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The effect of coating of Fe₃O₄/silica core/shell nanoparticles on T₂ relaxation time at 9.4 T

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Abstract. Magnetic Resonance Imaging (MRI) that provides superior soft tissue contrast is commonly used for diagnosis of many diseases. However specificity of MRI cancer diagnosis can be further increased by application of target contrast agents comprising superparamagnetic nanoparticles (NPs) synthesised with biological objects, which deliver the contrast to the specific cancer cells. These superparamagnetic NPs shorten T₂ relaxation time thus change contrast to noise ratio for tumor tissues. Therefore the impact of Fe₃O₄ size and silica coating for Fe₃O₄/silica core/shell superparamagnetic nanoparticles (NPs) on T₂ relaxation time was studied at 9.4 T. The magnetic resonance imaging (MRI) studies were performed using homogenous agar solution of NPs. Naked Fe₃O₄NPs with a mean core diameter of 10.0 ± 1.3 (mean ± SD), 15.0 ± 2.5 and 20.0 ± 0.9 nm were analyzed. Silica coated Fe₃O₄ NPs with core size of 10.0 ± 1.3 nm and the shell thickness of 16.7 ± 1.8, 25.3 ± 2.7 and 33.9 ± 4.0 nm were also investigated. The T₂ values of agar solutions with different NPs were calculated using a single slice multi echo method and single exponential fitting of the echo train. The measurements showed linear correlation between T₂ and Fe₃O₄ core diameter as well as shell thickness. Silica coating, while improving functionalization and potentially reducing toxicity of NPs, decreases the impact of the magnetic core on T₂, thus decreasing MRI contrast efficacy.

1 Introduction

Recent advances in nanotechnology have allowed reproducible production of superparamagnetic nanoparticles (NPs) with controlled physical and chemical attributes including their size, shape and surface properties. The high magnetic moments of NPs and their small size allows efficient manipulation thus enabling synthesis with targeting moieties such as antibodies [1], viruses [2], liposomes [3] or macrophages [4] for increased specificity. Moreover NPs exhibit a considerable potential as contrast agents for Magnetic Resonance Imaging (MRI) as they significantly decrease T₂ relaxation time of surrounding tissues, thus making them detectable with T₂-weighted MRI. Superparamagnetic NPs found recent application in the diagnosis of cancer [5,6], detection of inflammatory and degenerative disorders [7–9], lymph node metastases [9]

and unstable atherosclerotic plaque [10]. NPs have also been used for drug delivery [11–15] and treatment monitoring [4,16,17].

To increase specificity of the NPs many different biomolecules, such as single or monoclonal antibodies may be attached to them. Such targeted contrast agents used in MRI are the most common bio-application of NPs. An ideal MRI contrast agent based on superparamagnetic NPs should not only provide a strong decrease of T₂ within the target tissue [18–21] but must be biocompatible. While the NPs' core effects relaxivity (1/T₂), the coating (shell) is added to prevent aggregation and sedimentation of the particles in aqueous solution, provide chemical stability, allow functionalization and reduce potential toxicity [23].

The NPs most commonly used in MRI are iron oxide-based contrast agents [19,21–23]. As T₂ relaxivity increases with NPs concentration, a greater number of nanoparticles per unit volume would have a greater effect

on T_2 . However known toxicity of iron oxides prevents their clinical use in high concentrations [24,25]. Iron based nanostructures have been widely investigated due to their high magnetic moments. The experiments showed that the size and the coating affect their magnetic properties [21]. Physiochemical behaviour as well as the capacity to be internalized by bio-molecules is also core size dependant [19,26,27]. To enable functionalization and reduce toxicity the iron oxide core has been coated with gold [28] and silica [29,30]. Fe_3O_4 and $\text{Fe}_3\text{O}_4/\text{Au}$ NPs have been synthesized with a polymeric shell, such as poly(ethylene glycol) (PEG) [31] or dextran [32] due to their high binding affinity. Silica shell was found to improve reactivity of NPs, decrease toxicity and enable much higher surface concentration of amino groups [30], thus improving functionalization.

The relaxivity theory of solvent water proton in solutions of superparamagnetic NPs has not been fully developed yet for currently used in experimental MRI magnetic fields (0.2 T to 11.7 T). However relaxivity, and therefore potential as an MRI contrast agents, of some iron oxide based NPs have been tested [33] at relatively low frequencies (up to 4.7 T), but their behaviour at high magnetic fields and the influence of the shell have not been studied in detail. At the same time molecular MRI is currently being applied in animal models at high magnetic field (>7 T) to obtain maximum image resolution [34]. Therefore, studies of the impact of NPs architecture on relaxivity at high magnetic field are warranted. While the experiments and theory explaining the relationship between the concentration of the paramagnetics and relaxation times are well known (e.g. [35,36]), the measurements of the influence of the size of NPs, including their core and shell on T_2 relaxivity, are lacking. Therefore we investigated the relationship between the core and coating size of silica coated ($\text{Fe}_3\text{O}_4/\text{SiO}_2$) NPs and water proton T_2 at 9.4 T. Furthermore we also tested the quality of $\text{Fe}_3\text{O}_4/\text{SiO}_2$ production using T_2 as a measure of its reproducibility.

2 Experimental

2.1 Synthesis and characterization of Fe_3O_4 and $\text{Fe}_3\text{O}_4/\text{silica}$ NPs

Oleic acid (OA, 90%), 1-hexaneol anhydrous (99%), octyl ether (98%), ammonia solution (NH_4OH , 28–30 wt.% in water), Triton X-100, hexane (95%), cyclohexane (99.5%) and Tetraethoxysilane (TEOS, 99.999%) were purchased from Sigma-Aldrich Inc. Iron pentacarbonyl (99.5%) was purchased from Strem Chemicals, Inc. (Newburyport, MA).

The Fe_3O_4 NPs with various particle diameters of 10, 15, and 20 nm were synthesized based on a well-known process [37] which uses 10 nm Fe_3O_4 NPs as seeds for the subsequent silica coating. The core/shell $\text{Fe}_3\text{O}_4/\text{silica}$ NPs were synthesized by hydrolyzing TEOS in a water-in-oil microemulsion that consists of 0.5 mg Fe_3O_4 NPs, 1.77 g triton X-100, 1.6 ml 1-hexaneol anhydrous and 7.5 ml cyclohexane. The mixture was stirred for about 1 h, and

subsequently 0.5 ml of ~6% ammonia solution was added. After another 1 h, 25 μl TEOS (for the silica shells of 16.5 nm) or 25 μl + 25 μl TEOS (for the silica shells of 25 nm) or 25 μl + 25 μl + 25 μl TEOS (for the silica shells of 34 nm) were added, and allowed to react for 24 h between each interval (“+” indicates a reaction time interval of 24 h). The resultant products were separated by centrifugation at 9000 rpm, washed with ethanol, and then the procedure centrifugation/wash was repeated three times, and dried under vacuum, or directly dispersed in de-ionized water for characterizations. Transmission electron microscopy (TEM) samples were prepared by depositing hexane solution of the NPs on carbon-coated copper grids. The images were taken using Hitachi S-4700 with an accelerated voltage of 30 kV.

2.2 Sample preparation for MR measurements

For MRI, NPs were suspended in 1% agar solution to ensure homogenous distribution of the contrast agents. To prepare the solution, 1 g agarose was mixed in 100 ml deionized H_2O . The solution was boiled for homogeneity. Plastic 15 ml tubes containing water solutions of NPs were filled up to 10 ml with agar. The final NPs concentration was 0.03 mg Fe per 10 ml of agar for each tested NP. The solution was vortexed and transferred to 4 mm diameter NMR tube for MR measurements.

2.3 MRI ex vivo measurements

A 9.4 T/21 cm horizontal bore magnet (MagneX, UK) with a Biospec console (Bruker, Germany) was used. The volume radiofrequency coil (3 cm diam $\times$ 3 cm length) was applied for signal transition and reception. T_2 weighted MR images of the samples were acquired. Axial slices were positioned within the sample. A multi-slice, multi-echo sequence was used with the following parameters: repetition time (TR) = 7500 ms, 128 echoes, echo time (TE) = 4 ms; field of view (FOV) = 3×3 cm, matrix size 128×128 and slice thickness of 5 mm. T_2 values of the samples were measured using a single exponential fitting of the echo train from regions of interest (ROI).

3 Results and discussion

3.1 Structures and morphologies of nanoparticles

Figures 1a–1c show TEM images of synthesized Fe_3O_4 NPs with the mean particle diameters of 10.0 ± 1.3 , 15 ± 2.5 and 20.0 ± 0.9 nm respectively. The particles are spherical in shape with narrow size distributions and form self-assemble super-lattices. Figures 2a–2c show TEM images of $\text{Fe}_3\text{O}_4/\text{silica}$ NPs with the mean core size of 10.0 ± 1.3 nm and the mean total diameters of 43.4 ± 4.8 , 60.6 ± 5.8 and 77.8 ± 6.0 nm. The increased thickness of the silica shell improved dispersion of $\text{Fe}_3\text{O}_4/\text{silica}$ in

Fig. 1. TEM images of naked Fe_3O_4 nanoparticles with diameters of (a) 10.0 ± 1.3 nm, (b) 15.0 ± 2.5 nm and (c) 20.0 ± 0.9 nm.

aqueous solution, indicating that non-magnetic interactions between neighbour particles strongly depend on the steric hindrance of the shells. The synthesis process allowed production of consistently uniform structure of NPs and consistent distribution of the size of NPs in each sample. This controlled process resulted in reproducible MR measurements.

3.2 MR properties of nanoparticles

Three different sizes of naked Fe_3O_4 core NPs were examined. For each NP relaxation time T_2 was measured (Fig. 3). A linear relation ($R^2 = 0.929$) between T_2 and the core size was found. T_2 decreased from 59.0 ± 0.5 ms to 29.6 ± 0.5 ms for the Fe_3O_4 core size 10.0 ± 1.3 and 20.0 ± 0.9 nm respectively.

Three sizes of $\text{Fe}_3\text{O}_4/\text{SiO}_2$ core/shell NPs, 43.4 ± 4.8 nm, 60.6 ± 5.8 nm and 77.8 ± 5.9 nm diameter,

corresponding to shell thickness of 16.7 ± 1.8 , 25.3 ± 2.7 and 33.9 ± 4.0 nm were measured. The Fe_3O_4 core size was 10.0 ± 1.3 nm for each NP. A linear correlation ($R^2 = 0.975$) between the total core/shell NP size, and T_2 was observed (Fig. 4). T_2 decreased linearly from 82 to 60 ms (± 0.5 ms) for NPs with 43.4 ± 4.8 nm and 77.8 ± 5.9 nm shell/core diameter respectively. T_2 measurements of the tested NPs were compared with naked Fe_3O_4 of 10.0 ± 1.3 nm diameter ($T_2 = 55$ ms).

To illustrate the effect of the composition of NPs on MRI, T_2 -weighted MR images of tubes filled with agar solutions obtained with the 8th echo (TR/TE = 2000/32 ms) were also collected. Figure 5 shows MR images of agar tubes filled with naked Fe_3O_4 NP of (Fig. 5a) 20 ± 0.9 , (Fig. 5b) 15 ± 2.5 and (Fig. 5c) 10 ± 1.3 nm diameter respectively. Figure 6 shows MR images of agar tubes filled with $\text{Fe}_3\text{O}_4/\text{silica}$ NPs with Fe_3O_4 core of 10.0 ± 1.3 nm and the total diameters of core and shell NP 43.4 ± 4.8 , 60.6 ± 5.8 and 77.8 ± 6.0 nm. Both sets of images in Figures 5 and 6 were collected with the same pulse parameters. The agar tubes filled with naked and covered with a shell NPs provided different image intensity.

4 Discussion

Superparamagnetic NPs have proven beneficial contrast agents for in vivo molecular MRI. When selecting a nanoparticle for a specific application, its chemical composition, magnetic properties, biocompatibility, surface functionalization, size, and clearance from the organism have to be considered. One of the challenges facing molecular MRI is the development of NPs with strong magnetic properties, a surface that can be functionalized and minimal toxicity. Therefore this study analyzed the impact of the composition of $\text{Fe}_3\text{O}_4/\text{silica}$ core/shell NPs on T_2 relaxation time and thus their potential applications for molecular MRI. The influence of both the diameter of the naked Fe_3O_4 core as well as the thickness of the silica shell was examined.

Although superparamagnetic NPs have been of interest for few last decades the mechanisms of their impact on T_1 and T_2 relaxation times are not fully understood yet. As the Hamiltonian of NPs solution includes the exchange interaction, the Zeeman coupling and the anisotropy energy, relaxation times are a complex function of NP size, Larmour frequencies of the solute electronic and solvent proton magnetic moments, temperature, concentration and diffusion constant of solvent and solute [38]. Most authors refer to the outer sphere theory presented by Ayant et al. [39], who described dipolar interactions of protons of benzenic solutions for low magnetic fields. The sphere theory was extended up to 80 MHz for 5 to 50 nm diameter NPs by incorporating progressive magnetic saturation of solute NPs [23,38]. The authors stipulated that T_2 in solutions of magnetic NPs is reverse proportional to the square of the particles size. This theory was further improved by Roch et al. [21] by including anisotropy energy and showed a weaker relationship between T_2 and NP size

Fig. 2. (Color online) TEM images of the silica coated Fe_3O_4 nanoparticles and the histograms. TEM images and the corresponding histograms show the distribution of the sizes of the nanoparticles. The shown averaged particle diameter includes 10 nm Fe_3O_4 core and silica shell. The core/shell NPs of (a) 43.4 ± 4.8 nm, (b) 60.6 ± 5.8 nm and (c) 77.8 ± 5.9 nm diameters and their corresponding distributions are shown in (e)–(g), respectively. The mean particle diameters were obtained using dynamic light scattering measurement of 200 particles.

Fig. 3. Agar T_2 in the presence of naked Fe_3O_4 nanoparticles with diameters of 10.0 ± 1.3 , 15.0 ± 2.5 and 20.0 ± 0.9 nm.

for the proton Larmour frequency above 100 MHz and NP size diameter larger than 5 nm. The same results were reported by Chen et al. [40]. However the measurements of T_1 and T_2 of 50–150 nm core iron oxide NPs solutions at 0.47 T [41] showed no simple correlation between T_2 and the particle size. In our experiments, at 400 MHz (9.4 T) and the Fe_3O_4 core sizes of 10 to 20 nm, we found

Fig. 4. Agar T_2 in the presence of Fe_3O_4 /silica core/shell nanoparticles with core diameter of 10.0 ± 1.3 nm and shell thickness of 16.7 ± 1.8 , 25.3 ± 2.7 and 33.9 ± 4.0 nm.

a linear relationship between T_2 and the core size. However it should be noted that this relationship may be limited to the above boundaries. This relationship may be also caused by dispersion of sizes, that strongly impact T_2 time.

The presented observations indicate that the larger core size resulted in a greater decrease in T_2 . In principle, this faster T_2 relaxivity for larger iron core NPs is caused

(a)

(b)

(c)

Fig. 5. T_2 -weighted MR images (Spin Echo, TR/TE = 2000/32 ms) of agar tubes filled with naked Fe_3O_4 nanoparticles of (a) 20 ± 0.9 , (b) 15 ± 2.5 and (c) 10 ± 1.3 nm diameter, respectively.

by their larger magnetic moments [21]. The larger core has larger magnetic moment, thus greater distortion and fluctuation of the local magnetic field that causes faster spin dephasing and in a consequence shorter T_2 . However as stated above, the theoretical explanation includes complex mechanisms.

The results also showed that the thinner silica shell reduced T_2 relaxivity. While coating decreases T_2 efficacy of the naked core, it reduces toxicity. This toxicity was found to be dose and concentration dependent and significantly reduced by coatings, such as PEG, dextran, PVP [42] or silica [43]. Furthermore the nontoxic silica shell improves biocompatibility and enables NP functionalization [39]. The NP sell may also increase cellular uptake thus improve tissue MR contrast when compared to naked NP [44]. While the full theory of core/shell solutions is yet to be

(a)

(b)

(c)

Fig. 6. T_2 weighted MR images (Spin Echo, TR/TE = 2000/32 ms) of agar tubes filled with Fe_3O_4 /silica nanoparticles with Fe_3O_4 core of the mean size of 10.0 ± 1.3 nm and the total diameters of (a) 43.4 ± 4.8 , (b) 60.6 ± 5.8 and (c) 77.8 ± 6.0 nm, respectively.

developed, we stipulate that the non-magnetic silica shell introduces a shielding effect resulting in smaller magnetic field distortion, thus slower spin dephasing. Therefore T_2 in the presence of the coated NPs is longer than for naked NPs.

Based on the results we conclude that it may be possible to manipulate the composition of the iron oxide/silica shell NPs to produce a biocompatible MRI contrast agent that provides sufficient T_2 contrast and enables functionalization.

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