Supporting Information

Phycocyanin Functionalized Selenium Nanoparticles for Type I Photodynamic Antibacterial Therapy and Wound Healing

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Materials

All chemical reagents and solvents utilized in this study were of analytical grade and were used as received without further purification, unless otherwise specified. C-phycocyanin (C-PC) and 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) were procured from Macklin (China). Sodium selenite pentahydrate and Singlet Oxygen Sensor Green Reagent (SOSG) were obtained from Aladdin (China). Glutathione (GSH), 2,7-dichlorodihydrofluorescein (DCFH), and HPF ROS Probe were acquired from YUANYE (China). Dihydrorhodamine 123 (DHR123) was sourced from Coolaber. The L929 cell line was obtained from the Shanghai Cell Bank of the Type Culture Collection at the Chinese Academy of Sciences. Cell culture reagents, including Minimum Essential Medium (MEM), penicillin G (100 U/mL), streptomycin (100 U/mL), and a 0.25% trypsin-0.53 mM EDTA solution, were purchased from Gibco (Grand Island, USA). Horse serum was supplied by iCell Bioscience Inc. (China). Staphylococcus aureus, Escherichia coli, and Methicillin-resistant Staphylococcus aureus strains were provided by Nanfang Hospital in China. The SYTO9-PI Live/Dead Bacteria Stain Kit was purchased from Tianjingsha (China). Additional reagents included 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) sourced from Beyotime (China); the CCK-8 assay kit; 4',6-diamidino-2-phenylindole dihydrochloride (DAPI); FITC-phalloidin; Calcein acetoxymethyl ester (Calcein-AM); and Propidium iodide (PI), all acquired from Dojindo Co., Ltd. in Japan. Tumor necrosis factor-alpha (TNF-α) ; interleukin-6 (IL-6); Anti-CD31 antibody; Anti-TNF-alpha were purchased from Servicebio (china).

Characterization of Se@PC NPs

Particle size was measured using a NanoBrook 90PlusZeta. Transmission electron microscopy (TEM) images were obtained with a PHILIPS TECNAI 10. UV-vis absorption spectra were recorded on a Thermofisher Evolution 300 spectropolarimeter. The infrared spectrum was captured by a Nicolet iS50 FT-IR. X-ray photoelectron spectroscopy (XPS) analysis was performed with a Kratos Axis Ultra DLD. ICP-MS measurements were conducted on a ThermoFisher TQ-ICP-MS. UV-Vis diffuse reflectance spectrometry (DRS) were determined by Jasco V-550 UV–Vis spectrophotometer.

The detection of ROS generated from Se@PC NPs via DCFH

2′,7′-Dichlorodihydrofluorescein (DCFH) was used as the probe to evaluate ROS generation. Se@PC NPs (100 μg/mL) were dissolved in an aqueous solution, with H_2O and phycocyanin serving as control groups. Upon the addition of the probe, the mixture was subjected to visible light irradiation every 20 seconds at a power density of 100 mW/cm2. DCFH (10 μM) was introduced, and the

fluorescence intensity was monitored using a fluorescence spectrophotometer (Hitachi F-7000, Japan; excitation wavelength: 488 nm; emission wavelength: 500-650 nm). A curve was drawn to compare the fluorescence changes of DCFH in different solutions, so as to obtain the difference in ROS generation capacity.

The detection of superoxide anion radical ($O_2^{\bullet-}$) generated from Se@PC NPs via DHR123

Dihydrorhodamine 123 (DHR123) is used as an indicator of $O_2^{\bullet-}$, which can be converted to rhodamine 123 in the presence of $O_2^{\bullet-}$. Se@PC NPs (100 μg/mL) were dissolved in an aqueous solution, with H_2O and phycocyanin serving as control groups. Upon the addition of the probe, the mixture was subjected to visible light irradiation every 20 seconds at a power density of 100 mW/cm^2. DHR123 (10 μM) was added, and its fluorescence intensity was measured using a fluorescence spectrophotometer (excitation wavelength: 495 nm; emission wavelength: 500-620 nm). A curve was drawn to compare the fluorescence changes of DHR123 in different solutions, so as to obtain the difference in $O_2^{\bullet-}$ generation capacity.

The detection of hydroxyl radical (•OH) generated from Se@PC NPs via HPF

Hydroxyphenyl fluorescein (HPF) is used to monitor •OH production. Se@PC NPs (100 μg/mL) were dissolved in an aqueous solution, with H2O and phycocyanin serving as control groups. Upon the addition of the probe, the mixture was subjected to visible light irradiation every 20 seconds at a power density of 100 mW/cm^2. HPF (10 μM) was added, and its fluorescence intensity was also measured using the same spectrophotometer (excitation wavelength: 490 nm; emission wavelength: 500-620 nm). A curve was drawn to compare the fluorescence changes of HPF in different solutions, so as to obtain the difference in •OH generation capacity.

The detection of singlet oxygen generated from Se@PC NPs via SOSG

Specific single oxygen sensor green (SOSG) is used as an indicator of $^1O_2$. Se@PC NPs (100 μg/mL) were dissolved in an aqueous solution. SOSG (10 μM) was incorporated into the mixture, with fluorescence intensity being recorded under identical conditions (excitation wavelength: 504 nm; emission wavelength: 510-600 nm).

The detection of singlet oxygen generated from Se@PC NPs via ABDA

9,10-Anthracenediyl-bis(methylene)-dimalonic acid (ABDA) is used to monitor $^1O_2$ production. Se@PC NPs (100 μg/mL) were dissolved in an aqueous solution. ABDA (10 μM) were added to the solution, and the change in the absorption signal of the indicator ABDA at 380 nm was monitored by an ultraviolet–visible spectrometer to monitor the generation of $^1O_2$.

ROS generated from PDT detection with electron paramagnetic resonance (EPR)

DMPO was used as a trapping agent for •OH and $O_2^{\bullet-}$ were respectively dissolved in

H_2O/DMSO (Vwater/VDMSO = 99:1) mixed solution. DMPO was dissolved in CH_3OH or DMSO, which was further added into Se@PC NPs aqueous solution to reach a final concentration of 100 μM and 10 μM for DMPO and Se@PC NPs, respectively. The EPR spectra of the mixtures were recorded before and after light irradiation (white light, 5 min, 100 mW/cm^2). The electron paramagnetic resonance (EPR, Bruker A300) spectra were measured to evaluate ROS generation. For comparison, DMPO + Se@PC NPs (10 μM) group was tested by an electron paramagnetic resonance spectrometer.

Cytocompatibility Evaluation of Se@PC NPs.

L929 cells were cultured in 96-well plates at a density of 8 × 10³ cells per well. The cells were subsequently treated with Se@PC NPs at various concentrations: 10, 30, 50, 100, 150, and 200 μg/mL. Following the treatment, the cells underwent visible light irradiation for a duration of 10 minutes at an intensity of 100 mW/cm². After a subsequent incubation period of 24 hours, the cells were exposed to a CCK-8 solution (10%) and incubated in darkness for an additional two hours. All experiments were conducted in sextuplicate to ensure statistical reliability. Furthermore, after the incubation period, the cells were stained using Calcein-AM/PI solution and maintained in darkness for 30 minutes prior to imaging with an inverted optical microscope.

Hemocompatibility Evaluation of Se@PC NPs.

RBCs obtained from blood samples were subjected to centrifugation at 1500 rpm for a duration of 15 minutes. Following this, 800 μL of 0.9% normal saline (NS) containing Se@PC NPs at varying concentrations (10, 30, 50, 100, 150, and 200 μg/mL) was combined with 200 μL of diluted RBCs in the same solution (0.9% NS) and incubated for a period of two hours. A negative control was established using only the 0.9% NS solution, while distilled water served as a positive control. The supernatants were subsequently collected and analyzed using a microplate reader set to an absorbance wavelength of 540 nm. The hemolysis ratio of RBCs was calculated using the following equation: Hemolysis ratio percent $=(A_{sample}-A_{negative})/(A_{positive}-A_{negative})\times 100\%$. All groups were carried out in sextuplicate.

Antibacterial Activity Evaluation of Se@PC NPs.

The antibacterial activity of Se@PC NPs was evaluated using Staphylococcus aureus (Gram-positive), Escherichia coli (Gram-negative), and methicillin-resistant Staphylococcus aureus (MRSA) as bacterial models. The antibacterial efficacy of Se@PC NPs against these three bacterial strains was assessed utilizing the colony-forming unit (CFU) method. Bacterial cultures of S. aureus, E. coli, and MRSA at a concentration of 1×10^5 CFU/mL were incubated with the nanoparticles at varying

concentrations. For the groups without visible light irradiation, bacteria were maintained in darkness and incubated at 37 °C; conversely, for the visible light irradiation groups, samples were exposed to visible light at an intensity of 100 mW/cm² for 10 minutes. Following this treatment, all solutions were spread onto Luria-Bertani plates and incubated for an additional 12 hours at 37 °C. Each experiment was conducted in triplicate with six replicates per group. The morphological characteristics of the bacteria were examined using scanning electron microscopy (SEM). Nanoparticles were introduced into 48-well microplates containing 200 μL of medium with bacteria at a concentration of 10^8 CFU/mL. After exposure to visible light radiation (100 mW/cm² for 10 minutes), the bacteria were stained with SYTO-9 (6 μM) and propidium iodide (PI; 30 μM). After a subsequent incubation period of 15 minutes, bacterial detection was performed using an inverted optical microscope. For SYTO-9 detection, excitation occurred at a wavelength of 488 nm while emission was measured at a wavelength of 525 nm. For PI detection, excitation took place at a wavelength of 535 nm with emission recorded at a wavelength of 615 nm. Additionally, bacteria underwent incubation with the Reactive Oxygen Species Assay Kit under conditions set to maintain temperature at 37 °C. Following this incubation period lasting approximately forty minutes, imaging was promptly conducted using an inverted optical microscope employing excitation wavelengths set to both 488 nm and emission wavelengths set to 525 nm.

2.8. *In Vivo* Wound Healing Assay.

All animal experiments were conducted in accordance with protocols approved by the local Ethical Committee, adhering to Chinese laws regarding experimental animals and following the regulations set forth by the Institutional Animal Care and Use Committee of Southern Medical University. Female Balb/c mice aged 6-8 weeks were obtained from the Animal Center of Southern Medical University. Two 1 cm × 1 cm wounds were created on the back of each mouse using a punch tool. A total of 200 μL of Staphylococcus aureus (1×10^8 CFU/mL) was carefully applied to each wound site. After a period of 24 hours, Se@PC NPs solutions (200 μg/mL, 100 μL) and an equivalent volume of PBS were administered locally at the infection sites. Photodynamic therapy (PDT) treatment was performed by irradiating the infected areas with visible light at an intensity of 100 mW/cm² for a duration of 10 minutes. The NPs solutions were reapplied every two days for a total of two applications. Mice were euthanized on days 7 and 14 post-surgery; their skin samples were subsequently fixed in a solution containing 4% w/v paraformaldehyde. Hematoxylin and eosin (H&E) staining as well as Masson's trichrome staining on tissue sections were carried out to analyze

microscopic wound regeneration at day 14. Immunohistochemical staining for tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) was performed to assess inflammatory responses within granulation tissues seven days after surgery. Additionally, immunofluorescent staining for CD31 and α-smooth muscle actin (α-SMA) followed standard protocols to evaluate angiogenesis on day seven and day fourteen. The tissue slices were imaged using an Olympus VS120 slide scanner.

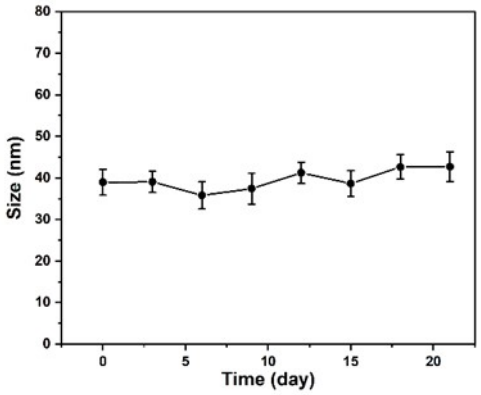

Figure S1 The size of Se@PC NPs after stored for 21 days in PBS.

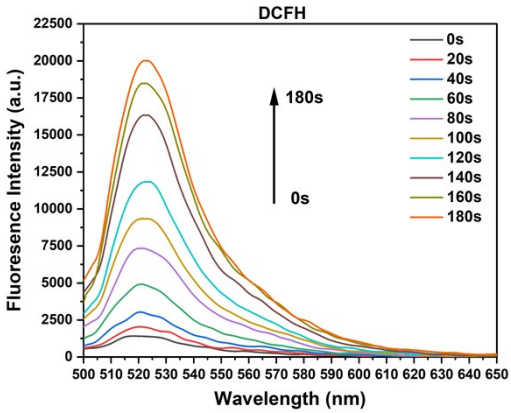

Figure S2 DCFH fluorescence intensity curves of Se@PC NPs after light irradiation (100 mW/cm^2) within 3 min.

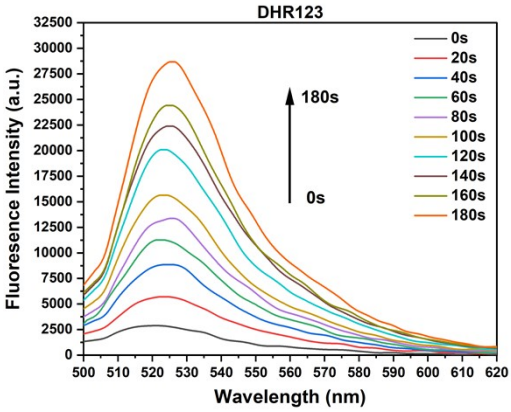

Figure S3 DHR123 fluorescence intensity curves of Se@PC NPs after light irradiation (100 mW/cm^2) within 3 min.

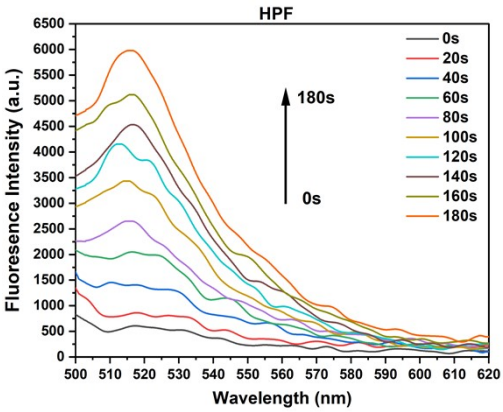

Figure S4 HPF fluorescence intensity curves of Se@PC NPs after light irradiation (100 mW/cm^2) within 3 min.

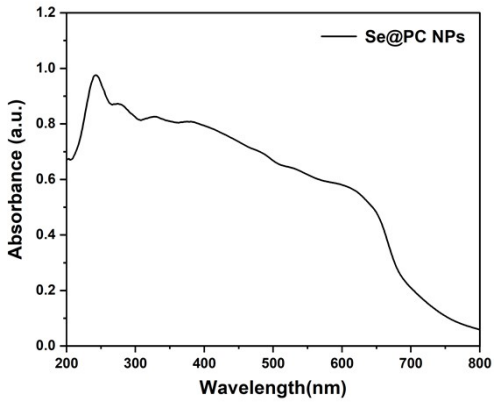

Figure S5 UV-vis-NIR reflection spectra of Se@PC NPs.

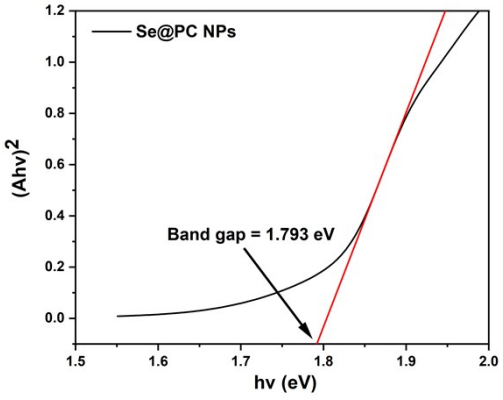

Figure S6 Energy bandgap of Se@PC NPs.

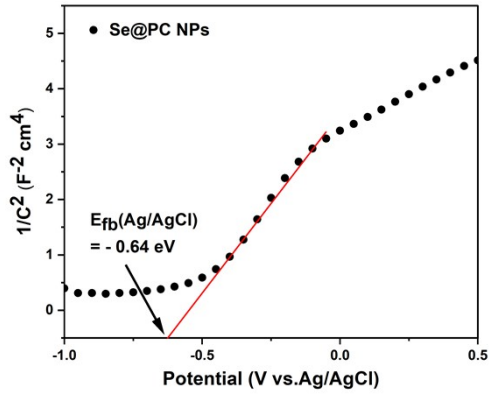

Figure S7 Mott-Schottky curve of Se@PC NPs.

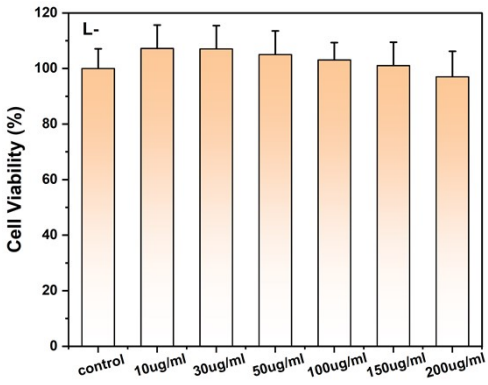

Figure S8 Bioactivity of L929 Cells in Response to Light Avoidance.

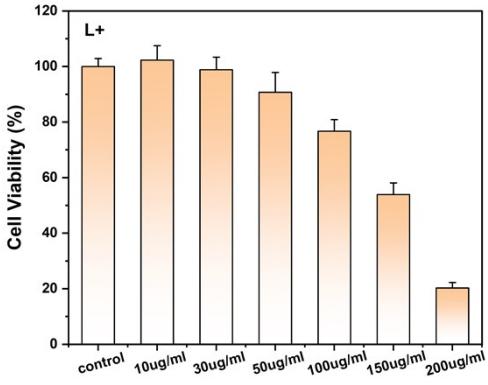

Figure S9 Biological Activity of L929 Cells Under White Light Irradiation.

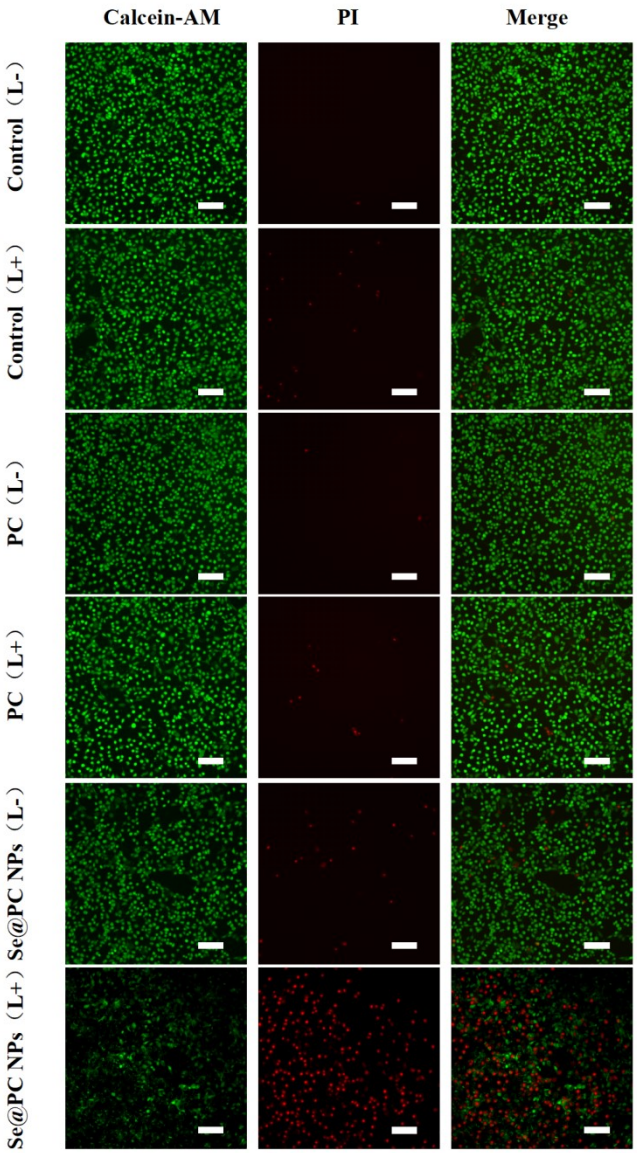

Figure S10 The live-dead staining assay, Scale =100μM.

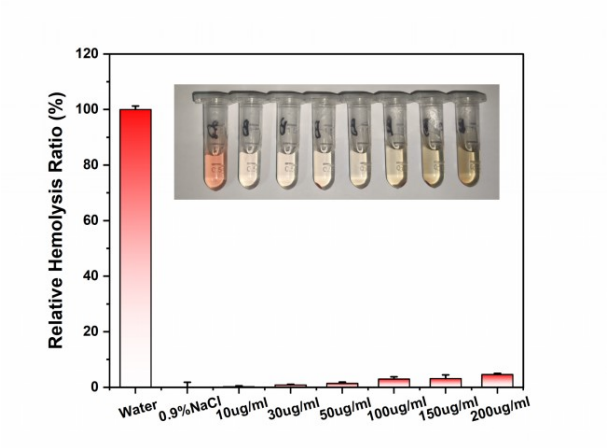

Figure S11 Hemocompatibility Evaluation of Se@PC NPs

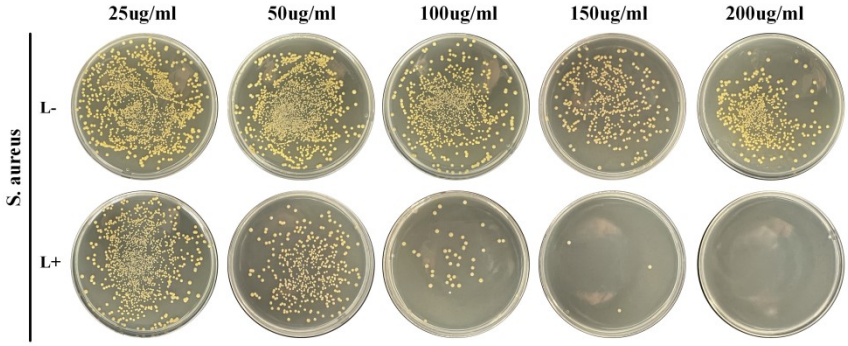

Figure S12 *In vitro* antimicrobial activity of Se@PC NPs against S. aureus, with and without visible light irradiation at various concentrations.

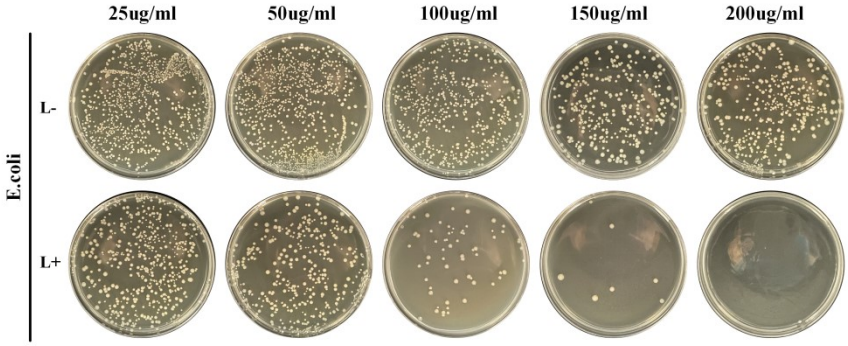

Figure S13 *In vitro* antimicrobial activity of Se@PC NPs against E.coli, with and without visible light irradiation at various concentrations.

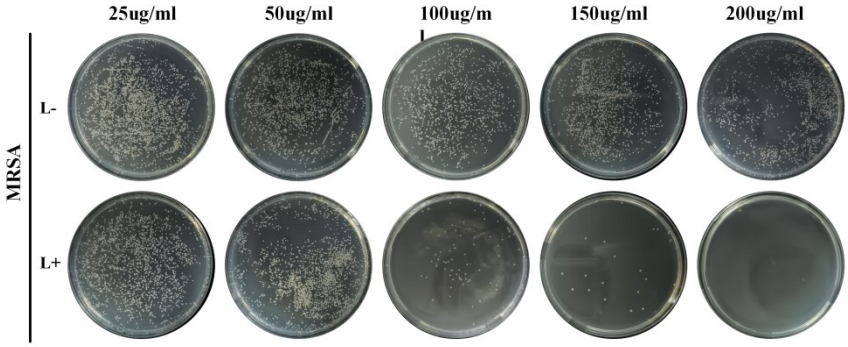

Figure S14 In vitro antimicrobial activity of Se@PC NPs against MRSA, with and without visible light irradiation at various concentrations.

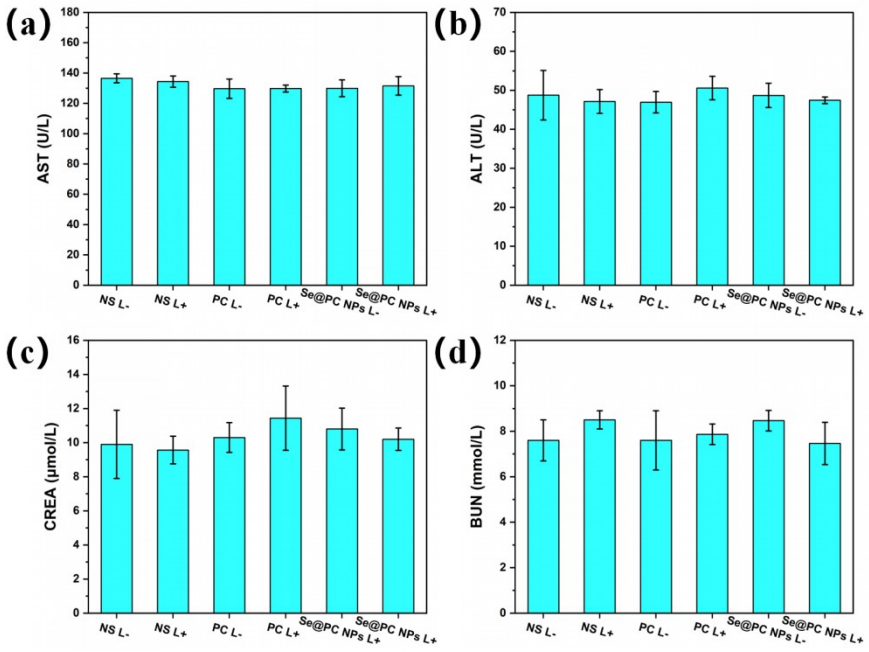

Figure S15 Blood panel analysis of mouse after injection of Se@PC NPs.

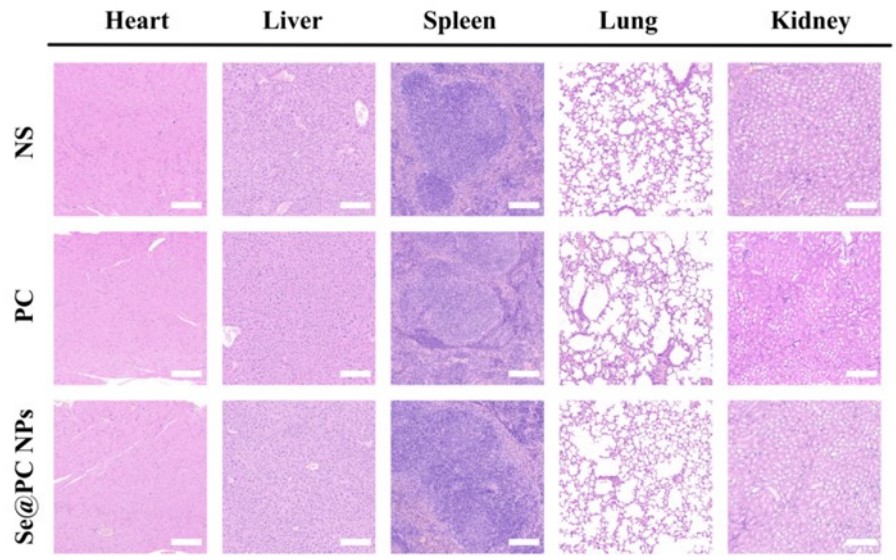

Figure S16 Major organs (heart, liver, spleen, lung, and kidney) after injection NS, PC and Se@PC NPs, Scale =200μm.