Published in final edited form as:

J Phys Chem C Nanomater Interfaces. 2009 December 10; 113(49): 20855–20860. doi:10.1021/jp907216g.

Ultrasmall, Water-Soluble Magnetite Nanoparticles with High Relaxivity for Magnetic Resonance Imaging

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Abstract

Ultrasmall (3, 4, 5, and 6 nm), water-soluble Fe_3O_4 magnetic nanoparticles were synthesized in diethylene glycol (DEG) via a facile one-pot reaction. Hydrodynamic size and relaxation time measurements did not show particle aggregation when Fe_3O_4 nanoparticles were dispersed in phosphate buffered saline, fetal bovine serum, or calf bovine serum for 1 week. Furthermore, the new Fe_3O_4 nanoparticles tolerated high salt concentrations (1 M NaCl) and a wide pH range from 5 to 11. Surface modification of the nanoparticles with poly(ethylene glycol) bis(carboxymethyl) ether (HOOC-PEG-COOH, 600 g/mol) was accomplished through a ligand-exchange reaction. The effects of PEG modification on magnetization and relaxivity of the Fe_3O_4 nanoparticles were investigated, and the results indicate that the increase in transverse relaxivity after PEG modification may be due to the increased volume of slowly diffusing water surrounding each nanoparticle. In vitro experiments showed that the DEG- and PEG-coated Fe_3O_4 nanoparticles have little effect on NIH/3T3 cell viability.

1. Introduction

Magnetic nanoparticles have shown great potential in biomedical applications such as magnetic separation, magnetic resonance imaging (MRI), targeted drug delivery, and hyperthermia treatment of cancer.^{1–11} In particular, magnetic nanoparticles are excellent MR contrast agents for noninvasive cellular and molecular imaging.^{4–9} Magnetic nanoparticles composed of Fe_3O_4 have been extensively investigated due to their excellent magnetic properties and biocompatibility.^{4–7} Recently, high-temperature decomposition strategies have been developed to produce monodisperse and highly crystalline Fe_3O_4 nanoparticles, but they are soluble only in nonpolar solvents.^{12,13} Consequently, their biological applications are limited without extensive surface modifications.^{14–16} To avoid sophisticated phase transfer processes, a modified thermal decomposition process for

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Supporting Information Available: Scheme of synthesis and surface modification of the Fe_3O_4 nanoparticles, low-magnification TEM image of 6 nm Fe_3O_4 nanoparticles, colloidal stability tests in NaCl solutions and in several pH, T_2 relaxation rates ($1/T_2$) plotted against the Fe concentrations for DEG- Fe_3O_4 and PEG- Fe_3O_4 (3, 4, and 5 nm) at 1.41 T and 37 °C and table of D_H , r_2 , r_1 , and r_2/r_1 at 1.41 T and 37 °C of DEG- Fe_3O_4 and PEG- Fe_3O_4 . This material is available free of charge via the Internet at <http://pubs.acs.org>.

preparing water-soluble and biocompatible Fe₃O₄ nanoparticles via a one-pot reaction was developed.^{5,17,18}

The polyol method is a versatile chemical approach for the synthesis of water dispersible nanoparticles with well-defined shapes and controlled sizes.^{19–22} Polyol can act as a solvent to dissolve inorganic compounds due to their high dielectric constants, and offers a wide operating-temperature range for preparing inorganic nanoparticles. It can act as a reducing agent, control particle growth, and prevent interparticle aggregation.²³ Nanoparticles prepared through this process can be dispersed in aqueous media and other polar solvents since the surface of the nanoparticles is coated by hydrophilic polyol ligands in situ. In addition, the high reaction temperature favors nanoparticles with high crystallinity and monodispersity. A polyol process has been developed to produce 8 nm nonaggregated Fe₃O₄ nanoparticles.²⁰ Most recently, an approach was developed to synthesize water-dispersible Fe₃O₄ nanoparticles by using FeCl₃ as the iron source and diethylene glycol (DEG) as the reductant and solvent in the presence of CH₃COONa.²¹ However, the poor colloidal stability of these nanoparticles at neutral pH makes them far from optimal for biomedical applications.

Enhancing in vivo targeting specificity is a great challenge encountered with iron oxide MR contrast agents. Commonly used iron oxide nanoparticle contrast agents generate local contrast through nonspecific uptake by mononuclear phagocytes, and with a hydrodynamic size of over 50 nm, these particles remain primarily intravascular and are taken up by the reticuloendothelial system (RES),^{24,25} which severely undermines their targeting specificity. Smaller hydrodynamic sizes are desired to overcome these problems.^{24,26} As shown in Table 1, despite recent synthetic progress, it remains challenging to prepare monodisperse ultrasmall magnetite nanoparticles with high relaxivity and good colloidal stability under physiological conditions via a one-pot reaction.^{5,19–21,27–30}

Herein, we present a direct synthesis of monodisperse, water-soluble, 3–6 nm Fe₃O₄ magnetic nanoparticles in DEG via a one-pot reaction, as illustrated in Scheme S1 (Supporting Information). The Fe₃O₄ nanoparticles show good colloidal stability in phosphate buffered saline (PBS), fetal bovine serum (FBS), and calf bovine serum (CBS). Furthermore, the Fe₃O₄ nanoparticles are stable in up to 1 M NaCl solution and against various pH (5–11). Surface modification with poly(ethylene glycol) bis(carboxymethyl) ether (HOOC-PEG-COOH) was accomplished through a facile ligand-exchange reaction, resulting in up to 95% increase in transverse relaxivity (r_2 , at 1.41 T) that may be due to the increased volume of slowly diffusing water surrounding each nanoparticle. Cell viability assays showed that the DEG- and PEG-coated Fe₃O₄ nanoparticles have little effect on NIH/3T3 cell viability. Due to their monodispersity, colloidal stability and high r_2 , these Fe₃O₄ nanoparticles are promising candidates for a variety of MR imaging applications.

2. Experimental Section

Synthesis of Fe₃O₄ Nanoparticles

The synthesis of 3 nm Fe₃O₄ nanoparticles is as follows. Iron (III) acetylacetonate [Fe(acac)₃, 1.2 mmol, 99.9+%, Aldrich] and diethylene glycol (DEG, 20 mL, 99%, Sigma-

Aldrich) were mixed and purged with nitrogen. The reaction mixture was heated at 140 °C for 1 h followed by 2 h at 200 °C, producing a black, homogeneous, colloidal suspension. After cooling to room temperature, 100 mL ethanol/ether (v/v = 1:8) was added to the reaction solution and resulted in a black precipitation of magnetite nanoparticles that were separated by centrifugation. After washing with ethanol/ether three times, the precipitate was redispersed in water for further investigation. Fe₃O₄ nanoparticles (4 and 5 nm) were prepared by extending the reaction time at 200 °C to 3 and 6 h, respectively. For 6 nm Fe₃O₄ nanoparticles, 2.4 mmol Fe(acac)₃ and 20 mL DEG were mixed followed by heating at 140 °C for 1 h and 200 °C for 8 h.

Surface Modification of Fe₃O₄ Nanoparticles with HOOC-PEG-COOH

Three milliliters of 6 nm (3, 4, or 5 nm) DEG-Fe₃O₄ aqueous solution (0.43 mg Fe/mL) was mixed with 84 mg poly(ethylene glycol) bis(carboxymethyl) ether (HOOC-PEG-COOH, Aldrich, 600 g/mol) in 27 mL of 10 mM phosphate buffer (PB) at pH 6.4 and ultrasonicated in Branson ultrasonication bath for 30 min. The reaction mixture was stirred at room temperature for 12 h. Excess HOOC-PEG-COOH molecules were removed by size filtration three times using Millipore Amicon Ultra 30 000 centrifugal filters. Purified PEG-Fe₃O₄ was redispersed in 10 mM PB.

Cell Culture

NIH/3T3 cells (ATCC, Manassas, VA) were grown in phenol red free DMEM (modified to contain 4.5 mg/L glucose, 2 mM L-glutamine, 1 mM sodium pyruvate, and nonessential amino acids) supplemented with 10% CBS. All cells were grown in a humidified incubator at 37 °C and 5% CO₂. All flasks and multiwell plates were tissue culture treated and sterile. Prior to each experiment, NIH/3T3 cells were plated in 24-well tissue culture plates and allowed to grow to ~50% before addition of nanoparticle solutions.

Cell Viability Experiments

Six nanometer DEG-Fe₃O₄ or 6 nm PEG-Fe₃O₄ (in 10 mM PB) were diluted in the aforementioned cell culture media to specified iron concentrations and vortexed. NIH/3T3 cells were incubated with Fe₃O₄ nanoparticles at 37 °C and 5% CO₂ for 24 h followed by two rinses with 0.50 mL of DPBS. Cells were detached using 0.25% trypsin/EDTA followed by addition of media (resulting in a 1:1 v/v trypsin-cell suspension/media solution) to neutralize the trypsin. Cells were then counted and percent cell viability determined using a Guava EasyCyte Mini personal cell analyzer (Guava Technologies, Hayward, CA). Specifically, 20 µL of cells were diluted 10-fold with Guava ViaCount reagent up to a total volume of 200 µL. Cells were allowed to stain at room temperature for 5–20 min. Samples were vortexed and analyzed using the Guava ViaCount software module (1000 trials per run).

Characterizations

TEM images and selected area electron diffraction patterns were obtained on a Hitachi H-8100 operated at an accelerating voltage of 200 kV. HR-TEM images were obtained on a JEOL JEM-2100F FAST TEM operated at an accelerating voltage of 200 kV. Samples for TEM and HRTEM analysis were prepared by spreading a drop of the solution sample on

copper grids coated with a carbon film followed by evaporation under ambient conditions. XRD measurement was performed on a powder sample of the Fe_3O_4 nanoparticles using a Scintag XDS2000 goniometer. DLS measurements were performed with a Malvern Instruments Zetasizer Nano Series Nano-ZS with Dispersion Technology Software 5.03 (Worcestershire, U.K.). FTIR spectra were obtained on a Thermo Nicolet NEXUS 870 Fourier transform spectrometer (Thermo Scientific, Waltham, MA). Magnetic susceptibility measurements were carried using a Quantum Design MPMS5 SQUID magnetometer (San Diego, CA). MR phantom images were collected at two field strengths, 14.1 and 3 T. 14.1 T images were acquired on a General Electric/Bruker Omega 600WB 14.1 T imaging spectrometer at ambient temperature ($\sim 25^\circ\text{C}$). A T_2 -weighted image was acquired using a multislice multi echo pulse sequence ($\text{TR} = 4294.3$ ms, $\text{TE} = 101.8$ ms, 16 echoes). Images were acquired with field of view (FOV) = 2×2 cm², data matrix = 256×256 , slice thickness = 1 mm, and four signal averages. T_2 was measured with a static TR (1169.9 ms) and variable TE (10.2, 20.4, 30.5, 40.7, 50.9, 61.1, 71.3, 81.4, 91.6, 101.8, 112, 122.2 ms) values. Three T images were acquired on a Siemens TIM Trio clinical imaging system using a 35 mm diameter volume RF coil. A T_2 weighted image was acquired using a spin echo pulse sequence ($\text{TR} = 1500$ ms, $\text{TE} = 75$ ms). Sequence parameters were as follows. $\text{FOV} = 7.5 \times 10$ cm², data matrix = 96×128 , slice thickness = 2 mm, and three signal averages. Transverse and longitudinal relaxation times were also measured at 1.41 T (60 MHz) and 37°C on a Bruker mq60 NMR analyzer equipped with the Minispec V2.51 Rev.00/NT software (Billerica, MA). An inversion–recovery pulse sequence was used to measure the longitudinal relaxation times, while both Carr–Purcell–Meiboom–Gill (CPMG) and spin–echo pulse sequences were used to measure transverse relaxation times. Iron concentrations of each sample for SQUID, relaxivity and MRI were determined via inductively coupled plasma atomic emission spectroscopy (ICP-AES, Varian).

3. Results and Discussion

A polyol process was designed to synthesize ultrasmall, watersoluble Fe_3O_4 magnetic nanoparticles by using $\text{Fe}(\text{acac})_3$ as the iron precursor and DEG as the solvent, reducing agent, and stabilizer. Under the reductive atmosphere provided by DEG at higher temperature,^{31,32} $\text{Fe}(\text{III})$ is partially reduced to $\text{Fe}(\text{II})$, and Fe_3O_4 nanoparticles are formed. Typically, 6 ± 0.6 nm Fe_3O_4 nanoparticles were prepared by heating a mixture of 2.4 mmol $\text{Fe}(\text{acac})_3$ and 20 mL DEG at 140°C for 1 h followed by 8 h at 200°C . Fe_3O_4 nanoparticles (3, 4, and 5 nm) were obtained using a lower concentration of $\text{Fe}(\text{acac})_3$ and shorter reaction times at 200°C . Figure 1a–d shows representative TEM and HR-TEM images of 3, 4, 5, and 6 nm Fe_3O_4 nanoparticles. The low-magnification TEM image of 6 nm Fe_3O_4 nanoparticles (Figure S1, Supporting Information) clearly shows their good monodispersity and high dispersity. The histogram of particle size based on statistical results of 6 nm Fe_3O_4 nanoparticles is shown in Figure 1e. Ten percent relative standard deviation demonstrates the high monodispersity of the Fe_3O_4 nanoparticles prepared by this procedure. Selected area electron diffraction reveals that the nanoparticles are of high crystallinity (Figure 1f). The lattice spacings (calculated on the basis of the diffraction patterns) are in agreement with those of bulk magnetite (JCPDS 19-0629). The crystal structure was further determined by powder X-ray diffraction (XRD). As shown in Figure 2, the position and relative

intensity of all diffraction peaks of 6 nm Fe₃O₄ nanoparticles match well with those from the JCPDS card (19-0629) for magnetite. The average particle size calculated using Debye–Scherrer’s formula is 6.1 nm, which is quite close to the TEM results.

Colloidal stability under physiological conditions is one of the most important issues relating to the biological applications of nanomaterials. The Fe₃O₄ nanoparticles synthesized by this procedure can be readily and stably dispersed in aqueous media, including PBS, FBS, and CBS, without further surface modification. The colloidal stability of the Fe₃O₄ nanoparticles in PBS was investigated by measuring their hydrodynamic size (D_H) for 1 week. As shown in Figure 3, the D_H of 6 nm Fe₃O₄ nanoparticles was stable during the 1 week measurement period representing excellent particle stability. Since the presence of serum will affect the D_H of the Fe₃O₄ nanoparticles, the colloidal stability of the Fe₃O₄ nanoparticles in FBS and CBS was investigated by measuring the transverse relaxation times (T_2) over a period of 1 week. Figure 3 shows the temporal evolution of T_2 of 6 nm Fe₃O₄ nanoparticles in FBS and CBS. The T_2 of the Fe₃O₄ nanoparticles changed only slightly after 1 week, demonstrating good colloidal stability in FBS and CBS. To investigate the effects of pH and ionic strength on colloidal stability, Fe₃O₄ nanoparticles were exposed to 0–1 M NaCl and pH 3–11 solutions, as shown in Figure S2 (Supporting Information). The Fe₃O₄ nanoparticles were stable with salt concentrations as high as 1 M NaCl and tolerated a wide pH range from 5 to 11.

Surface modification of Fe₃O₄ nanoparticles is necessary to realize their biocompatibility and surface functionality.^{2,4,7,15,26} The relatively weak coordinating ability of DEG molecules makes it possible to modify the nanoparticle surface through a ligand-exchange reaction. Previous studies have shown that carboxylate groups provide excellent ligation for Fe₃O₄ nanoparticles.¹² In addition, PEG is a widely used biocompatible material for modifying magnetic nanoparticles due to its nontoxic, nonimmunogenic, and nonantigenic characteristics.^{4,5,16,18} Therefore, commercially available HOOC-PEG-COOH (600 g/mol) was chosen to modify the Fe₃O₄ nanoparticle surface. Typically, a mixture of DEG-Fe₃O₄ and HOOC-PEG-COOH molecules in 10 mM phosphate buffer at pH 6.4 was ultrasonicated in Branson ultrasonication bath for 30 min and stirred for 12 h under room temperature. Extensive size filtration by centrifugation was used to remove excess HOOC-PEG-COOH molecules.

The surface chemical structure of both DEG-Fe₃O₄ and PEG-Fe₃O₄ was characterized by Fourier-transform infrared (FTIR) spectroscopy. Figure 4 shows the spectra of DEG-Fe₃O₄, PEG-Fe₃O₄, DEG, and HOOC-PEG-COOH. The characteristic bands of DEG at 2936 (CH₂ asymmetric stretch), 2872 (CH₂ symmetric stretch), 1459 (CH₂ scissor), 1132 (C–O–C asymmetric stretch), and 1075 cm^{−1} (C–O–C symmetric stretch) appear in the spectrum of DEG-Fe₃O₄, indicating DEG is present on the Fe₃O₄ nanocrystal surface.^{33,34} The band at 1633 cm^{−1} has been shown in tri(ethylene glycol)-coated Fe₃O₄ nanoparticles²⁰ and might be ascribed to the byproducts produced in the reaction system under high temperature.²¹ After PEG modification, the C–O–C stretching band coalesced into one peak at 1106 cm^{−1}, instead of two peaks at 1132 and 1075 cm^{−1} in DEG-Fe₃O₄, which matches well with the spectrum of HOOC-PEG-COOH. This spectral change indicates that HOOC-PEG-COOH molecules have been modified to the Fe₃O₄ nanoparticle surface. In addition, IR spectra of

DEG-Fe₃O₄ and PEG-Fe₃O₄ exhibit characteristic peaks centered at 590 cm⁻¹ that are attributed to lattice absorption of the magnetite.^{27,35–37}

The presence of PEG on the nanoparticle surface was further confirmed by DLS. The D_H of 6 nm Fe₃O₄ nanoparticles increased from 10 to 20 nm following PEG modification. The D_H of 10 nm for 6 nm DEG-Fe₃O₄ is slightly larger than the superposition of 6 nm core and ~1.5 nm DEG coating since counterions and water form a diffuse layer which moves with the Fe₃O₄ nanoparticles.³⁸ This result reveals no particle aggregation in aqueous solutions. Similarly, the D_H of 6 nm PEG-Fe₃O₄ is 20 nm, slightly larger than the simple superposition of 6 nm core and ~10 nm PEG coating, indicating that some of the HOOC-PEG-COOH molecules on the Fe₃O₄ nanoparticle surface are coordinated with Fe₃O₄ nanoparticles with only one carboxyl group, and therefore, another carboxyl group can be used for conjugation with dyes or biomolecules. Similarly, PEG modification of 3, 4, and 5 nm Fe₃O₄ nanoparticles resulted in an increase in D_H : from 8 to 16, 9 to 17, and 9 to 19 nm, respectively.

Magnetic properties of these Fe₃O₄ nanoparticles were measured using a superconducting quantum interference device (SQUID). Magnetization vs applied magnetic field was measured at 250 K for each sample. As shown in Figure 5, both DEG-Fe₃O₄ and PEG-Fe₃O₄ exhibit superparamagnetic behavior without magnetic hysteresis and remanence. There are no differences in magnetization between DEG-Fe₃O₄ and PEG-Fe₃O₄, demonstrating that the Fe₃O₄ core is intact during the surface modification with HOOC-PEG-COOH. The saturation magnetizations of 6 nm DEG-Fe₃O₄ and 6 nm PEG-Fe₃O₄ are 71 emu/g Fe.

MR contrast agents are evaluated on the basis of their relaxivity or the change in the relaxation rates of water protons in the presence of the agent per unit concentration. MR contrast agents with higher relaxivities can be detected at lower concentrations, improving sensitivity. Figure 6A shows a T_2 -weighted MR image of 6 nm DEG-Fe₃O₄ and 6 nm PEG-Fe₃O₄ at different Fe concentrations (14.1 T, 25 °C). Increasing the concentration of DEG-Fe₃O₄ or PEG-Fe₃O₄ led to a large change in signal intensity in the T_2 -weighted MR image. PEG-Fe₃O₄ shows a much higher transverse relaxation rate than DEG-Fe₃O₄. The r_2 of the Fe₃O₄ nanoparticles was determined by calculating the slope of a plot of $1/T_2$ versus Fe concentration. Figure 6B displays the r_2 s of 6 nm DEG-Fe₃O₄ and 6 nm PEG-Fe₃O₄ (14.1 T, 25 °C). PEG-Fe₃O₄ has a 116% larger r_2 (119 mM⁻¹ s⁻¹) than DEG-Fe₃O₄ (55 mM⁻¹ s⁻¹), as indicated by the steeper slope in Figure 6B. The MR signal enhancement properties of these Fe₃O₄ nanoparticles at clinical field strength (3 and 1.41 T) were further investigated. As shown in Figure 6C, a T_2 -weighted image at 3 T (25 °C) shows the same trend. T_2 s of 6 nm DEG-Fe₃O₄ and 6 nm PEG-Fe₃O₄ at different Fe concentrations were measured on a 1.41 T (60 MHz) relaxometer at 37 °C. As shown in Figure 6D, the r_2 of 6 nm DEG-Fe₃O₄ and 6 nm PEG-Fe₃O₄ are 61 and 119 mM⁻¹ s⁻¹, respectively. Therefore, the r_2 of 6 nm Fe₃O₄ nanoparticles increases 95% after PEG modification. These relaxivity results are comparable to those measured at 14.1 T (25 °C). It is worth noting that the r_2 (119 mM⁻¹ s⁻¹, 1.41 T) of 6 nm PEG-Fe₃O₄ is higher than 8 nm tri(ethylene glycol)-coated Fe₃O₄ nanoparticles (82.68 mM⁻¹ s⁻¹, 1.5 T) even though the relaxivity of Fe₃O₄ nanoparticles decreases accompanying with the decrease of Fe₃O₄ core size (Table 1).^{4,20}

Similarly, PEG modification of 3, 4, and 5 nm Fe₃O₄ nanoparticles resulted in r_2 increases from 29 to 47, 42 to 69, and 48 to 86 mM⁻¹ s⁻¹, respectively (Supporting Information, Figures S3–5). Compared to a 95% r_2 increase, longitudinal relaxivity (r_1) weakly decreased (20%) after PEG modification. Therefore, r_2/r_1 , an important parameter to estimate the efficiency of T_2 contrast agents, increased significantly (80%) after PEG modification (Supporting Information, Table S1).

The basis of the T_2 MR contrast effect is that a magnetic dipole moment is induced in superparamagnetic nanoparticles under an applied magnetic field. When water molecules diffuse into the periphery (outer-sphere) of the induced dipole moment, the magnetic relaxation processes of the water protons are perturbed and the spin–spin relaxation time (T_2) is shortened.⁴ This results in a signal decrease relative to other water protons, and appears as a dark region on a T_2 -weighted MR image. The effects of particle size and composition on relaxivity have been extensively investigated.⁴ SQUID results showed that there are no differences in magnetization between DEG-Fe₃O₄ and PEG-Fe₃O₄; therefore, the r_2 increase after PEG modification is probably induced by the surface modification. The effects of surfactants on relaxivity are not well understood.^{23,38–40} The effect of the nanoparticle magnetic moment on water is stronger within the coating layer because of its closer proximity to the Fe₃O₄ core than the surrounding bulk water. It has been demonstrated that the diffusion coefficient of water within a PEG coating is slower than in bulk water.⁴¹

On the basis of this finding, computational simulation results showed that as the thickness of the PEG coating is increased, r_2 increases due to the increased volume of slowly diffusing water surrounding each nanoparticle.³⁸ PEG-Fe₃O₄ forms a larger hydrophilic layer than DEG-Fe₃O₄, as has been shown by DLS. As a result of the larger hydrophilic layer, the r_2 of the PEG-Fe₃O₄ is much higher than that of DEG-Fe₃O₄, which fits well with the simulation results.

Due to their monodispersity, excellent colloidal stability and high r_2 , DEG-Fe₃O₄ and PEG-Fe₃O₄ are promising candidates for a variety of MR imaging applications. Initial experiments were conducted to determine the effects of these nanoparticles on NIH/3T3 cell viability. Specifically, cells were incubated with 8.75, 17.5, 35.0, or 70.0 mg Fe L⁻¹ of 6 nm DEG-Fe₃O₄ or 6 nm PEG-Fe₃O₄ (6 nm nanoparticles were chosen due to their higher r_2 values) for 24 h. After nanoparticle incubation, cells were washed twice with DPBS and detached using trypsin. A fraction of the cell suspension was used for cell counting and viability analysis via flow cytometry. NIH/3T3 cell viability was >95% after 24 h incubation with 6 nm DEG-Fe₃O₄ or 6 nm PEG-Fe₃O₄ (Figure 7), demonstrating that these Fe₃O₄ nanoparticles have little effect on NIH/3T3 cell viability. Further experiments using other cell lines and more sensitive molecular approaches to assess toxicity and labeling efficiency are under investigation.

4. Conclusions

In conclusion, monodisperse, ultrasmall, water-soluble Fe₃O₄ nanoparticles of varying size were synthesized in DEG via a one-pot reaction. The Fe₃O₄ nanoparticles show excellent

colloidal stability in PBS, FBS, and CBS. Furthermore, the Fe₃O₄ nanoparticles tolerated high salt concentrations (1 M NaCl) and a wide pH range from 5 to 11. PEG modification on the surface of Fe₃O₄ nanoparticles via a ligand-exchange reaction provided a facile surface modification method for DEG-stabilized nanoparticles and results in up to 95% increase in r_2 . DLS, SQUID, and relaxivity results indicate that the r_2 increase might be due to the increased volume of slowly diffusing water surrounding each nanoparticle. In vitro experiments showed that the DEG- and PEG-coated Fe₃O₄ nanoparticles have little effect on NIH/3T3 cell viability, indicating that the Fe₃O₄ nanoparticles have potential as high-performance MR contrast agents for in vitro and in vivo MR imaging experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Prof. Vinayak P. Dravid and Dr. George H. Chan for helpful discussions. This work was supported by the National Cancer Institute Center for Cancer Nanotechnology Excellence initiative at Northwestern University Award No. U54CA119341 and the National Institute of Biomedical Imaging and Bioengineering Award No. 1R01 EB005866-01. We acknowledge NUANCE and IMSERC at Northwestern University, and the Center for Advanced Magnetic Resonance Imaging at Northwestern University's Feinberg School of Medicine for use of their instrumentation.

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Figure 1. TEM (inset is HR-TEM) images of (a) 3, (b) 4, (c) 5, and (d) 6 nm Fe_3O_4 nanoparticles; (e) histogram and (f) electron diffraction patterns of 6 nm Fe_3O_4 nanoparticles. The scale bars are 20 nm in TEM images and 2 nm in HR-TEM images.

Figure 2.

Powder X-ray diffractogram of 6 nm Fe_3O_4 nanoparticles. All peaks are well matched to the JCPDS card (19-0629) data for magnetite (bars).

Figure 3.
Temporal evolution of hydrodynamic size of 6 nm Fe_3O_4 nanoparticles in PBS and T_2 of 6 nm Fe_3O_4 nanoparticles in FBS and CBS.

Figure 4.

FTIR spectra of (a) 6 nm DEG-Fe₃O₄ and (b) 6 nm PEG-Fe₃O₄. Spectra c and d were recorded from the parent (c) DEG and HOOC-PEG-COOH (600 g/mol).

Figure 5. Magnetization (M) loops for 6 nm DEG- Fe_3O_4 and 6 nm PEG- Fe_3O_4 measured at 250 K.

Figure 6.

(A–B) High field strength MRI analysis: (A) T_2 -weighted MR image of aqueous solutions of 6 nm PEG-Fe₃O₄ and 6 nm DEG-Fe₃O₄ at different Fe concentrations at 25 °C and 14.1 T; (B) T_2 relaxation rates ($1/T_2$) of 6 nm PEG-Fe₃O₄ and 6 nm DEG-Fe₃O₄ at different Fe concentrations, measured at 25 °C and 14.1 T. (C–D) Clinical field strength MRI analysis: (C) T_2 -weighted MR image of aqueous solutions of 6 nm PEG-Fe₃O₄ and 6 nm DEG-Fe₃O₄ at different Fe concentrations, measured at 25 °C and 3.0 T; (D) T_2 relaxation rates of 6 nm PEG-Fe₃O₄ and 6 nm DEG-Fe₃O₄ at different Fe concentrations, measured at 37 °C and 1.41 T.

Figure 7.
Cell viability of NIH/3T3 cells after 24 h incubation with 6 nm DEG-Fe₃O₄ or 6 nm PEG-Fe₃O₄ at various Fe incubation concentrations.

TABLE 1
Properties of Iron Oxide Nanoparticles Reported in Relative Literature and Our Work

synthesis method	composition	size (nm)	colloidal stability	r_2 (mM ⁻¹ s ⁻¹)	refs
thermal decomposition	Fe ₃ O ₄	10.9	H ₂ O and PBS	–	5
polyol	Fe ₂ O ₃	30–200	diethylene glycol	–	19
polyol	Fe ₃ O ₄	3–11	H ₂ O (pH 5 or pH 9)	–	21
polyol	Fe ₃ O ₄	8	H ₂ O and PBS	8 nm: 82.68 (1.5 T)	20
thermal decomposition	Fe ₃ O ₄	4–12	H ₂ O and PBS (phase transfer needed)	6 nm: 106 (1.5 T)	27
coprecipitation	5 mol % Eu USMIO	6.6	H ₂ O	6.6 nm: 33.9 (0.47 T)	28
coprecipitation	MION	4.6	–	4.6 nm: 34.8	29
coprecipitation	USPIO	4.9	0.9% normal saline	4.9 nm: 53.1 (0.47 T)	30
polyol	Fe ₃ O ₄	3–6	H ₂ O, PBS and serum (0–1 M NaCl, pH 5–11)	5 nm: 86.6 nm: 119 (1.41 T)	our work