analysis. The wound tissues, tumors and main organs (heart, liver, spleen, lung and kidney) were harvested and fixed in 4% paraformaldehyde solution. Then they were

paraffined, sectioned, and then analyzed by H&E staining. Masson's trichrome staining was applied to the wound tissues.

Part II. Supplemental figures

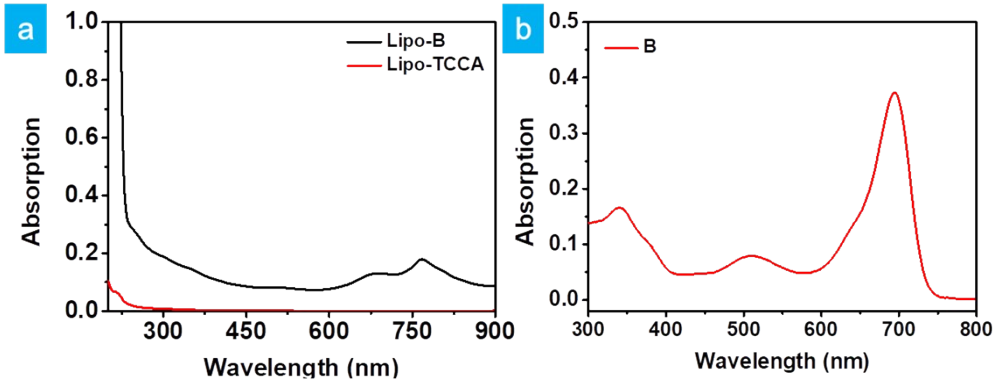


Figure S1 (a) UV-vis absorption spectra of Lipo-B and Lipo-TCCA; (b) UV-vis absorption spectra of B 10 μM.

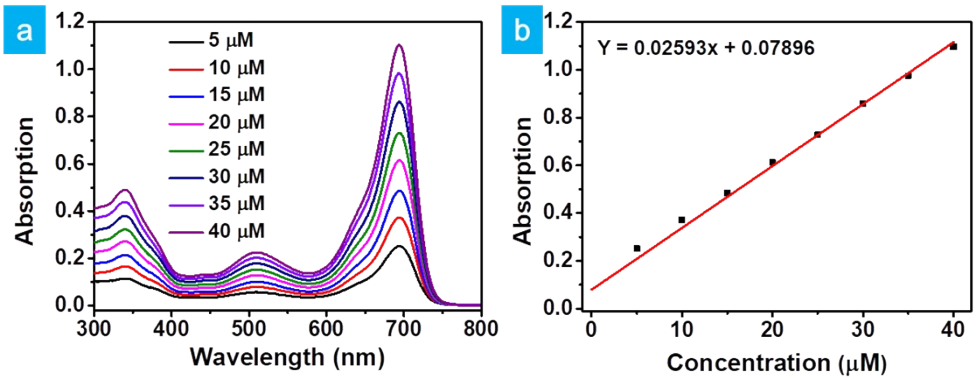


Figure S2 (a) UV-vis absorption spectra of B with different concentrations in dichloromethane. b) Absorbance standard linear fit of different concentrations B in dichloromethane recorded by UV-vis absorption spectra.

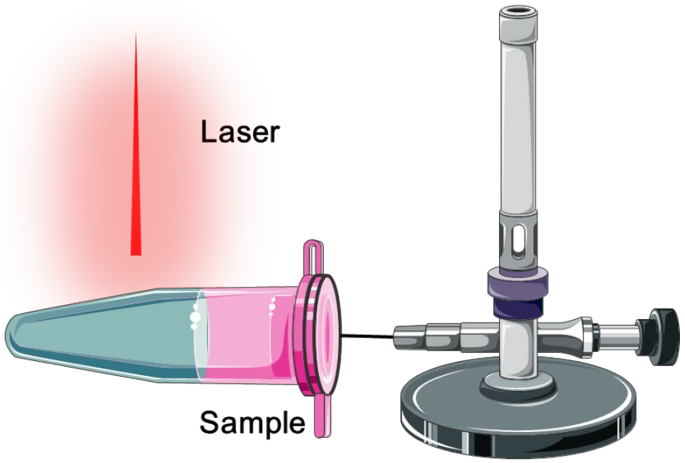


Figure S3 Schematic illustration of contactless phothermal testing system.

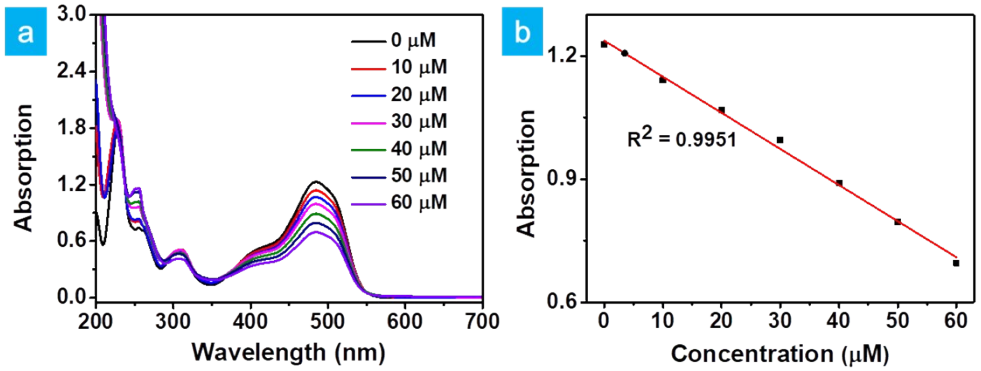


Figure S4 (a) UV-vis absorption spectra of AO7 with different concentrations of trichloroisocyanuric acid in dichloromethane. b) Absorbance standard linear fit of different concentrations AO7 with different concentrations of trichloroisocyanuric acid in dichloromethane recorded by UV-vis absorption spectra.

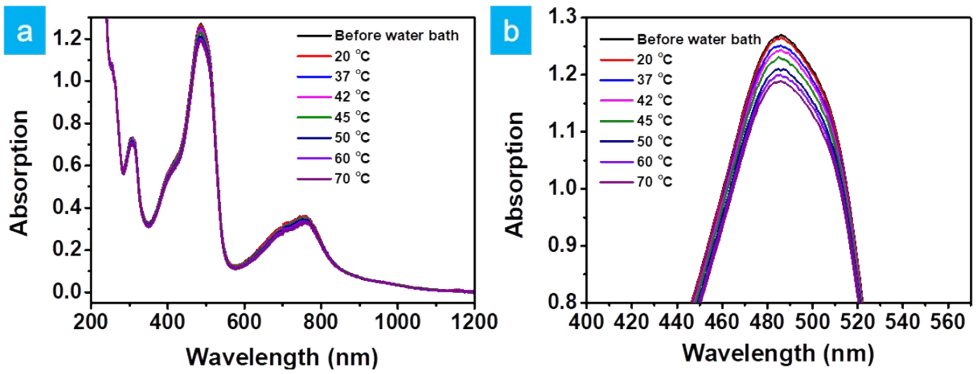


Figure S5 UV-vis absorption spectra of AO7 with **Lipo-B-TCCA** solution in water bath with different temperature. (a) (wavelength 200-1200 nm) (b) (wavelength 400-570 nm).

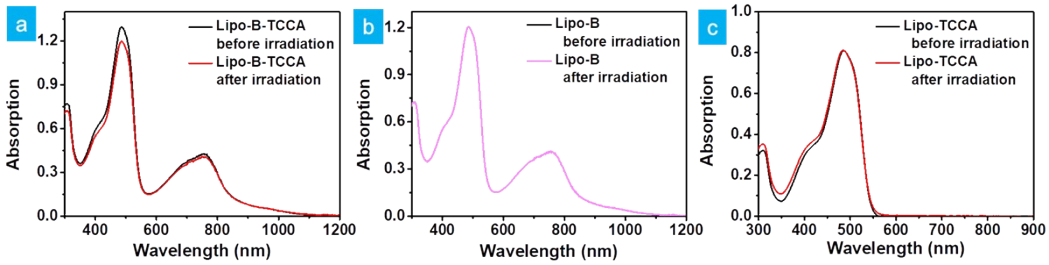


Figure S6 UV-vis absorption spectra of AO7 with (a) **Lipo-B-TCCA**, (b) Lipo-B, (c) Lipo-TCCA before and after irradiation.

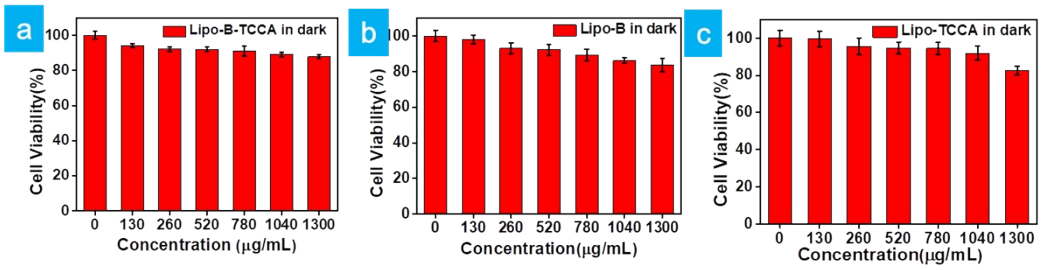


Figure S7 The cell viability of the Hela cells which treated with **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA in dark.

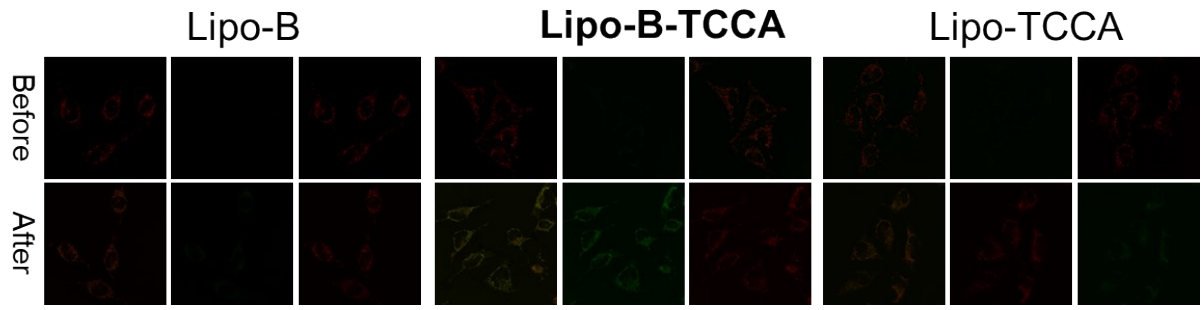


Figure S8 The mitochondrial membrane potential (MMP) change induced by **Lipo-B-TCCA**, Lipo-B and Lipo-TCCA before and fluorescence.

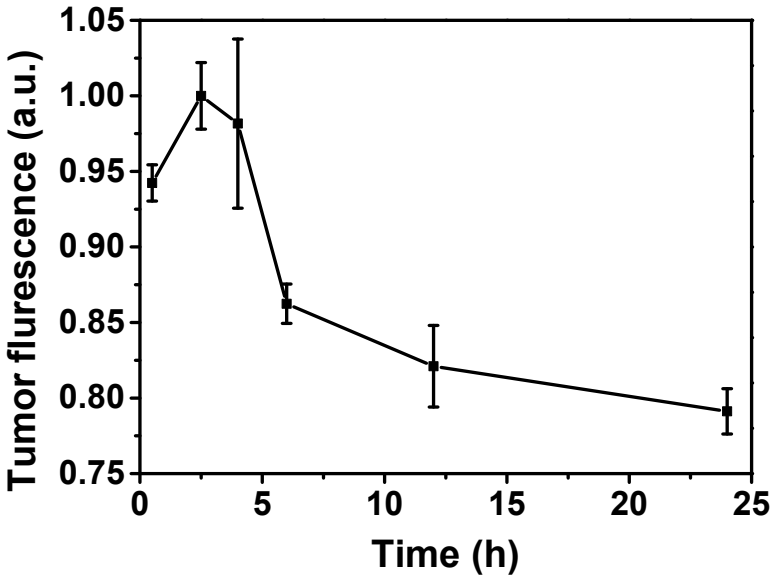


Figure S9 Relative fluorescence intensities of tumor at various time intervals based on *in vivo* fluorescence images shown in Figure 3a.

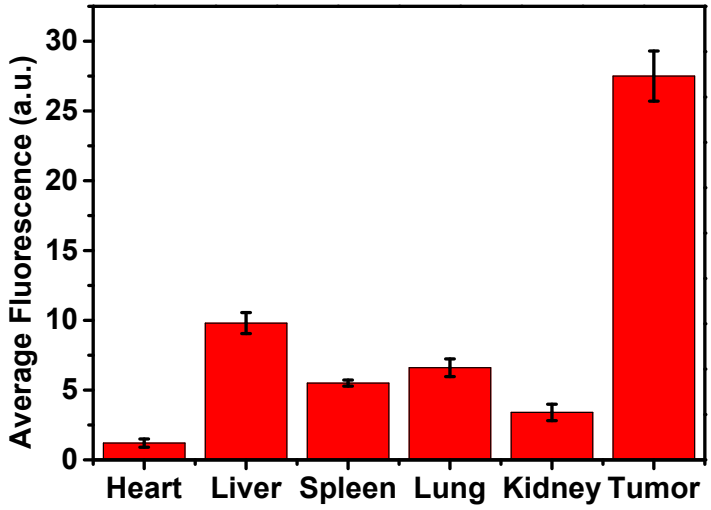


Figure S10 Fluorescence intensities of tumor and the major organs based on *in vivo* fluorescence images shown in Figure 3b.

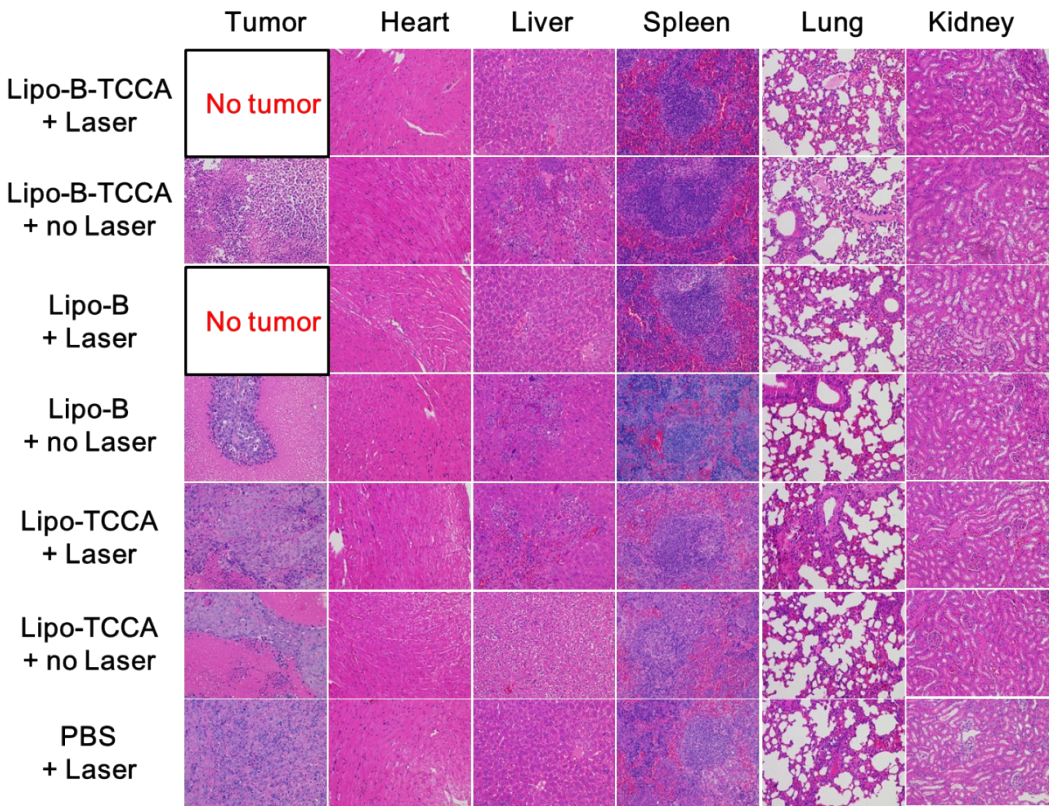


Figure S11 H&E staining of tumor, heart, liver, spleen, lung, kidney tissue of each group.

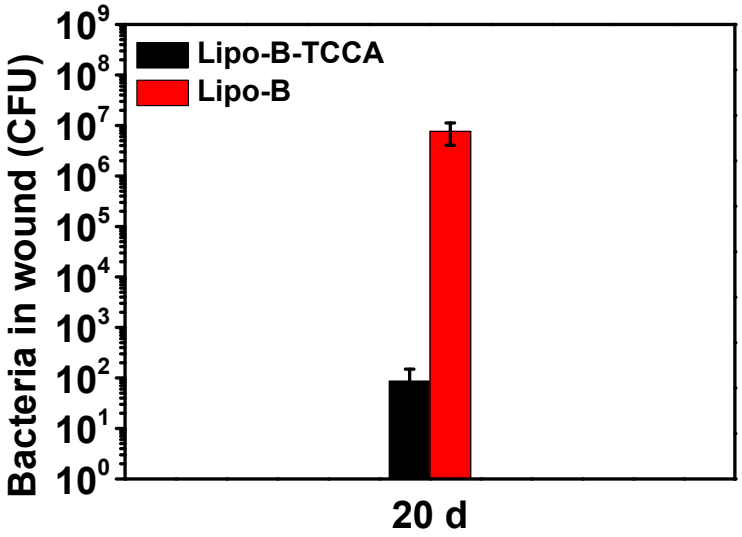


Figure S12 Number of the surviving bacteria in the infected wound tissues.

Part III. 3. Supplemental references

[1] M. L. Zhao, Y. J. Xu, M. J. Xie, L. Zou, Z. L. Wang, S. J. Liu, Q. Zhao, Adv. Healthcare Mater., 2018, **7**, 1800606.

[2] H. Zhu, J. L. Fan, J. Y. Wang, H. Y. Mu, X. J. Peng, J. Am. Chem. Soc., 2014, **136**, 12820.

[3] M. John, L. Barbara, M. K. Alhallak, J. Sun, K. Wasden, N. Guenthner, P. Puente, C. Federico, A. K. Azab, Pharm. Res., 2019, **36**, 144.