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Water-soluble superparamagnetic manganese ferrite nanoparticles for magnetic resonance imaging

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ABSTRACT

We report here a thermal decomposition approach to the synthesis of water-soluble superparamagnetic manganese ferrite ($MnFe_2O_4$) nanoparticles (NPs) for magnetic resonance (MR) imaging applications. In this approach, tetraethylene glycol was utilized as a coordination and stabilization agent, rendering the NPs water-soluble and stable. The formed NPs had a diameter of 7 nm with a narrow size distribution, and were superparamagnetic with a saturated magnetization (Ms) of 39 emu/g. *In vitro* cytotoxicity test revealed that the $MnFe_2O_4$ NPs were biocompatible at a particle concentration below 200 μg/mL. The transverse relaxivity of $MnFe_2O_4$ NPs in water and cells after incubation were determined to be 189.3 $mM^{-1}s^{-1}$ and 36.8 $mM^{-1}s^{-1}$ based on iron concentration, respectively. *In vivo* MR imaging studies in conjunction with inductively coupled plasma-atomic emission spectroscopy showed that the $MnFe_2O_4$ NPs were preferentially accumulated in liver after intravenous injection for 4 h. This suggests that the developed $MnFe_2O_4$ NPs can serve as a sensitive MR imaging contrast agent for liver imaging. By appropriately modifying or functionalizing the surface of the NPs, these particles may be used for MR detection of other diseases.

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1. Introduction

Magnetic resonance (MR) imaging is currently one of the most powerful *in vivo* imaging technologies due to its noninvasive property and multidimensional tomographic capabilities coupled with high spatial resolution [1–10]. However, the contrast difference between biological tissues is subtle [11]. With the help of efficient imaging agents, it is possible to provide the higher contrast and obtain the information-rich images for disease detection. In general, MR imaging contrast agents must have a strong effect to accelerate longitudinal relaxation (T_1) of water and exhibit bright or positive contrast where they are localized, or to accelerate the transverse relaxation (T_2) and produce dark or negative-contrast images.

Compared with conventional paramagnetic gadolinium complex-based contrast agents such as Magnevist, Prohance, and Dotarem, superparamagnetic nanoparticles (NPs) are emerging as the next generation magnetic probes for MR imaging because of their excellent magnetism and long circulating times [3]. During the past twenty years, magnetic iron oxide NPs have received considerable attention since it was firstly reported as a liver contrast agent in 1986 [12,13]. Since then, many researchers have developed novel nanoparticulate contrast agents including new magnetic NPs with improved contrasting ability, and surface-functionalized magnetic NPs with improved biocompatibility, targeting ability, and multifunctionality [14–16]. For instance, Cheon reported an innovative approach to developing magnetism-engineered iron oxide NPs with high and tunable nanomagnetism, and systematically elucidated the magnetic characteristics and the MR signal enhancement effect based on their magnetic spin, size and type, and applied for the ultra-sensitive detection of target biological molecules [17]. Shi and coworkers developed multifunctional dendrimer-modified iron oxide NPs that were used for specific MR imaging of cancer cells *in vitro* and *in vivo* [18–20].

As a potential candidate of contrast agents in MR imaging, the superparamagnetic manganese ferrite ($MnFe_2O_4$) NPs have been found to have a very high magnetization and large relaxivity owing

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to their large magnetic spin magnitude [17]. To synthesize high quality $MnFe_2O_4$ NPs, thermal decomposition has been considered as one of the most effective methods [21,22]. However, most of the NPs prepared by this approach are only soluble in organic solvents, thereby limiting their biological applications. In this case, sophisticated post-preparative processes have been devoted to making the particles water-soluble and biocompatible [15]. For example, Weller and coworkers developed three different approaches including ligand exchange of oleic acid using water-soluble polymer, coating of individual NPs with amphiphilic polymer, and embedding the NPs into lipid micelles to achieve the water solubility of the NPs [23]. Very recently, Ai et al. utilized amphiphilic diblock copolymer micelles to encapsulate manganese doped superparamagnetic iron oxide nanocrystals for the formation of clustered nanocomposites [24]. In general, a polymer coating can reduce the aggregation and improve the colloidal stability of the magnetic NPs for MR imaging applications. However, in some cases, the polymer shell could significantly increase the overall size of the NPs, leading to limited tissue distribution, penetration, and metabolic clearance of the particles [25]. Development of small molecule-coated magnetic NPs may be promising to attain the overall small size of the NPs. Up to now, only a few examples related to non-polymer-coated $MnFe_2O_4$ NPs have been reported. For instance, Bahadur successfully prepared lauric acid-coated nanoferrites (Fe_3O_4, $MnFe_2O_4$, and $CoFe_2O_4$) to evaluate their uses in hyperthermia treatment of cancer [26].

In this present study, in virtue of the advantage of the thermal decomposition method, we developed a facile synthetic route to prepare water-soluble superparamagnetic $MnFe_2O_4$ NPs directly *via* a one-pot approach [27,28]. Our strategy is to replace the commonly used nonpolar high-boiling-point solvents with water-soluble tetraethylene glycol (TEG) molecules (Fig. 1). The TEG solvent used can serve as a stabilizer to control the particle growth and to prevent the aggregation of particles in high temperature reaction medium. The morphology, structure, and magnetic property of the NPs were investigated by transmission electron microscopy (TEM), X-ray diffraction (XRD), fourier transform infrared spectroscopy (FTIR), superconducting quantum interference device (SQUID), and relaxivity measurements. The *in vitro* biocompatibility of the prepared $MnFe_2O_4$ NPs was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. To further explore the potential use of the magnetic NPs for MR imaging applications, the *in vitro* cellular uptake and the *in vivo* biodistribution of the particles in the KM mice were thoroughly investigated.

2. Experimental section

2.1. Materials

Tetraethylene glycol (TEG) was purchased from Aldrich. Iron chlorides, manganese chlorides, acetylacetone, and triethylamine were purchased from Sinopharm Chemical Reagent Co., Ltd. Iron (III) acetylacetonate and manganese acetylacetonate were synthesized according to the literature [29].

2.2. Preparation of $MnFe_2O_4$ NPs

A mixture of $Mn(acac)_2$ (0.25 g, 1.0 mmol) and $Fe(acac)_3$ (0.71 g, 2.0 mmol) dissolved in TEG (20 mL) was continuously heated to 110 °C under vigorous magnetic stirring and nitrogen atmosphere. After heating for 1 h, the solution was then heated to 210 °C and kept for 2 h at this temperature. The system was then refluxed at 295 °C for 1 h and finally the black–brown mixture was cooled down to room temperature by removing the heat source. Then, ethanol was added and the solution was centrifuged at 8000 rpm for 15 min to remove the solvent. A black precipitate was obtained and washed by ethanol for three times, which could be easily dispersed in water.

2.3. Characterization

XRD was performed using a Rigaku DMAX 2000 diffractometer equipped with Cu/K_α radiation at a scanning rate of 1°/min in the 2θ range from 20 to 80° ($\lambda = 0.15405$ nm) (40 kV, 40 mA). The size and morphology of the $MnFe_2O_4$ NPs were characterized using a JEOL JEM-2010 transmission electron microscope operating at 200 kV. The samples were dispersed in water, dropped onto carbon-coated copper grids, and air-dried before TEM measurements. FTIR spectra were collected using a Nicolet Avatar 370 spectrometer. The samples were pelletized with KBr before measurements. Hysteresis loop was measured with a Quantum Design SQUID MPMS XL-7 magnetometer. MR relaxometry were performed using an NMI20-Analyst NMR Analyzing & Imaging system (Shanghai Niumag Corporation). The instrumental parameters were set as follows: a 0.5 T magnet, point resolution = 156 × 156 μm, section thickness = 0.6 mm, TE = 60 ms, TR = 4000 ms, number of acquisitions = 1.

2.4. Cell culture

A human cervical carcinoma cell line (HeLa cells) was provided by Shanghai Institute for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS, China). Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS at 37 °C and 5% CO_2. Cells were plated in tissue culture flask under the 100% humidity.

2.5. In vitro cytotoxicity assay

In vitro cytotoxicity of the $MnFe_2O_4$ NPs was evaluated by MTT assay of HeLa cells. The cells were seeded into a 96-well cell culture plate at 5×10^4/well and cultured in DMEM with 10% FBS at 37 °C and 5% CO_2 for 24 h. Then, the cells were incubated with $MnFe_2O_4$ NPs with different particle concentrations (0, 10, 20, 60, 100, and 200 μg/mL NPs diluted in DMEM) for another 12 h at 37 °C under 5% CO_2. Thereafter, MTT (10 μL, 5 mg/mL) was added to each well and the plate was incubated for an additional 4 h at 37 °C under 5% CO_2. Then, 10% sodium dodecyl sulfate (SDS, 100 μL/well) was added and the plate was maintained at room temperature over night. The OD570 value of each well, with background subtraction at 690 nm, was measured by Tecan Infinite M200 monochromator-based microplate reader.

2.6. In vitro MR imaging and cellular uptake of $MnFe_2O_4$ NPs

Hela cells (5×10^6) were separately incubated with 150 μg/mL of $MnFe_2O_4$ NPs for different times at 37 °C in cell culture medium. The cells were then washed with PBS buffer three times and the cells (1×10^6 cells/mL) were suspended in PBS buffer before MR imaging.

The amount of iron uptake in the cells was determined using a high-resolution sector field inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (VARIAN TECHNOLOGIES CHINA). Data were acquired at medium resolution (4000) using rhodium (5 ppb) as an internal standard. Digestion of the cells was performed

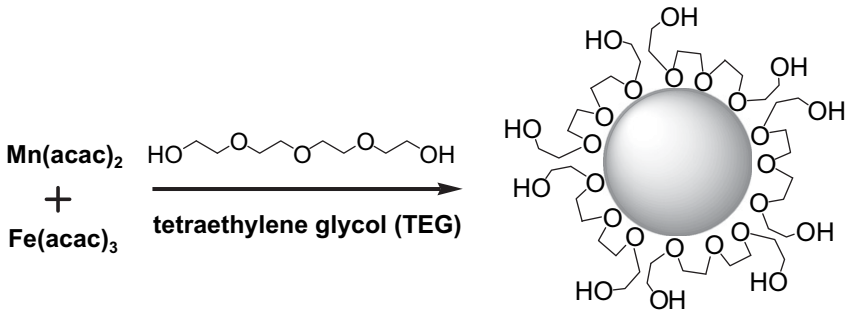

Fig. 1. Schematic illustration of the formation process of water-soluble $MnFe_2O_4$ nanoparticles.

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