Electronic Supplementary Information

Amphiphilic Janus Nanoparticles for Nitric Oxide Synergistic Photodynamic Eradication of MRSA Biofilms

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1. Materials and methods

1.1 Reagents

Calcium hydroxide ($Ca(OH)_2$), Rhodamine 6 G (purity: 95%) were purchased from Sigma Aldrich (Shanghai) Trading Co. Ltd. Dopamine hydrochloride was purchased from Sa'en Chemical Technology (Shanghai) Co., Ltd. Ammonia solution (25%) was purchased from Tianjin Beichen Fangzheng Reagent Factory (Tianjin, China). Sodium phosphate dibasic dodecahydrate ($Na_2HPO_4.12H_2O$), S-Nitrosoglutathione (GSNO) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). chlorin e6-polyethylene $glycol_{2K}$-thiol (Ce6-PEG_{2K}-SH), Chlorin e6 (Ce6) were purchased from Ruixi Biological Technology Co., Ltd (Xi'an, Shanxi, China). L-ascorbic acid and Glutathione reduced were purchased from Tianjin Heowns Biochemical Technology Co., Ltd (Tianjin, China).

CheKine™ Micro Reduced Glutathione (GSH) Assay Kit was purchased from Abbkine Biotechnology Co., Ltd (Wuhan, China). Nitric Oxide Assay Kit was purchased from Shanghai yuanye Bio-Technology Co., Ltd (Shanghai, China). 2',7'-dichlorodifluorescein diacetate (DCFH-DA) was purchased from MedChemExpress (Monmouth Junction, NJ, USA). A LIVE/DEAD® BacLight™ Bacterial Viability Kit (L7012) was purchased from Thermo Fisher Scientific Inc (Shanghai, China).

1.2 Bacteria and cells

MRSA USA300 were purchased from Hangzhou BIOSCI Company (Hangzhou, China). Vero cells were purchased from American Type Culture Collection (Manassas, VA, USA), and cultured in DMEM media (Gibco BRL, Grand Island, NY) containing 10% FBS.

1.3 Animals

BALB/c mice were purchased from Pengyue Laboratory Animal Breeding Co., Ltd (Jinan, Shandong, China). The New Zealand white rabbits were purchased from Qingdao Kangda Biotechnology Co., Ltd (Qingdao, Shandong, China). The animal studies were conducted according to the experimental protocols by Institutional Animal Care and Use Committee of Binzhou Medical University.

1.4 Acute toxicity test

The *in vivo* acute toxicity of Ce6-PDA/CaP-GSNO was evaluated using Maximal Tolerance Dose (MTD) method here. Twenty-four BALB/c mice of both sexes were equally divided into two

groups (n=12, 6 male and 6 female mice). Each animal was *i.v.* injected with 200 μL of Ce6-PDA/CaP-GSNO with an equivalent Ce6 dose of 2.0 mg/kg, which was 10-fold to the clinical dose used in this study. The control group was treated in an identical manner except that a similar volume of saline was used instead. The body weights of the mice were measured every 2 days. At 14th day, all animals were sacrificed. Major organs (heart, liver, spleen, lung and kidneys) were stained with haematoxylin-eosin (H&E) for histopathologic examination. In addition, the serum samples were collected for serological analysis on automatic biochemical analyzer Chemray 420 (China).

2. Results

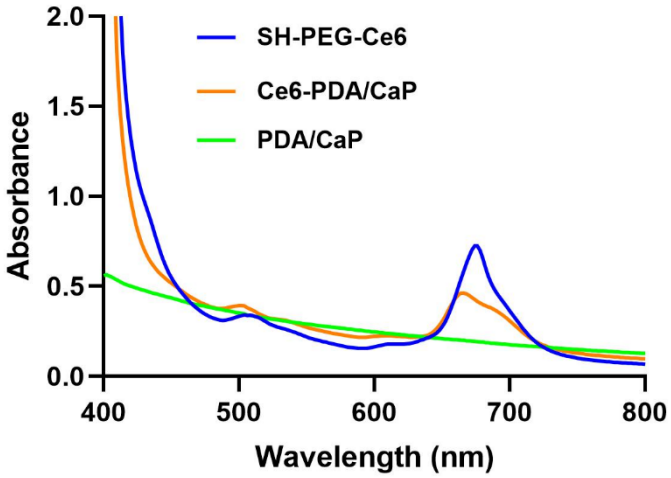


Fig. S1 The UV-vis spectra of free Ce6-PEG-SH, PDA/CaP, Ce6-PDA/CaP JNPs.

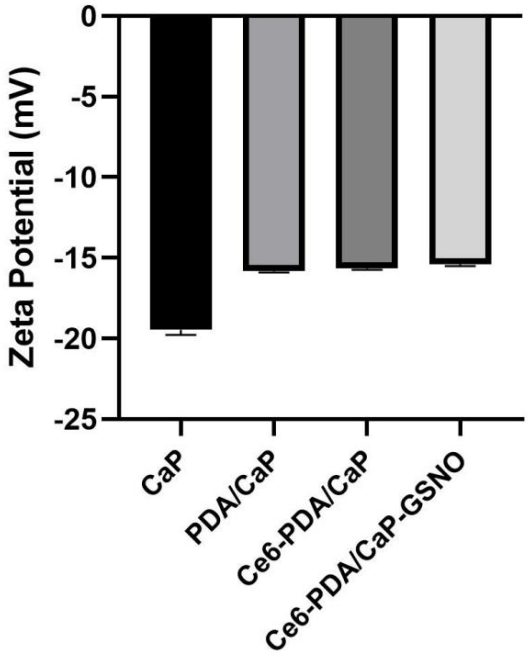


Fig. S2 Zeta potentials of CaP NPs, PDA/CaP NPs, Ce6-PDA/CaP JNPs and Ce6-PDA/CaP-GSNO JNPs

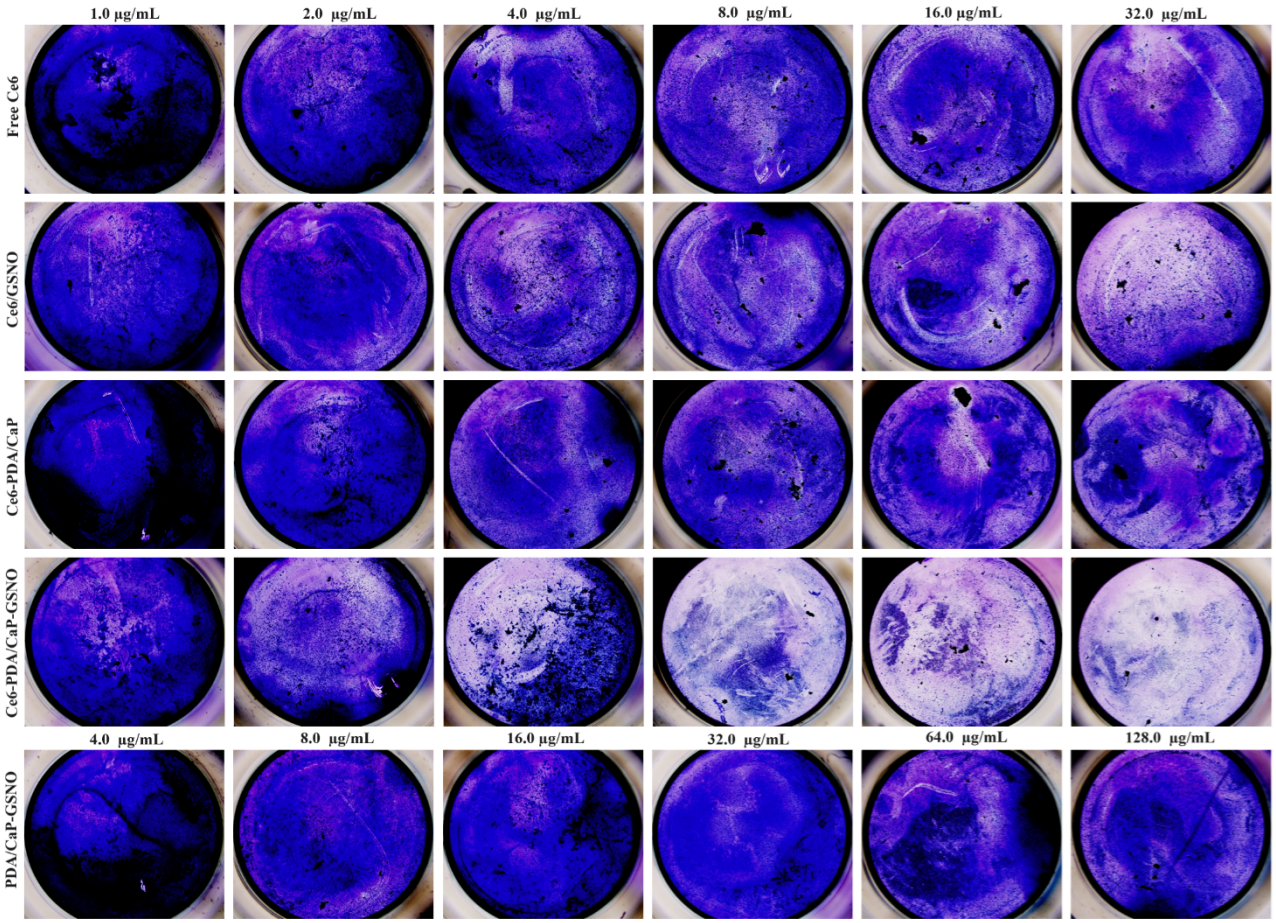
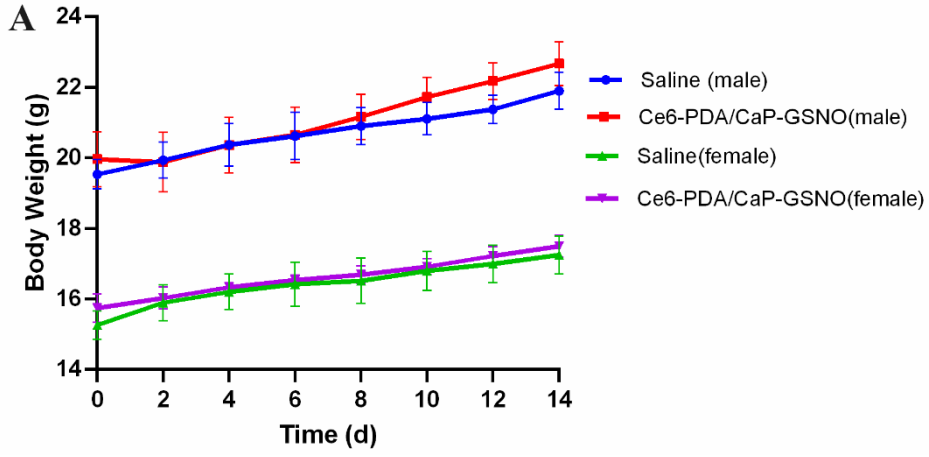


Fig. S3 Crystal violet staining images of MRSA biofilms after treated with free Ce6, Ce6/GSNO, Ce6-PDA/CaP, PDA/CaP-GSNO and Ce6-PDA/CaP-GSNO at different concentrations.

Table S1 Hepatorenal toxicity parameters in BALB/c mice (n=6)

Formulations	ALT (U/L)	AST (U/L)	UA (μmol/L)	CR (μmol/L)
Saline (male)	63.09 ± 1.15	113.07 ± 2.88	73.48 ± 1.95	17.34 ±1.25
Ce6-PDA/CaP-GSNO (male)	64.06 ±1.49	116.85 ± 2.66	67.33 ± 4.84	19.18 ± 2.14
Saline (female)	53.86 ± 2.43	129.56 ± 6.97	76.44 ± 8.00	21.64 ± 3.25
Ce6-PDA/CaP-GSNO (female)	54.34 ± 2.96	146.78 ± 9.70	81.89 ± 5.58	20.69 ± 2.29



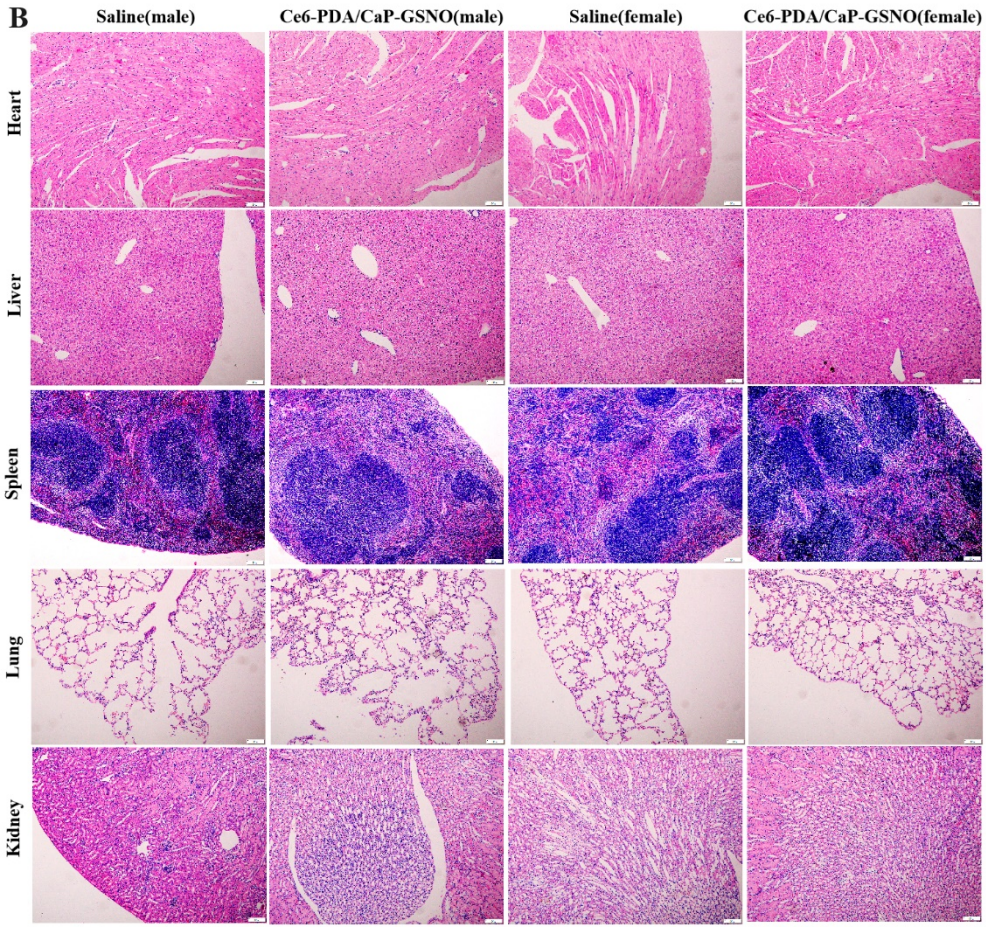


Fig. S4 *In vivo* acute toxicity of Ce6-PDA/CaP-GSNO. Body weight changes of mice (A) and photographs of major organs (B) after single *i.v.* injection of Ce6-PDA/CaP-GSNO at an equivalent Ce6 dose of 2.0 mg/kg in healthy BALB/c mice (n=12, 6 male and 6 female mice). Scale bars: 100 μm.