Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Integration of Highly Monodispersed Covalent Organic Framework Photosensitizer with Cation Exchange Synthesized Ag_2Se Nanoparticles for Enhanced Phototherapy

Chunling Hu,[a, b] Lihan Cai,[a, b] Sainan Liu,[a, b] and Maolin Pang*[a, b]

[a] State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China.
[b] University of Science and Technology of China, Hefei 230026, PR China.

* Corresponding author. E-mail address: mlpang@ciac.ac.cn.

Experimental Section

Materials and Reagents. 1,3,5-Triformylbenzene ($C_9H_6O_3$, AR, 98.0%, Yuhao Chemical), 1,4-diaminobenzene ($C_6H_8N_2$, AR, 97.0%, Aladdin), Acetic acid (CH_3COOH, AR, 99.5%, Beijing Chemical Works), Copper(Ⅱ) nitrate hydrate ($Cu(NO_3)_2 \cdot 3H_2O$, AR, 99%, Aladdin), Selenium (Se, AR, 99.99%, Aladdin), Sodium borohydride ($NaBH_4$, AR, Guangfu Works), Silver nitrate ($AgNO_3$, 99.8%, Shanghai Chemical Reagent), Acetonitrile (CH_3CN, 99.8%, Vetec), Ethanol (AR, Beijing Chemical Works), Polyvinyl pyrrolidone-40000 (AR, Vetec), and Hyaluronic acid (HA, 97%, Macklin). All of the chemicals and solvents are analytical grade and used as received without further purification.

Characterization. SEM images were obtained using a field emission scanning electron microscope (FE-SEM, S-4800, Hitachi). TEM images and HAADF-STEM-EDS mapping images of samples were acquired by FEI Tecnai G2 S-Twin with a field emission gun operating at 200 kV. Powder X-ray diffraction (PXRD) measurements were performed on Rigaku MiniFlex 600 at a scanning rate of 5° min^{-1} in the 2θ range from 3 to 60°, with graphite monochromatized Cu Kα radiation (λ= 0.15405 nm). Thermogravimetric analysis (TGA) data were recorded with Thermal Analysis Instrument (SDT 2960, TA Instruments, New Castle, DE) with a heating rate of 10 ^{o}C min^{-1} in air flow of 100 mL min^{-1}. Fourier transform infrared (FT-IR) spectra were measured on a Vertex PerkinElmer 580BIR spectrophotometer (Bruker) with KBr pellet technique. The UV-vis absorption spectra were measured on a Hitachi U-3100 spectrophotometer. The X-ray photoelectron spectra (XPS) were taken on a VG ESCALAB MK II electron energy spectrometer using Mg KR (1253.6 eV) as the X-ray excitation source. MTT experiments were carried out using a microplate reader (Thermo Multiskan MK3).

Synthesis of COF-LZU-1. 1,3,5-Triformylbenzene (12.5 mg, 0.01849 mmol) and 1,4-diaminobenzene (15 mg) were dissolved in 10 mL of acetonitrile in a 16 mL capped vial, and acetic acid (0.05 mL) was added. When the solution turned yellow, PVP-4000 (0.1 g mL^{-1} in acetonitrile) was added. Then the mixture was stirred at room temperature for 24 hours. The products were harvested by centrifugation at 8000 rpm for 15 min, and washed with ethanol for three times, finally dried at 50 ^{o}C for 12 hours.

Synthesis of COF-LZU-1-CuSe. COF-LZU-1 (1 mg) was mixed with $Cu(NO_3)_2$ (0.1 mL, 1.208 g mL^{-1}) and stirred for 16 hours. Then the precipitate was collected by centrifugation and washed with deionized water. 0.04 mL of Se^{2-} (7.89 mg mL^{-1}) solution was added into the above solution. After stirred for 0.5 h, the precipitate was collected by centrifugation and washed with deionized water to obtain COF-LZU-1-CuSe.

Synthesis of COF-LZU-1-Ag_2Se. COF-LZU-1-CuSe (10 mg) was suspended in 20 mL distilled water, then silver-ammonia solution (0.5 mL, 10 mg mL^{-1}) was added and stirred for 15 min in dark. The precipitate was collected by centrifugation and washed with deionized water.

Modification of the as-synthesized products with hyaluronic acid. In a typical procedure, COF-LZU-1 or COF-LZU-1-Ag_2Se (10 mg) was mixed with hyaluronic acid (5 mg) in 5 mL of deionized water. After stirred overnight, the precipitates were collected by centrifugation and

washed with anhydrous ethanol several times. All the experiments were carried out at room temperature.

***In vitro* photodynamic effect of COF-LZU-1 and COF-LZU-1-Ag_2Se.** 1,3-Diphenylisobenzofuran (DPBF) was employed as a chemical probe to evaluate the $^1O_2$ generation. Firstly, 10 mg of DPBF was dissolved in 1 mL of DMSO to form a stable solution (37 mmol L^{-1}). Secondly, an amount of COF-LZU-1 or COF-LZU-1-Ag_2Se was dispersed evenly in 2 mL of deionized water in a quartz cell, and then 10 μL of DPBF-DMSO solution was added. Before laser radiation, the mixed solution was tested *via* UV-Vis spectroscopy to confirm the initial absorption intensity of DPBF around 410 nm. Then the mixed solution was irradiated under a 650 nm laser for different times (0-12 min). The decrease in the DPBF absorption around 410 nm was monitored *via* UV-Vis spectroscopy at different time intervals. All experiments were carried out at room temperature under continuous stirring.

Intracellular ROS assay. HeLa cells were seeded into confocal culture plates and adhered for 24 hours. Then cells were incubated in culture medium containing 100 μg mL^{-1} of COF-LZU-1@HA or COF-LZU-1-Ag_2Se@HA NPs (2 mL) for another 24 hours in the dark. After that, the cells were incubated in the DCFH-DA (10 μM, 10 μL) in the dark for 20 min. After the laser irradiation, cell imaging was recorded with a confocal laser scanning microscope (CLSM) (excited wavelength: 405 nm, collection from 420 to 520 nm).

***In vitro* and *in vivo* photothermal effect of hyaluronic acid modified COF-LZU-1-Ag_2Se.** Hyaluronic acid modified COF-LZU-1-Ag_2Se aqueous solutions with different concentrations (3.75, 7.5, 15, 31.25, 62.5, 125, 250, and 500 μg mL^{-1}) were suspended in different wells of a 96 well plate, and irradiated by an 808 nm CW laser with a power density of 1.2 W cm^{-2} for different times. The temperature was carefully measured by a digital thermometer with a thermocouple probe. The photothermal stability was evaluated on COF-LZU-1-Ag_2Se solution (500 μg mL^{-1}), and the heating and cooling (ON-OFF) cycle could be repeated for six times upon CW laser irradiation (808 nm, 1.5 Wcm^{-2}). In each heating-cooling cycle, CW laser irradiation lasted for 10 min followed by a 15 min cooling period until it reached room temperature again. The *in vivo* photothermal effect was conducted by intratumoral injection of hyaluronic acid modified COF-LZU-1-Ag_2Se (500 μg mL^{-1}) into the tumor on a Balb/C mouse with a power density of 1.5 W cm^{-2}, and the temperature was recorded every 1 min by a R300SR-HD infrared camera (NEC). The control group (only injected with PBS) was also tested as a comparison.

Photothermal conversion efficiency of COF-LZU-1-Ag_2Se. The photothermal efficiency was determined based on the protocol reported before. To examine the photothermal behavior, the aqueous solution of COF-LZU-1-Ag_2Se with a concentration of 500 μg mL^{-1} was irradiated using an 808 nm laser (BWT Beijing Ltd., China) with a power density of 1.5 W cm^{-2} over a period of 10 minutes. The temperature of solution was recorded every 30 s by a digital thermometer with a thermocouple probe. The photothermal conversion efficiency (η) of COF-LZU-1-Ag_2Se or COF-LZU-1-Ag_2Se@PEG was calculated according to the following equation:

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

Where *h* is the heat transfer coefficient, *s* is the surface area of the container, T_{max} is the equilibrium temperature after 10 min's irradiation, T_{Sur} is ambient temperature, Q_{Dis} expresses the heat dissipation by the test cell, *I* is 808 nm laser power (1.5 W cm^{-2}), and A_{808} is the absorbance of the solution at 808 nm. The value of *hs* is determined according to the following equation:

$$hS = \frac{m_d C_d}{\tau_s}$$

Where m_d is the mass (1 g) and C_d is the heat capacity (4.2 J g^{-1}) of the aqueous solution, τ_s is the sample system time constant.

Cell culture and incubation conditions. The HeLa cells line was cultured in DMEM culture medium supplemented with 1% (v/v) penicillin, 1% (v/v) streptomycin, and 10% (v/v) fetal bovine serum at 37 °C in 5% CO_2. L929 cells line was cultured in DMEM culture medium supplemented with 1% (v/v) penicillin, 1% (v/v) streptomycin, and 10% (v/v) fetal bovine serum at 37 °C in 5% CO_2.

Cellular uptake. Cellular uptake was certified by intracellular fluorescence. For staining, Hela cells seeded into 6-well plates at a density of 1.5 × 10^5 cells per well and incubated with DMEM culture medium at 37 ^oC for 24 h. After that, DMEM was replaced with fresh media containing COF-RhB, and COF-Ag_2Se-RhB (100 μg mL^{-1}), and then incubated for 0h, 2h, and 4h, respectively. Finally, after removing DMEM, the HeLa cells were washed with fresh PBS for three times, and then fixed with 4% paraformaldehyde solution for 20 min. The cell membrane was stained with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) for 10 min and washed with PBS for three times. A fluorescence microscope was utilized to monitor the cells for qualitative evaluation.

In vitro cytotoxicity evaluation. The biocompatibility of hyaluronic acid modified COF-LZU-1-Ag_2Se was evaluated using a standard [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) test. The L929 cells were seeded in a 96-well plate (8000 cells per well) and incubated in a humidified atmosphere of 5% CO_2 overnight to ensure that the cells had attached to the wells. After that, hyaluronic acid modified COF-LZU-1-Ag_2Se with different concentrations (3.75, 7.5, 15, 31.25, 62.5, 125, 250, and 500 μg mL^{-1}) were added into each well. After 24 h incubation in the dark condition, the medium containing nanoparticles was removed and 10 μL of MTT solution was added into each well and cultured for another 4 h. Finally, the supernatant was replaced with 150 μL of dimethyl sulfoxide (DMSO) each well. The plate was shaken for 10 min and examined using a microplate reader (Therom Multiskan MK 3) at the wavelength of 490 nm. Cell viability values were calculated by the equation of Cell viability (%) = absorbance of experimental group / the absorbance of control group ×100%.

***In vitro* photo-induced cancer cell Killing.** For photodynamic therapy at the cellular level, HeLa

cells (8000 per well) were placed in each well of a 96-well plate and incubated overnight. Then the cells were treated with at different concentrations (0, 3.75, 7.5, 15, 31.25, 62.5, 125, 250, and 500 μg mL^{-1}) of COF-LZU-1@HA or COF-LZU-1-Ag2Se@HA for 6 h at 37 °C in the dark. Followed by removing the medium containing non-internalized nanoparticles, the cells were washed thrice with PBS, and then exposed to a 650 nm laser with a density of 0.5 W cm^{-2} for 5 min. For photothermal therapy at the cellular level, HeLa cells were exposed to an 808 nm laser with a density of 1.2 W cm^{-2} for 5 min. While for the combined photodynamic and photothermal therapy, HeLa cells were irradiated with a 650 nm (0.5 W cm^{-2}) laser for 5 min first, and then by an 808 nm (1.2 W cm^{-2}) for another 5 min. After the photo-induced therapy process, the cells were further incubated for 24 h in the dark, and the cell viability relative to the untreated cells was evaluated using MTT assay.

Cell apoptosis. To study the cell apoptosis process of COF and COF-Ag_2Se, the Annexin V-FITC/PI Apoptosis Detection Kit was used. HeLa cells were treated with PBS, 650 nm laser, 808 nm laser, COF (125 μg mL^{-1}), COF-Ag_2Se (125 μg mL^{-1}), COF under a 650 nm laser irradiation (125 μg mL^{-1}, 5 min, 0.5 W cm^{-2}), COF under a 808 nm laser irradiation (125 μg mL^{-1}, 5 min, 1.2 W cm^{-2}), COF-Ag_2Se under a 650 nm laser irradiation (125 μg mL^{-1}, 5 min, 0.5 W cm^{-2}), COF-Ag_2Se under a 808 nm laser irradiation (125 μg mL^{-1}, 5 min, 0.5 W cm^{-2}), and COF-Ag_2Se under a 650 (5 min, 0.5 W cm^{-2}) and 808 nm (5 min, 1.2 W cm^{-2}) laser irradiation respectively, and then the cells were incubated in 6-well plates overnight. After that, the cells were harvested and washed with PBS, and then resuspended with binding buffer (400 μL). At last, 5 μL of Annexin V-FITC and 10 μL of PI were utilized to stain the samples for 15 min and 5 min in the dark, respectively. The cell apoptosis process was monitored *via* a flow cytometer.

Live/dead cell viability assay. Hela cells with a density of 50000 mL^{-1} (1 mL) were plated onto twelve-well plate for 24 h. Then the cells were treated with PBS, 650 nm laser, 808 nm laser, COF (125 μg mL^{-1}), COF-Ag_2Se (125 μg mL^{-1}), COF under a 650 nm laser irradiation (125 μg mL^{-1}, 5 min, 0.5 W cm^{-2}), COF under a 808 nm laser irradiation (125 μg mL^{-1}, 5 min, 1.2 W cm^{-2}), COF-Ag_2Se under a 650 nm laser irradiation (125 μg mL^{-1}, 5 min, 0.5 W cm^{-2}), COF-Ag_2Se under a 808 nm laser irradiation (125 μg mL^{-1}, 5 min, 0.5 W cm^{-2}), and COF-Ag_2Se under a 650 (5 min, 0.5 W cm^{-2}) and 808 nm (5 min, 1.2 W cm^{-2}) laser irradiation respectively, and then the cells were incubated in twelve-well plates overnight. The medium was substituted with the mixed solution of calcium AM for 30 min and PI for 5min. Then the cells were washed and immersed with PBS and imaged by a fluorescence microscope.

***etc*anti-tumor efficacy evaluation.** Female Balb/c mice (18-20 g weight) were purchased from the Center for Experimental Animals, Jilin University. All animal studies were conducted in accordance with the guidelines of the National Regulation of China for Care and Use of Laboratory Animals. The H22 tumor models were successfully established by subcutaneous injection of 4×10^6 cells suspended in 100 μL of PBS into the left axilla of each mouse. The mice were treated when the tumor volumes reached 80-100 mm^3. The tumor-bearing mice were randomly divided into ten groups (n=5, each group), and treated with i, control; ii, 650 nm; iii, 808 nm; iv, COF; v, COF-Ag_2Se; vi, COF + 650 nm; vii, COF + 808 nm; viii, COF-Ag_2Se + 650 nm; ix, COF-Ag_2Se + 808 nm; x, COF-Ag_2Se + 650 + 808 nm, respectively. For group iv, vi and vii, the

tumors were exposed to a 650 nm laser (0.5 W cm^{-2}) for 10 min after the injection for 12 h. While for group v, viii and ix, the tumors were exposed to an 808 nm laser (1.5 W cm^{-2}) for 10 min after the injection for 12 h. As for group x, the tumors were irradiated by a 650 nm laser (0.5 W cm^{-2}) for 5 min first, then by an 808 nm laser (1.2 W cm^{-2}) for 5 min. The body weight and tumor volume of each mouse were monitored every two days, and after 14 days treatment, the tumors were dissected and weighed to evaluate the therapeutic efficacy. In a typical calculation, the tumor volume was calculated by V = 4/3 x Length x $width^2/8$. The relative tumor volume was calculated as V/V_0, where V_0 was the tumor volume before the treatment. Finally, the major organs, such as liver, spleen, heart, lung, and kidney, were removed and fixed in 4% paraformaldehyde solution for histological examination in order to further investigate the biocompatibility of COF-LZU-1 and COF-LZU-1-Ag_2Se.

Results and Discussion

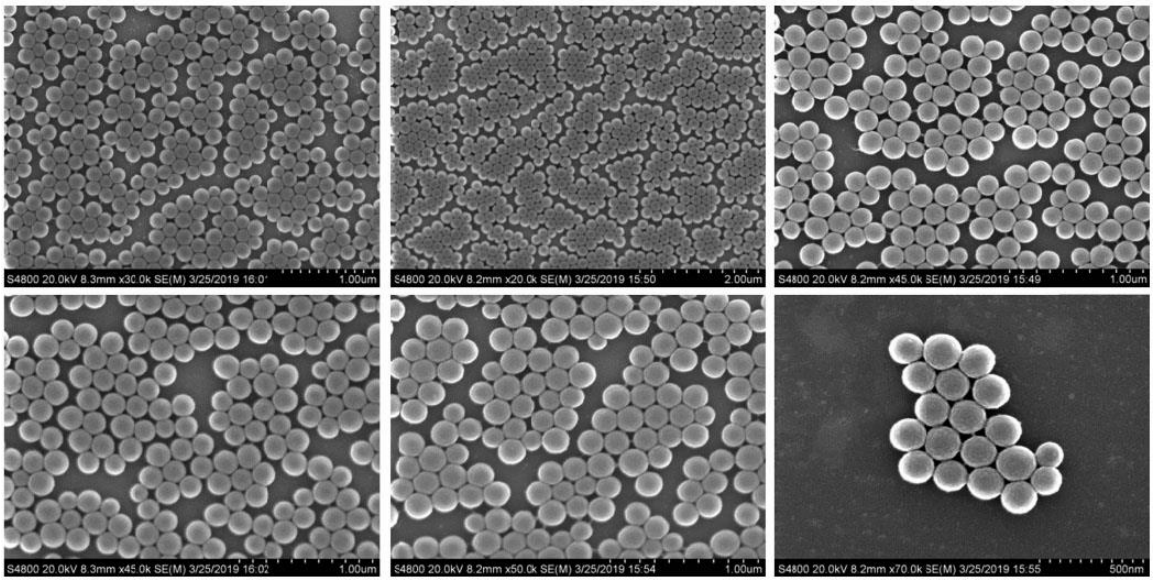

Fig. S1 SEM images of COF-LZU-1 nanoparticles.

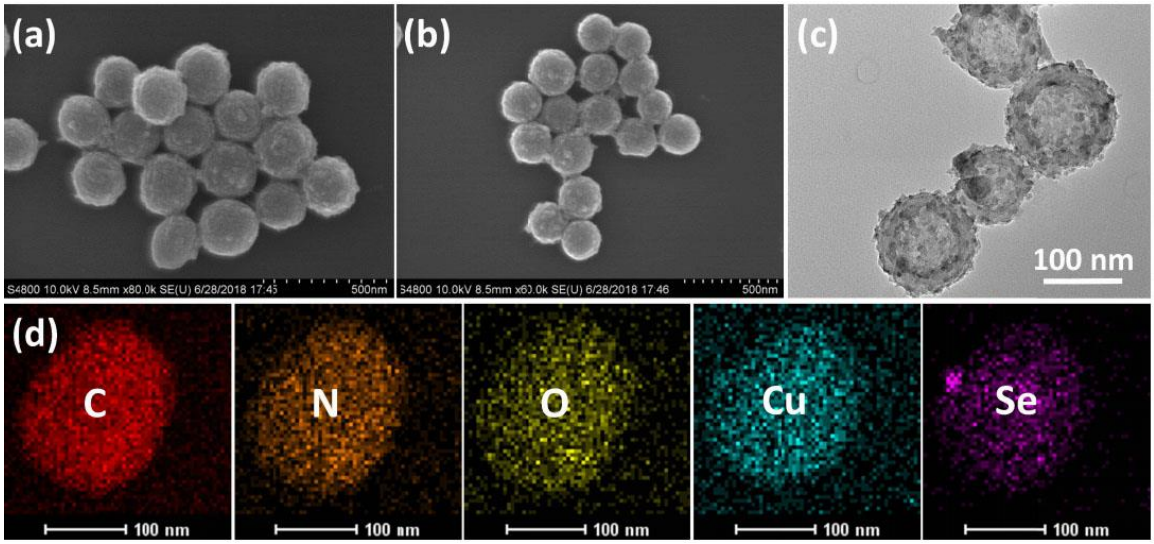

Fig. S2 (a, b) SEM images, (c) TEM image, and (d) HAADF-STEM-EDS mapping images of COF-CuSe nanoparticles.

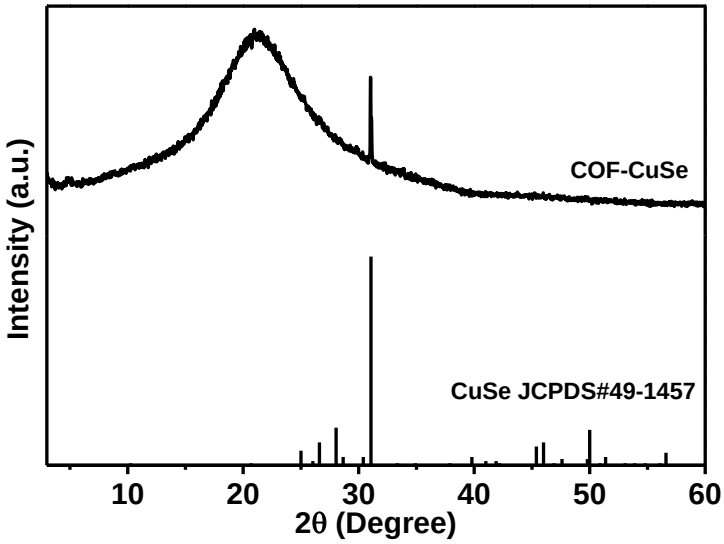

Fig. S3 XRD pattern of COF-CuSe.

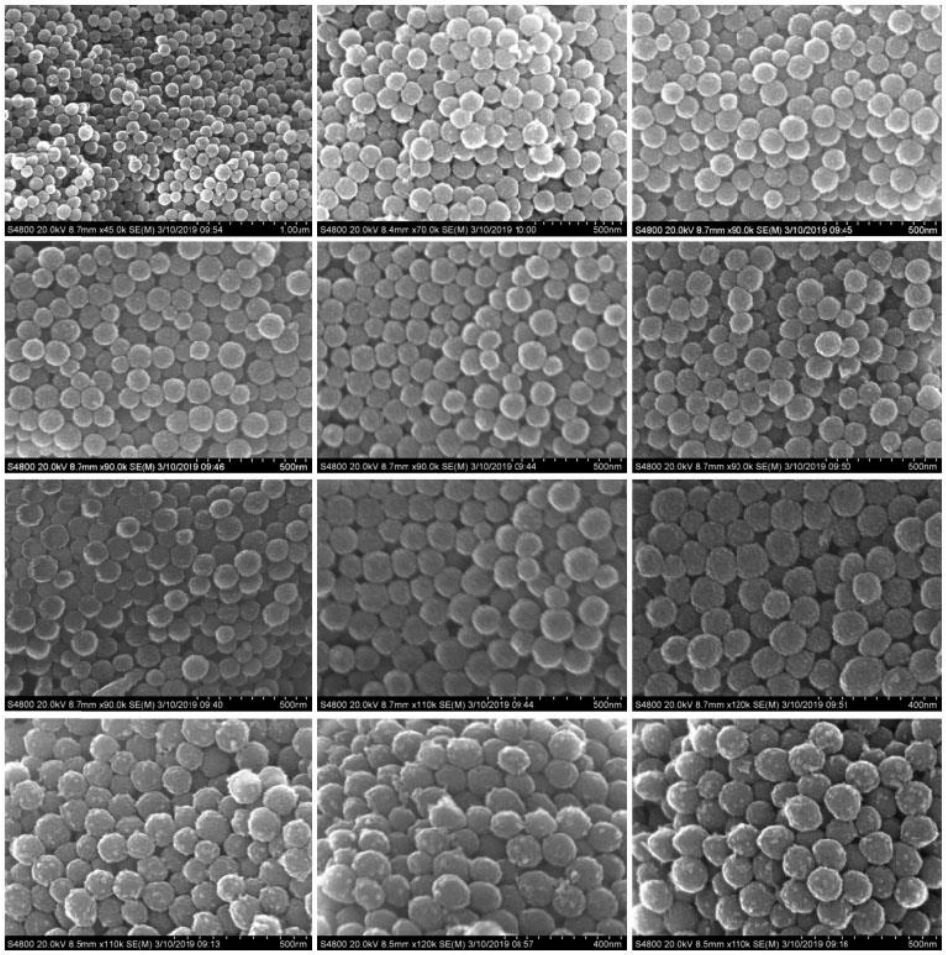

Fig. S4 SEM images of COF-Ag_2Se nanoparticles.

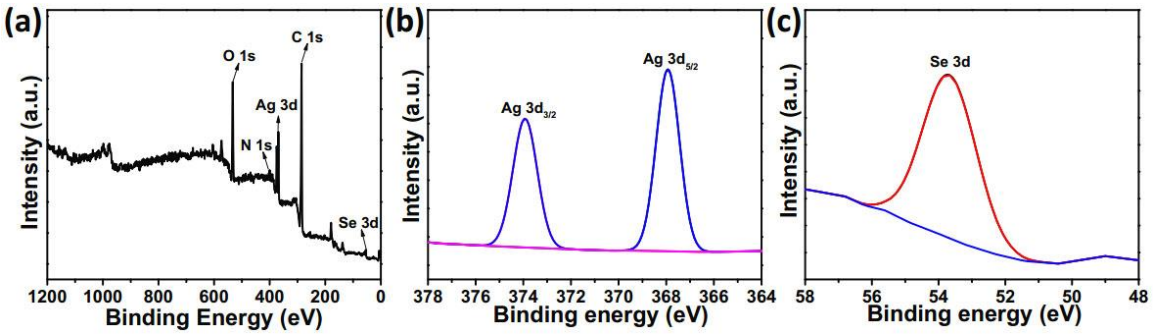

Fig. S5 XPS spectra of COF-Ag_2Se nanoparticles: (a) Whole spectrum, (b) Ag 3d, and (c) Se 3d spectrum.

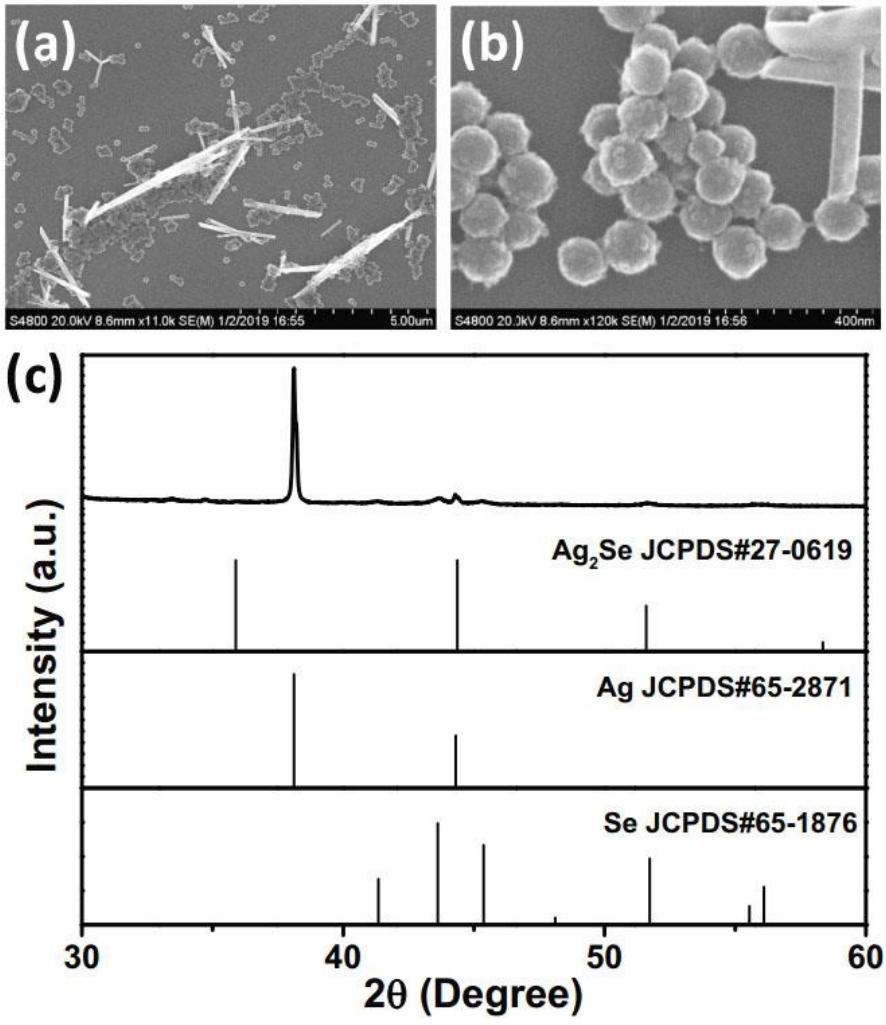

Fig. S6 (a, b) SEM images and (c) XRD patterns of COF nanoparticles mixed with Ag^+ and Se^{2-} directly.

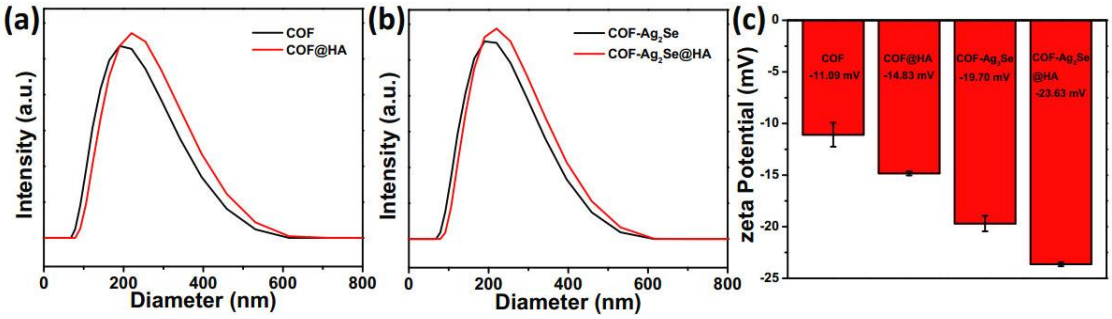

Fig. S7 DLS analysis of (a) COF and COF@HA, and (b) COF-Ag_2Se and COF-Ag_2Se@HA. (c) Zeta potentials of COF and COF-Ag_2Se nanoparticles before and after modification with HA.

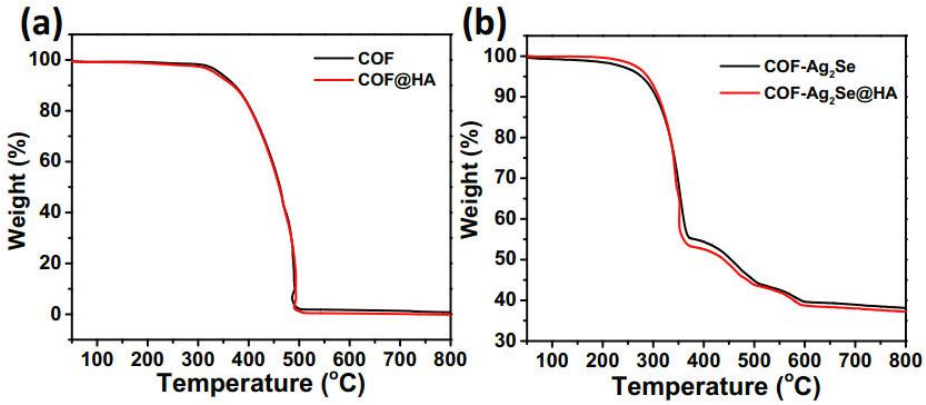

Fig. S8 TGA curves of (a) COF and COF@HA, and (b) COF-Ag_2Se and COF-Ag_2Se@HA.

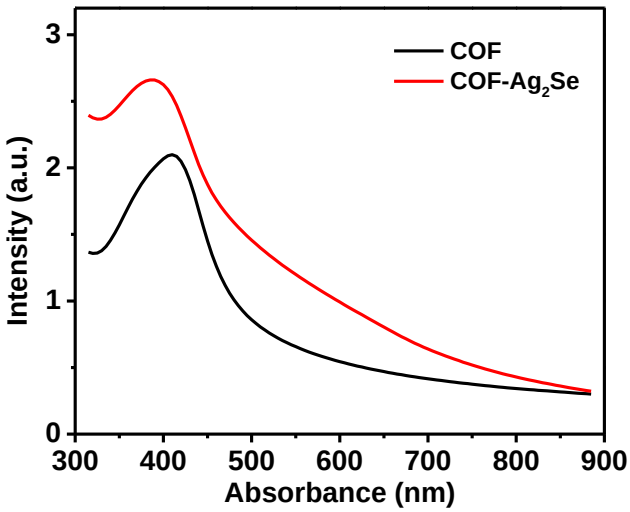

Fig. S9 UV-absorption spectra of COF and COF-Ag_2Se aqueous solution.

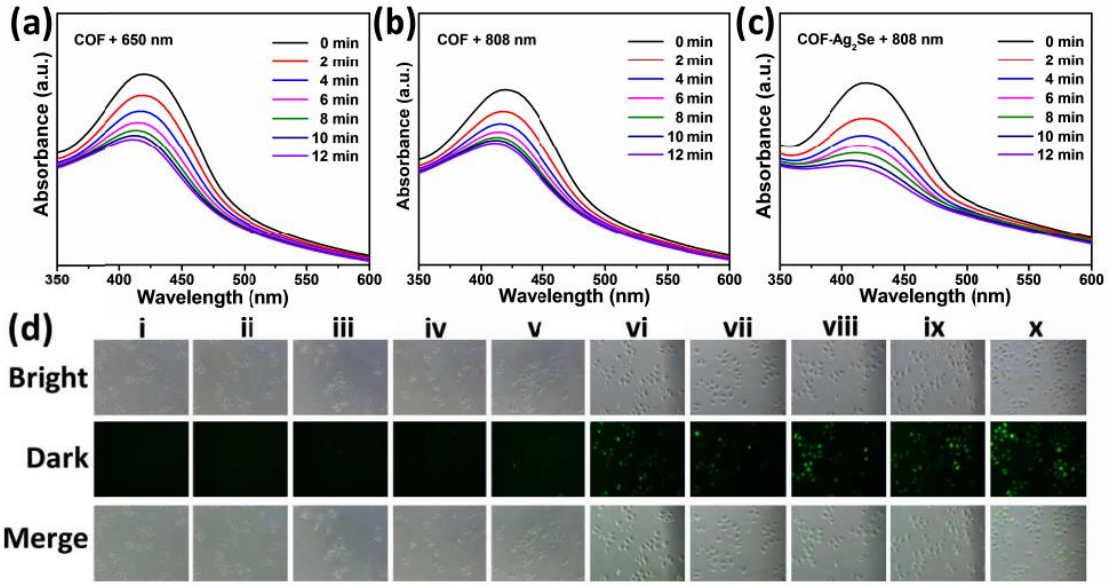

Fig. S10 Time dependent absorption variation curves of DPBF (20 mM, 10 μL) treated by (a) COF + 650 nm laser, (b) COF + 808 nm laser, and (c) COF-Ag_2Se + 808 nm laser (125 μg mL^{-1}, 12 min, 0.5 W cm^{-2}). (d) Fluorescence images of HeLa cells incubated under different conditions: i, control; ii, 650 nm; iii, 808 nm; iv, COF; v, COF-Ag_2Se; vi, COF + 650 nm; vii, COF + 808 nm; viii, COF-Ag_2Se + 650 nm; ix, COF-Ag_2Se + 808 nm; x, COF-Ag_2Se + 650 + 808 nm.

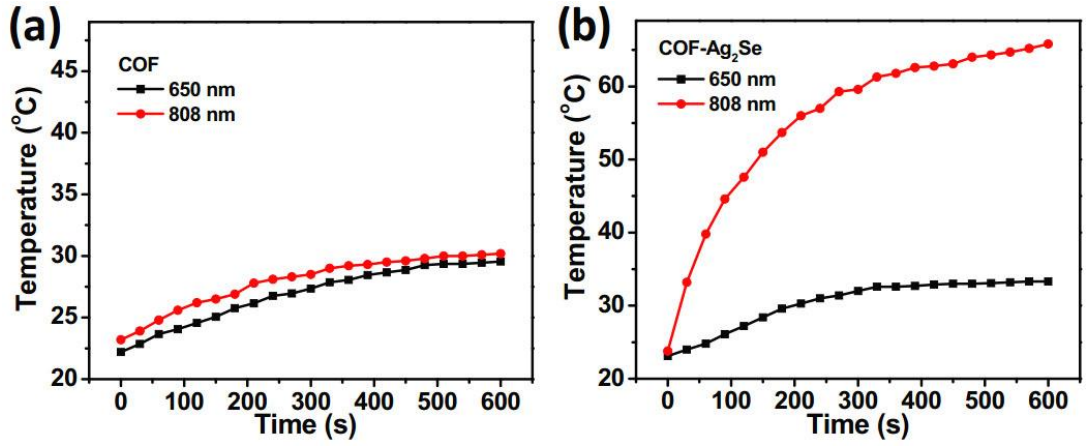

Fig. S11 Temperature variations of (a) COF and (b) COF-Ag_2Se aqueous solution (250 μg mL^{-1}) under 650 or 808 nm laser irradiation (1.2 W cm^{-2}).

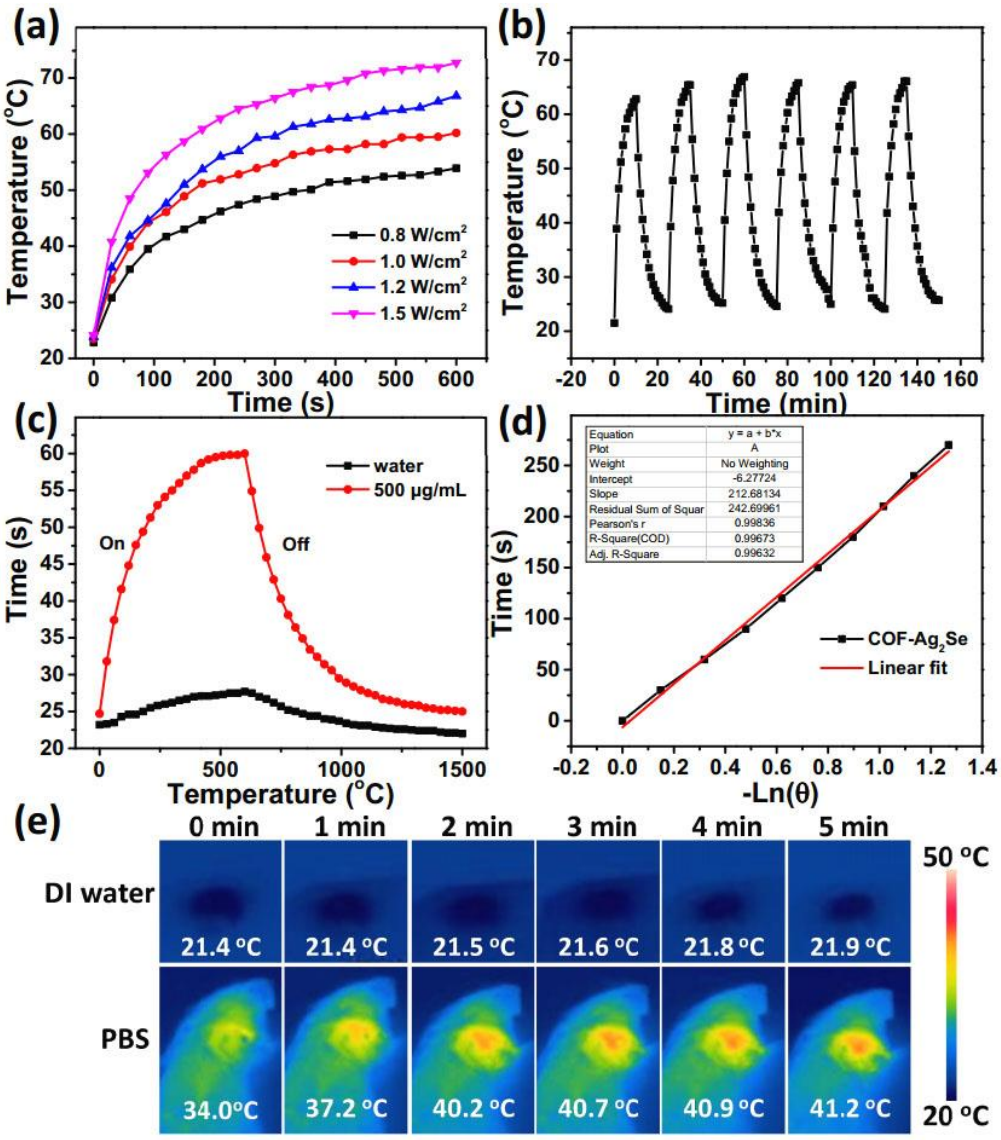

Fig. S12 (a) COF-Ag_2Se aqueous solution (500 μg mL^{-1}) irradiated with different laser power densities. (b) Photostability characterization of COF-Ag_2Se (500 μg mL^{-1}, 1.5 W cm^{-2}) for 6 cycles. (c) Temperature variation curves of COF-Ag_2Se (500 μg mL^{-1}) and deionized water during one heating-cooling cycle (808 nm laser, 1.5 W cm^{-2}), and (d) linear fit of -ln(θ) vs. time acquired from the cooling process. (e) Infrared thermal images of DI water and tumor bearing mice injected with PBS upon 808 nm irradiation (500 μg mL^{-1}, 1.5 W cm^{-2}).

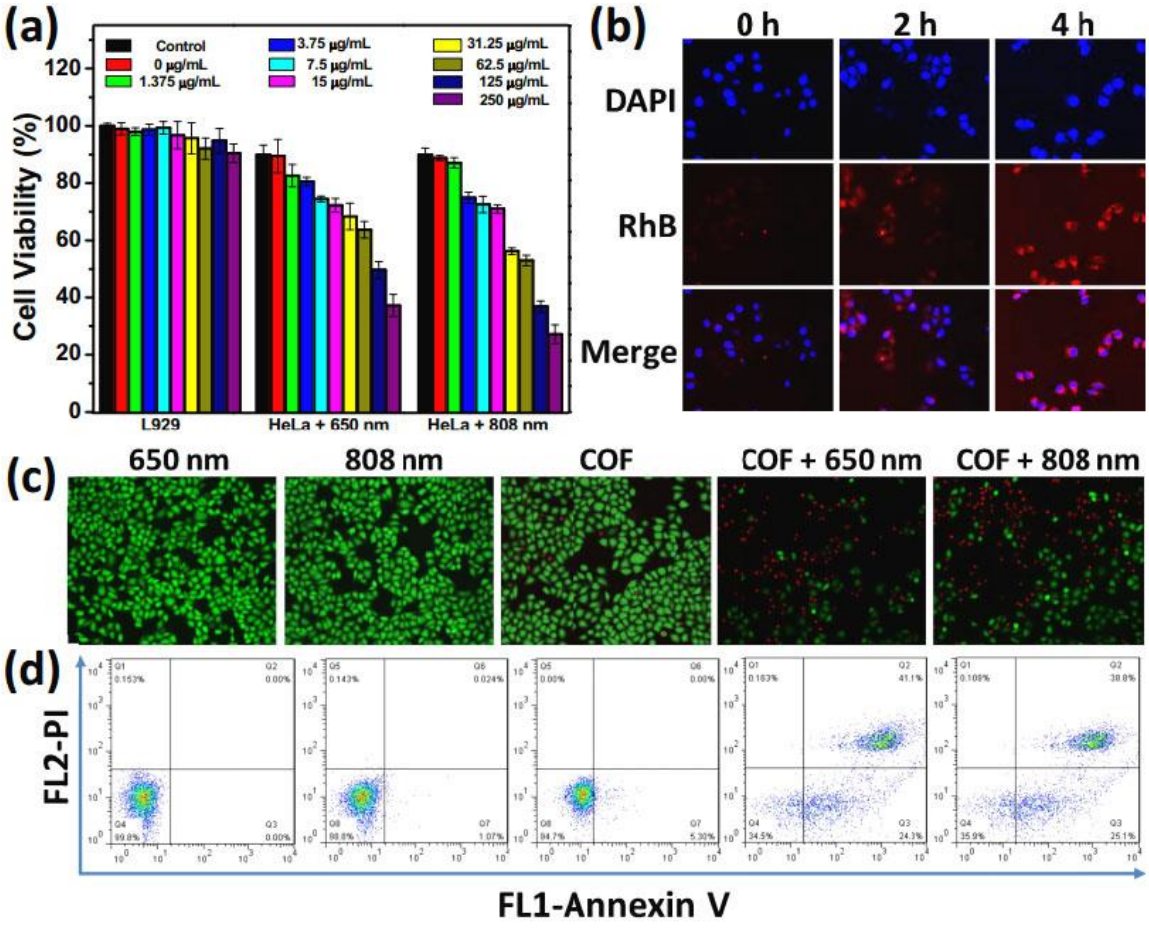

Fig. S13 (a) Cell viability of COF against L929 and HeLa cells. (b) Fluorescence images of HeLa cells treated with COF for different times. (c) Fluorescence images of calcein-AM/PI stained HeLa cells after different treatments. (d) Flow cytometry test of HeLa cells apoptosis with different treatments.

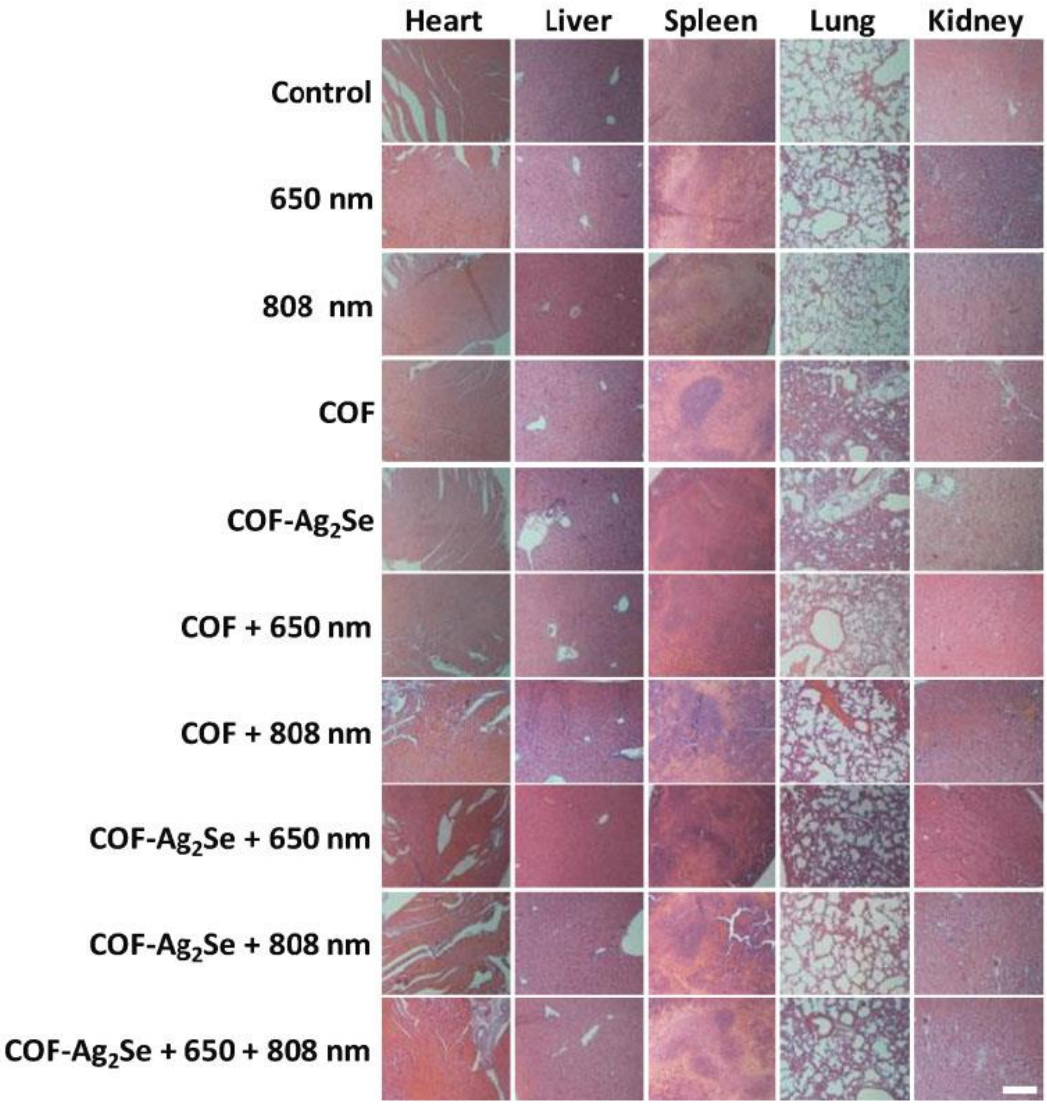

Fig. S14 Hematoxylin and eosin (H&E) stained images of major organs (heart , liver, spleen, lung and kidney) for all groups. The scale bar is 20 μm.

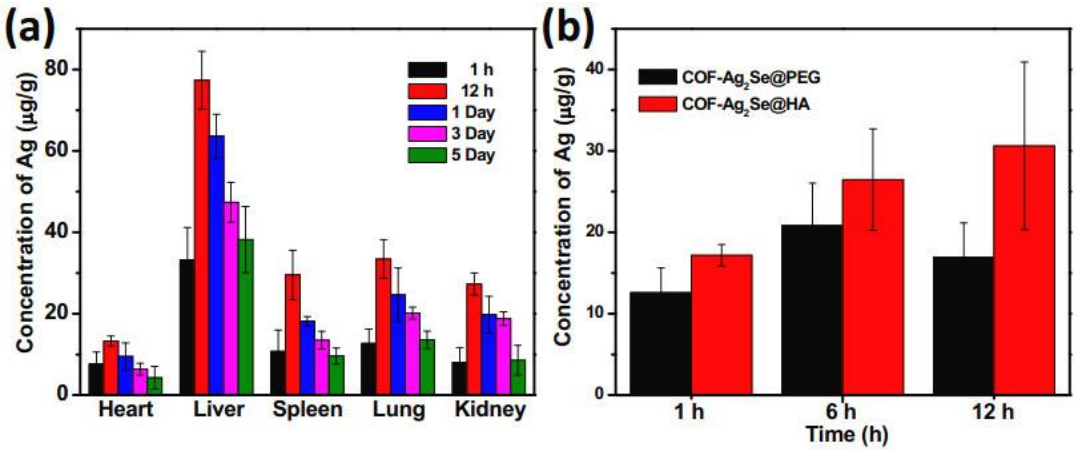

Fig. S15 (a) The biodistribution of COF-Ag_2Se@HA nanoparticles in major organs. (b) Accumulation of HA or PEG modified COF-Ag_2Se nanoparticles at the tumor site.