Supporting Information Available

Self-delivery nanomedicine for chemotherapy sensitized photodynamic therapy

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Experimental Section

1. Materials and Instruments.

Chlorine e6 (Ce6) was obtained from Frontier Scientific (Shanghai, China). Curcumin (Cur) and chromatographic grade acetic acid were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. Singlet oxygen sensor green (SOSG) was provided by Meilunbio. 2′,7′-dichloroflorescein diacetate (DCFH-DA), Hoechst 33342, methylthiazolyldiphenyl-tetrazolium bromide (MTT), Calcein-AM/PI Cell Viability/Cytotoxicity Assay Kit and Annexin V-FITC Apoptosis Detection Kit were obtained from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Thioredoxin reductase (TrxR) Activity Detection Kit was provided by Solarbio. Sodium dodecyl sulfate (SDS) and sodium chloride (NaCl) were purchased from Aladdin. Cell culture relevant reagents such as Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin, antibiotics and Dulbecco's phosphate buffered saline (PBS) were obtained from Gibco Invitrogen Corp. Chromatographic grade acetonitrile and methyl alcohol were purchased from Oceanpak. The instruments used in this work included DLS (Brookhaven, NanoBrook 90plus PALS), UV-vis spectrophotometer (Thermo Scientific, Evolution 300), fluorescence spectrophotometer (Thermo Scientific, Lumina), small animal imaging system (Bruker, FX Pro), microplate reader (BioTek, SYNERGY H1), CLSM (Carl Zeiss, LSM880), inverted microscope (Nikon, Eclipse Ni-U), HPLC (SHIMADZU, SPD-M20A), TEM (JEOL, JEM 1400plus).

2. Preparation and Characterization.

To prepare CeCu, Ce6 (0.45 mg) and Cur (0.9 mg) were dissolved in 165 μL of DMSO. After stirring for 4 h, the above DMSO solution was diluted by 2 mL of pure water. Subsequently, the solution was stirred for 6 h and left to set for another 12 h to obtain CeCu. The unassembled Ce6 and Cur were removed by centrifugation (4000 rpm/min) for 5 min. Different formulations were prepared using the similar method at various feed ratio of Ce6 and Cur. Drug contents of Ce6 and Cur were respectively calculated according to the standard curves obtained by using UV-vis spectrum and high performance liquid chromatography (HPLC). The particle size and zeta potential were measured by the method of dynamic light scattering (DLS). The morphology of particles was observed by transmission electron microscopy (TEM).

3. Exploration of Assembling Mechanism.

To explore the self-assembling mechanism, CeCu (8 mg/L) was treated with 0.2% SDS for 2 h and the UV-vis spectrum was detected. The spectra of CeCu and 0.2% SDS were also recorded as the controls. Moreover, the spectra of Cur, CeCu and Ce6 were also measured after being dispersed in water or DMSO.

4. Drug Release of Ce6

3 mL of CeCu (0.266 g/L) was placed in the dialysis tube (3500 Da) for dialysis against 15 mL of PBS with the pH value of 7.4 or 5.5. To detect the drug release, 1mL of PBS was taken out at the predetermined time for fluorescence analysis while isopyknic fresh PBS was added.

5. Cell Culture.

Murine mammary carcinoma (4T1) cells were cultured in DMEM medium containing 10% FBS and 1% antibiotics at 37℃.

6. ROS Detection.

ROS production was detected by using fluorescence spectrum and confocal laser scanning microscopy (CLSM). On the one hand, Ce6 (5 mg/L), Cur (8.3 mg/L) and CeCu (13.3 mg/L) were respectively mixed with SOSG (5 mg/L) in PBS. In the presence or absence of light (635 nm, 80 $mW \cdot cm^{-2}$), their fluorescence spectra were recorded every 10 s to detect the fluorescence changes of SOSG. On the other hand, after cultured for 24 h, 4T1 cells were incubated with Ce6 (1 mg/L), Cur (1.66 mg/L) or CeCu (2.66 mg/L) for 4 h. Then the cells washed by PBS and treated with DCFH-DA (10 μM) for 0.5 h. The intracellular fluorescence was observed by CLSM after irradiated with light (630 nm, 29.8 $mW \cdot cm^{-2}$) for 30 s or incubated in the shield of light.

7. TrxR Activity Evaluation.

TrxR activity was detected by TrxR Activity Detection Kit. In brief, 4T1 cells were seeded and cultured for 24 h. Then the cells were treated with Ce6 (6 mg/L), Cur (10 mg/L) or different concentrations of CeCu (2, 4, 8, 16 mg/L). 24 h later, the cells were washed with PBS and collected to be resuspended in reagent 1 at the concentration of 5×10^6 cells per mL. After ultrasonic decomposition, the cells in reagent 1 were centrifuged to obtain the supernatant. Subsequently, 100 μL of the supernatant was mixed with 2 μL of reagent 4 for 0.5 h at 37℃. After that, the supernatant (100 μL) was further treated with reagent 2 (100 μL), reagent 3 (100 μL) and reagent 1 (700 μL)

for 10 s. The absorbance (A_1) of the mixed solution was measured at 412 nm. After further incubation for 5 min at 37℃, the absorbance (A_2) was measured again at 412 nm. The difference value between A_2 and $A_1$was denoted by ΔA. The supernatant was replaced by the reagent 1 to be used as the blank control. The TrxR activity (U/10^4 cells) was calculated as 147 × (ΔA_{sample} - $\Delta A_{control}$)/N, where N represented the number of cells.

8. Cellular Uptake.

4T1 cells were seeded and cultured for 24 h. Then the cells were incubated with Ce6 (10 mg/L), Cur (16.6 mg/L) or CeCu (26.6 mg/L) for 3, 6 or 12 h. After that, the cells were washed by PBS and stained with Hoechst 33342 for 15 min. Intracellular fluorescence was observed by CLSM. Besides, the cells were also harvested for quantitative fluorescence analysis by flow cytometry.

9. MTT Assay.

4T1 cells were seeded and cultured in 96-well plates for 24 h. Then the cells were treated with gradient concentrations of Ce6, Cur or CeCu for 4 h. Subsequently, the cells were irradiated (630 nm, 29.8 mW·cm^{-2}) for 30 s or cultured in the shield of light. 20 h later, 20 μL of MTT (5 g/L) was added into each well to incubate for 4 h. After that, the culture medium of each well was replaced by 150 μL of DMSO. The absorbance at 570 nm was detected by a microplate reader. The cell viability was calculated as follows: cell viability (%) = OD_{sample} × 100/$OD_{control}$. In which, OD_{sample} and $OD_{control}$ represented the absorbance in the presence and absence of samples.

10. Live/Dead Cell Staining Assay.

After incubated for 24 h, 4T1 cells were treated with Ce6 (1 mg/L), Cur (1.66 mg/L) or CeCu (2.66 mg/L) for 4 h. Subsequently, the cells in light groups were irradiated (630 nm, 29.8 $mW \cdot cm^{-2}$) for 1 min. While the cells in dark groups were cultured in the shield of light. Then all of the cells were washed by PBS and treated with calcein-AM/PI for 0.5 h. At last, the cellular fluorescence was observed by CLSM.

11. Cell Apoptosis Detection.

4T1 cells were seeded and cultured in 6-well plates for 24 h. Then the cells were treated with Ce6 (1 mg/L), Cur (1.66 mg/L) or CeCu (2.66 mg/L) for 4 h. Subsequently, the cells were irradiated (630 nm, 29.8 $mW \cdot cm^{-2}$) for 30 s or cultured in the shield of light. Then the cells were harvested and stained with Annexin V-FITC/PI for 20 min. The cellular fluorescence was analyzed by flow cytometry.

12. Fluorescence Imaging *in Vivo*.

To construct the tumor model, 4T1 tumor cells (1×10^6) were subcutaneously injected into the right hind limb of the BALB/c mice. Afterwards, the mice were intravenously injected with Ce6 (1.69 mg/kg) or CeCu (4.5 mg/kg). At a predetermined time, the mice were performed for fluorescence imaging. 12 h later, the mice were sacrificed to collect the tumors and main organs for *ex vivo* imaging.

13. Antitumor Study *in Vivo*.

4T1 tumor-bearing mouse model was established by subcutaneously injecting 4T1 cells (1×10^6) into the right hind leg of female Balb/c mouse. Moreover, the treatment was started after 7 days of tumor growth. 4T1 tumor-bearing mice were randomly

divided into 6 groups, including PBS group, Cur group, Ce6 group, Ce6 (+) group, CeCu group as well as CeCu (+) group. In which, "(+)" represented the addition of light irradiation (635 nm, 0.80W cm^{-2}). To carry out the antitumor study, the mice were intravenously injected with Ce6 (1.69 mg/kg), Cur (2.81 mg/kg) or CeCu (4.5 mg/kg). 6 h later, the mice in light groups were irradiated for 10 min. During the treatments, the tumor volume and body weight of the mice were monitored every other day. Tumor volume (V) was calculated as $W^2L/2$, where W and L represented the shortest and longest diameter of tumors. Relative tumor volume was calculated as V/V_0, in which V_0 represented the initial tumor volume before treatments. After 15 days, the mice were sacrificed to harvest the tumors and main organs for H&E staining. In brief, the sacrificed tissues were fixed in formalin for 2 days, which were subsequently embedded in paraffin and sectioned into slices with 5 μm thickness. And then, the tissue slices were stained with haematoxylin and eosin. Finally, the stained sections of the tissue slices were photographed by using inverted microscope. Additionally, the tumors were photographed and weighed.

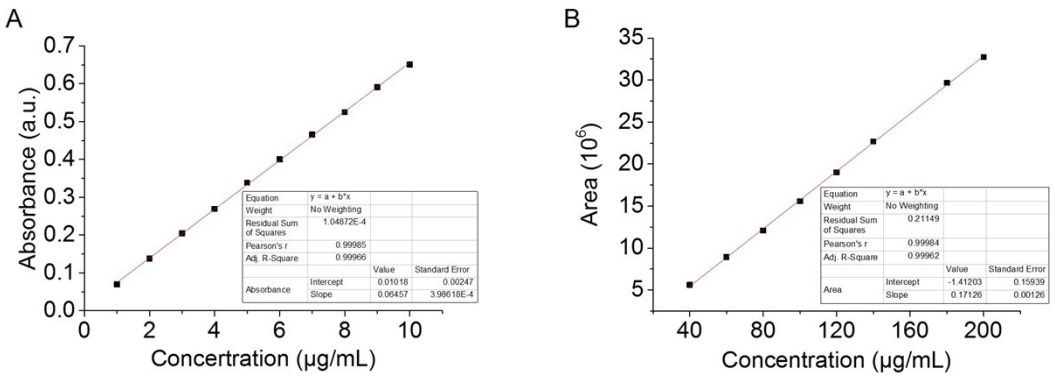
14. Statistical Analysis.

Statistical analysis was carried out by a Student's t-test. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.005$ were considered to be significant differences.

15. Live Subject Statement.

The *in vivo* experiments were performed in compliance with the guidelines of animal experiments established by Laboratory Animal Center of Southern Medical University (Guangzhou, China) as well as the Regulations of Guangdong Province on

the Administration of Experimental Animals.



C

Ce6/Cur	EE (%Ce6)	EE (%Cur)	LE (%Ce6)	LE (%Cur)
2/1	57.3	54.1	67.9	32.1
1/1	16.2	21.8	42.7	57.3
1/2	27.1	11.2	37.6	62.4
1/4	78.8	88.1	18.3	81.7

Fig. S1 Stand curves of (A) Ce6 and (B) Cur detected by UV-vis spectrum and HPLC. (C) Encapsulation efficiency (EE) and loading efficiency (LE) of Ce6 and Cur in various formulations obtained at different feed ratios.

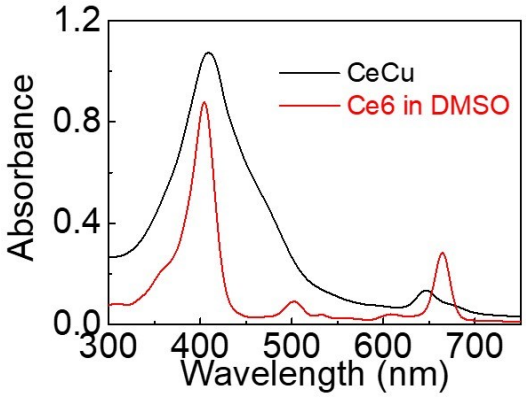


Fig. S2 The UV-vis spectrum of Ce6 and CeCu.

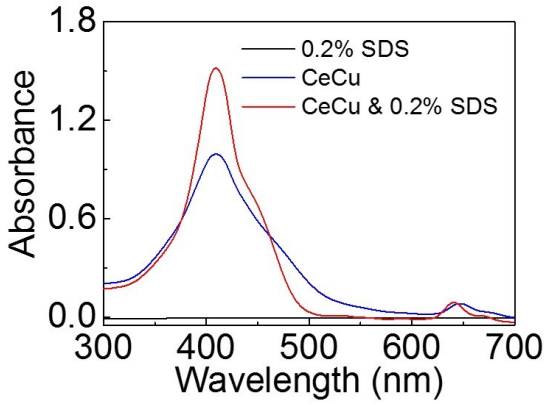


Fig. S3 The UV-vis spectrum of CeCu in the presence or absence of 0.2% SDS.

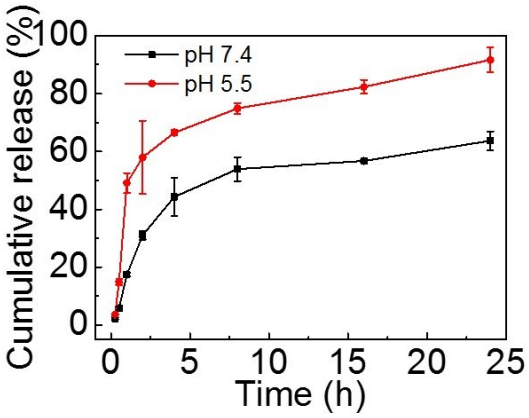


Fig. S4 The drug release behaviors of Ce6 from CeCu at pH 7.4 and 5.5.

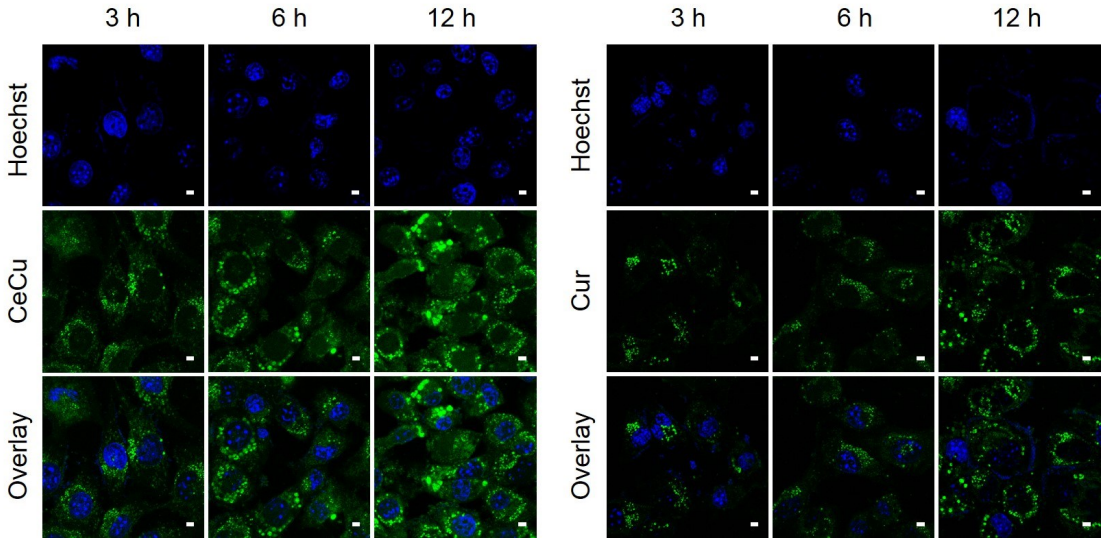


Fig. S5 CLSM images of 4T1 cells after treatment with CeCu and Cur for 3, 6 or 12 h and then stained by Hoechst 33342. Scale bar: 5 μm.

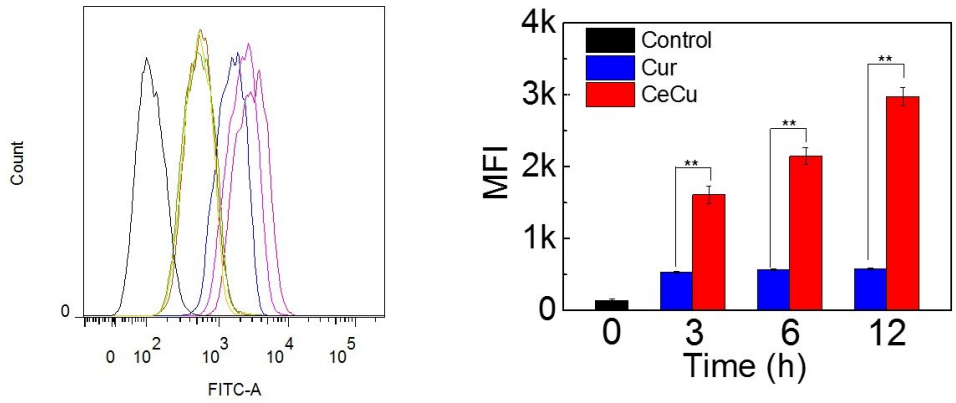


Fig. S6 Flow cytometry analysis of the Cur fluorescence in 4T1 cells after treatment with CeCu and Cur for 3, 6 and 12 h.

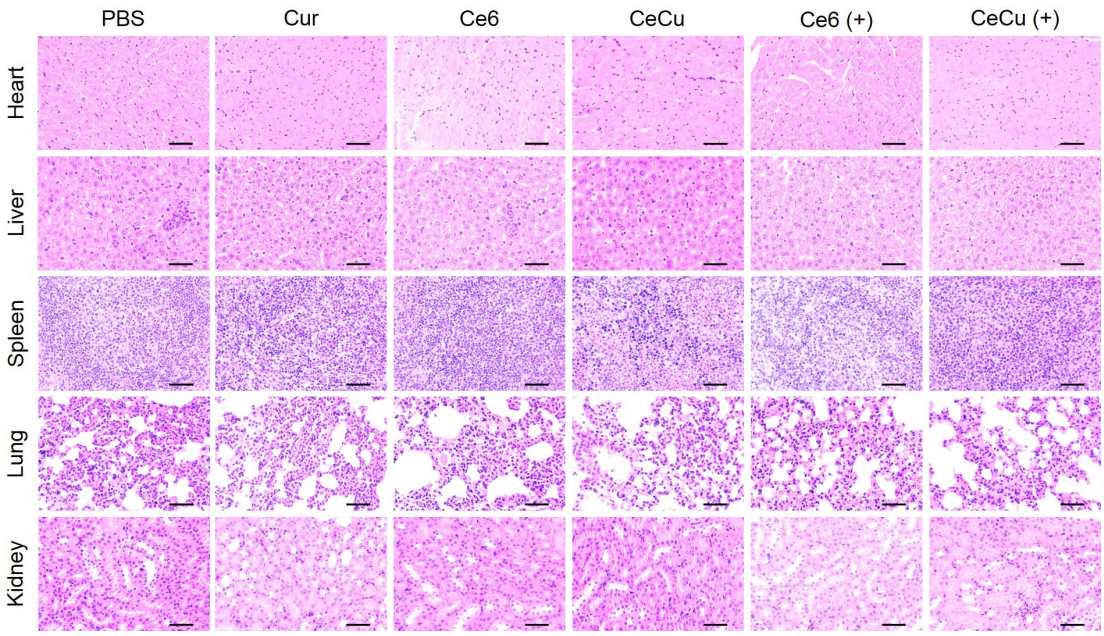


Fig. S7 H&E staining of heart, liver, spleen, lung and kidney.