Electronic Supplementary Information

Ultra-high loading of sinoporphyrin sodium in ferritin for single-wave motivated photothermal and photodynamic co-therapy

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1. Materials and method

1.1. Preparation of DVDMS loaded ferritin

The protocols for producing ferritins and encapsulation of DVDMS into the R-Fn was achieved following our established methods.[1] Briefly, DVDMS was added into R-Fn solution at the room temperature. To dissociate R-Fn into its subunits, the pH of solution was adjusted to 2 with 1M HCL and kept stirring about 30 min. Afterwards, the pH was turned back to 7.4 with 1M NaOH. The resulting solution was still stirred for 2 h to make R-Fn reconstituted fully. PD-10 column was used to purify the above mixed solutions to obtain R-Fn-DVDMS.

1.2. Characterization of R-Fn-DVDMS

The morphologies of R-Fn before and after loading DVDMS were characterized by transmission electron microscope (TEM). Furthermore, the size and zeta potential were characterized by Zetasizer Nano ZS (Marvins). The UV-vis spectra and fluorecence emission spectra of DVDMS and R-Fn-DVDMS were monitored with an F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) under 385nm excitation and Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc.), respectively. The in vitro and in vivo fluorecent images were measured by Carestream FX Pro (Caliper Life Sciences, Hopkinton, MA).

The protein concentration of R-Fn or R-Fn-DVDMS was detected by BCA kit and

the standard curve of protein concentrations was built by pre-prepared conference BSA solution: y=1.473x-0.328. We also built the standard curve for DVDMS concentration detection: y=4.375x+0.039, based on the characteristic absorption peak at 630 nm. The amount of DVDMS was calculated by detecting the unloaded DVDMS in the removed solutions after ultrafiltration through 100 KDa MWCO filters according to the built standard curve. The DVDMS-loading efficiency (DLE) was calculated by equation (1): $\mathrm{DLE\%} = \frac{M_{DVDMS}}{M_{Ferritin} + M_{DVDMS}} \times 100\%$.

Where M_{DVDMS} is the calculated weight of loading DVDMS in ferritin and $M_{Ferritin}$ is the weight of the remained R-Fn in the final solution.

1.3. *In vitro* PDT and PTT effect

The generation of $^1O_2$ was detected according reported procedure.[2] In the experiment, 2',7'-Dichlorofluorescein (DCFH) was used as a probe for the ROS fluorescent response. As the DCFH was an unstable agent and can't be stored, the DCFH solution used in the experiment was obtained by mixing the 10 uL of DCFH-DA solution (10 mM) with 400 uL of NaOH solution (10 mM) for 30 min. Then, 2ml 1×PBS was added as the stock solution for further use. To study the PDT effect of the R-Fn-DVDMS and DVDMS, 100 uL R-Fn-DVDMS or DVDMS solution (DVDMS, 20 ug/mL) and 100 uL above-prepared DCFH solution were mixed. The mixture was irradiated from 0 to 5 min by 630 nm 300 mW lasers (diode-pumped solid-state laser system (LASERGLOW Technologies, Toronto, Canada). The fluorescence of the DCFH was measured with fluorospectrophotometer using an excitation wavelength of 480 nm.

For PTT effect measurement, R-Fn-DVDMS or DVDMS at different concentrations and water solutions were irradiated with 630 nm laser for 10 min. The real-time temperature and thermal image was acquired by FLIR Ax5 camera (FLIR Systems Inc., Wilsonville, OR).

1.4. Cell uptake

For *in vitro* cell experiments, we applied 4T1 cells, obtained from the American Type Culture Collection (ATCC, Manassas, VA), were cultured in Dulbecco's Modified Eagle Medium containing 10% FBS and 1% penicillin/streptomycin at 37 °C under 5% CO_2. In cell uptake assay, 4T1 cells were seeded in Lab Tek II 6-well chamber slides (Nalge Nunc Internationl, Rochester, NY) with a density of 1 x 10^4 cells/mL. When cells grew to 60 - 80% confluency, medium was replaced and cells were incubated in the dark with R-Fn-DVDMS or Fn-DVDMS at DVDMS concentration of 5 μg/mL for 2 h, 6 h or 12 h. Then, the medium was removed and cells were washed with PBS for three times. After being mounted with a mounting solution containing DAPI (SlowFade® Gold Antifade Reagent with DAPI, Molecular Probes, Invitrogen) for nuclear staining, the cells were observed through an IX81 epifluorescence microscope (Olympus, Japan).

1.5. MTT assay

4T1 cells were seeded in 96-well plates at 10^4/well for overnight and incubated with predetermined concentrations of free DVDMS, the R-Fn and the R-Fn-DVDMS for another 12 h. Then, the cell activity was determined by standard MTT assay. For in-vitro PTT/PDT study, 4T1 cells incubated with R-Fn-DVDMS were exposed to 630 nm laser at 0.5 W/cm^2 for 5 min after adding NAC as ROS scavengers for PTT group and ice bathing for PDT group. Then, the cells were incubated for another 24 h and cell viability was measured using MTT assay.

1.6. Animal model

All animal experiments were approved by the Animal Management and Ethics Committee of Xiamen University. For *in vivo* study, athymic nude mice

(5 weeks old, femal,18-24 g) were obtained from animal breeding center of Xiamen university and the 4T1 tumor models were constructed by subcutaneously injecting 5×10^6 cells in PBS.

1.7. In vivo fluorescence imaging.

When tumor volume reached ~100 mm^3, 100 μL R-Fn-DVDMS solution or free DVDMS solution were injected at 8mg/kg for DVDMS. Fluorecence imagings were performed by Carestream FX pro at a series point and the fluorescence intensities of tumors were recorded. To analysis the distribution difference of R-Fn-DVDMS and free DVDMS, the mice were sacrificed at 48 h post-injection. Tumor and major organs including heart, liver, spleen, lung, kidney and muscle were collected and visualized by Carestream FX pro. The fluorescence intensity ratio (tumor/muscle) was calculated.

1.8. In vivo PTT/PDT efficacy

Mice bearing 4T1 tumor were randomized into 5 groups, when tumor volume reached ~100 mm^3, including R-Fn-DVDMS+laser group, R-Fn-DVDMS group, DVDMS laser group, PBS+laser group and PBS group. The mice of PBS laser group and PBS group received only 100 μL PBS whereas other groups received R-Fn-DVDMS or DVDMS at the equivalent DVDMS dose (8 mg/kg). For all laser groups, the tumor was irradiated with 630 nm laser (0.5 W/cm^2) for 5 min at the 48 h post injection and the themal images was acquired by FLIR Ax5 camera (FLIR Systems Inc., Wilsonville, OR).

The tumor sizes and mouse body weights was recorded every other day. Tumor volume were calculated as follows: tumor volume=$A \times B^2/2$, where A is the largest and B is the smallest diameter of tumor. The tumor volumes were normalized as V/V0 (V0 is the original tumor volume). The mice were sacrificed at the 16 d post injection and tumors and major organs were collected for Haematoxylin and eosin (H&E) staining.

2. Results and discussion

Table S1. The loading rate of R-Fn to drugs reported in literatures.

Drug	Loading Rate (wt%)	References
IR820	29.53	1
Doxorubicin	42.36	3
$ZnF_{16}PC$	40	4
DVDMS	66.67	This work

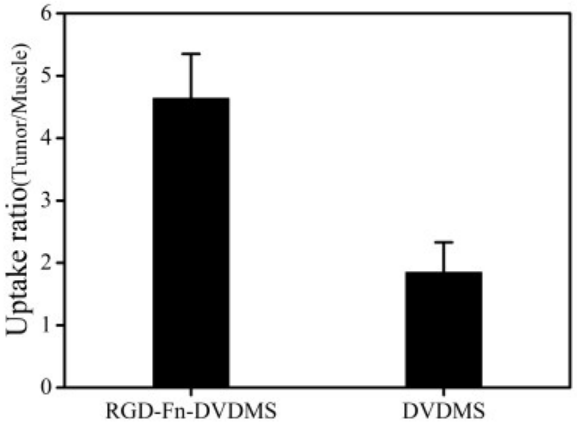

Fig S1. The fluorescence intensity ratio of ex vivo tumor to muscle 48h post-injection.

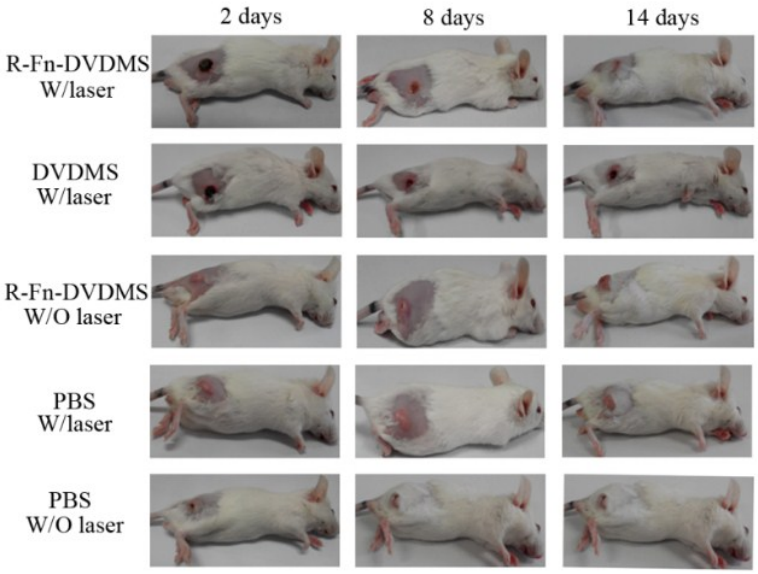

Fig S2. Representative photos of 4T1 tumor-bearing mice from different groups after treatment.

References

1. P. Huang, P. Rong, A. Jin, X. Yan, M. G. Zhang, J. Lin, H. Hu, Z. Wang, X. Yue, W. Li, G. Niu, W. Zeng, W. Wang, K. Zhou, X. Chen, *Adv. Mater*., 2014, **26**, 6401-6408.
2. L.-S. Lin, Z.-X. Cong, J. Li, K.-M. Ke, S.-S. Guo, H.-H. Yang and G.-N. Chen, *J. Mater. Chem. B*, 2014, **2**, 1031-1037.
3. Z. Zhen, W. Tang, H. Chen, X. Lin, T. Todd, G. Wang, T. Cowger, X. Chen and J. Xie, *ACS Nano*, 2013, 7, 4830-4837.
4. Z. Zhen, W. Tang, Yen-Jun Chuang, Trever Todd, Weizhong Zhang, Xin Lin, Gang Niu, Gang Liu, Lianchun Wang, Zhengwei Pan, Xiaoyuan Chen, and Jin Xie , ACS Nano, 2014, 8, 6004–6013 .