FePt@CoS₂ Yolk-shell Nanocrystals as A Potent Agent to Kill HeLa Cells

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Supporting Information

General: oleic acid (99%), oleylamine (99%), 1, 2-hexadecanediol (tech. 90%), dioctyl ether (99%), phenyl ether (99%), trioctylphosphine oxide (tech 90%), Sulfur (99.998%), Co₂(CO)₈, Fe(acac)₃ and 1, 2-dichlorobenzene are purchased from Sigma-Aldrich; Fe(CO)₅ and Pt(acac)₂ (98%) from Strem Chem; All the chemical reactions were carried out under inert atmosphere in glove box unless otherwise stated.

Synthesis of FePt@CoS₂ yolk-shell nanocrystals: Under inert atmosphere, ~20 mg of as-prepared FePt nanoparticles (~2 nm in diameter), 0.1 g of trioctylphosphine oxide (TOPO) and 0.05 mL of oleic acid were dissolved in 15 mL of 1, 2-dichlorobenzene. 0.3 g of Co₂(CO)₈ dissolved in 1.5 mL of 1, 2-dichlorobenzene was injected in the refluxing bath of the above solution. After 30 min, 0.12 g of sulfur dissolved in 2 mL of 1, 2-dichlorobenzene was injected into the hot solution immediately. The reaction was cooled down to the room temperature after 30 min. The addition of ethanol (30 mL) to the reaction mixture afforded a black produce by centrifuging. The product was further washed by hexane (5 mL) and ethanol (20 mL) two more times in order to removed the surfactant completely. The final product can be redispersed in water very well by a brief ultrasonic. The nanostructures were characterized by means of transmission electron micrographs (JEOL 2010, 200 kV), high-resolution TEM and EDX (JEOL 2010F, 200 kV), X-ray photoelectron spectroscopy (XPS) and X-ray fluorescence spectrum (XRF). The magnetic properties of the nanostructures were measured by SQUID.

Synthesis of FePt nanoparticles:¹ Under argon condition, platinum acetylacetonate (197 mg, 0.5 mmol), 1, 2-hexadecanediol (390 mg, 1.5 mmol), and dioctyl ether (20 mL) were mixed and heated to 100 °C. Oleic acid (0.16 mL, 0.5 mmol), oleylamine (0.17 mL, 0.5 mmol) were added, After ~5 min, when the color of solution was changed from yellow to brown, Fe(CO)₅ (0.13 mL, 1 mmol) was added immediately, then the mixture was heated to reflux (297 °C). The reaction was cooled down to the room temperature after 1 hour. The addition of ethanol (30 mL) to the reaction mixture afforded a black produce by centrifuging. The FePt nanoparticles were dispersed in hexane and stored in nitrogen.

Synthesis of CoS₂ hollow nanospheres:² 0.2 g of trioctylphosphine oxide (TOPO) and 0.1 mL of oleic acid were dissolved in 15 mL of 1, 2-dichlorobenzene. 0.6 g of Co₂(CO)₈ dissolved in 3.0 mL

of 1, 2-dichlorobenzene was injected in the refluxing bath of the above solution. After 30 min, 0.25 g of sulfur dissolved in 3 mL of 1, 2-dichlorobenzene was injected into the hot solution immediately. The reaction was cooled down to the room temperature after 45 min. The addition of ethanol (30 mL) to the reaction mixture afforded a black produce by centrifuging. The product was further washed by hexane (5 mL) and ethanol (20 mL) two more times in order to removed the surfactant completely. CoS₂ hollow nanospheres also can be dispersed in water very well without any surface modification.

Synthesis of FePt@Fe₃O₄ core-shell nanoparticles:³ After the formation of FePt nanoparticles, keeping the high temperature, Fe(CO)₅ (0.08 mL) was injected into the refluxing solution. The reaction was cooled down to the room temperature and exposed in air condition after 30 min. FePt@Fe₃O₄ core-shell nanoparticles were separated by centrifuging after the addition of ethanol.

Surface modification of FePt nanoparticles using cysteine: In ambient environment, 2 mL of hexane solution containing ~10 mg of FePt nanoparticles was added into 2 mL of water solution containing ~2 mg of cysteine (adjusted pH~7). These two solutions were mixed by vigorous stirring and kept stirring for about 24 hours in order to let FePt nanoparticles completely transfer from hexane layer into water layer. The as-prepared FePt-Cys sample was washed by DI water for three times, then was redispersed in DI water and stored under nitrogen.

MTT assay: We chose HeLa cells for the cytotoxicity analysis. HeLa cells were seeded into a 96-well plate at a concentration of 3×10^3 cells/well in 100 μ L of MEM medium with 10% FBS. FePt@CoS₂ yolk-shell nanocrystals with different concentration (1.0, 2.0, and 4.0 μ g/mL) were added when cells were plated. Then, the cell cultures were incubated for 3 days at 37 °C and 5% CO₂. MTT assay was carried out every 24 hours to measure the proliferation of HeLa cells. For each MTT assay, after the incubation period, 10 μ L of the MTT labeling reagent (final concentration: 0.5 mg/mL) was added to each well. After incubating the 96-well plate for 4 h in a humidified atmosphere (e.g., 37 °C, 5% CO₂), 100 μ L of the solubilization solution was added into each well, allowing the plate to stand overnight in the incubator. Finally, complete solubilization of the purple formazan crystals was checked and the spectrophotometrical absorbance of the samples was measured by using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product was 595 nm. In order to prepare cells sample for TEM examinations, after 3 days incubation with FePt@CoS₂ yolk-shell nanocrystals, the medium was removed and the cells were washed with PBS (5 mL) three times until the totally removal of the non-uptaken nanocrystals. Then the cells were trypsinized, centrifuged, and suspended in 1 mL of DI water. A single drop of the aqueous cells mixture after ultrasonication was placed onto a carbon-coated copper grid and dried under vacuum for several hours at room temperature.

MIC value determination: The MIC of FePt@CoS₂ nanocrystals for the strains tested was determined under conditions identical to those used for bacterial culture with serial twofold dilutions of antibiotic. Specifically, FePt@CoS₂ nanocrystals were incubated with *Bacillus subtilis* ATCC 11774 in 2 mL LB broth or NB broth at a concentration of 1×10^7 cells/mL each tube respectively. The tubes were then shaken at 200 rpm at 37 °C for 4.5 h and thereafter OD600 values were measured. The minimum concentrations of FePt@CoS₂ nanocrystals of those tubes that have

clear solution and the OD600 values below 0.5 are considered as the MIC values of FePt@CoS₂ yolk-shell nanocrystals.

Figure S1. TEM images of (A) as-synthesized FePt nanoparticles with ~2-3 nm in diameter (Inset: HRTEM image) and (B) FePt@Co core-shell intermediate nanoparticles (Inset: HRTEM image).

Figure S2. XPS analysis of FePt@CoS₂ yolk-shell nanocrystals. The signals of the shell composition cobalt and sulfur are strong, but the signals of the core composition iron and platinum are very weak

Figure S3. (A) XRF analysis of FePt@CoS₂ yolk-shell nanocrystals indicates the ratio of FePt and CoS₂ is about 1 : 65; (B) Temperature-dependent of the ZFC/FC magnetization measured with a magnetic field of 100 Oe (Inset: The hysteresis loop obtained at 5 K for FePt@CoS₂ yolk-shell nanocrystals).

Figure S4. The optical image of HeLa cells incubated (37 °C and 5% CO₂) with 5 µg/mL of FePt@CoS₂ nanocrystals for 72 h. The HeLa cells are almost dead. Upon death, the smooth cells detach from the surface and sprout out multiple white small buds around the surface of the cells, suggesting the apoptosis of the cells.

Figure S5. Bacterial inhibition analysis (*Bacillus subtilis* ATCC 11774).

Figure S6. TEM images and EDX analysis of FePt@CoS₂ nanocrystals when incubated with phosphate buffer solution (pH = 4.98) at 37 °C and 5% CO₂ after 1 day (A, B), 2 days (C, D), and 3 days (E, F): The FePt parts are destroyed and disappeared day by day. After 3 days, Fe atom parts are disappeared completely and Pt atom parts are almost disappeared.

Figure S7. MTT assay of CoS₂ hollow nanospheres (A), FePt@Fe₃O₄ core-shell nanoparticles (B), and cysteine-coated FePt nanoparticles (C) on HeLa cells at 5.0, 10.0, 20.0 µg/mL, respectively.

References

- (1) Sun, S. H.; Murray, C. B.; Weller, D.; Folks, L.; Moser, A. *Science* **2000**, *287*, 1989-1992.
- (2) Yin, Y. D.; Rioux, R. M.; Erdonmez, C. K.; Hughes, S.; Somorjai, G. A.; Alivisatos, A. P. *Science* **2004**, *304*, 711-714.
- (3) Chen, M.; Liu, J. P.; Sun, S. H. *J. Am. Chem. Soc.* **2004**, *126*, 8394-8395.