Supporting Information

Trojan-Horse Biomimetic Delivery Strategy using Mesenchymal Stem Cells for PDT/PTT Therapy Against Lung Melanoma Metastasis

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1. Supplementary Methods

1.1. Characterization of PDA-Ce6

The morphology of nanoparticles was acquired using transmission electron microscope (TEM, JEM-1230, JEOL, JP) without staining. Particle size and zeta potential were determined by laser light scattering (Zetasizer NanoZS, Malvern, UK).

The content of Ce6 in supernatant was determined by HPLC. The grafting efficiency (GE) and drug loading rate (DL) were calculated using the following equations:

$$GE(\%) = \frac{M_{feeding} - M_{free}}{M_{feeding}} \times 100\% \quad (1)$$

$$DL(\%) = \frac{M_{feeding} - M_{free}}{M_{PDA} + M_{feeding} - M_{free}} \times 100\% \quad (2)$$

where $M_{feeding}$ is the feeding amount of Ce6, M_{free} is the amount of Ce6 in the supernatant, M_{PDA} is the feeding amount of PDA.

1.2. *In vitro* release

The release of Ce6 *in vitro* was investigated using the dialysis method. Nanoparticles conjugated with 70 μg of Ce6 in dialysis bags were immersed into buffers (pH = 4.0, 6.0, 7.4) containing 0.2% Tween 80 and shaken at 37 °C. The

medium was collected and analyzed by the fluorescence spectrophotometer (RF-5301pc, Shimadzu, JP, Ex = 400 nm, Em = 660 nm).

1.3. TEM analysis of MSC-PDA-Ce6

MSCs were incubated with PDA-Ce6 (4 μg/mL, Ce6-equiv.) for 4 h and harvested. The cell pellets were fixed with 4% paraformaldehyde overnight and post fixed with osmium tetroxide. The specimens were dehydrated in series of graded alcohol solutions and embedded in Araldite and polymerized for 24 h. Sections were cut and observed by TEM.

1.4. Intracellular distribution of PDA-Ce6

MSCs were incubated with PDA-Ce6. Then, cells were washed and stained with LysoTracker Green and DAPI.

1.5. Intracellular and cell-membrane associated Ce6

Both trypan blue staining and trypsinization method was applied to distinguish the absorbed PDA-Ce6 from the internalized ones. Trypan blue was used to quench the extracellular fluorescent signal. MSCs (5×10^4 cells/well) were incubated with PDA-Ce6 (4 μg/ mL). Then, cells were washed with PBS and incubated with trypan blue (0.4%, PBS) for 3 min. The cells were washed, fixed, and stained with DAPI before CLSM observation. For quantitative determination, MSC-PDA-Ce6 before or after typan blue wash was lysed by 0.5% Triton X-100 solution. The cellular and intracellular Ce6 levels were measured by the fluorescence spectrophotometer.

To validate the trypan blue staining method, the MSC-PDA-Ce6 were trypsinized. The cell pellets (internalized PDA-Ce6) and supernatant (adsorbed PDA-Ce6) were collected and assayed.

1.6. Ce6 and PDA-Ce6 identification in MSCs

MSC-PDA-Ce6 was culture for 12 h after culture medium was renewed. Cell lysate and conditioned medium (CM) was collected for HPLC assay.

Sephadex G50 column was also used to separate particulate Ce6 and free Ce6 which might be degraded from PDA-Ce6 intracellularly. Cell lysate dissolved in PBS (0.5 mL) was eluted using PBS containing 0.02% Tween 80 and 0.1% Triton 100, and 0.1 mL fractions were collected. The fractions were monitored with the fluorescence

spectrophotometer. The control sample contained 5 μg/mL PDA-Ce6 and 5 μg/mL free Ce6.

2. Supplementary Figures

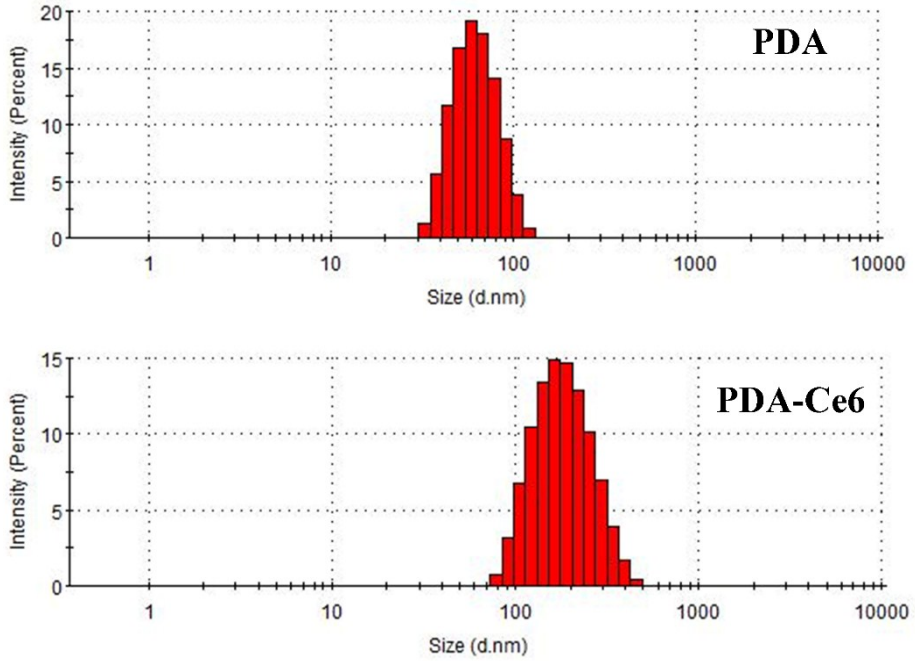

Fig. S1. Particle size distribution of PDA and PDA-Ce6.

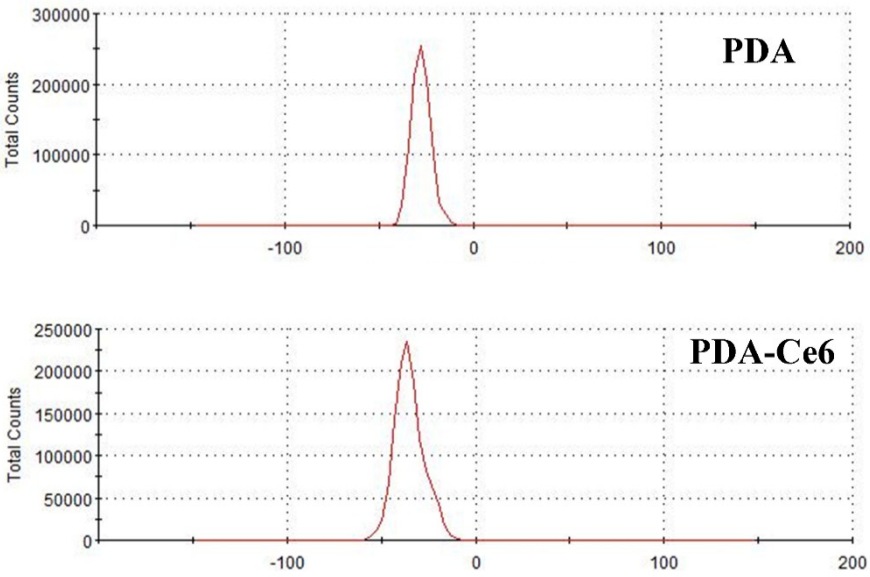

Fig. S2. Zeta potential of PDA and PDA-Ce6.

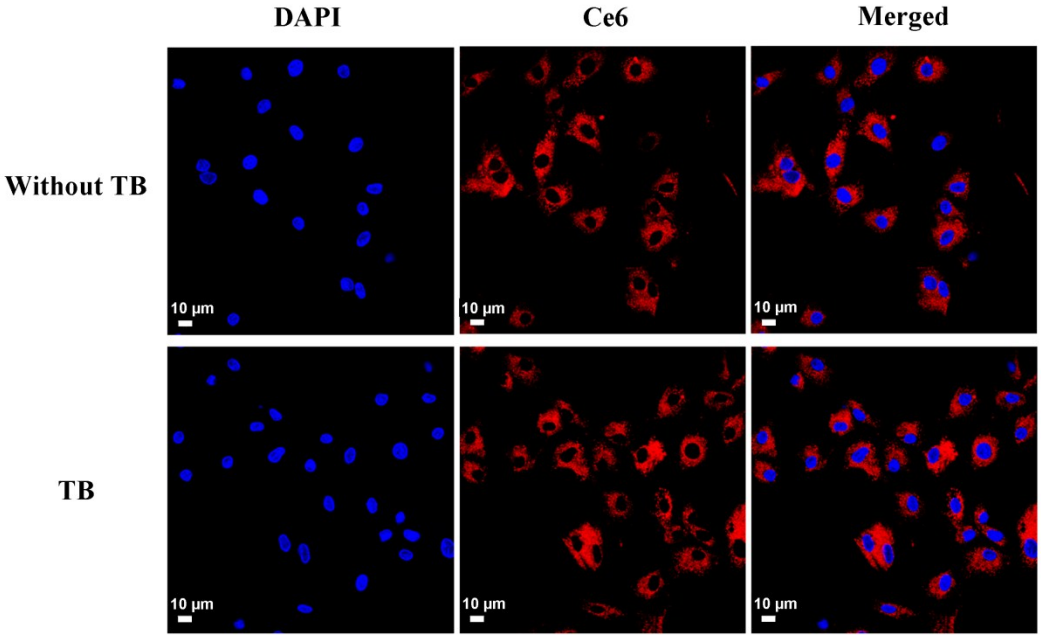

Fig. S3. Cellular and intracellular PDA-Ce6 (red) in MSCs. MSC-PDA-Ce6 was prepared by incubating MSCs with PDA-Ce6 (4 μg/mL) for 4 h. CLSM images of MSC-PDA-Ce6 were acquired before and after trypan blue wash to quench the extracellular fluorescence. The nuclei were stained with DAPI (blue). Scale bar: 10 μm. No obvious attenuation of Ce6 fluorescence intensity was observed after quenching. The membrane associated PDA-Ce6 was insignificant.

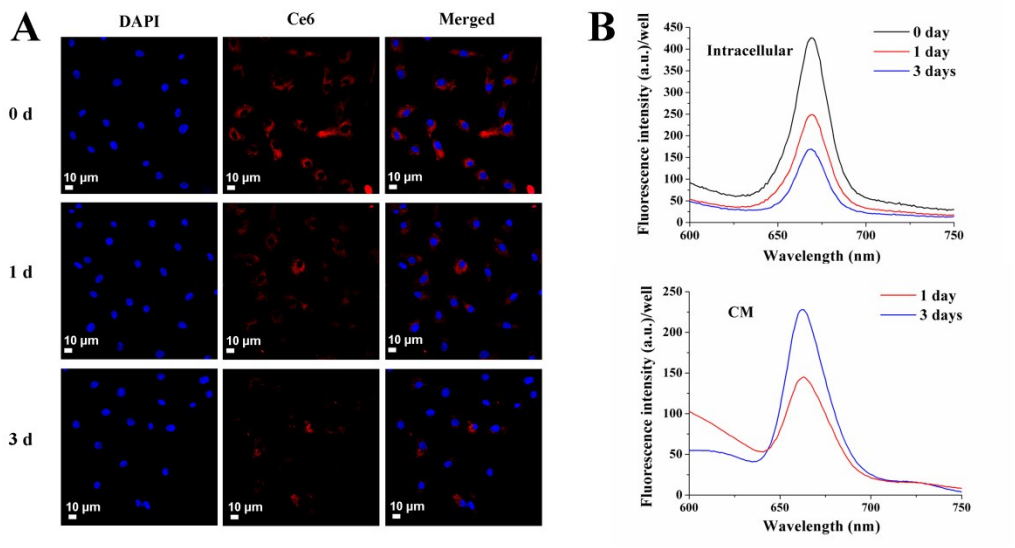

Fig. S4. Exocytosis of PDA-Ce6 from MSC to conditioned medium (CM). (A) CLSM images of MSCs when Ce6 was released from MSCs-PDA-Ce6 after PDA-Ce6 withdraw. 5×10^4 cells/well MSCs incubated with PDA-Ce6 (4 μg/mL, Ce6-equiv.) for 4 h. Then, fresh CM was renewed and replaced every day. Cells were fixed, stained and observed by CLSM at 0 d, 1 d and 3 d latter, respectively. Scale bar: 10 μm. (B) Fluorescent spectra of Ce6 in cell lysate and CM. Cells were lysed after drug withdraw on day 0, day 1 and day 3. CM was collected on day 1 and day 3. Significant amount of Ce6 was transferred from MSC to the culture medium.