Supporting Information

Bovine Serum Albumin Loaded Nano-selenium/ICG Nanoparticles for Highly Effective Chemo-photothermal Combination Therapy

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Experiment Section:

1. Materials and methods

1.1 Materials

Sodium selenite, Indocyanine green (ICG), GSH were purchased from Sigma-Aldrich. Hochest33258, Calcein-AM and propidium iodide (PI), Cell counting kit-8 were obtained from Invitrogen (USA). Bovine serum albumin (BSA) was obtained from Beijing Biosynthesis Biotechnology CO., Ltd. Fetal bovine serum (FBS), DMEM, trypsin-EDTA and penicillin-streptomycin were purchased from Gibco Life Technologies (AG, Switzerland). All other chemicals used in this study were of analytical reagent grade and used without further purification. Amicon ultra-4 centrifugal filter with a molecular weight cutoff of 10 kDa was bought from Millipore (USA). BALB/c athymic nude mice were maintained under aseptic conditions in a small animal isolator.

1.2. BSINPs generated by programmed assembly

10 mL of 20 mM sodium selenite solution was mixed with 40 mL of 25 mM glutathione containing 825 mg of BSA, and followed by an addition of 1.5mL of 1M NaOH (pH=9.0). Thus instantaneously forming red colloidal Se nanoparticles. After one hour of reaction, the red solution was dialyzed against with double distilled water for 24 hours, and the water changed every four hours to separate GSSG from the SeNPs. After centrifugation at 7000 rpm/min for 10 min, the supernatant was discarded. The precipitate (SeNPs) was diluted 3 mL 0.2 M Na_2HPO_4 (pH 8.6), and it was incubated with ICG-Sulfo-OSu (250 μg) at room temperature for one hour. Finally the BSINPs were washed three times using an amicon ultra-4 centrifugal filter.

1.3 Characterization of BSINPs

The size, surface charge, polydispersity of the NPs were obtained using Beckman Coulter DelsaTM Nano C (Beckman, USA) at room temperature. The fresh BSINPs suspension were transferred onto a 200 mesh copper grid coated with carbon, stained with 2% (w/v) phosphotungstic acid, dried at room temperature, then analyzed by TEM (FEI Tecnai G2 F20 S-Twin, USA). UV/vis absorption spectra were obtained from a PerkinElmer Lambda 25 UV/vis spectrophotometer. The PL spectra were obtained by a FL spectrometer (LS55, PerkinElmer, Inc., UK) with at excitation at 760 nm, and the ICG fluorescence intensity at 820 nm was measured at different time.

1.4. Cell culture

Human malignant glioma cell (U87L) were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) penicillin, and 1% (v/v) streptomycin. Cells were incubated in a humidified incubator at 37 °C with 5% CO_2.

1.5. *In vitro* cell uptake

U87L cells (1×10^4) were seeded into 8-well chambered cover glasses (Lab-Tek, Nunc, USA) in 200 μL of medium, respectively. After 24 h, BSINPs (containing 10μg/mL ICG) or 10 μg/mL free ICG was incubated with cells for 4 h at 37 °C. The cells were washed thrice with PBS and fixed with 4% paraformaldehyde solution for 10 min, then washed thrice with PBS. The nuclear dye hochest 33258 was used as a positive control to stain nuclei in the experiment. Finally the fixed cells were observed by confocal laser scanning microscope (Leica TCS SP5, GER).

For cellular uptake experiment, the cells (1×10^5 cells per well) were seeded in 6-well plates and incubated overnight, and then incubated with BSINPs (containing 5μg/mL ICG) or 5μg/mL free ICG. After incubation 4 h, cells were rinsed with PBS three times, tripsinized, and resuspended with medium. Afterward, the cells were collected by FACSCantoTM II Gallios flow cytometer (BD Biosciences) and analyzed by CFlow Plus software (BD, Ann Arbor, MI).

1.6. *In vitro* chemo-photothermal treatments

To observe the chemo-photothermal therapeutic efficacy *in vitro*. The U87L cells were seeded into 96-well plate (1×10^4 cells) in 200 μL of medium incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. The medium was replaced by the medium with different concentration of BSINPs, and the medium without NPs as control. After 24 h incubation. The cells were irradiated with a 0.8 W/cm^2 808 nm laser for 5 min for chemo-photothermal treatments, whereas for chemotherapy alone, the cells were not exposed to laser. After another 2 h incubation, the standard CCK-8 assay was carried out to evaluate the cell viability.

The chemo-photothermal combined effects of BSINPs on U87L cells were further verified using calcein AM and propidium iodide (PI) co-staining. U87L cells (5×10^4 cells/well) were seeded in 8-well chambered and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere. After being rinsed with PBS, the cells were incubated with different concentration of BSINPs, SeNPs or free ICG for 4 h at 37 °C under the same conditions. Subsequently irradiation using an 808 nm laser with energy density of 0.8 W/cm^2 for 5 min. Another 4 h incubation cells were stained with calcein-AM for visualization of live cells and with PI for visualization of dead/late apoptotic cells, according to the manufacturer's suggested protocol (Invitrogen).

1.7. Animals and tumor model

Female BALB/c nude mice (6 weeks old) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (China). Animals received care in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals. The procedures were approved by Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences Animal Care and Use Committee. Six week old male BALB/c athymic nude mice were maintained under aseptic conditions in a small animal isolator and were housed in a group of five in standard cages with free access to food and water and a 12 h light/dark cycle. U87L cells (5×10^6) were administered by subcutaneous injection into the hind legs region of the mice. Tumor volume was calculated as (tumor length) (tumor width)^2/2.

1.8. *In vivo* imaging and biodistribution analysis

The nude mice were divided into two groups (three per group) randomly. Mice in group 1 and group 2 were respectively intratumoral injected with 100 μL of 48 μg/mL free ICG and BSINPs (containing 48 μg/mL ICG). Images and FL semiquatitative analysis of ICG were taken at 0, 6, 12, and 24 h after injection using the *ex*/*in vivo* imaging system (CRi maestro, USA) with a 745 nm excitation wavelength and a 820 nm filter to collect the FL signals of ICG. The mice after injection at 24 h were sacrificed and the organs including heart, liver, spleen, lung, kidneys and tumor were collected for imaging and semiquatitative biodistribution analysis.

1.9. *In vivo* thermal imaging and *in vitro* temperature measurements during laser irradiation.

PBS (1mL), free ICG (1mL, containing 48 μg/mL ICG), BSINPs (1 mL, containing 48 μg/mL ICG) was added to centrifuge tubes. When the tumor size reached 100 mm^3， the nude mice bearing U87L tumors were intratumoral injected with 100 μL of 48 μg/mL free ICG, and BSINPs (containing 48 μg/mL ICG). Mice bearing with the U87L tumor were also injected with 100 μL of PBS as control. The tubes and tumors were irradiated by the 808 nm laser at 0.8W/cm^2 for 8 min. Thermal imaging was captured by an infrared thermal imaging camera (Ti27, Fluke, USA).

1.10. *In vivo* chemotherapy, photothermal and chemo-photothermal treatments

The mice were divided into five groups (seven mice per group) that were intratumoral injected with 100 μL of PBS, PBS + laser, 48 μg/mL free ICG + laser, SeNPs, BSINPs (containing 48 μg/mL ICG) + laser. For the laser treatment groups, the tumors of mice were irradiated by the 808 nm laser at 0.8 W/cm^2 for 5 min. The tumor volumes and changes in body weight of each mouse were recorded. Mice with tumor sizes exceeding 1000 m^3 were euthanatized according to the animal protocol.

1.11. H&E staining

H&E staining was performed according to a protocol provided by the vendor (BBC Biochemical). Briefly, 8 μm cryogenic slides were prepared and fixed with 10% formalin for about 30 min at room temperature. After washing with running water for 5 min, the slides were treated with gradient concentrations of alcohol (100%, 95%, and 70%), each for 20 s. The hematoxylin staining was performed for about 3 min and washed with water for 1 min. The eosin staining was performed for about 1 min. The slides were washed, treated with xylene, and mounted with Canada balsam. The images were acquired on a Nikon Eclipse 90i microscope.

1.12. Statistical analysis.

Statistical analysis was performed by Student's t-test for two groups and one-way analysis of variance for multiple groups. All results were expressed as the mean ± s.d. unless otherwise noted. A value of $p = 0.05$ was considered statistically significant. (*) $P < 0.05$, (**) $P < 0.01$.

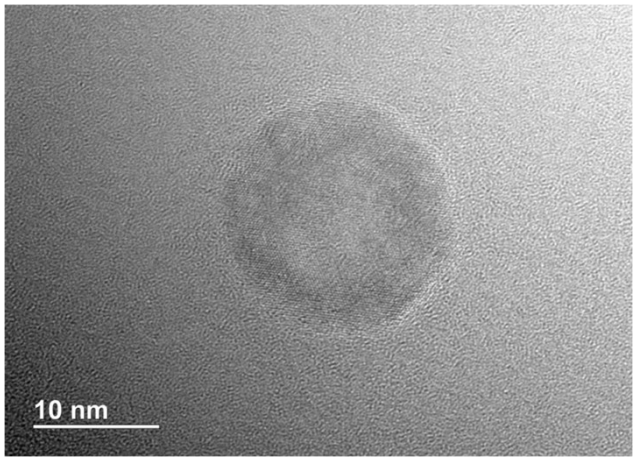

Figure S1. High resolution transmission electron microscopy (HRTEM) images of SeNPs.

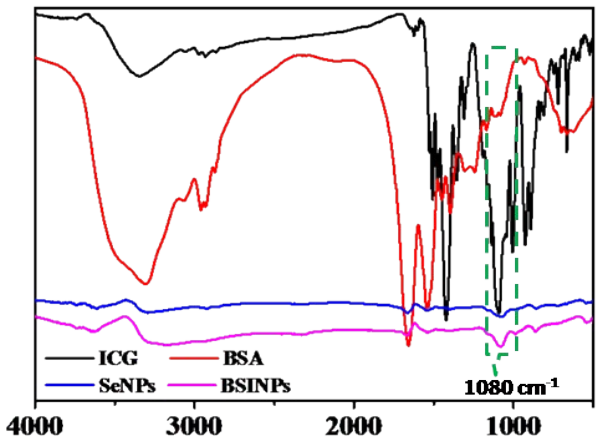

Figure S2. FT-IR mapping of BSINPs (Green dashed box represent the peak of ICG at 1080 cm^{-1})

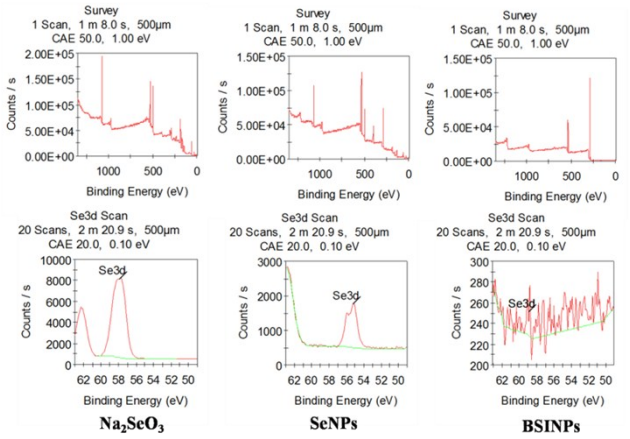

Figure S3. XPS mapping of BSINPs

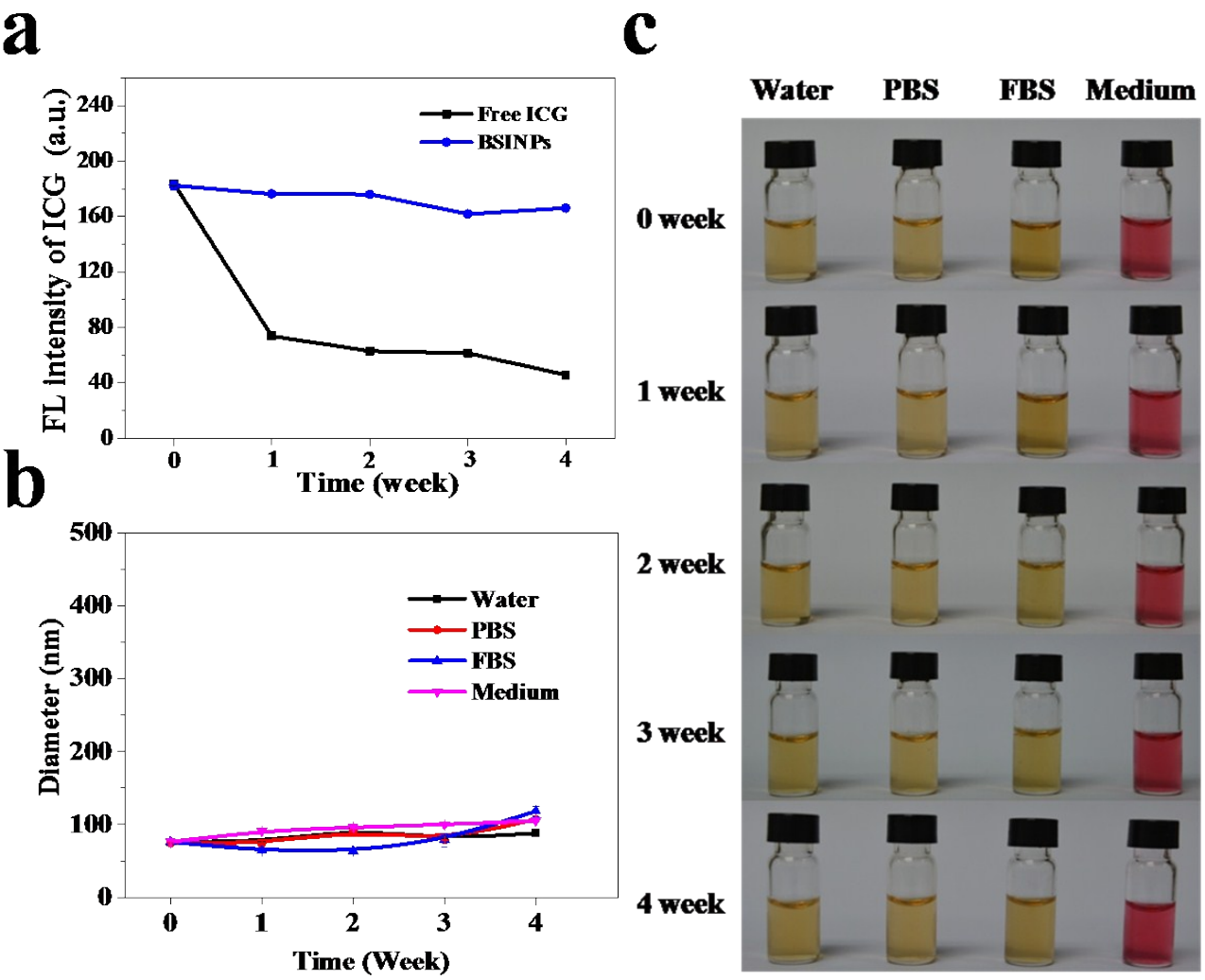

Figure S4. The stable properties of BSINPs. (a) ICG FL stability of BSINPs and free ICG. (b) Size stability of BSINPs. (c) The photos of BSINPs dispersed in Water, PBS, FBS, medium at different store times.

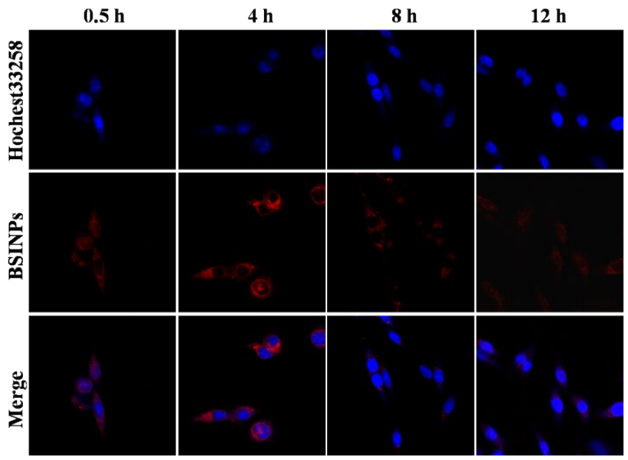

Figure S5. The uptake of BSINPs in U87L cells in an observation period of 12 h.

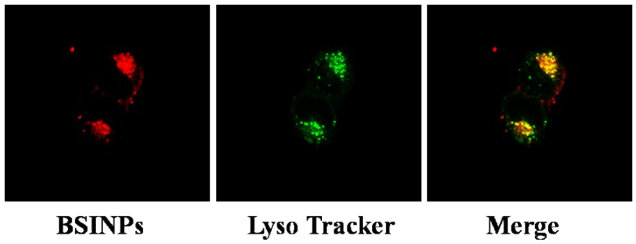

Figure S6. Subcellular localization of BSINPs

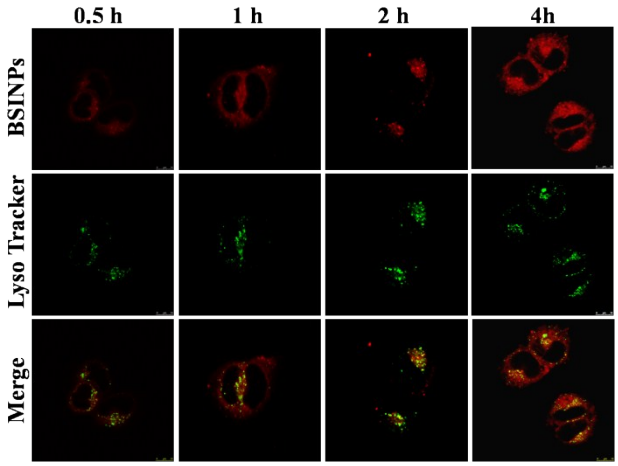

Figure S7. The endocytosis process of BSINPs

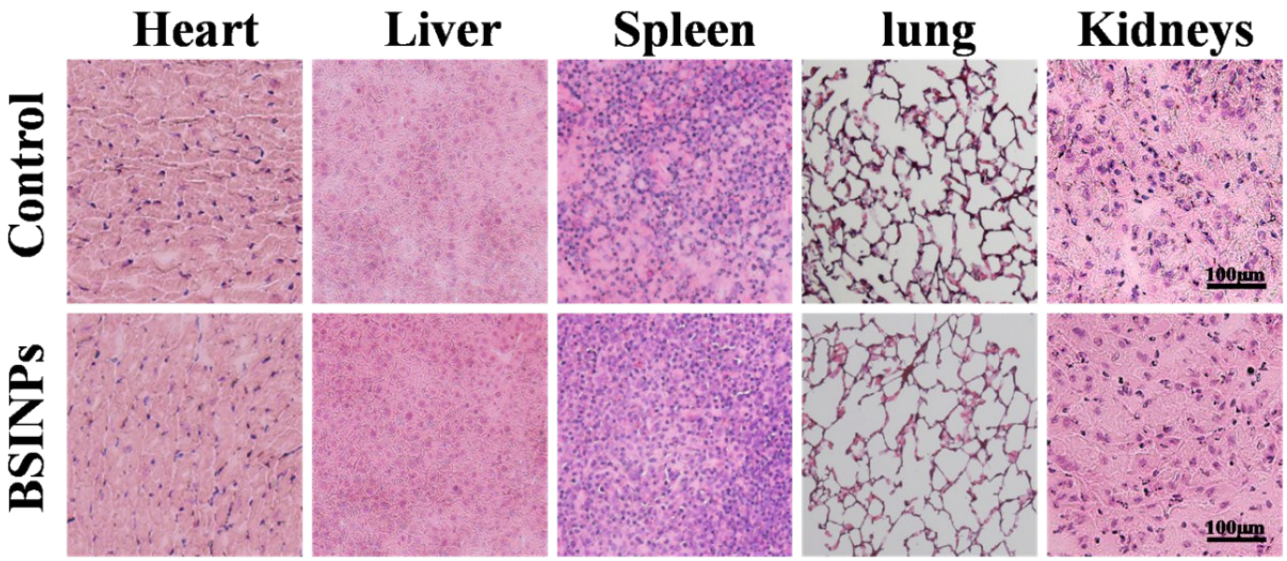

Figure S8. H&E staining images of major organs collected from control and BSINPs administrated and irradiated mice.