Supporting information for

From Biomaterials to Biotherapy: Cuttlefish Ink with Protoporphyrin IX Nanoconjugates for Synergistic Sonodynamic-Photothermal Therapy

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1. Experiment section

2.1 Materials

Protoporphyrin *IX* (PpIX), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), *N*-Hydroxysulfosuccinimide (NHS), *N, N*-dimethylformamide (DMF), and 1.3-diphenyl isobenzofuran (DPBF) were brought from Sinopharm Chemical Reagent Co., Ltd. Cell counting kit-8 (CCK-8), Calcein-AM, propidium iodide (PI), and 2,7-Dichlorofluorescin diacetate (DCFH-DA) were purchased from Beyotime Biotechnology. Cuttlefish ink nanoparticles were harvested from cuttlefish and washed heavily with water/ethanol solution.

2.2 Characterizations

The morphology, phase and composition of samples were investigated by using a field emission scanning electron microscope (SEM), and an IRPrestige-21 spectrometer. Zeta potentials were measured by using a Malvern Nano-S90 Zetasizer, which were repeated 3 times for each solution. UV-Vis-NIR absorption spectra were measured on a Lambda 950 UV-visible spectrophotometer. The US-triggered singlet oxygen ($^1O_2$) generation was quantified by a JES-

FA200 electron spin resonance (ESR).

2.3 Cytotoxicity test

HUVEC cells (human umbilical vein endothelial cells), 4T1 cells (murine breast cancer cells) and CT26 cells (colon cancer cells) were grown in DMEM medium under the standard conditions. HUVEC, 4 T1 and CT26 cells were cultured in 96-well plate and 24 h latter cell medium was discarded and added with a new medium containing CIPs at a series of concentrations. After 24 or 36 h, these cells were washed with PBS and tested by using the standard CCK-8 assay.

2.4 Fluorescence of intracellular CIPs

For the fluorescence of intracellular CIPs, 4 T1 cells were cultured with CIPs (300 μg mL^{-1}) or melanin (300 μg mL^{-1}) for 4 h, and cells were washed three times and imaged by a digital microscope.

2.5 Fluorescence of intracellular $^1O_2$ generation

For the fluorescence of intracellular $^1O_2$ generation, 4 T1 cells were incubated with CIPs (300 μg mL^{-1}) for 4 h. Subsequently, cells were cultured with the serum free medium with DCFH-DA (100 μL, 20 μM). 2 h later, cells were exposed to US (1.8 W cm^{-2}) for 5 min and imaged.

2.6 Cell therapies

For SDT of 4 T1 cells, they were pre-cultured with CIPs (300 μg mL^{-1}) for 4 h and excited by US (1.8 W cm^{-2}) at different time. For PTT of 4 T1 cells, the CIPs-mediated 4 T1 cells were excited with NIR laser (808 nm, 1 W cm^{-2}) at different time. For the combined SDT-PTT, 4 T1 cells were cultured with CIPs (300 μg mL^{-1}) for 4 h and then simultaneously exposed to US and laser for different time. After irradiation of US and/or NIR laser, the cell viability was evaluated through CCK-8 assay. Meanwhile, cells after same treatments were also performed with Calcine

AM/PI assay.

2.7 Blood and histological analysis

Healthy Balb/c mice (n = 3) were intravenously injected with saline (80 μL) or CIPs saline dispersion (80 μL, 50 mg kg^{-1}), and they were sacrificed on the 10th day. The blood samples were collected for biochemistry analysis, and major organs were cut into slices for H&E staining.

2.8 Tumor therapies

The tumor-bearing mice were allocated to five groups (n = 5) as following: (1) Control (saline, 80 μL), (2) CIPs (80 μL, 50 mg kg^{-1}); (3) CIPs (80 μL, 50 mg kg^{-1})+US (10 min); (4) CIPs (80 μL, 50 mg kg^{-1}) + NIR (10 min) and (5) CIPs (80 μL, 50 mg kg^{-1}) + US (10 min) + NIR (10 min). After treatments, mice body weight and their tumor volume were recorded and calculated every 3 days. At the 15th day, mice were sacrificed, and the tumors were extracted which further cut into tumor slice for staining with H&E assay. The tumor volume was determined by the following equation: volume = $width^2 \times length/2$. The efficiency in tumor suppression was calculated according to the below equation: tumor-inhibition efficiency (%) = $(1\text{-}V/V_0) \times 100$ (Where V_0 and V are the tumor volume of the control group and the other groups).

2.9 Statistical analysis

The data were expressed as the mean value ± standard deviation (SD), and any statistical comparison between two groups was analyzed using the Student's two-tailed test. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. All animal investigations conformed to the guide for the Care and Use of Laboratory Animals by the U.S. National Institutes of Health (NIH Publication no. 86-23, revised 1985) and per the protocols approved by the Animal Welfare and Research Ethics Committee of China Three Gorges University.

2. Supplementary figures

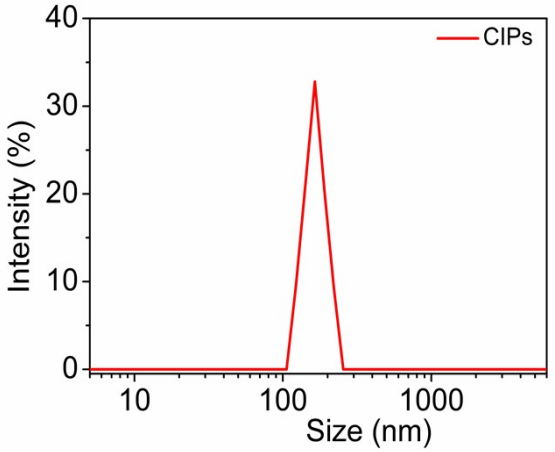

Figure S1. Hydrodynamic sizes of CIPs dispersed in PBS.

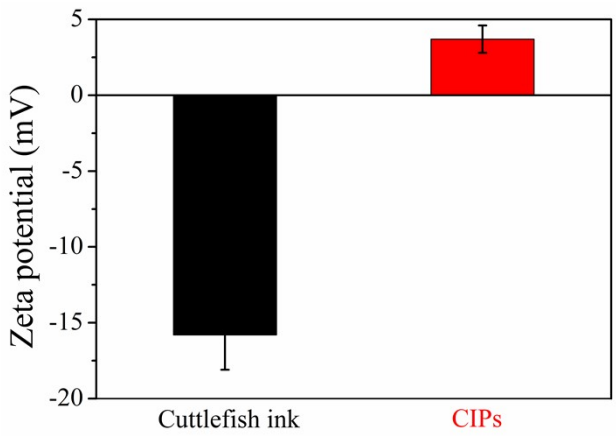

Figure S2. Zeta potential of Cuttlefish ink and CIPs.

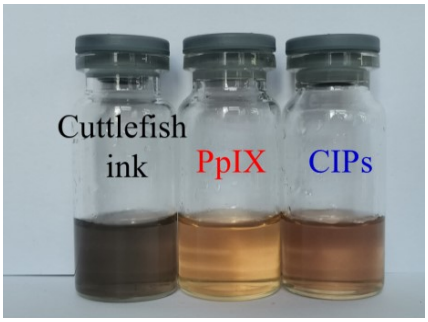

Figure S3. The photographs of cuttlefish ink, PpIX and CIPs dispersions.

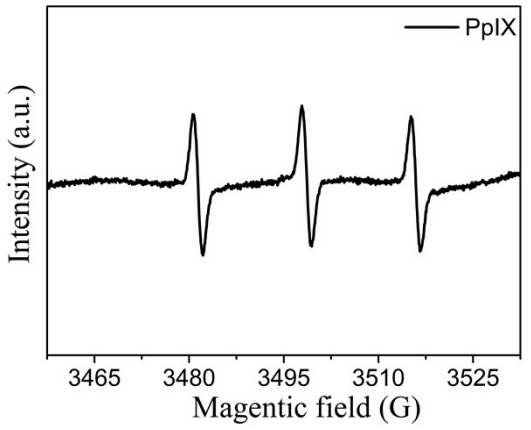

Figure S4. ESR signal of PpIX upon US with TEMP.

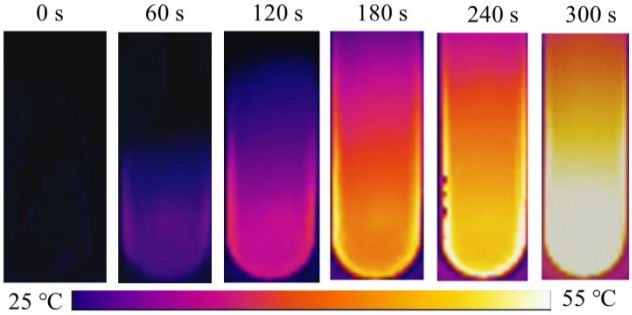

Figure S5. Thermographic images of CIPs (10 mg mL^{-1}).

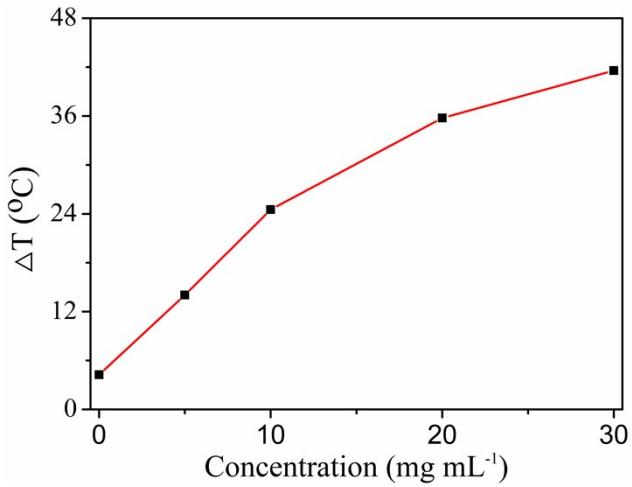

Figure S6. Temperature elevation (ΔT) of CIPs aqueous dispersions after laser irradiation (808, 1 W cm^{-2}) for 10 min.

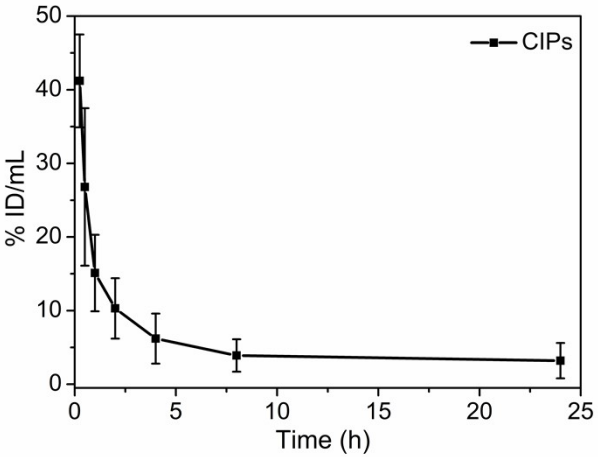

Figure S7. Pharmacokinetics of CIPs over a span of 24 h.

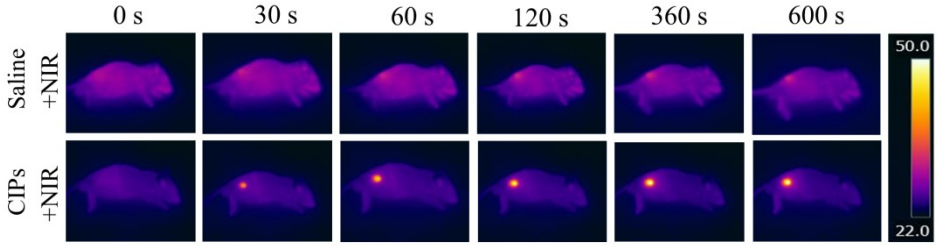

Figure S8. Thermographic images in tumor site of mice intravenous injected with the saline, or CIPs solution, under the irradiation of 808 laser at 1.0 W cm^{-2} for 600 s.

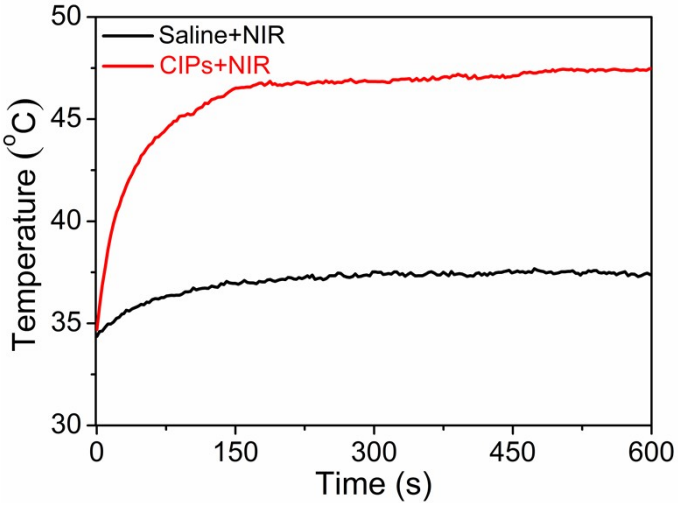

Figure S9. The temperature of tumors for mice injected with saline or CIPs and two hours later irradiated with 808 nm laser.