Application of Singlet Oxygen-Activatable Nanocarriers to Boost X-ray-Induced Photodynamic Therapy and Cascaded Ferroptosis for Breast Cancer

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**Experimental methods**

**Materials**

RPMI 1640 medium, fetal bovine serum (FBS) and trypsin were purchased from Gibco BRL (Gaithersberg, MD; USA). Cell counting kit-8 (CCK-8) and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Beyotime Biotechnology Co., Ltd. (Nantong; China). Liperfluo was obtained from Beijing Dojindo Biotechnology Co., Ltd (Beijing, China). Annexin V-FITC/PI was purchased from Invitrogen Corporation (IVGN; USA). All other solvents were of analytical grade.

**Characterization**

The proton nuclear magnetic resonance (1H NMR) spectra were recorded in CD$_3$SOCD$_3$ on a 400-MHz spectrometer (Avance III, Bruker, Germany). The mean particle size, polydispersity, and zeta potential were measured by dynamic light scattering (DLS) with a Malvern Zetasizer (Nano-ZS, Malvern Instruments, UK). The morphology of nanoparticles was observed by transmission electron microscopy (TEM, Hitachi HT7700). The UV/Vis absorptions of nanoparticles and drug loading efficiency were detected on a UV/Vis spectrophotometer (UV-3600 Shimadzu, Japan). The fluorescence of SOSG was measured using a Hitachi F7000 fluorescence spectrophotometer. *In vivo* imaging of small animals was captured by Xenogen IVIS Lumina system (AMI-934M, USA). *In vivo* imaging of small animals was captured by Xenogen IVIS Lumina system (AMI-934M, USA).

**Cellular internalization of nanocarriers in vitro**

For confocal laser scanning microscope (CLSM) observations, 4T1 cells were
seeded onto 14-mm coverslips at a density of $2 \times 10^4$ cells per well in 1.0 mL of 1640 medium, and cultured at 37 °C with 5% CO$_2$ for 12 h. The cells were then incubated with NP$_{VR}$ or D-NP$_{VR}$ for 2, 4 or 6 h, washed with PBS, fixed with 4% paraformaldehyde, and then stained with phalloidin and 4′,6-diamidino-2-phenylindole (DAPI) sequentially according to the manufacturer's protocol. The cellular internalization was then visualized on a Zeiss LSM 810 confocal microscope.

**Biosafety Evaluation**

Twenty healthy BALB/c mice were randomly divided into four groups and received i.v. injection daily for three times with PBS, free VP + RLS3, NP$_{VR}$, or D-NP$_{VR}$, respectively. The orbital plexus blood sampling was collected on day 7 for routine blood count, creatinine, urea nitrogen, alanine aminotransferase, and aspartate aminotransferase detection.

**Statistical Analysis**

Statistical significance was analyzed using a t-test. One-way analysis of variance (ANOVA) was used for comparisons between more than two groups. Unless specially noted, data are shown as mean ± SD ($p<0.05$).
Figure S1. Synthetic route of 1,2-bis(2-hydroxyethylthio)ethylene bridged D-HPE.
Figure S2. $^1$H NMR spectrum of D-HPE in CDCl$_3$ recorded on an AVANCE III 400 MHz spectrometer at 25 °C.

Figure S3. $^1$H NMR spectrum of HPE in CDCl$_3$ recorded on an AVANCE III 400 MHz spectrometer at 25 °C.
**Figure S4.** The diameter (A) and morphology (B) change of NP\textsubscript{VR} and D-NP\textsubscript{VR} with X-ray irradiation. The scale bar is 200 nm.

**Figure S5.** Bis(2-hydroxyethythio)ethylene linker degradation of D-NP\textsubscript{VR} with or without 4 Gy of X-ray radiation.
Figure S6. Fluorescent imaging of lipid peroxides in 4T1 cells. The cells were treated by various formulations including VP&RSL3, NP$_{VR}$, and D-NP$_{VR}$.

Figure S7. Relative viabilities after incubation with NP$_{VR}$ or D-NP$_{VR}$ on 4T1, MCF-7 and NIH-3T3 cells.
Figure S8. Following i.v. injections, the doxorubicin fluorescence observed by CLSM at 24 h with (+) or without (-) X-ray radiation.

Figure S9. Body weight change following multiple i.v. injections of PBS, free VP&RSL3, NPVR or D-NPVR.
Figure S10. Complete blood count in BALB/c mice after i.v. injections (n = 5).

Table S1. Drug loading contents (DLC) and encapsulation efficiencies (EE) of VP and RSL3 for NP_{VR} and D-NP_{VR}.

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<tr>
<th></th>
<th>DLC (%)</th>
<th>EE (%)</th>
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<tr>
<td></td>
<td>VP</td>
<td>RSL3</td>
</tr>
<tr>
<td>NP_{VR}</td>
<td>3.39</td>
<td>3.22</td>
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<tr>
<td>D-NP_{VR}</td>
<td>3.53</td>
<td>3.07</td>
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