Synthesis of heterodimer radionuclide nanoparticles for magnetic resonance and single-photon emission computed tomography dual-modal imaging

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Experimental Section

Materials

All chemicals involved in this work were analytical grade and used without further purification. Iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), silver acetate and sodium oleate were purchased from Shanghai Chemical Industrial Co. Oleyl amine (OAm) and 1-Octadecene (ODE) were purchased from Sigma-Aldrich. Lipoic acid (LA) and N,N'-dicyclohexylcarbodiimide (DCC) were also purchased from Sigma-Aldrich. mPEG-NH_2 (5k) was purchased from biomatrik. Sodium bicarbonate, dichloromethane, triethylamine were all purchased from sinopharm chemical reagent Co. Ltd. $Na^{125}I$ was purchased from Chengdu Gaotong Isotope Co. Ltd. High Q water was used throughout the research.

Methods

Synthesis of iron oxide nanoparticles

In a typical procedure[1], iron-oleate complex was used as the precursor for the synthesis of iron oxide nanoparticles. 10 mmol $FeCl_3 \cdot 6H_2O$, 30 mmol sodium oleate, 20 ml ethanol, 15 ml deionized water and 35 ml hexane were mixed and refluxed at 70°C for 4 h, then the upper hexane solution containing iron-oleate complex was separated, and washed three times with deionized water. Hexane was evaporated in a rotary evaporator, yielding iron-oleate complex. To synthesis iron oxide nanoparticles, 10 mmol iron-oleate complex, 25 g 1-octadecene, 5 mmol sodium oleate were mixtured in three-necked bottle and then heated to 320 °C for 30 min under Ar atmosphere. The resulting black nanocrystal solution was cooled to room temperature, and 2-propanol was added to precipitate the magnetic nanoparticles. After centrifugation, nanoparticles were washed with hexane and ethanol three times, and then redispersed in toluene.

Synthesis of Fe_3O_4-Ag heterostructured nanoparticles.

40 mg Fe_3O_4 nanoparticles, 40 mg silver acetate, 1ml OAm, 20 ml toluene were mixed and heated to 60 °C for 2 h, then 20 mg silver acetate were added into the mixture and keep at 60 °C for another 10 h. A dark yellowish brown solid was collected by centrifugation at 6000 rpm, washed twice with hexane and ethanol, The final product was redispersed in hexane.

Synthesis of mPEG-LA

mPEG-LA polymer was synthesized following a previous protocol[2]. In brief, 500 mg of 5k mPEG-NH_2 were reacted with 45 mg lipoic acid (LA) (mPEG : LA molar ratio = 1:2) in 2 ml dichloromethane for one day in the presence of 10 mg of N,N'-dicyclohexylcarbodiimide (DCC, Sigma-Aldrich) and 6 μl triethylamine. The reaction solution was blown-dry by nitrogen, yielding a solid in the bottle to which 10 ml water was added. The insoluble solid was removed by filtration. After adjusting the solution pH to 8 with sodium bicarbonate (0.1M), the filtrate solution was extracted by dichloromethane for three times. After evaporating the organic solvent, the product was dissolved in water and then lyophilized. The final product was mPEG-LA.

Surface modification of Fe_3O_4-Ag heterostructured nanoparticles.

0.5 ml stock solution of Fe_3O_4-Ag heterostructured nanoparticles were precipitated by centrifugation and redispersed in hexane. Another solution of 10 mg mPEG-LA polymer in 2ml water was added. The mixture was then stirred for 2 h. Hexane was evaporated in a rotary evaporator. The resultant solution was filtered through a 0.22-μm syringe filter to remove large aggregates. PEG-Fe_3O_4-Ag heterostructured nanoparticles was centrifugated to remove coating polymers, re-dispersed in water, and stored under 4 °C for further use.

Radiolabeling of Fe_3O_4-Ag heterostructured nanoparticles.
$Na^{125}I$ (5 μL) was added into the Fe_3O_4-Ag heterostructured nanoparticles (2 mL, 1 mmoL Fe/L). The sample were protected from light with aluminum foil under rapid agitation with a vortex for 30 min. The solution was filtered through sterile membrane with 0.22 μm pore size to filter out the bacteria. Then excess 125I was removed by sterile membrane (MWCO-100 kDa).

Characterization
The Fe_3O_4-$Ag^{125}I$ heteromider nanoparticles were characterized by X-ray diffraction (XRD), transmission electron microscope (TEM), X-ray photoelectron spectroscopy (XPS). XRD was carried out using a X'Pert-Pro MPD X-ray diffractometer at 40 KV and 40 Ma with Cu Kα radiation. TEM (Tecnai G220, FEI, USA) was performed using a Gatan CCD794 camera operated at 200 kV. XPS data was obtained using a KRATOS Axis ultra-DLD X-ray photoelectron spectrometer with monochromatic Mg X-ray.

***In vitro* cytotoxicity assay by methylthiazoletetrazolium (MTT)**
The effect of Fe_3O_4-Ag heterodimer nanoparticles on the cell viability was carried out by the MTT assay. The HUVEC and Hela cells seeded in 96-well plates (1×10^4 cells per well) for 24 h, respectively. Afterwards a series of concentrations of Fe_3O_4-Ag nanoparticles were added into the two cell cultures. After 24 h incubation, 20 ml of MTT solution was mixed in each well for another 4 h. After that, dimethyl sulfoxide (DMSO) was added into the well. Finally, the cell viability was measured by a microplate reader (Model 680 Bio-RAD).

***In vitro* MRI phantom study**
The MR imaging of Fe_3O_4-Ag heterodimer nanoparticles in all samples were scanned using a 1.5 T clinical MRI scanner (GE Medical systems, Signa HDX) at room temperature. The T_2 values at different iron concentrations were measured by a manually drawn region-of-interest (ROI) after obtaining the T_2-weighted MR imaging.

***In vivo* SPECT of Kunming mice**
The study was approved by our institutional review board/local ethics committee, and the experiments followed the guidelines on experiments with animals. The Kunming mice (n=5) were first anesthetized with isoflurane. Fe_3O_4-$Ag^{125}I$ nanoparticle solution (120 μCi, 100 μL) was injected via tail vein. Equivalent amount of $Na^{125}I$ solution was injected intravenously into mice for comparison. *In vivo* SPECT were carried out on an IRIX3 SPECT scanner (PHILIPS, the Netherland). Planar scintigraphy was performed with the energy window being centered at 37 keV, and opened by ±30 %. Images were acquired with 1×10^5 counts on a 128×128 matrix equipped with a parallel-hole collimator for low-energy and high-sensitive imaging. SPECT scans were performed at 1 h after injection.

***In vivo* biodistribution study**
The mice were sacrificed and the major organs were collected and wet weighted after 1 h injection. The radioactivity organs was measured using γ-counter, and calibrated against a known aliquot of the injection and normalized with body weighted. The radioactivity uptake in the major organs was expressed as A%ID/g.

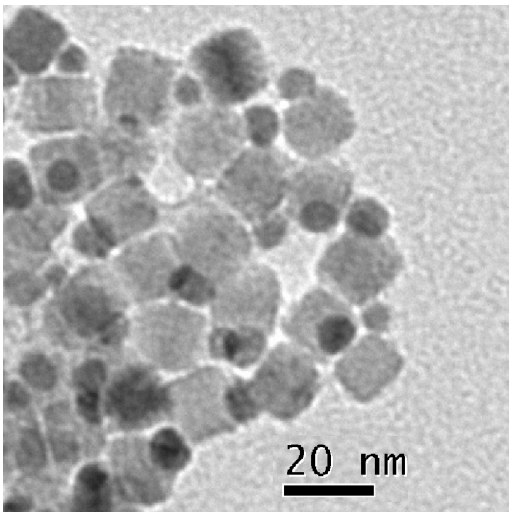

Figure S1. TEM image of PEG-Fe_3O_4-Ag in water.

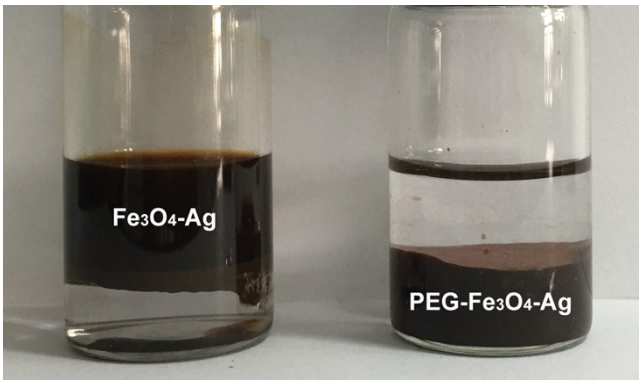

Figure S2. The optical image of PEG-Fe_3O_4-Ag nanoparticles.

Organ	A%ID/g
Blood	1.288±0.09
Heart	0.430±0.07
Liver	31.98±2.44
Spleen	41.87±4.41
Lungs	2.364±0.55
Pancreas	1.498±0.70
Kidney	1.393±0.36
Stomach	4.523±0.62
Sausage	0.860±0.12
Muscle	0.353±0.10
Bone	1.083±0.04
Brain	0.112±0.11
Thyroid	2.149±0.21

Table S1. Quantitative radioactive results of major organs for Fe_3O_4-Ag^{125}I nanoparticles uptake *in vivo*. Kunming mice (n=5) were intravenously injected with Fe_3O_4-Ag^{125}I nanoparticles (100 μL, 120 μCi) and the uptake was analyzed 1 h later after injection. Values were corrected for radiodecay and expressed as %ID/g along with the corresponding standard deviation.

Organ	A%ID/g
Blood	8.33±2.66
Heart	3.30±0.99
Liver	2.99±0.60
Spleen	5.16±0.82
Lungs	6.09±1.89
Pancreas	4.71±1.04
Kidney	5.92±1.48
Stomach	55.19±3.52
Sausage	5.05±1.57
Muscle	2.21±0.69
Bone	3.80±0.78
Brain	0.46±0.12
Thyroid	32.74±4.35

Table S2. Quantitative radioactive results of major organs for Fe_3O_4-Ag^{125}I nanoparticles uptake *in vivo*. Kunming mice (n=5) were intravenously injected with free ^{125}I (100μL, 120 μCi) and the uptake was analyzed 1 h later after injection. Values were corrected for radiodecay and expressed as %ID/g along with the corresponding standard deviation.

[1] J. Park, K. Hwang, G. Park, J. Noh, Y. Kim, H. Park, M. Hwang, T. Hyeon, *Nat Mater*, 2004, **3**, 891.

[2] C. Wang, L. Cheng, Z. Liu, *Biomaterials,* 2011, **32**, 1110.