Supporting Information

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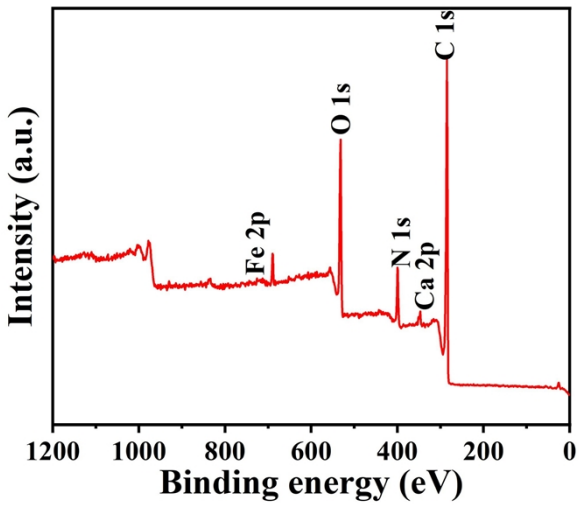

Fig. S1 XPS survey spectrum of PB@PAA-CaO_2@PDA NPs.

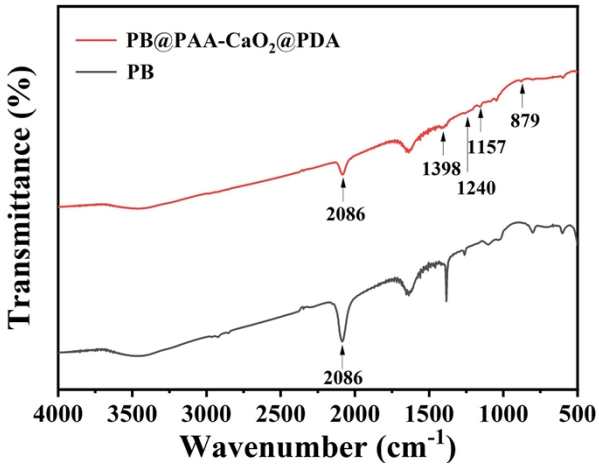

Fig. S2 The FT-IR spectra of PB NPs and PB @PAA-CaO_2@PDA NPs.

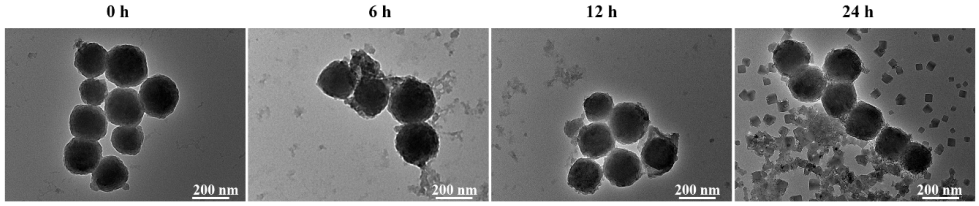

Fig. S3 TEM images of NPs at different reaction times in a 37 ℃ water bath.

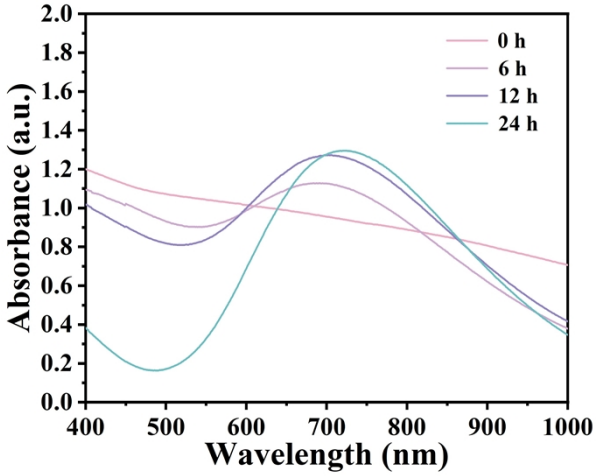

Fig. S4 UV-vis absorption spectra of NPs at different degradation times.

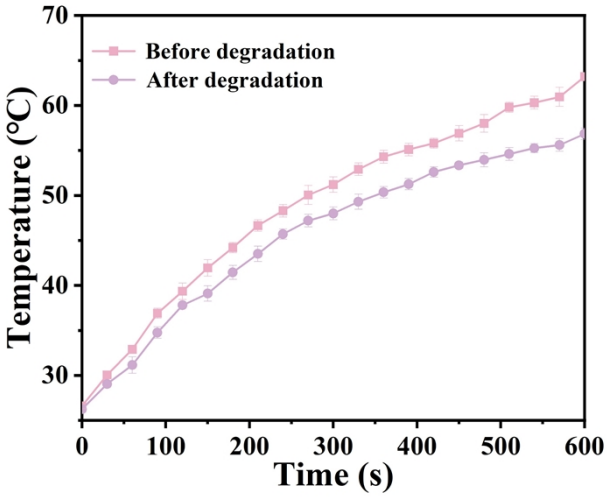

Fig. S5 Photothermal curves of NPs before and after degradation under 808 nm laser irradiation.

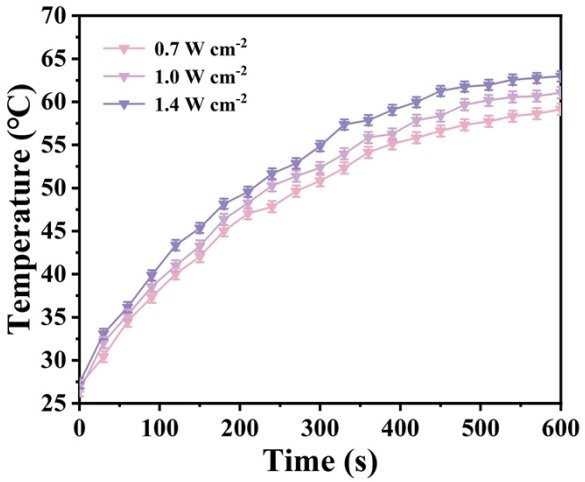

Fig. S6 Photothermal conversion of PB@PAA-CaO_2@PDA NPs (0.2 mg mL^{-1}) irradiated with NIR laser at different power densities.

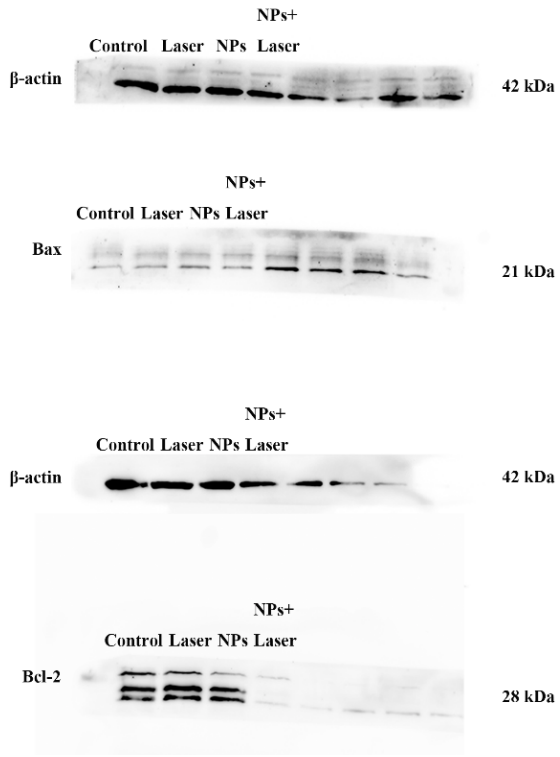

Fig. S7 Western blot images corresponding to Fig. 4E have been labeled with the closest molecular mass markers and lane designations.

Materials and methods

Materials

Potassium hexacyanoferrate (III) ($K_3[Fe(CN)_6]$), polyacrylic acid (PAA), and dopamine hydrochloride (DA) were purchased from Sigma-Aldrich (USA). Polyvinylpyrrolidone (PVP, K29-32) and calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) were obtained from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), ammonium hydroxide ($NH_3 \cdot H_2O$), and hydrochloric acid (HCl) were sourced from Beijing Chemical Works (Beijing, China).

Synthesis of PB NPs

PB NPs were synthesized according to a previously reported method[34]. 3 g PVP was dissolved in 80 mL deionized water under vigorous stirring. Followed by adding 0.8 mL of HCl (1 mol L^{-1}) and 0.264 g $K_3[Fe(CN)_6]$. The mixture was then placed in a thermostatic oil bath and heated to 80 ℃ for 20 h. Finally, the as-prepared PB NPs were obtained and washed with deionized water and absolute ethanol, respectively.

Synthesis of PB@PAA NPs

6 mg of PAA was dispersed into 20 mL of absolute ethanol. Then PB solution (6 mg in 6 mL of absolute ethanol) was added dropwise to the ethanol dispersion of PAA under moderate stirring and the mixture was further stirred at room temperature for 12 h.

Synthesis of PB@PAA-CaO_2 NPs

Prepared PB@PAA NPs were dispersed into 20 mL of absolute ethanol. Then, dissolved 2.4 mg $CaCl_2 \cdot 2H_2O$ into the above PB@PAA ethanol solution and reacted for 30 min. Afterward, 54 μL of 0.6 mol L^{-1} NaOH was added and stirred for 15 min. Finally,17 μL of H_2O_2 solution (30%) was introduced into the solution to initiate the reaction. After reacting for 30 min at room temperature, the products were collected by centrifugation at 9600 rpm/min, washed three times with absolute ethanol.

Synthesis of PB@PAA-CaO_2@PDA NPs

The as-prepared PB@PAA-CaO_2 NPs were dispersed into 20 mL of absolute ethanol, and then adjusted the pH of the PB@PAA-CaO_2 solution to 8-9 with $NH_3 \cdot H_2O$ (25%~28%), and mixed with 1 mL of dopamine hydrochloride (4 mg mL^{-1}). The mixture was further stirred at room temperature for 12 h. The products were collected after thorough washing with absolute ethanol.

Measurement of photothermal effect

PB@PAA-CaO_2@PDA solutions at varying concentrations (0.05, 0.1, 0.2, and 0.4 mg mL^{-1}) were subjected to irradiation using an 808 nm NIR laser at a power density of 1 W cm^{-2} for 10 min, during which the temperature was monitored. Subsequently, the PB@PAA-CaO_2@PDA suspension with a concentration of 0.2 mg·mL^{-1} was irradiated with an 808 nm laser at power densities of 0.7, 1.0, and 1.4 W·cm^{-2} for 10 min, and the temperature changes were recorded. In addition, the photothermal stability of the composite material was evaluated at the same concentration. The test consisted of four consecutive irradiation cycles, each comprising 10 min of laser exposure followed by a 10 min cooling period without irradiation The photothermal conversion

efficiency (η) was determined according to the following equation:

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{808}})} \quad (1)$$

In Equation 1, I represents the incident laser power (1 W cm^{-2}), A_{808} denotes the absorbance at 808 nm of the PB@PAA-CaO_2@PDA NPs, h is the heat-transfer coefficient, S stands for the surface area of the container, T_{max} indicates the equilibrium temperature, T_{Surr} corresponds to the ambient temperature of the surroundings, and Q_{dis} refers to the heat associated with the light absorbance by the solvent.

The value of hS is derived according to Equation (2):

$$\tau_s = \frac{m_D C_D}{hS} \quad (2)$$

In this equation (2), the τs means the sample system time constant, m_D and C_D express the mass and heat capacity (4.2 J g^{-1}) when DI water was used as the solvent. Pure water in a test tube was utilized to measure the Q_{dis}.

DOX loading and release

The drug-loaded nanoparticles were prepared by combining an aqueous solution of DOX (10 mg mL^{-1}, 50 μL) with 950 μL of a PB@PAA-CaO_2@PDA NPs suspension (1 mg mL^{-1}), followed by shaking the mixture for 24 h. The drug loading efficiency (LE%) was subsequently calculated using Equation (3):

$$LE\% = \frac{W_{(original\ DOX)} - W_{(residual\ DOX)}}{W_{(original\ DOX)}} \times 100\% \quad (3)$$

The pH/NIR dual-stimuli-responsive controlled release of DOX was assessed by monitoring the absorbance at 480 nm using UV-vis spectroscopy. Specifically, three identical aliquots of DOX-loaded PB@PAA-CaO_2@PDA NPs were incubated in phosphate-buffered saline (PBS) (1 mL) at pH 7.4 and pH 5.0, maintained at 37 °C. At predetermined time points, the supernatant was collected and replaced with an equal volume of fresh buffer. To evaluate the enhancement of drug release under photothermal stimulation, selected samples were irradiated with an 808 nm NIR laser for 5 min at specific intervals.

Hemolysis assay

A hemolysis assay was conducted using human blood to assess the in vitro hematocompatibility of PB@PAA-CaO_2@PDA NPs. Red blood cells (RBCs) were isolated through repeated washing with physiological saline and centrifugation at 1500 rpm for 10 min to remove serum components. Subsequently, 0.5 mL of the purified RBCs were resuspended in 4 mL of 0.9% NaCl solution. Then, 0.9 mL aliquots of this cell suspension were mixed with: (1) 0.1 mL of 0.9% NaCl (negative control), (2) 0.1 mL of distilled water (positive control), or (3) 0.1 mL of nanoparticle suspensions at different concentrations. All seven sample groups were incubated for 2 h at room temperature. After incubation, the samples were centrifuged at 6000 rpm for 10 min, and the absorbance of the supernatant was measured at 540 nm. The hemolysis ratio was calculated according to the following equation:

$$\text{Hemolysis}\ (\%) = \frac{A_{sample} - A_{control}}{A_{control(+)} - A_{control(-)}} \times 100\% \qquad (4)$$

In vitro cytotoxicity study

A standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed to evaluate the in vitro cytotoxicity. MCF-7 cells were subjected to the following treatments: 808 nm laser irradiation alone, free DOX, PB@PAA-CaO_2@PDA NPs alone, PB@PAA-CaO_2@PDA NPs with 808 nm laser irradiation, DOX-loaded PB@PAA-CaO_2@PDA NPs, and DOX-loaded PB@PAA-CaO_2@PDA NPs with 808 nm laser irradiation. The concentrations of PB@PAA-CaO_2@PDA NPs ranged from 6.25 to 100 μg mL^{-1} (6.25, 12.5, 25, 50, and 100 μg·mL^{-1}). After 24 h of incubation, the groups designated for laser treatment were exposed to NIR irradiation for 5 min. Subsequently, 10 μL of MTT solution was added to each well, and the cells were further incubated for 3 h. The medium was then replaced with 150 μL of dimethyl sulfoxide (DMSO) prior to measuring the absorbance. Cell viability was calculated using Equation (5):

$$Cell\ viability\ (\%) = \frac{A_{(test\ cell)}}{A_{(control\ cell)}} \times 100\% \qquad (5)$$

MCF-7 cancer cells were treated with the following respective conditions: free DOX, PB@PAA-CaO_2@PDA NPs (100 μg mL^{-1}), DOX-loaded PB@PAA-CaO_2@PDA NPs (100 μg mL^{-1}), laser irradiation alone, PB@PAA-CaO_2@PDA NPs (100 μg mL^{-1}) combined with laser irradiation, and DOX-loaded PB@PAA-CaO_2@PDA NPs (100 μg mL^{-1}) combined with laser irradiation. Following 24 h of incubation, the groups designated for laser treatment were subjected to NIR irradiation (808 nm) for 6 min. The cells were then stained using a calcein-AM/propidium iodide (PI) double-staining kit and visualized under a confocal laser scanning microscope (CLSM) to acquire fluorescence images.

Determination of Ca^{2+} content

MCF-7 cells were seeded in small dishes and cultured for 24 h. Subsequently, 1 mL of PB@PAA-CaO_2@PDA NPs (50 μg mL^{-1}) was introduced, and the cells were further incubated for 0.5, 2, 4, and 8 h. Following this, 1 mL of the calcium-sensitive fluorescent probe Fluo-4 AM was added, and the cells were incubated for an additional 30 min. After incubation, the cells were washed twice with PBS, and fluorescence images were acquired using CLSM.

Detection of mitochondrial membrane potential

Briefly, MCF-7 cells were treated with the following respective groups: Control, Laser, PB@PAA-CaO_2@PDA NPs, and PB@PAA-CaO_2@PDA NPs combined with Laser irradiation, for a duration of 8 h. Subsequently, the cells were washed with PBS and stained using the JC-1 probe from a mitochondrial membrane potential assay kit (Beyotime) for 20 min. Mitochondrial membrane potential changes, indicative of mitochondrial damage, were finally evaluated by CLSM.

In vivo anticancer efficacy

All animal procedures were performed in accordance with the guide- lines for Care and Use of Laboratory Animals of Northeast Normal University (202402045), and approved by the Animal Ethics Committee of Northeast Normal University.

To establish the tumor model, mice were first subcutaneously inoculated with 4T1 cells. After one week, the mice were randomly assigned to seven experimental groups (n=3 per group): 1. Control, 2. Laser alone, 3. Free DOX, 4. PB@PAA-CaO_2@PDA

NPs, 5. PB@PAA-CaO_2@PDA NPs+Laser, 6. PB@PAA-CaO_2@PDA NPs+DOX, 7. PB@PAA-CaO_2@PDA NPs+DOX+Laser. Throughout the treatment period, the respective agents were administered via tail vein injection every other day. For groups receiving laser treatment, the tumor region was irradiated with an 808 nm NIR laser at 24 h post-injection, and the temperature changes at the tumor site were monitored using an infrared thermal camera. Body weight and tumor volume were recorded every two days for a total of 10 days. On day 10, all mice were euthanized, solid tumors were harvested and photographed, and major organs were collected for subsequent histological staining.