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Supporting Information

Multimodal Imaging Contrast Property of Nano Hybrid $\text{Fe}_3\text{O}_4@Ag$ Fabricated by Seed-Growth for Medicinal Diagnosis

The Tam Le,* Thi Ngoc Linh Nguyen,* Hoa Du Nguyen, Thi Hong Tuyet Phan, Hong Nam Pham, Duc Giang Le, Thanh Phong Hoang, Thi Quynh Hoa Nguyen, Trong Lu Le, and Lam Dai Tran

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Experimental section

Chemicals

All chemicals used in the experiments were purchased from Sigma-Aldrich (Singapore), including iron (III) acetylacetonate ($\text{Fe}(\text{acac})_3$), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, silver nitrate (AgNO_3), oleylamine (OLA), sodium oleate (SOA), 1-octadecanol (OCD-ol), poly (maleic anhydride-alt-1-octadecene) (PMAO), 1-octadecene (ODE), dibenzyl ether (DBE), chloroform (CHCl_3), absolute ethanol ($\text{C}_2\text{H}_5\text{OH}$) and n-hexane (C_6H_{14}). They were used as received without further purification.

Preparation of the hybrid nanomaterials

The fabrication of the hybrid structure materials between Fe_3O_4 and Ag for the biological application takes three steps as depicted in (**Scheme 1**). In the early stage, the Fe_3O_4 NPs were prepared by the decomposition of the $\text{Fe}(\text{acac})_3$ and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ precursors in ODE solvent at high temperatures. The second step was the preparation of $\text{Fe}_3\text{O}_4@Ag$ hybrid NPs by seeded-growth method using Fe_3O_4 NPs as seeds and AgNO_3 as a precursor. The as-synthesized $\text{Fe}_3\text{O}_4@Ag$ hybrid NPs were encapsulated by a hydrophobic layer of OLA, such that they dispersed in ODE solvent. In the third step, the obtained $\text{Fe}_3\text{O}_4@Ag$ hybrid NPs were transferred into the water phase by treatment with the PAA polymer as shown in (**Scheme 1**).

Synthesis of Fe_3O_4 NPs seeds by thermal decomposition method

Fe_3O_4 nanoparticles were synthesized by the thermal decomposition method described in previous work,^[1] with modifications. Sodium oleate (SOA) was added to the reaction solution, SOA is used as an alternative to oleic acid. SOA acts as an intermediate complexing agent with Fe(II) and Fe(III). In a typical synthesis, a mixture of $\text{Fe}(\text{acac})_3$ (120 mM), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (60 mM, OLA (720 mM) and OCD-ol (300 mM)) is dissolved in 40 mL of DBE in 100 mL three-neck flask under nitrogen atmosphere. Different amounts of SOAs were added in different reactions to ensure concentrations in the range of 300 - 750 mM. The reaction mixture was de-gassed at room temperature for 30 min and dehydrated at 100 °C for 30 min. Then, the reaction temperature was increased and kept at 200 °C under reflux heating for 30 min. The reaction was heated to 315 °C for 60 min with the speed 5 °C min⁻¹. After the reaction, the resulting mixture was cooled to room temperature by removing the heat source. The synthesized nanoparticles were purified using absolute ethanol with the help of a magnetic bar to collect the nanoparticles and were redispersed in n-hexane solvent. The obtained Fe_3O_4 NPs were dried at room temperature. (**Scheme 1**).

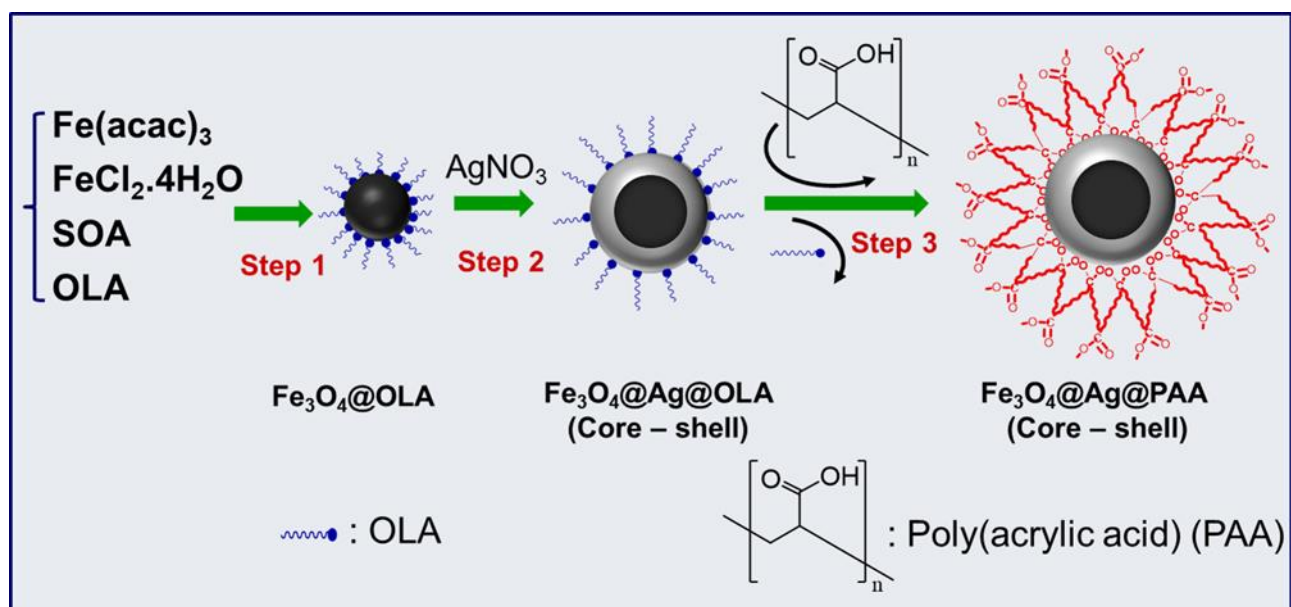
Synthesis of $\text{Fe}_3\text{O}_4@Ag$ core-shell HNPs by seed-mediated growth method

The $\text{Fe}_3\text{O}_4@Ag$ hybrid nanostructure was fabricated by the seeded-growth method using as-synthesized Fe_3O_4 NPs as seeds. In brief, 0.3 g of AgNO_3 , 3 mL of OLA and 0.15 g of OCD-ol were dissolved in 20 mL DBE in a three-neck flask. The 2 mL n-hexane containing 0.5 g of the purified Fe_3O_4 NPs was injected into the mixture while continuously stirred under nitrogen flow.

The reaction mixture was heated to 80 °C and kept at this temperature for 30 min to remove n-hexane. Afterwards, the system was heated to 200 °C with a rate of 5 °C min⁻¹, and then refluxed for 60 min before being cooled down to room temperature. The as-synthesized Fe₃O₄@Ag hybrid NPs were washed by ethanol and n-hexane solvents, using a magnetic bar to remove free Ag NPs from the hybrid product (**Scheme 1**).

Prepare water fluid based on hybrid nanomaterials Fe₃O₄@Ag

A simple procedure using hydrophilic PAA polymer can support the transfer of the hydrophobic NPs into an aqueous solvent by forming a layer of PAA on the surface of as-synthesized Fe₃O₄@Ag. [2] In a typical process, 2 g PAA was dissolved in 40 mL triethylene glycol (TEG) (Solution A) and heated the mixture up to 110 °C. Then, 100 mg Fe₃O₄@Ag hybrid NPs were dissolved in 5 mL n-hexane (solution B) and added quickly into solution A. The obtained mixture was heated up to 280 °C and the temperature was kept for six hours. After the reaction, the products were cooled to room temperature. Fe₃O₄@Ag@PAA was separated from TEG solution by adding diluted acid HCl and centrifuge at 12000 rpm. The final product was dispersed in water by sonication to obtain Fe₃O₄@Ag@PAA fluid in water (**Scheme 1**).



Scheme 1. Schematic three steps of the fabrication of Fe₃O₄@Ag@PAA hybrid nanoparticles.

Materials characterization

The particle size and shape were determined using a transmission electron microscope (TEM, JEOL JEM-1010, Japan). The average size and the size distribution of nanoparticles were analyzed with using ImageJ software. Infrared and UV-Vis spectra were obtained by the FT-IR NEXUS 670 (Nicolet, USA) and the UV-Vis spectrophotometer (Jasco V-670, Japan). The elemental composition of the fabricated materials was checked using an energy dispersive X-ray spectrometer (EDS, Jeol 6490 - JED 2300). The magnetic properties of the materials at room temperature were measured using a vibrating sample magnetometer (VSM) with an applied field up to 10 kOe. The stability and hydrodynamic size of the magnetic fluids were examined by a dynamic light scattering (DLS) measurements on the Zetasizer equipment (Malvern, UK). Inductively coupled plasma mass spectrometry (ICP-MS, Agilent technologies 7900) was used to determine the amount of silver and iron in the investigated samples.

In Vitro Cytotoxicity test by SRB assay method

Cell culture

Vero (Cercopithecus aethiops monkey kidney) and Hep-G2 (Human hepatocellular carcinoma) cell lines were cultured at 37 °C, 5% CO₂ and maintained in DMEM (Dulbecco's Modified Eagle Medium) included L-Glutamine, Sodium piruvat, NaHCO₃, NAA (Non-Essential Amino Acids), 1% PSF (Penicillin - Streptomycin sulfate - Fungizone), and 10% BCS (Bovine Calf Serum). Trypsin-EDTA was used for cell detachment and medium was changed every 2 days.

SRB assay

Cell survival in presence of Fe₃O₄@Ag@PAA were assessed by SRB assay. Cells were seeded in triplicate in 96-well flat-bottomed plates, followed by a treatment with different concentration of Fe₃O₄@Ag@PAA (5 - 100 ug/ml). Ellipticine (5 ug/ml) and DMSO (10%) were chosen as positive control and solvent control. After 3 - 48hrs of incubation at 37 °C and 5% CO₂, cells were fixed and stained with SRB solution before the OD (optical density) value was recorded by ELISA Plate Reader at wavelength of 495 - 515 nm. Inhibitory concentration IC₅₀ and graph were obtained by using GraphPad Prism 5 software. All data is presented as mean ± standard deviation (SD).

In Vitro MR/CT Imaging Studies

MRI Measurements

The MRI measurements were performed in an 1.5 T (Siemens MAGNETOM Avanto). The different amount of the Fe₃O₄@AgPAA HNPs were dispersed in 1 mL agarose aqueous solution and then loaded into the microtubes for MRI measurements. The final concentration (as Fe, mM) of tested materials were 0 (water), 0.01, 0.02, 0.03, 0.05, and 0.08, respectively.

Image acquisition was performed in the Spin Echo mode with following parameters: repetition time (TR) = 3000 ms, echo time (TE) = 10 - 200 ms, imaging matrix = 192×192 , slice thickness = 2 mm, field of view (FOV) = $2.0 \times 2.0 \text{ cm}^2$, and number of averages = 2. By fitting the reciprocal of T_2 relaxation time (s^{-1}) against the concentration of Fe in a line, the r_2 relativity values were derived from the slope of the formula representing the line. Measurements were performed (region of interest-ROIs, cm^2) on eFilm workstations (Merge Healthcare, Chicago, IL, USA).

CT Measurements

The $\text{Fe}_3\text{O}_4@ \text{AgPAA}$ NPs fluids at a concentration range of 0 - 0.06 M were poured into 1000 μL tubes and analysed using 128-Somatom Perspective CT scanner (Siemens, Germany) to examine the X-ray attenuation property of $\text{Fe}_3\text{O}_4@ \text{AgPAA}$ NPs. The CT scanning parameters were as follows: Source voltage 100kV, 80 mA, slice thickness 0.5 mm, field of view (FOV) x-y $278 \times 295 \text{ mm}^2$, matrix size 541×510 . Measurements were performed (region of interest-ROIs, cm^2) on eFilm workstations (Merge Healthcare, Chicago, IL, USA).

References

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