Surporting Information

Platelet membrane-based biochemotactic targeted nanoplatform combined PDT with EGFR inhibition therapy for the treament of breast cancer

Guoyun Wan [a,†], Xuheng Chen [a,†], Ruiling Gou [a], Chenguang Guan [a], Jiayu Chen [a], Qian Wang [a], Wenjie Wu [a,b], Hongli Chen [a,b,*], Qiqing Zhang [a,*], Haijiao Wang [a,*]

[a] *The Key Laboratory of Biomedical Material, School of Life Science and Technology, Xinxiang Medical University, Xinxiang 453003, China*
[b] *The Third Affiliated Hospital of Xinxiang Medical University, Xinxiang 453003, China*

† These authors contributed equally to this work.
*Corresponding authors: E-mail: zhangqiq@126.com (Q. Zhang); WangHJ@xxmu.edu.cn (H. Wang); chenhl@xxmu.edu.cn (H. Chen).

Contents

Experimental section

Materials and methods

Materials

Chlorin e6 (Ce6) was bought from Aikonchem (Jiangsu, China), Lapatinib (Lap), calcein-AM, JC-1, DiO, and PI were purchased from Meilun Biotechnology (Dalian, China). Tetraethyl orthosilicate (TEOS), Cetyl trimethyl ammonium bromide (CTAB), Triethanolamine (TEA), and Sodium salicylate (NaSal) were bought from Heowns Biochem Technologies (Tianjin, China). 9.10-anthracenyl-bis (methylene) dimalonic acid (ABDA), 2′, 7′-dichloro-dichlorofluorescein diacetate (DCFH-DA), and singlet oxygen sensor green (SOSG) were purchased from Sigma-Aldrich (Shanghai, China). Coomassie brilliant blue methyl thiazolyl diphenyl-tetrazolium bromide (MTT) and Annexin-V-FITC/PI apoptosis kits were obtained from Solarbio Science & Technology (Beijing, China). Alexa Flour 488-labeled secondary antibodies and cytochrome C (Cyt-C) were bought from BIOSS Biotechnology (Beijing China).

Preparation of DLMSN/IR820 nanoparticles

The preparation method of DLMSN/IR820 nanoparticles was similar to that of DLMSN/Ce6/Lap nanoparticles. Briefly, 100 μL IR820 DMSO solution (50 mg/mL) was added to the 10 mL PBS solution containing 20 mg DLMSN and stir at room temperature for 12 h away from light. After that, DLMSN/IR820 nanoparticles were collected by centrifugation, washed once with PBS, and then dispersed in deionized water and stored at 4 ºC away from light for later use.

Preparation of RM@DLMSN/Ce6/Lap and RM@DLMSN/IR820 nanoparticles

Erythrocytes were obtained from normal mouse blood, and the fragments of red blood cell membrane (RM) were extracted through repeated centrifugation and lysis as previously reported,[1] and then added to PBS containing Cocktail and suspended at -80 ºC for storage. The RM droplets isolated from 50 μL of whole blood were added to 400 μg DLMSN/Ce6/Lap nanoparticles and sonicated for 1 min in an ice bath probe (power 100 W, sonication on for 3 s, off for 5 s) to obtain RM@DLMSN/Ce6/Lap biomimetic nanoparticles. The RM@DLMSN/IR820 nanoparticles were prepared the same as RM@DLMSN/Ce6/Lap nanoparticles.

Total protein profile analysis and cell membrane fluorescence labeling by DIO

The surface proteins on DLMSN/Ce6/Lap, PM@DLMSN/Ce6/Lap, RM@DLMSN/Ce6/Lap,

PM, and RM were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and protein staining was performed using coomassie brilliant blue reagent, and the gels were placed on a scanner for image scanning and saving pictures of the protein bands.[2] For cell membrane fluorescence labeling by DIO 40 μg of PM@DLMSN/Ce6/Lap and RM@DLMSN/Ce6/Lap biomimetic nanoparticles were incubated with a 5 μM DiO fluorescent probe for 30 min at 37°C. The DiO fluorescence signal in the biomimetic nanoparticles was detected by flow cytometry.

In vitro evaluation of ROS production

The PDT efficiencies of PM@DLMSN/Ce6/Lap were assessed by determining the $^1O_2$ generation levels using ABDA as a UV probe after different times of laser irradiation.[3] The free Lap, free Ce6, DLMSN/Ce6/Lap, and PM@DLMSN/Ce6/Lap nanoparticle solutions contained ABDA were laser irradiated for different time points at a power of 100 mW/cm^2, respectively. The UV absorption spectra of ABDA in each group of sample solutions were characterized by UV-Vis spectrometer.

Cell culture

4T1 cell and Human Umbilical Vein Endothelial Cells (HUVECs) were obtained from the National Biomedical Laboratory Cell Resource Bank (Beijing，China). The cells were cultured at 37 ℃ and 5% CO_2 in RPMI 1640 medium containing 10% FBS and 1% penicillin-streptomycin.

Mitochondrial integrity study

4T1 cells were cultured overnight at a density of 5×10^4 cells/well in a 24-well plate with 14 mm slides. Then the cell culture medium was replaced with fresh media containing free Ce6, DLMSN/Ce6/Lap, RM@DLMSN/Ce6/Lap and PM@DLMSN/Ce6/Lap nanoparticles (the concentration of Ce6 in each group was 2 μg /mL). After incubation for 4 h, each group was irradiated with a near-infrared 650 nm laser at laser power of 28 mW/cm^2 for 1 minute. In addition, the nanoparticles-treated cells without light irradiation were used as the control. After another 24 h incubation, the culture medium was replaced with 10 μM JC-1 staining solution according to the manufacturer's protocol.[4] Then cells were washed with pre-cooled PBS and the nucleus was stained by DAPI. Subsequently, the cells were imaged with a confocal microscope (Leica SP8, Germany).

For Cytochrome c (Cyt c) staining, after the nanoparticle treated cells were irradiated with 650 nm laser, the cells were further incubated for 24 h and then stained with MitoTracker Green (M7514). Next, the cells were fixed with paraformaldehyde, permeabilized with 0.5 % (v/v) Triton X-100, blocked with 3% BSA, and treated with a primary anti-Cyt c monoclonal antibody (1:250) overnight at 4°C and a secondary Alexa-647-Sheep Anti-Rabbit Secondary Antibody (1:400) according to the manufacturer's protocol.[5, 6] After that, the cells were stained with DAPI and then imaged with a confocal microscope (Leica SP8, Germany). Similarly, the nanoparticles-treated cells without light irradiation were used as the control.

***In vitro* HUVEC cell damage effect**

HUVEC cells were seeded into 96-well plates at a density of 8×10^3 cells/well and cultured overnight. After 60% confluence, the cell culture medium was replaced with fresh media containing free Ce6, free Lap, DLMSN/Ce6/Lap and PM@DLMSN/Ce6/Lap nanoparticles at various dosages. After incubation for 4 h, the cells were irradiated with a near-infrared 650 nm laser at laser power of 28 mW/cm^2 for 1 minute. After further cultured for 20 h, the culture medium was replaced by fresh culture medium containing MTT (500 μg/mL). After incubation at 37 ºC for 2.5 h, the culture medium was discarded and 150 μL DMSO was added. Finally the cell viability was measured by a microplate reader at 490 nm. In addition, the nanoparticles-treated cells without light irradiation were used as the control.

***In vivo* PDT mediated vascular damage**

4T1 tumor-bearing mice were intravenously injected with PM@DLMSN/Ce6/Lap nanoparticles via tail vein when the tumor volume was about 500 mm^3 (The Ce6 contents in each mice were 2 mg/kg). After 12 h post injection, the tumor was irradiated with a 650 nm near-infrared laser (the laser power was 300 mW/cm^2, and the irradiation time was 5 min). After 12 h post laser irradiation, the mice injected with 300 uL Ivans Blue solution at a dose of 6 mg/kg through the tail vein. 8 h later, all mice were euthanized and the tumors were isolated and harvested to observe by fluorescence microscope. The isolated tumor was then dried and cut into pieces in a solution containing formamide (the ratio of tumor mass to formamide was 100 mg: 1 mL), was incubated at 37 ºC for 24 h. After centrifugation at 3000 rpm for 10 min, the supernatant was taken, and the absorbance at 624 nm was detected by using a microplate reader, and the content of Evans blue in the tumor was calculated by using the standard concentration

curve of Evans Blue. In addition, the PBS and PM@DLMSN/Ce6/Lap bionic nanoparticles-treated cells without light irradiation were used as the control.

Hemolysis test

The hemolysis assay was employed to evaluate the biosafety of PM@DLMSN/Ce6/Lap. Erythrocyte solutions extracted from Balb/c mice were incubated with different concentrations of free Lap, free Ce6, DLMSN/Ce6/Lap, and PM@DLMSN/Ce6/Lap at 37°C for 4 h. After centrifugation at 10000 g for 5 min, the hemolysis was photographed and recorded, and the absorbance of the supernatant of each sample group at 577 nm was measured by enzyme marker to calculate the hemolysis rate, and the positive control was 100%.

Supplementary References

1. M. Gao, C. Liang, X. Song, Q. Chen, Q. Jin, C. Wang and Z. Liu, *Adv. Mater.*, 2017, **29**, 1701429.
2. H. Ye, K. Wang, M. Wang, R. Liu, H. Song, N. Li, Q. Lu, W. Zhang, Y. Du, W. Yang, L. Zhong, Y. Wang, B. Yu, H. Wang, Q. Kan, H. Zhang, Y. Wang, Z. He and J. Sun, *Biomaterials*, 2019, **206**, 1-12.
3. M. Xuan, J. Shao, J. Zhao, Q. Li, L. Dai and J. Li, *Angew. Chem. Int. Ed.*, 2018, **57**, 6049-6053.
4. H. Dai, H. Yan, F. Dong, L. Zhang, N. Du, L. Sun, N. Li, G. Yu, Z. Yang, Y. Wang and M. Huang, *Biomater. Sci.*, 2022, **10**, 1456-1469.
5. G. Wan, Y. Cheng, J. Song, Q. Chen, B. Chen, Y. Liu, S. Ji, H. Chen and Y. Wang, *Chem. Eng. J.*, 2020, **380**, 122458.
6. Z. Li, W. Pan, E. Shi, L. Bai, H. Liu, C. Li, Y. Wang, J. Deng and Y. Wang, *ACS Biomater. Sci. Eng.*, 2021, **7**, 772-786.

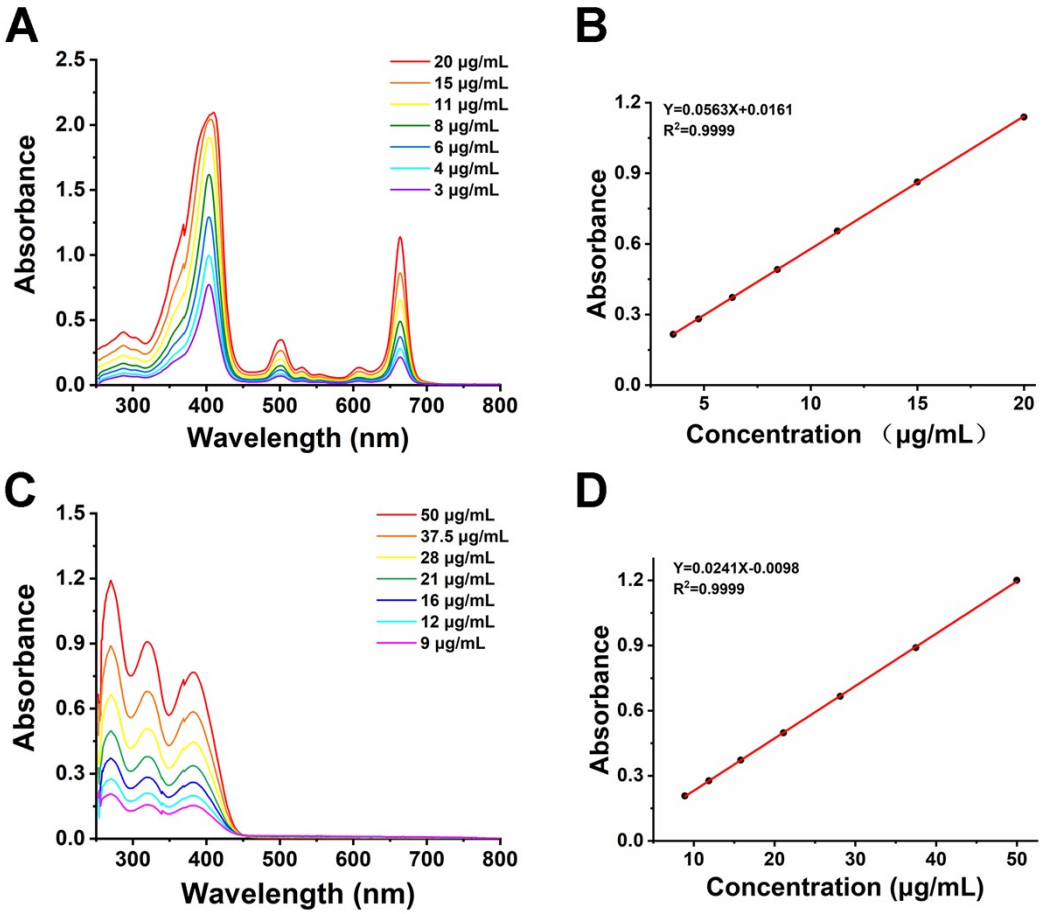

Fig. S1 (A) UV-visible absorption of Ce6 at different concentrations and (B) standard curve of Absorbance at 664 nm versus different Ce6 concentrations. (C) UV-visible absorption of Lap at different concentrations and (D) standard curve of of Absorbance at 270 nm versus different Lap concentrations.

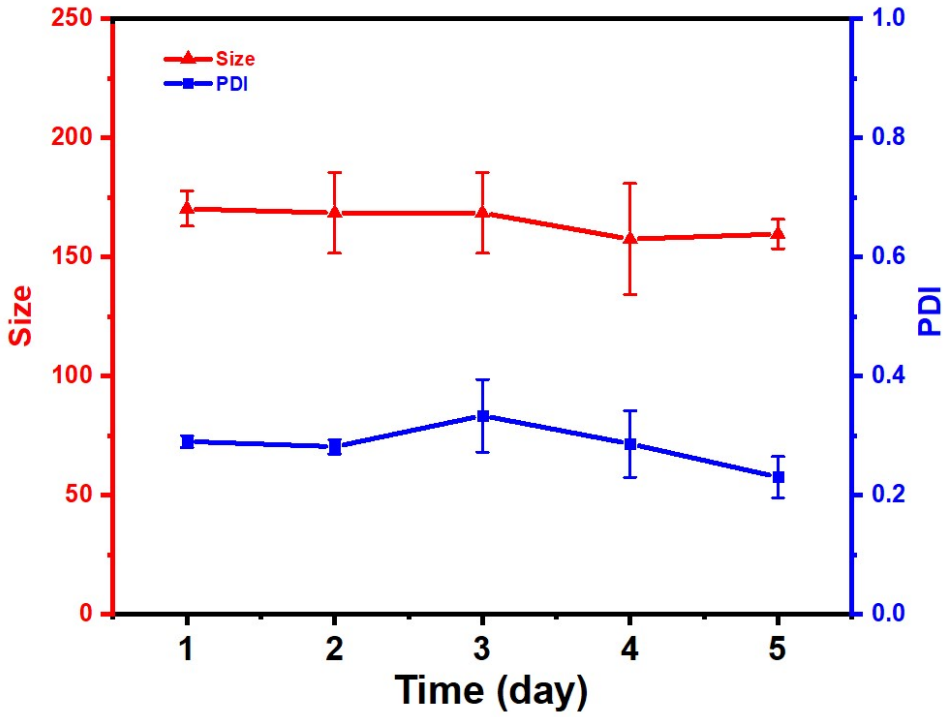

Fig. S2 The change curves of the size and PDI of PM@DLMSN/Ce6/Lap storagein PBS containing 10% FBS.

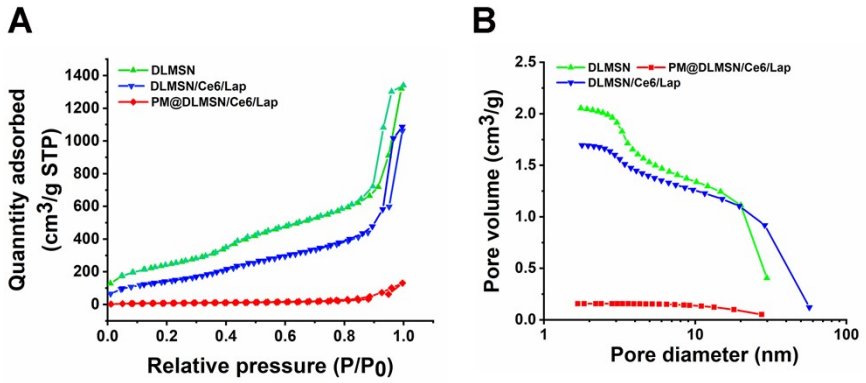

Fig. S3 (A) Nitrogen adsorption-desorption isotherm curves of various nanoparticles. (B) Pore volume distribution curves of various nanoparticles.

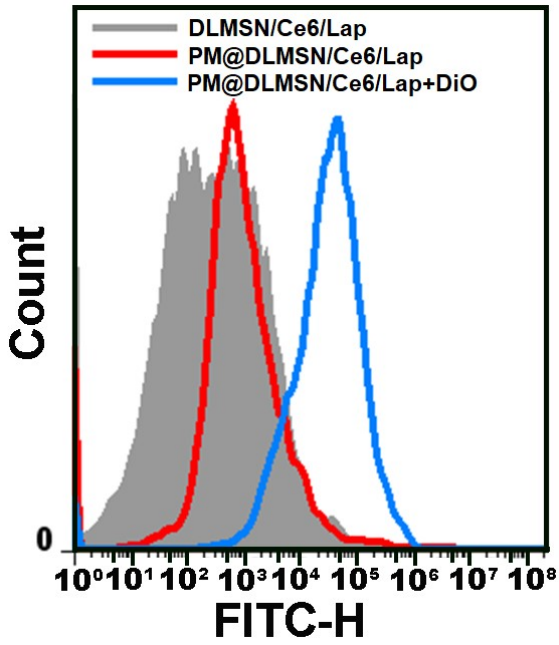

Fig. S4 Flow cytometry analysis of Dio stained PM@DLMSN/Ce6/Lap nanoparticles.

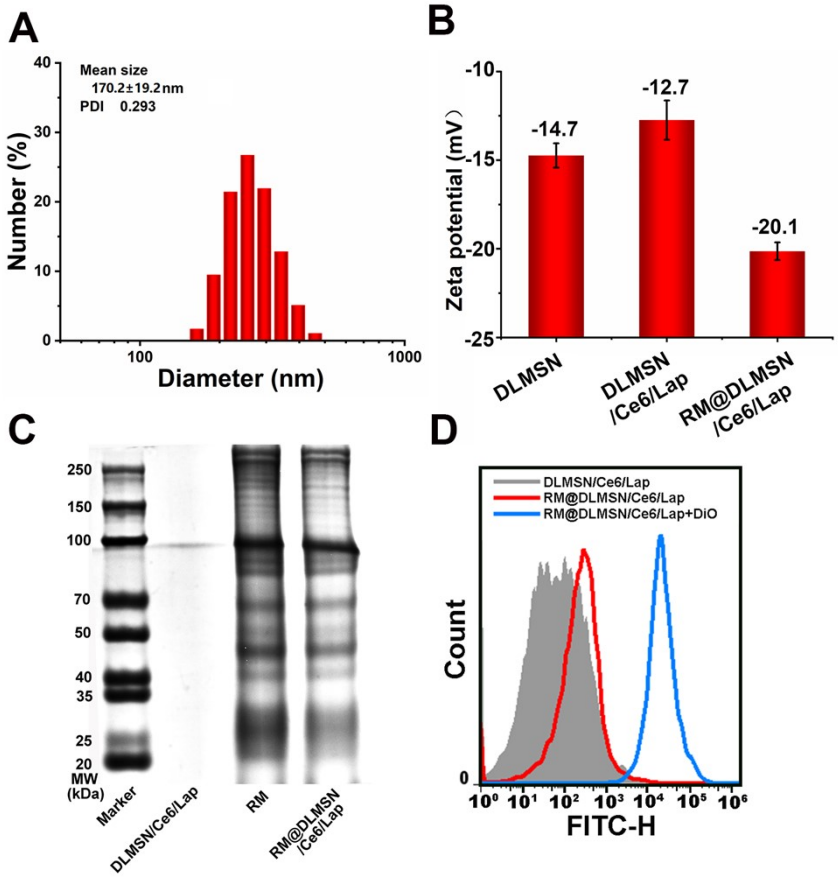

Fig. S5 (A) DLS diameter distribution of RM@DLMSN/Ce6/Lap biomimetic nanoparticles and (B) Zeta potentials of DLMSN, DLMSN/Ce6/Lap and RM@DLMSN/Ce6/Lap nanoparticles. (C)

Coomassie brilliant blue stained proteins bands isolated from DLMSN/Ce6/Lap, Rm and RM@DLMSN/Ce6/Lap nanoparticles. (D) Flow cytometry analysis of Dio stained RM@DLMSN/Ce6/Lap nanoparticles.

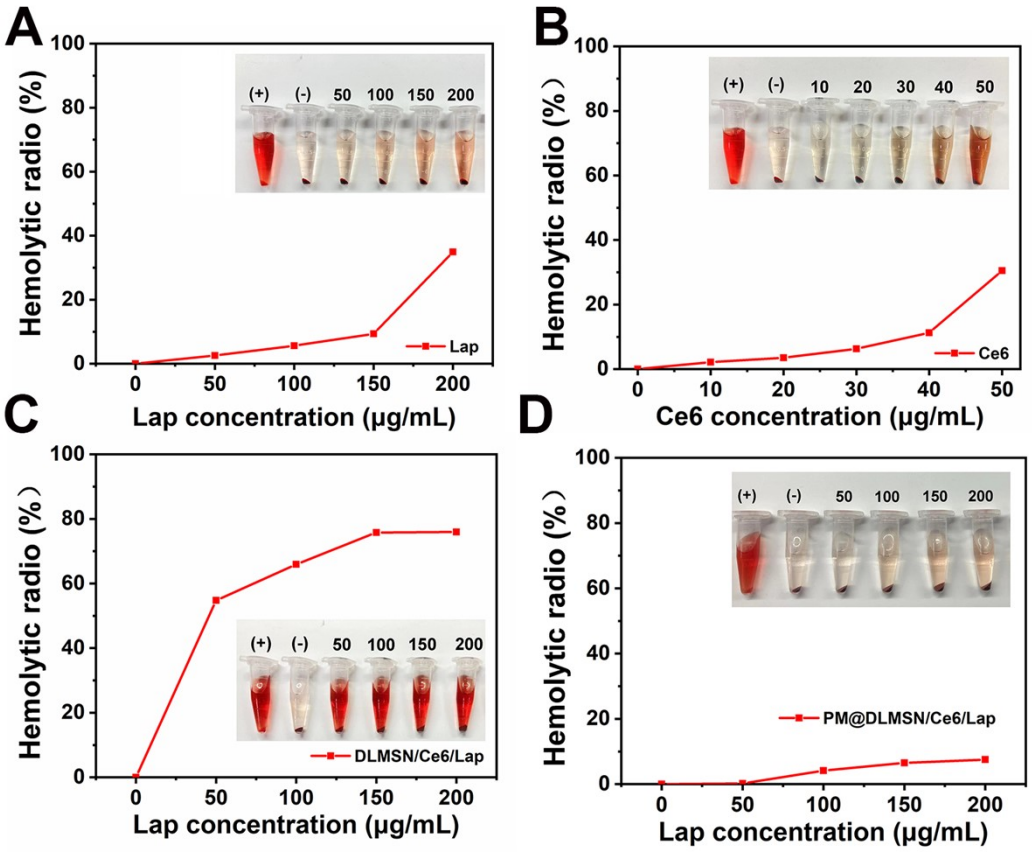

Fig. S6 Curve of hemolysis rate of (A) free Lap, (B) free Ce6, (C) DLMSN/Ce6/Lap and (D) PM@DLMSN/Ce6/Lap at different concentrations (insert image showing the supernatant of incubated red blood cells)

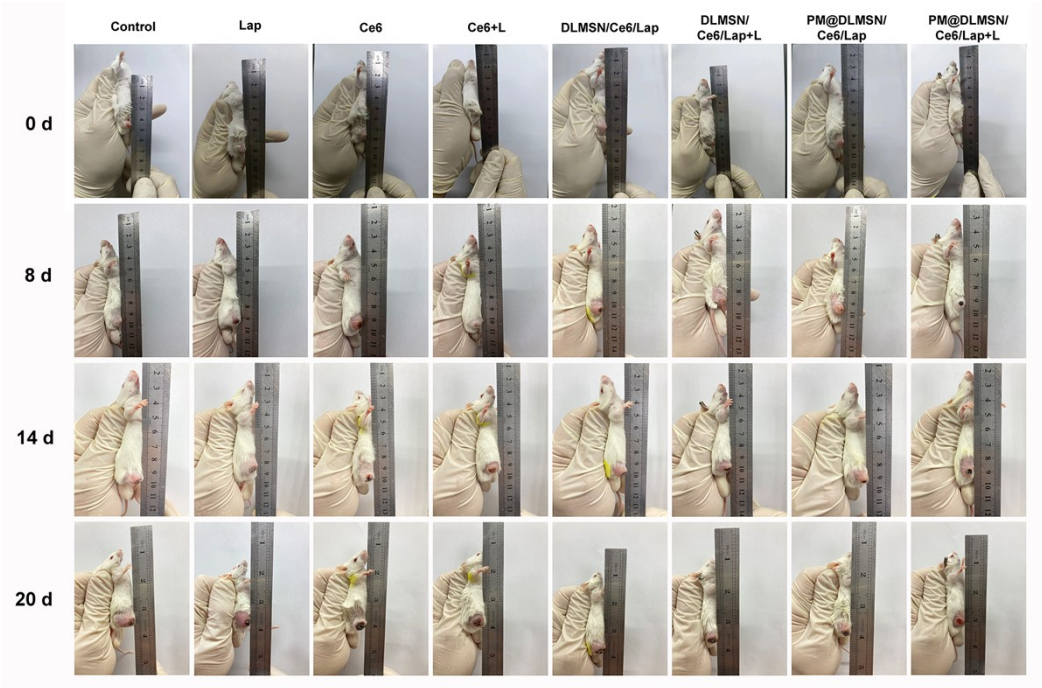

Fig. S7 Photographs of the mice at different stages of treatments.

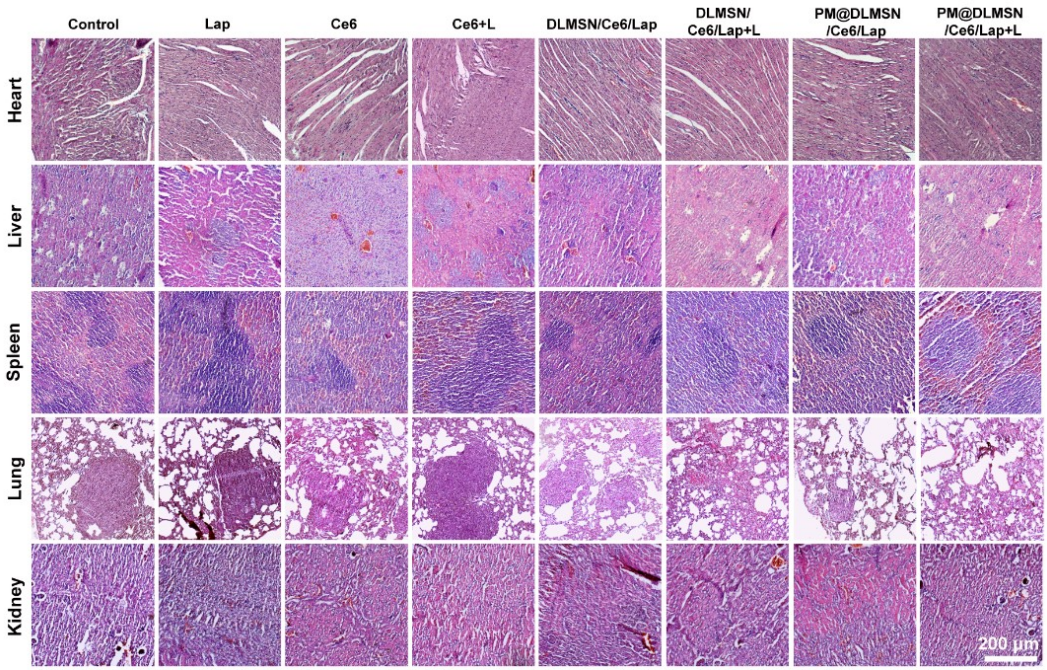

Fig. S8 H&E staining of isolated organ tissues after treatment.

Table S1. Parameters of blood biochemstry analysis

Items	Normal saline	PM@DLMSN/Ce6/Lap	Reference range	Unit
WBC	3.8±0.4	5.6±0.5	0.8-6.8	10^9/L
Lymph#	1.2±0.2	2.0±0.3	0.7-5.7	10^9/L
RBC	8.3±0.8	10.4±1.6	6.36-9.42	10^{12}/L
HGB	131±6.0	138±4.6	110-143	g/L
HCT	41.2±6.5	46.7±1.4	34.6-44.6	%
MCV	45.7±1.5	46.0±2.2	48.2-58.3	fL
MCH	16.7±1.7	18.7±2.4	15.8-19	pg
MCHC	354±28.7	313±15.4	302-353	g/L
RDW	12.7±0.4	16.3±0.5	13-17	%
PLT	543.2±32.3	547±52.6	450-1590	10^9/L

Five Balb/c mice were separately received intravenous injections of Normal saline and PM@DLMSN/Ce6/Lap on day 0 and day 3 with the same dose as the treatments use. All mice were sacrificed at day 6 and then the blood samples were collected for blood panel test. WBC: white blood cell; Lymph#: lymphocyte; RBC: red blood cell; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: Red blood cells distribution width; PLT: platelets.