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Microwave-assisted synthesis of magnetite nanoparticles for MR blood pool contrast agents

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ABSTRACT

Microwave-assisted polyol process was developed for the synthesis of magnetite nanoparticles with precisely controlled size, high crystallinity and high water solubility. The process is simple, time-saving and low energy-consuming due to the advantages of polyols and microwave irradiation combined. The crystal phases of the nanoparticles were determined by transmission electron microscopy, X-ray powder diffraction and Raman spectrum. The coating materials of the nanoparticles were analyzed by Fourier transformed infrared spectroscopy and thermal gravimetric analysis. Precise size tuning enables an easier way to adjust the relaxation properties of the magnetite nanoparticles. The colloid nanoparticles with high longitudinal relaxivity (r_1) and low ratio of transverse relaxivity (r_2) to r_1 have a potential application in magnetic resonance angiography.

1. Introduction

Magnetite nanoparticles (MNPs) have been widely used as contrast agents for magnetic resonance imaging [1–3]. Contrast agents work by shortening the relaxation time T_1 and T_2, which are described as longitudinal and transverse relaxation of excited protons, respectively. The efficiency of contrast agents is usually expressed as their relaxivities r_1 or r_2, respectively. T_1-weighted contrast agents (positive agents) mainly shorten T_1 while only have a moderate shortening on T_2, generating a bright image with very low ratio of r_2/r_1. On the other hand, T_2-weighted agents (negative agents) mainly shorten T_2, leading to a dark image with a high ratio of r_2/r_1. Several commercialized or clinical used iron oxide nanoparticles were designed with high r_1 relaxivity and low ratio of r_2/r_1 before they were used in high-resolution contrast-enhanced magnetic resonance angiography (MRA) [3–6]. High r_1 and low r_2/r_1 require that the MNPs have suitable core and hydrodynamic size, high water solubility, uniform particle size and high magnetization, which mainly depend on the development of the particle synthesis technology [1–3]. Co-precipitation method is widely used to fabricate MRI contrast agents [1,2,7] but it has some intrinsic drawbacks such as poor crystallinity or broad particle size distribution. High-temperature decomposition of iron precursors in nonpolar solvents produces nanoparticles with hydrophobic surfaces and complicated phase transfer into water is required before they are used for T_1 or T_2 contrast agents [8–11]. High-temperature decomposition of iron precursors in polar solvents is capable of producing nanoparticles with hydrophilic surface but which are more suitable for T_2 contrast agents because of high ratio of r_2/r_1 [12–18].

Heat transfer during the high-temperature method for the synthesis of MNPs by traditional methods is time consuming or very low energy efficiency, resulting in potentially inhomogeneous particles. This could be improved by microwave assisted chemistry, which has been widely applied in the polar liquid synthesis of nanoparticles with monodisperse size, well-controlled shape and high crystallinity [19–22]. Since microwave heating is not suitable for high temperature reactions in the nonpolar solutions, this technique is still not successfully introduced into the synthesis of high quality superparamagnetic Fe_3O_4 nanoparticles. Polyols as solvents have been used to prepare water soluble MNPs at high temperature by traditional heating methods. Recently, our group synthesized magnetite nanoclusters with tunable size between 40 and 300 nm by a polyol method with urea as a homogeneous precipitator [23]. Other groups also developed polyol methods heated by traditional ways to prepare MNPs [18,24–27]. Polyol methods offer great opportunities to merge microwave technique into the synthesis of MNPs towards direct use in biomedicine, especially for application in MRA. Based on this idea, we developed a simple and rapid microwave assisted polyol process to synthesize superparamagnetic Fe_3O_4 monodisperse nanoparticles especially suitable for MRA application.

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This process combines the advantages of microwave heating and polyols. The homogeneous volumetric heating leads to the MNPs with more uniform size due to the same nucleation and growth history.

2. Experimental section

2.1. Materials

Anhydrous iron trichloride ($FeCl_3$, $>97\%$, Sinopharm Chemical Reagent Co. Ltd.), diethylene glycol (DEG, $(HOCH_2CH_3)_2O$, 99%, Sigma–Aldrich), sodium hydroxide (NaOH, $>96\%$, Shanghai Lingfeng Chemical Reagent Co. Ltd.), poly acrylic acid (PAA, 50 wt%, solution in water, MW=5000, $d=1.18$, Acros) were used as received. Ultrafiltration membranes (Normal Molecular Weight Limited (NMWL)=300,000) and membrane filters of 0.22 and 0.1 μm were purchased from Millipore.

2.2. Preparation of Fe_3O_4 nanoparticles

The synthetic reactions were performed inside the microwave reactor with controllable program of focused microwave synthesis system (Discover SClass, CEM, USA). The synthetic reaction was modified according to literature [24]. In our process, Fe_3O_4 nanoparticles were produced by fast injection of 2.4 mL hot NaOH solution (50 mmol NaOH in 20 mL diethylene glycol (DEG) at 80 °C) into the iron precursor solution (1.2 mmol $FeCl_3$ in 9 mL DEG, kept at 220 °C for 5 min) in the presence of poly acrylic acid (PAA, 2.4 mmol) under magnetic stirring. After further heating for 10 min by microwave and quickly cooling to room temperature by high pressure air flow, the nanoparticles were precipitated by the mixture of ethanol and acetic ether. The final nanoparticles were harvested by subsequently washing with ethanol three times, and redispersed in water for further purification with millipore ultrafiltration device. The particle size can be easily tuned by adding different amount of NaOH solution. Total four samples were marked MNP-2.4, MNP-2.0, MNP-1.6 and MNP-1.2 with the amount of NaOH solution at 2.4, 2.0, 1.6 and 1.2 mL, respectively. All four samples were filtered first with a 0.22 μm and then a 0.1 μm membrane filter. Solid samples were prepared from colloid nanoparticles by freeze dryer (BETA 1-8, Christ, Germany).

2.3. Characterization

Transmission electron microscopy (TEM, JEOL 2010F) observation was used for observing the size and morphology of the MNPs. Hydrodynamic size was measured on high performance particle sizer (HPPS 5001, Malvern). X-ray diffraction (XRD) was recorded on Rigaku D/max 2550 VB/PC with CuKα radiation (1.54056 Å) at 40 kV, 200 mA to confirm the nanocrystal phase and particle size. Fourier transformed infrared spectroscopy (FTIR) was measured on a Perkin-Elmer spectrum 100. The mass fraction of magnetite nanoparticles powder was determined by a Mettler Toledo TGA/DSC 1/1600 thermogravimetric analyzer (heating nanoparticles powders from 30 to 1000 °C at 10 °C/min under a nitrogen flow). Magnetization parameters were measured on a Lakeshore 7400 vibration sample magnetometer (VSM). Iron concentrations were determined by inductively coupled plasma emission spectroscopy (ICP, ICA P6300, Thermo Fisher, USA). The T_1 and T_2 relaxation time was measured on NMR spectrometer (Minispec, mq60, Brucker, Germany) and the proton resonance frequency is 60 MHz, corresponding to the applied magnetic field $\mu_0 H_0=1.41$ T. The Carr–Purcell–Meiboom–Gill (CPMG) spin echo sequence was used in T_2 measurements and saturation recovery sequence was used in T_1 measurements.

2.4. In vitro cytotoxicity assay

Mouse NSC line (C17.2, Cell bank of Chinese Academy of Science) was maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (Sigma Co.), 5% horse serum (Gibco Co.), 1 mM glutamine, 100 U/mL penicillin and 100 ppm streptomycin. Cells were seeded in tissue culture flasks at 3×10^5 cells/mL and incubated in a standard humidified atmosphere at 37 °C with 5% CO_2. The cells were subcultured when they reached a density of 5×10^6 cells/mL or greater. The mitochondrial function of NSCs exposed to Fe_3O_4 nanoparticles was assessed using the cell counting kit-8 (CCK-8, Cell bank of Chinese Academy of Science). The cells were seeded into a 96-well plate at 3×10^3/well and seeded in DMEM at 37 °C and 5% CO_2 for 12 h. Then, the cells were incubated with Fe_3O_4 Nanoparticles with different particle concentrations (10, 25, 50, 75 and 100 μg/mL nanoparticles diluted in DMEM) for different time (3, 6, 12 and 24 h) at 37 °C under 5% CO_2. Thereafter, CCK-8 solution was added to each well. After the cells were incubated for an additional 1.5 h, the absorbance of each well at 450 nm was measured with a standard microplate reader (Varioskan flash, Thermo Scientific). Each sample was done in quadruplicates.

The relative mitochondrial activity (%) of cells exposed to nanoparticles was calculated by $[A]_{test}-[A]_{blank1}/[A]_{control}-[A]_{blank2}\times100$. $[A]_{test}$ was the absorbance of the sample containing cells, Nanoparticles and DMEM, $[A]_{blank1}$ was the absorbance of the sample containing Nanoparticles and DMEM, $[A]_{control}$ was the absorbance of the sample containing cells and DMEM, while $[A]_{blank2}$ was the absorbance of the sample only containing DMEM.

2.5. MR imaging

Animal experiments were approved by the animal care and used by the committee at the Shanghai Zhongshan Hospital, Fudan University. MR experiments were performed with three male rabbits (weight, 2.5–3.0 kg) on a 1.5 T MR scanner (Magnetom Avanto, Siemens AG, Erlangen, Germany) using a surface coil with 6 independent receiver channels. T_1-weighted images of coronal orientation were obtained from the 3D fast low angle shot (FLASH) sequence and the imaging parameters were as follows: TR/TE 2.8 ms/1.1 ms; field of view (FOV) 200×162 mm^2; spatial resolution $1\times1\times1$ mm^3 and scanning time of each phase 19 s. After pre-contrast image acquired for baseline measurement of intravascular signal, MNP-2.0 dispersed in saline was injected through ear and vein at a dose of 40 μmol Fe/kg body weight (b.w.) during 2–3 s followed by 3 mL saline flush. Postcontrast measurements were initiated simultaneously. The signal of abdominal aorta and inferior vena cava was measured at different phase and time–signal curves of each sample were illustrated.

3. Results and discussion

3.1. Size tuning and crystal phase determination

As shown in Fig. 1, the nanoparticles have uniform size of several nanometers without aggregation. The average sizes (d_w) of MNP-2.4, MNP-2.0, MNP-1.6 and MNP-1.2 counted from TEM images are 8.3, 6.9, 5.4 and 4.6 nm, respectively. The particle size increases when more amounts of NaOH solution added while keeping the other parameters fixed and the size between 3 and 10 nm can be easily and exactly controlled. As shown in Fig. 1