Electronic Supplementary Information for:

High Quality β-FeOOH Nanostructures Constructed from Biomolecule-assisted Hydrothermal Approach and Their pH-responsive Drug Delivery Behaviors

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Experimental Section

Materials

Ferric chloride hydrates ($FeCl_3 \cdot 6H_2O$, 99.5 %, Tianjin Zhiyuan Chemical Company), Gelatin ($C_{102}H_{151}N_{31}O_{39}$, G7041-500G 99.0 % Sigma-Aldrich), other type Gelatin (G2500-500G 99.0 % Sigma-Aldrich), Urea ($CO(NH_2)_2$, 99.9 % Sigma-Aldrich), and Ethanol (C_2H_6O, > 99.7 %) were used as received without further purification.

Synthesis of FeOOH1 Nanostructures

A typical synthetic procedure for **FeOOH1** was described: First, 10 mL of pure water containing 27.0 g (100 mmol) of $FeCl_3 \cdot 6H_2O$ were added into a 100 mL round-bottom flask with vigorous magnetic stirring at room temperature for ~45 min. Then, another 9 mL of pure water containing 1.0 g of gelatin were added into a centrifuge tube, which was heated in an electric oven at 80 °C for 20 min formed transparent solution to inject into the $FeCl_3 \cdot 6H_2O$ system stirring at room temperature for ~30 min. After that, 6.0 g (100 mmol) of urea was dissolved in the $FeCl_3$/gelatin mixture stirring at room temperature for ~30 min, subsequently; elevating the temperature to 80 °C and reaction lasted for 21 h. After it cooling down to room temperature, the as-formed **FeOOH1** were collected by centrifugation at 8000 rmp and washed with ethanol for several times, and then dried in a vacuum at 60 °C overnight. **FeOOH2** nanostructures synthetic procedure was **s**imilar to **FeOOH1** except urea was not joined into the reaction system. **FeOOH3** nanostructures synthetic procedure was similar to **FeOOH1** except the increase of the urea to 300 mmol and prolonging the reaction time for 46 h.

Characterization. Powder X-ray diffraction (XRD) patterns of the dried powders were recorded on Riau D/MAX-RB (Japan) at a scanning rate of 2 ° min^{-1} from 10 ° to 80 °, using Cu Ka radiation (λ = 1.5406 Å). The lattice parameters were calculated with the least-squares method. The transmission electron microscopy (TEM) analysis was performed with a Hitachi HT-7700 (Japan) transmission electron microscope operating at 100 kV. Selected area electron diffraction (SAED) and scanning transmission electron microscopy (STEM) characterizations were performed with a FeiTecnat G^2 F20S-Twin (USA) operated at 200 kV. The UV-vis absorption spectra of the β-FeOOH nanostructures

were obtained on a Lambda 35 UV-vis Spectrometer at room temperature (Japan). The Fourier transforms infrared absorption (FTIR) spectra of the β-FeOOH nanostructures were carried on NICOLET 6700 FT-IR (USA). The zeta potentials of β-FeOOH nanostructures were recorded by the Beckman Coulter delsa nano particle analyzer system (USA). Nitrogen adsorption–desorption isotherms were recorded on a Micrometrics TriStar 3000 porosimeter (mesoporous characterization) and Micrometrics ASAP 2020 microporous characterization) at 77 K. All β-FeOOH samples were outgassed at 160 °C for 6 h under the vacuum state prior to measurements. The specific surface areas were calculated based on the Brunauer–Emmett–Teller (BET) method. X-ray Photoelectron Spectra were obtained using a Escalab 250 xi photoelectron spectrometer using Al K radiation (15 kV, 225 W, base pressure $\approx 5 \times 10^{-10}$ Torr).

Anti-cancer Drug Loading

5-fluorouracil (5-Fu) (Sigma), as an anti-cancer drug, was loaded in β-FeOOH nanostructures. Briefly, 5-Fu was dissolved in deionized water to obtain a 10 mg/mL drug solution. 50 mg of β-FeOOH samples were soaked in 5-Fu solution for 24 h at 37 °C. After that, the β-FeOOH samples with drugs were obtained by centrifugation and washing. The drug loading efficiency (DLE) was calculated by the following formula: DLE % = ((weight of drug in feed-weight of drug in medium after loading)/weight of drug in feed) × 100 %. The weights of drug before and after loading were determined by UV-vis analysis.

Evaluation of pH-controlled Release

In vitro pH-controlled releases of β-FeOOH nanostructures were carried out at 37 °C in PBS of pH 7.4 and acetic buffer solutions (ABS) of pH 4.0. At pre-determined time points, the release medium containing samples was centrifuged and 1 mL solution was taken out for further analysis. Then the fresh medium with the same volume was added into release solution. The drug concentrations and accumulated release was determined using UV-vis spectrophotometer (Lambda 35 UV-Vis) by measuring the maximum absorbance wavelengths at 266 nm.

Cell Culture

Fibroblasts (L929 cells) line and human liver hepatocellular carcinoma cell line (HepG2 cells) was employed to study the cytotoxicity of as-prepared β-FeOOH nanostructures and anticancer ability of 5-Fu loaded β-FeOOH nanostructures respectively. The L929 and HepG2 cells were provided by the State Key Laboratory for Manufacturing Systems Engineering, Xi'an Jiaotong University. Cells were incubated at 37 °C in a humidified atmosphere containing 5 % CO_2. The complete growth medium was Dulbecco's Modified Eagle Medium (DMEM, GIBCO) supplemented with 10 % fetal bovine serum (FBS, GIBCO), 1.0×10^5 U/L penicillin (Hyclone) and 100 mg/L streptomycin (Hyclone).

Cytotoxicity Assessment

The cytotoxicity of β-FeOOH nanostructures against L929 cells was determined by an AlamarBlue kit (Invitrogen, USA). L929 with a concentration of 5.0×10^4 per mL was seeded in 96-well plate. After culture for 24 h, the culture medium for L929 was changed into growth medium containing β-FeOOH nanostructures at different concentrations (25 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL, and 800 μg/mL). At predetermined time intervals (24 h and 72 h), the culture medium was replaced by 100 μL growth medium containing 10 μL of the AlamarBlue® reagent. After incubation for another 5 h, the fluorescence intensity related as cell viability was tested at 560/600 nm by a microplate reader (Molecular Devices). Cells cultured on tissue culture plate (TCP) without nanomaterials treating were used as the positive control which was conceded as 100 % of cell viability. Six species for each group were tested.

Anticancer Ability Investigation

The anticancer ability of 5-Fu loaded β-FeOOH nanostructures against HepG2 cells was also evaluated by the alamarblue assay as described. 100 μL of medium containing 5000 HepG2 cells was added in a 96-well plate. After 24 h, the culture medium was changed into growth medium containing β-FeOOH nanostructures and 5-Fu loaded β-FeOOH nanostructures at different concentrations (25 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL, 800 μg/mL) respectively. Cells cultured on tissue culture plate (TCP) without nanomaterials and pure 5-Fu at different concentrations served as the negative and positive control. Six species for each group were tested.

Cellular Uptake of β-FeOOH Nanostructures

Pure β-FeOOH nanoparticles do not show any fluorescent properties. Therefore, to observe the cellular uptake of β-FeOOH nanostructures, samples were stained by Nile red dye. Briefly, 10 mg of β-FeOOH nanostructures were immersed into a certain concentration of Nile red solution for several hours, then collected by centrifugation at 8000 rpm and washed with ethanol and water for several times, then dried in a vacuum for 2 h. Cells were seeded on sterile glass coverslips at the density of 5000 cells/cm^2. After 12 h of incubation, the cells were treated with Nile red loaded β-FeOOH nanostructures (50 μg/mL) for 4 h. The fluorescence images were observed under the confocal microscope (Olympus).

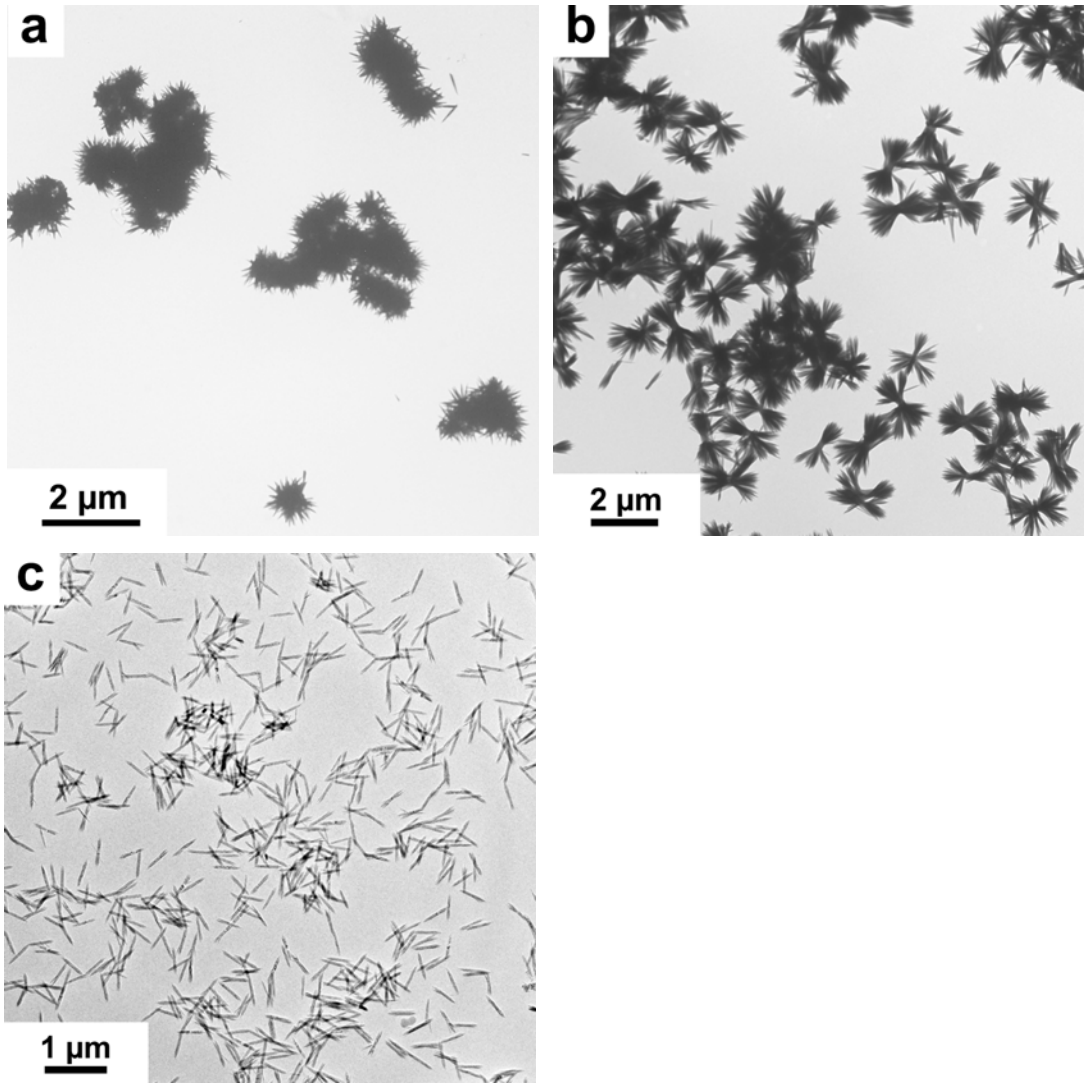

Figure S1. Low magnified TEM images of as-obtained (a) **FeOOH1**, (b) **FeOOH2** and (c) **FeOOH3** nanostructure.

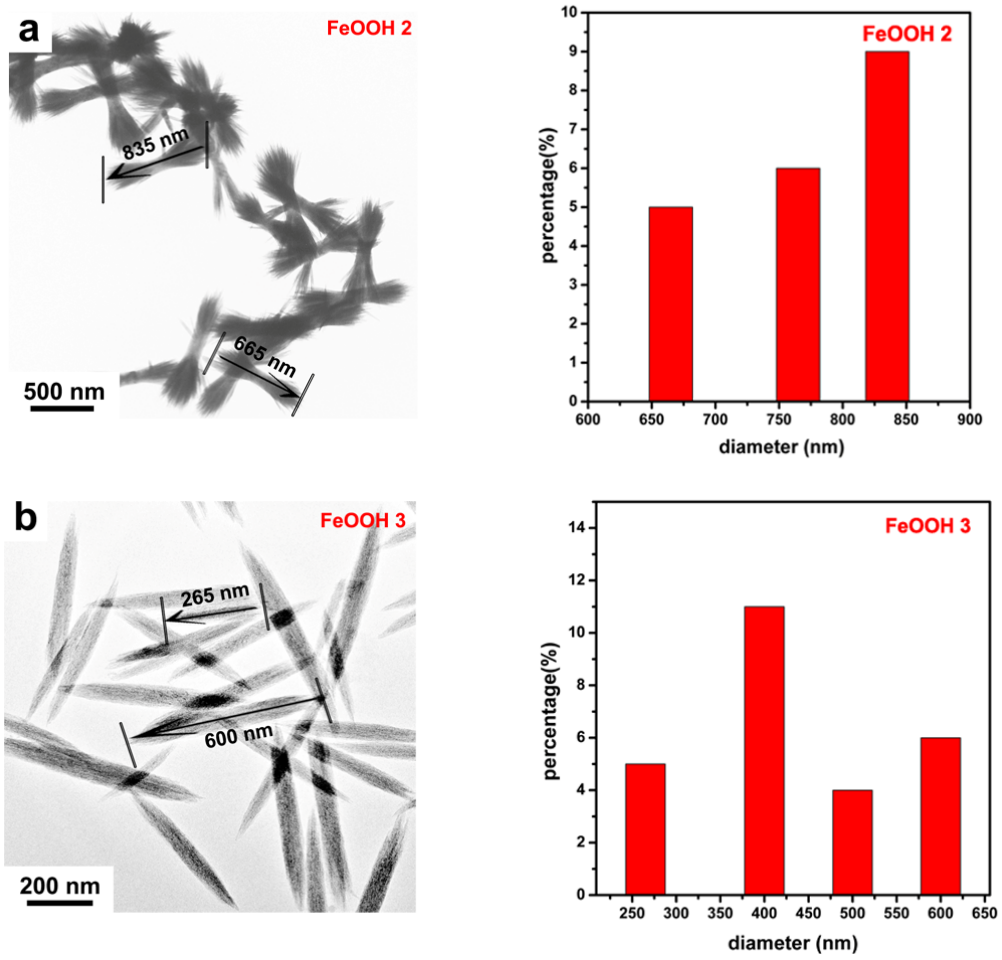

Figure S2. TEM images and size distribution of the as-obtained (a) **FeOOH2** and (b) **FeOOH3** nanostructures.

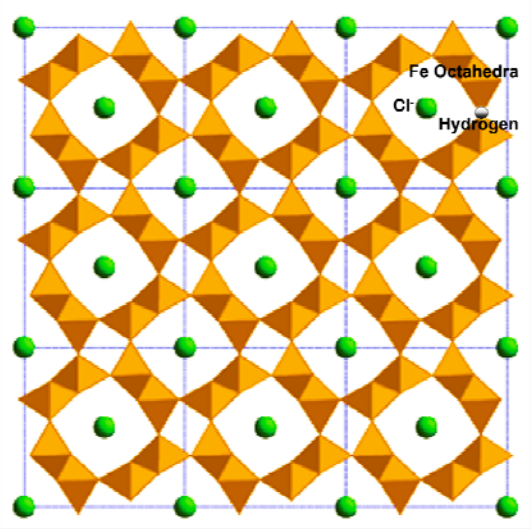

Figure S3. Structural model of akaganeite. The unit cell consists of $FeO_3(OH)_3$ octahedra forming a double ring structure, and there is cavity with a diameter of 0.5 nm (where it is normally for Cl^- to incorporate). The akaganeite has a structure in which four double chains of $FeO_3(OH)_3$ octahedral form a 0.5 nm × 0.5 nm tunnel running along [010]. The tunnel is partly occupied by chloride anions, which substitute for hydroxide and are suggested to play an vital role in stabilizing the structure.

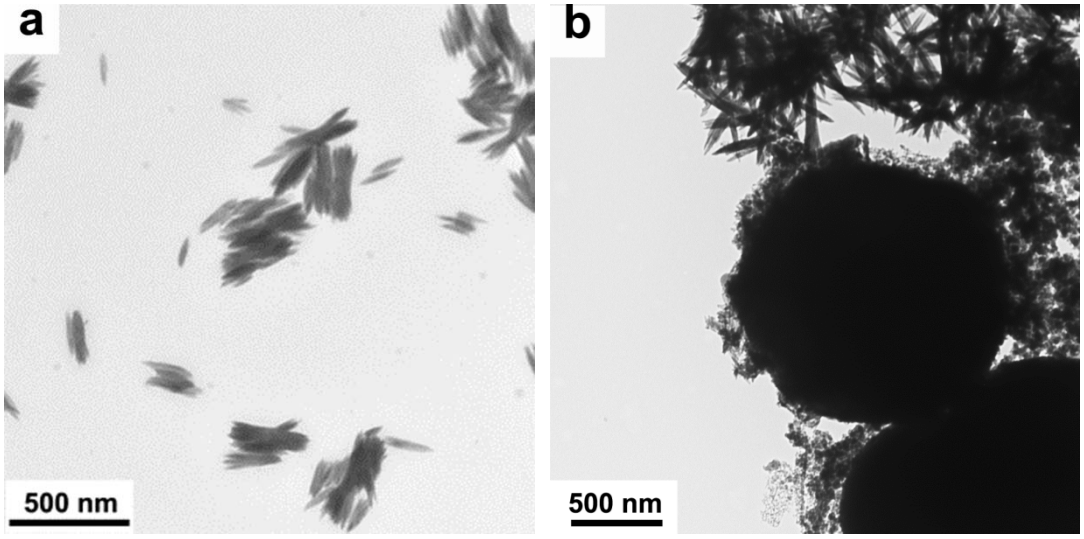

Figure S4. TEM images of the obtained **FeOOH1** *via* different concentration of $FeCl_3 \cdot 6H_2O$ with 1.0 g gelatin, 100 mmol urea at 80 °C for 21 h: (a) 10 mmol, (b) 1mol.

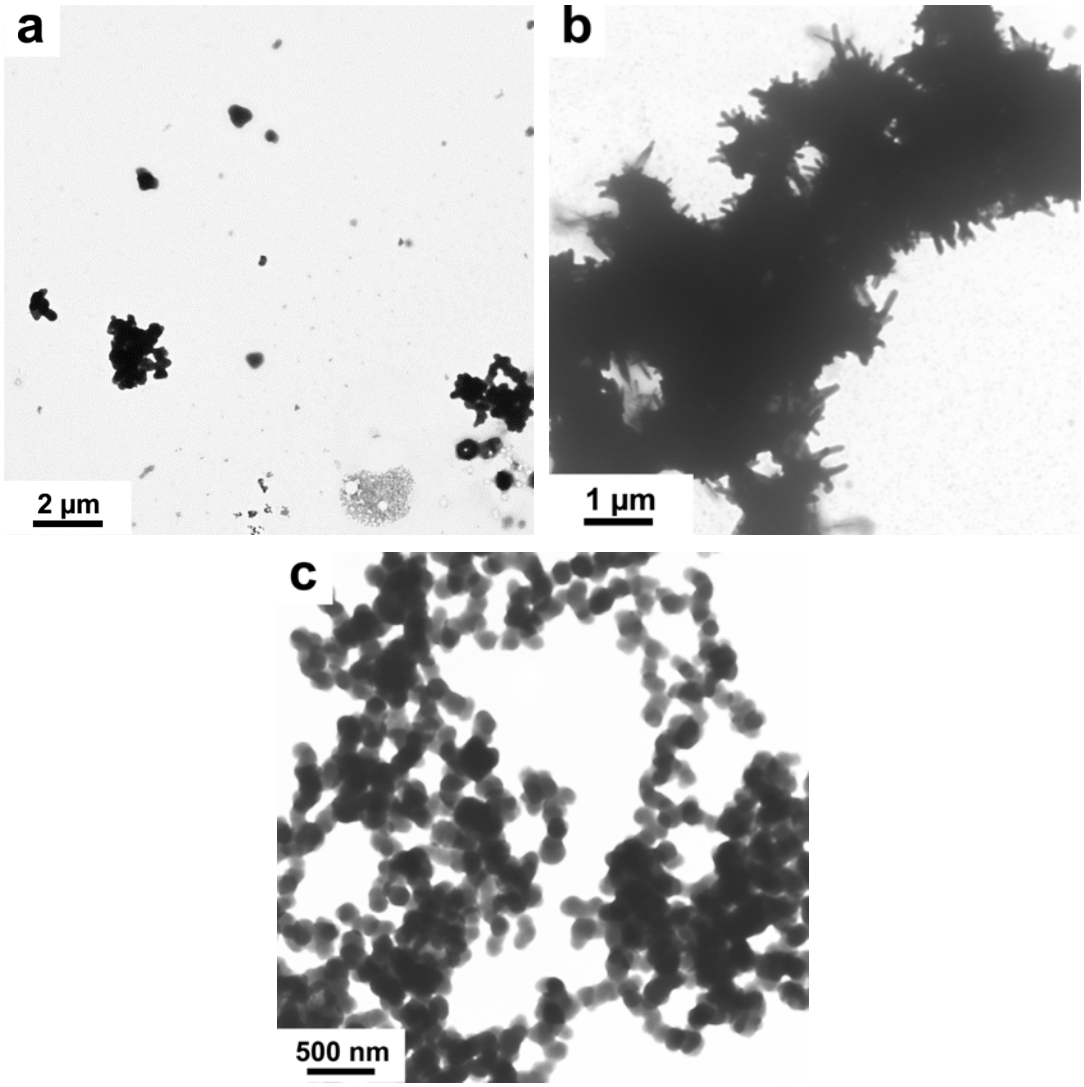

Figure S5. TEM images of the obtained **FeOOH1** *via* hydrothermal reaction of 100 mmol $FeCl_3 \cdot 6H_2O$ and 100 mmol urea under different surfactants at 80 °C for 21 h: (a) no gelatin, (b) 5 g gelatin, (c) other types of gelatin (G2500-500G).

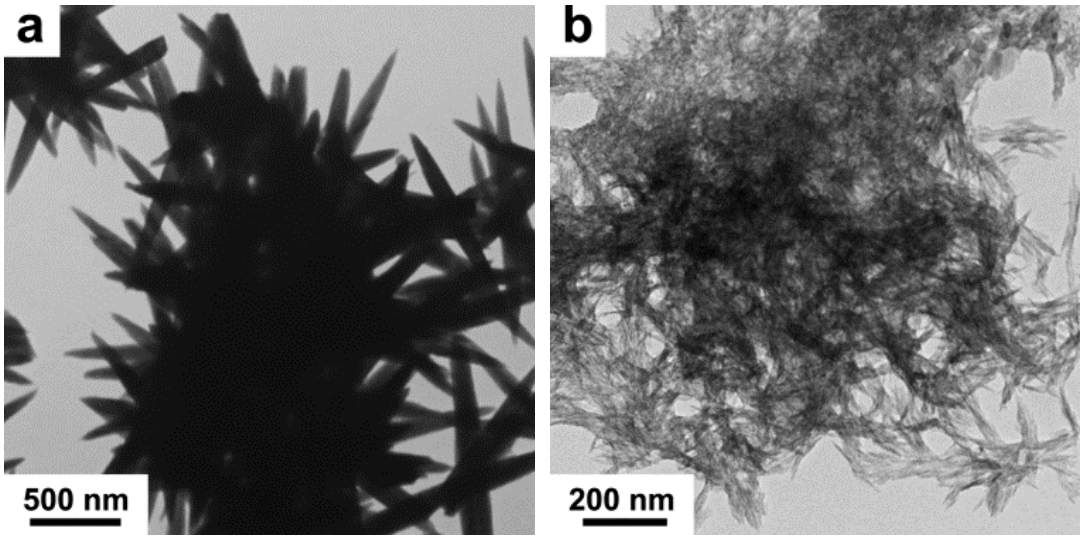

Figure S6. TEM images of the obtained **FeOOH1** *via* hydrothermal reaction of 100 mmol $FeCl_3 \cdot 6H_2O$, 1.0 g gelatin and 100mmol urea at different temperatures for 21 h: (a) 120 °C, (b) 40 °C.

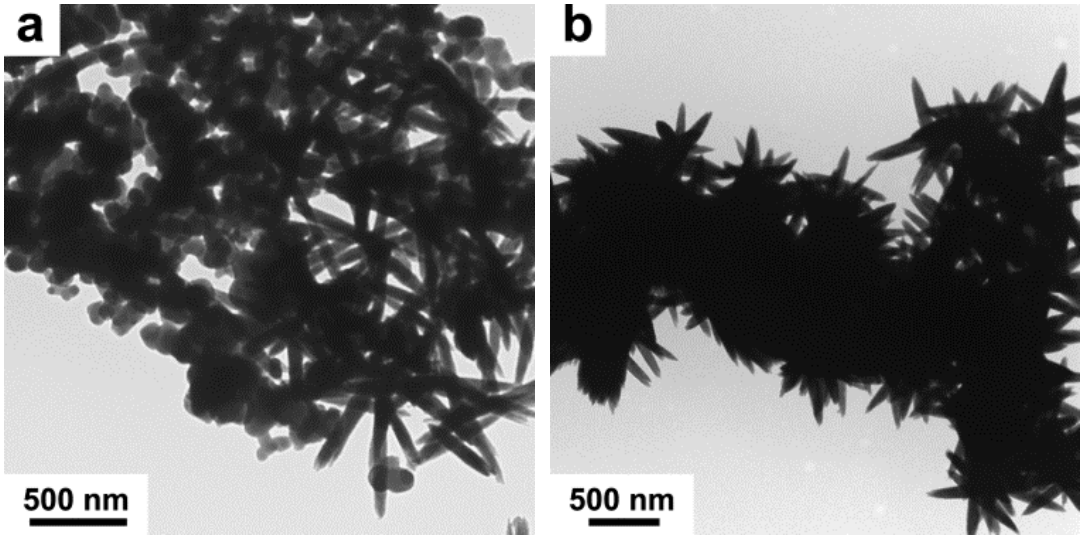

Figure S7. TEM images of the obtained **FeOOH1** *via* hydrothermal reaction of 100 mmol $FeCl_3 \cdot 6H_2O$, 1.0 g gelatin and 100 mmol urea at 80 °C with different time: (a) 10 h, (b) 50 h.

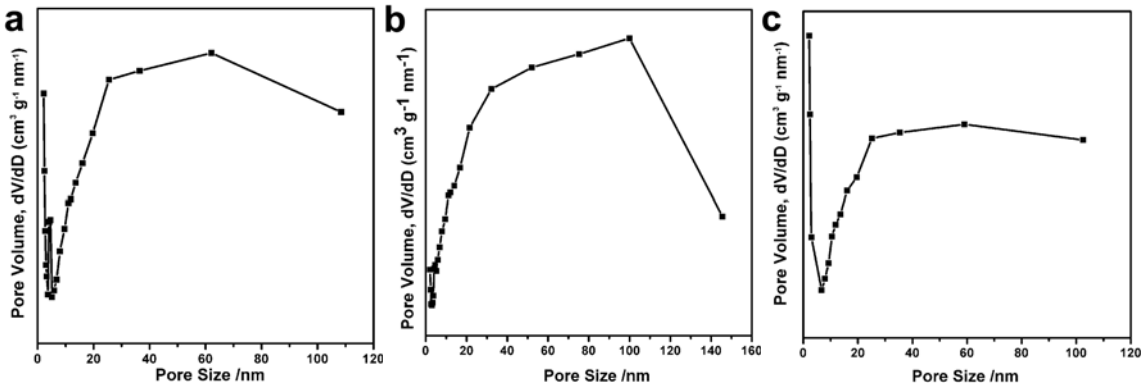

Figure S8. BJH pore size distribution curves of (a) **FeOOH 1** (b) **FeOOH 2** and (c) **FeOOH3** nanostructures.

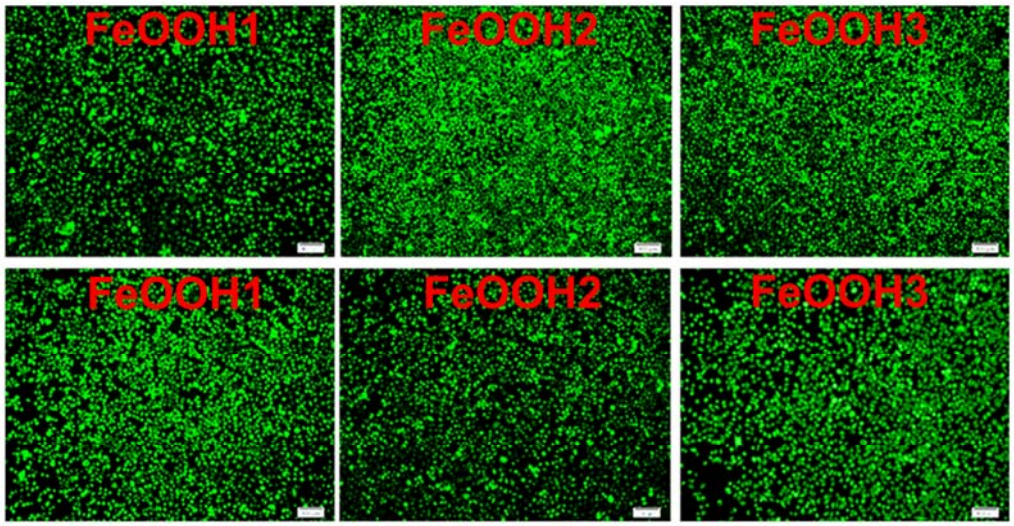

Figure S9. Live cell morphology after incubation with nanostructures at 100 μg/mL.

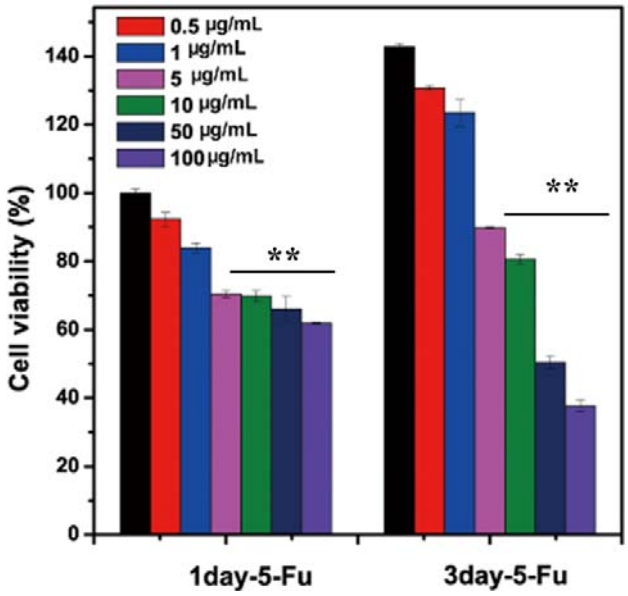

Figure S10. Cancer cell viability after incubation with pure 5-Fu at different concentrations. **p<0.01 represents the significant difference relative to the cell viability at low 5-Fu concentrations.

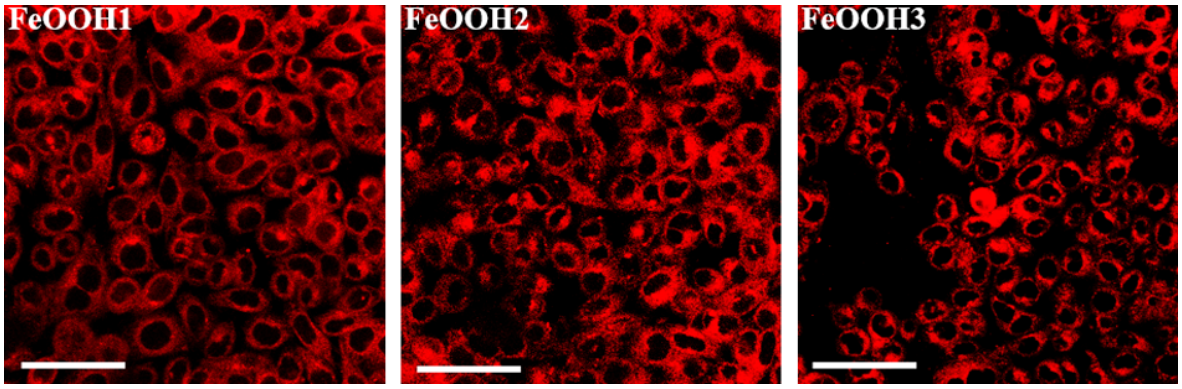

Figure S11. Cellular uptake study of *β*-FeOOH nanostructures. Fluorescent images of HepG2 cells after incubated with Nile red-loaded *β*-FeOOH nanostructures. Scale bar = 100 μm.

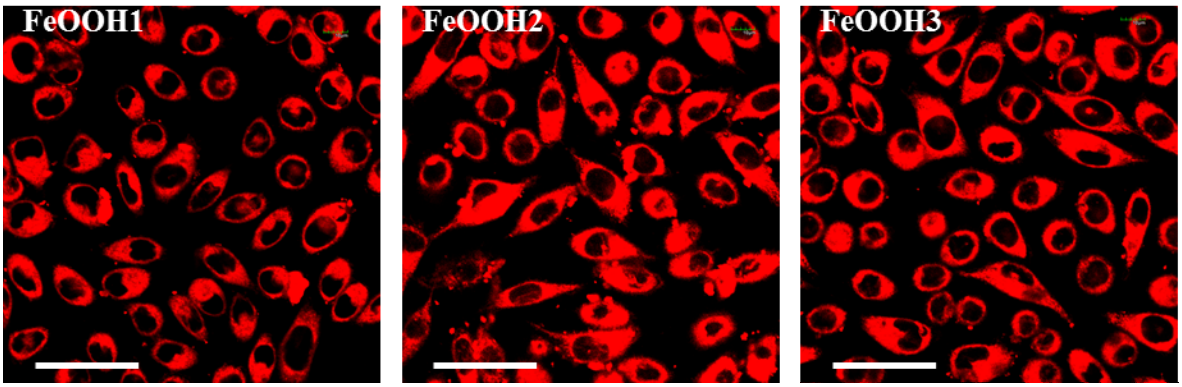

Figure S12. L929 cellular uptake study of β-FeOOH nanostructures. Fluorescent images of cells after incubated with Nile red-loaded β-FeOOH nanostructures. Scale bar= 100 μm.