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Magnetic resonance imaging contrast of iron oxide nanoparticles developed for hyperthermia is dominated by iron content

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Abstract

Purpose—Magnetic iron oxide nanoparticles (MNPs) are used as contrast agents for magnetic resonance imaging (MRI) and hyperthermia for cancer treatment. The relationship between MRI signal intensity and cellular iron concentration for many new formulations, particularly MNPs having magnetic properties designed for heating in hyperthermia, is lacking. In this study, we examine the correlation between MRI T₂ relaxation time and iron content in cancer cells loaded with various MNP formulations.

Materials and methods—Human prostate carcinoma DU-145 cells were loaded with starch-coated bionised nanoferrite (BNF), iron oxide (Nanomag[®] D-SPIO), Feridex[™], and dextran-coated Johns Hopkins University (JHU) particles at a target concentration of 50 pg Fe/cell using poly-D-lysine transfection reagent. T₂-weighted MRI of serial dilutions of these labelled cells was performed at 9.4 T and iron content quantification was performed using inductively coupled plasma mass spectrometry (ICP-MS). Clonogenic assay was used to characterise cytotoxicity.

Results—No cytotoxicity was observed at twice the target intracellular iron concentration (~100 pg Fe/cell). ICP-MS revealed highest iron uptake efficiency with BNF and JHU particles, followed by Feridex and Nanomag-D-SPIO, respectively. Imaging data showed a linear

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Declaration of interest

Robert Ivkov is an inventor on several issued and pending patents that disclose nanoparticle formulations for imaging and hyperthermia. All patents on which Robert Ivkov is an inventor are assigned to either Johns Hopkins University or Aduro Biotech, LLC. All other authors report no conflicts of interest.

correlation between increased intracellular iron concentration and decreased T_2 times, with no apparent correlation among MNP magnetic properties.

Conclusions—This study demonstrates that for the range of nanoparticle concentrations internalised by cancer cells the signal intensity of T_2 -weighted MRI correlates closely with absolute iron concentration associated with the cells. This correlation may benefit applications for cell-based cancer imaging and therapy including nanoparticle-mediated drug delivery and hyperthermia.

Keywords

Magnetic iron oxide nanoparticles; magnetic nanoparticle hyperthermia; magnetic resonance imaging; cancer

Introduction

Magnetic iron oxide-based nanoparticles (MNPs) continue to generate significant interest for their utility in numerous biomedical applications [1–5]. Central to the appeal of iron oxide MNPs is the unique combination of inherent biocompatibility, and responsiveness to magnetic fields that give rise to multiple applications [1]. When suitably formulated, the magnetic properties of MNPs can be adjusted for use with static magnetic fields to provide contrast for magnetic resonance imaging (MRI) [1,3]. Use of MNPs with gradient magnetic fields extends to high-throughput cell sorting applications, and magnetic vectorisation for targeted drug delivery [4,5]. When exposed to an alternating magnetic field (AMF), MNPs having appropriate magnetic anisotropy can generate heat [1,2,6,7]. The amount of heat generated by MNPs under AMF can be modulated by adjusting the magneto-structural properties of MNPs [7]. Magnetic nanoparticle-mediated heat therapy or hyperthermia is of particular interest for targeted and image-guided therapy of various diseases including cancer.

Magnetic nanoparticle (or magnetic fluid) hyperthermia was first proposed by Gilchrist in 1957 to treat metastatic lymph nodes in recurrent breast cancer by heating the ferrofluid infused into the draining lymph nodes with AMF [8]. Since this first example, efforts have been devoted to developing clinically relevant AMF devices and nanoparticle constructs to enable clinical applications for cancer hyperthermia [1,2,9–12]. In addition, molecular targeting approaches have been explored to enable remote and non-invasive therapy for cancer following systemic (intravenous) delivery [13]. Despite this considerable effort, only one application has been successfully translated into clinical trials for cancer hyperthermia of prostate and brain (glioblastoma multiforme) [14–16].

The paucity of this type of clinically viable technology may, in part be due to the historical lack of nanoparticle formulations that provide sufficient heat at clinically relevant nanoparticle concentrations and AMF frequency and strength [1,6,7]. It has been established that the interactions of AMF with conductive tissues induce eddy currents, which deposit power causing Joule heating of tissues non-selectively in the absence of MNPs [17–21]. The extent of power deposition in tissue, or specific absorption rate (SAR, measured as W/kg tissue) scales with the cross-section of exposed tissue (eddy current path), and AMF

frequency and amplitude [17,22]. Thus, for a given volume of a patient exposed to the AMF, both frequency and amplitude are constrained to ensure patient safety. This requirement places a significant burden on MNP performance, requiring that the specific loss power (SLP) normalised to the mass has to be high at low frequency (~100–200 kHz) and for amplitudes <20 kA/m [6,22].

Early work with magnetic iron oxide nanoparticles was dominated largely by a desire to produce superparamagnetic nanoparticles (SPIOs) as MRI contrast agents, because it was believed that superparamagnetism ensured colloid stability [2]. Stabilised superparamagnetic constructs were desired for MRI contrast, largely because of their inherently high T2/T2* relaxivity and straightforward surface modification chemistry that would enable functionalised MNPs with targeting molecules and/or use them as carrier platforms [23–26]. For hyperthermia, however, superparamagnetism is undesirable because superparamagnetic materials exhibit no hysteresis (this is by definition, hence the terminology ‘paramagnetism’) with alternating magnetic fields yielding no work and thus no heating [7]. Recent efforts have been directed to synthesise magnetic nanoparticle formulations containing iron oxide nanocrystalline compositions that exhibit enhanced magnetic anisotropy through modified crystalline properties, controlled aggregation of crystals to form a collective magnetic state, or some combination [27–32].

Grüttner et al. [28] described synthesis of nanoparticles in which nanocrystalline shape was controlled during crystal growth using high-pressure homogenisation to form anisotropic structures. These crystals were aggregated under high-energy conditions leading to controlled aggregates forming nanoparticle cores comprising 5–9 parallelepipeds that exhibit a collective magnetic state leading to particles exhibiting significant uniaxial anisotropy and thus enhanced heating with AMFs [29–31]. Interestingly, once coated with polysaccharides (i.e. dextran or starch) or surfactant (i.e. citrate) the MNPs form a stable suspension in water or phosphate-buffered saline (PBS) through either steric or ionic mechanisms. This demonstrates that superparamagnetism is not a pre-requisite requirement to ensure colloid stability [28–31].

More recently, Hedayati et al. [29] have described methods and particle synthesis based on high-gravity controlled precipitation and subsequent hydrothermal processing to produce MNP constructs having reduced uniaxial anisotropy while still retaining significant magnetic properties at saturation to enable heating at clinically relevant magnetic field conditions [7,29]. MNP constructs designed for heating, as described above are generally not characterised for their MR imaging contrast, and conspicuously absent are comparisons with formulations designed for MRI contrast.

Non-invasive imaging techniques such as MRI are commonly utilised clinical tools for the diagnosis of many types of cancer. Image contrast is most commonly improved using contrast agents such as gadolinium-based complexes. It is, however, important to recognise that MNPs are inherently MR contrast agents that are uniquely suited to be theranostic agents, i.e. therapeutic and diagnostic, hence ‘theranostic’, because of their multifunctional capability when coupled with magnetic fields. This versatility results from their efficient contrast mechanisms (both negative and positive) that can be exploited, and the influence a

local particle distribution can have within a voxel on MRI contrast [33–35]. When MNPs are internalised in cells, the T_1 effect is strongly reduced due indirectly to the switch from a dispersed state (freely suspended colloid) to an aggregated state (sequestered in endosomes or other organelles within the cell) [36]. MNP aggregation thus modifies susceptibility contrast and water access to the iron core, thereby reducing the T_1 effect. Additionally, T_2 and T_2^* relaxivity effects are modulated by a diffusion-mediated contrast mechanism that depends upon the size of the MNPs or MNP clusters [37,38].

Several MNP constructs and formulations have been studied in clinical trials for multiple indications [39], and some have gained regulatory approval such as Feridex I.V.[®] (Advanced Magnetix, Cambridge, MA) [39,40] and Resovist[®] (Bayer Schering Pharma Pharmaceutical, Pittsburgh, PA) [41] for detection and characterisation of liver lesions. MNPs have also been used to visualise liver, spleen, bone marrow, lymph nodes, and renal flow due to non-specific uptake of the reticuloendothelial system (RES) in these organs [4]. Indeed, current and past clinical applications of imaging contrast are based and rely upon the non-specific uptake or phagocytic processes by macrophages and other components of the innate immune system. Future applications may be based upon molecular targeting of the nanoparticles for direct cancer cell detection. Regardless of mechanism or route by which the nanoparticles become associated with the target cell – SPIOs make possible MR imaging of cells when sufficient nanoparticle concentration is associated with the cell. Stated another way, it is established that MRI contrast depends principally upon cell-associated Fe concentration when SPIOs are used as the contrast agent [35–39]. Consequently, the efficacy of particles designed for imaging (i.e. SPIOs) is largely determined by *physicochemical* properties (e.g. size, surface coating, surface hydrophobicity, surface charge) that in turn affect their *physiochemical* properties (e.g. toxicity, stability and aggregation, protein adsorption) [1,4,39,42]. Thus considerable attention has been directed to surface modifications using various coatings, targeting ligands, and other biologically active molecules to assist with selective tissue accumulation [43,44]. Most iron oxide nanoparticle formulations developed as contrast agents comprise some biopolymer (polysaccharide such as dextran or carboxydextran) coating of microparticles or nanoparticles of magnetite/maghemite (Fe_3O_4/Fe_2O_3). The magnetic properties of these compounds have been thoroughly documented [26,39,41,43,44].

Thus we may ask the question whether MNP formulations designed for hyperthermia, i.e. enhanced anisotropy, will exhibit different or enhanced contrast properties than the SPIOs designed for use as contrast agents only. Recent reports of characterisation studies suggest that the magnetic properties of MNPs can influence their MRI contrast properties [32,45]. In this work we aim to characterise and compare the MRI contrast with heating properties of several MNP formulations designed for hyperthermia or T_2 -weighted contrast. The particles studied comprise Feridex I.V., an approved (SPIO) contrast agent that does not heat at clinically relevant AMF conditions [6,40]; BNF (Micromod Partikeltechnologie, Rostock, Germany) that heats very well at high amplitude AMF [6,46]; Nanomag-D-SPIO (Micromod), a predominantly superparamagnetic formulation that produces modest heat [6,47,48]; and, JHU MNPs [29]. The latter are citrate-stabilised MNPs produced by high-gravity controlled precipitation with hydrothermal ageing as previously described. All

particles studied produced measurable amplitude-dependent heat when exposed to alternating magnetic fields having frequency 140–160 kHz. Feridex produced measurable but negligible amplitude-dependent heating that has no clinical relevance at field conditions tested [6]. Other magnetic nanoparticle constructs are available, and it may be desirable to perform a comprehensive future study. The set chosen for the current study, however, presents a focused and representative sampling of MNPs for which structural and heating properties have been previously measured, and which have demonstrated significant variations of amplitude-dependent SLP at our chosen AMF conditions [6,29]. The MNPs were loaded into human prostate cancer cells (DU-145) which were then suspended in agarose gel for T_2 -weighted contrast measurements. All data were normalised for total sample iron content measured by inductively coupled plasma mass spectrometry (ICP-MS). T_2 -weighted contrast correlated with measured total iron (MNP) concentration among the samples and was essentially independent of magnetic properties among MNPs as determined by their heating properties.

Materials and methods

For clarity, a simplified schematic of the experimental design is illustrated in Figure 1. The specific steps are described in detail following this summary. Human prostate carcinoma DU-145 cells were cultured and exposed to four iron oxide nanoparticle formulations, each having distinct magnetic properties. Standard cell transfection reagent, a synthetic amino acid poly-D-lysine, was used to modify the surface charge of the particles to aid cellular internalisation, following previously described methods [49]. After exposure for 24 h, cells were washed and pelleted to remove any excess particles that had not been internalised. Iron concentration of supernatant was not measured. After re-suspending the cells in PBS, serially diluted aliquots of cells were immobilised in agarose gel for T_2/T_2^* MR relaxivity measurement and one aliquot from each sample was used for iron quantification to co-register the result with the obtained relaxation times. A sample of nanoparticle-loaded cells was also seeded for clonogenic survival assay and stained for ferric iron using Perls' (Prussian blue) reaction.

Magnetic iron oxide nanoparticles (MNPs)

Four aqueous iron oxide magnetic nanoparticle formulations – starch-coated BNF MNPs, Nanomag-D-SPIO (both from Micromod), Feridex I.V., and dextran-coated JHU (NanoMaterials Technology, Singapore). MNPs were obtained and characterised (Table I). The hydrodynamic particle diameter of each particle type was determined using dynamic light scattering (DLS) with a Zetasizer Nano ZS-90 (Malvern Instruments, Malvern, UK). Physical and magnetic properties of the particles have been previously reported in several publications [6,28–31,40,41,46–49]. SLP of each formulation over a range of field amplitudes is illustrated in Figure 2, providing a surrogate comparison of differing total anisotropy energy relevant for hyperthermia at 155 kHz used in this study.

Nanoparticle loading

Human prostate carcinoma DU-145 cells (ATCC HTB-81, American Type Culture Collection, Manassas, VA, USA) were cultured in flasks with GIBCO® Roswell Park

Memorial Institute (RPMI)-1640 medium containing 2mM L-glutamine and 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA). Aliquots of 75 μg Fe/mL of each particle type and 1 μg /mL poly-D-lysine (PDL, Sigma-Aldrich, St. Louis, MO) were added to the cell culture media exposing cells for 24 h at 37°C and 5% CO₂ [49,50]. Then each flask was washed three times with Dulbecco's PBS to remove any remaining free particles. Trypsin EDTA (0.25%) was added to detach the cells which were then re-suspended in RPMI for counting (with a Nexcelom Bioscience Cellometer Auto T4, Lawrence, MA).

Clonogenic survival assay

Cells were loaded at twice the target iron concentration, 150 μg /mL and incubated for 24 h. Samples were diluted and plated in triplicate in 100-mm culture dishes. After 14 days, the plates were stained with a crystal violet solution in 50% methanol. Colonies were counted and the survival fraction was determined relative to control unlabelled cell samples.

Intracellular iron quantification

An aliquot of each sample collected was set aside for iron quantification, using methods previously described [49]. Briefly, aliquots reserved for iron quantification were centrifuged, supernatant removed, and the cell pellets were suspended in nitric acid (HNO₃) for digestion in a MARS5 Xpress microwave (CEM, Matthews, NC). Cells were digested using a two-stage ramp to temperature method and the cellular digests were diluted for analysis in an Agilent 7500ce inductively coupled plasma mass spectrometer (Agilent Technologies, Santa Clara, CA). Total iron content of each cell sample was calculated based on an eight point calibration curve, blank correction, and the recovery of a standard reference material, SRM 2709a San Joaquin Soil (National Institute of Standards and Technology, Gaithersburg, MD). Total iron content per cell was calculated based on known cell number. Unlabelled controls were included to account for the endogenous iron present in cancer cells.

MR imaging

An aliquot of each sample was collected and set aside for MR imaging. Each aliquot was centrifuged and the cells were suspended in PBS and embedded in 3% agarose gel (1:10 dilution) in 200 μm micro-centrifuge tube. Up to 10 tubes were positioned in a plastic holder in a 40-mm diameter volume RF coil and imaged on a 9.4 T horizontal bore AvanceBiospec system (Bruker, Billerica, MA) at room temperature using a standard multi-slice spin echo imaging pulse sequence. Acquisition parameters used in the experiments were: repetition time TR = 4,000 ms, four spin echo times of TE = 7, 14, 21, and 28 ms, coronal slices with a slice thickness of 0.5 mm. Quantitative T₂ maps were reconstructed from the imaging data using a fitting routine developed with IDL programming environment (Exelis, McLean, VA). The final analysis consisted of selecting an appropriate region of interest (ROI) and determining the mean T₂ and standard deviation for the ROI using ImageJ software (<http://rsbweb.nih.gov/ij>). T₂* weighted gradient echo MR images were also obtained but were not used for quantitative analysis due to the very rapid signal decay.

Perls' (Prussian blue) reaction

Perls' reagent was used to detect ferric iron and ferritin. DU-145 cells were seeded on glass coverslips in flasks and incubated with identical particle concentrations as detailed above. The coverslips were removed, washed with PBS and fixed with 3% formaldehyde at room temperature. Fresh Prussian blue solution prepared from a 1:1 mixture of 4% potassium hexacyano ferrate (II) w/v in PBS and 4% HCl was added. Coverslips were then washed with PBS and incubated with nuclear fast red dye (diluted 1:5 in PBS), washed again and mounted.

Results

Experimental design and nanoparticle properties

In this study, our aim was to compare the T_2/T_2^* relaxivity and MRI contrast properties of several MNP formulations developed for different applications: Feridex as an MRI contrast agent, Nanomag D-SPIO for biomedical applications such as cell sorting, and magnetic nanoparticle BNF and JHU for hyperthermia. The physicochemical and magnetic properties of these four representative iron oxide MNPs have been well described and differ significantly as measured by characterisation of their heating rates and SLP values measured at a frequency of 155 ± 10 kHz (Table I and Figure 2). Feridex SLP values, which are measurable but negligible at this frequency, have been previously reported and those values are shown in Figure 2 for comparison [6]. Nanomag-D-SPIO MNPs have intermediate SLP, which is at least 100-fold above those of Feridex. Both JHU and BNF MNPs have the highest SLP of the panel of the nanoparticles used in the study, exceeding Feridex by a factor of 400 to 500. Although maximum SLP measured from JHU particles is similar to that observed with BNF particles, the field amplitude (H) dependence appears exponential and qualitatively similar to that of the Nanomag-D-SPIOs. Uniquely, the BNF particles display a strong 'sigmoidal' SLP with field at this frequency, consistent with previously reported results [6]. The MNPs used in this study have comparable chemical composition (magnetite/maghemite) and similar hydrodynamic diameter (~ 100 nm), but varying iron oxide crystal size and structure, surface coating and surface charge [6,28,29–31,39,40,46–48]. Thus, Feridex and Nanomag D-SPIO are composed of iron oxide nanocrystals embedded into a dextran matrix, whereas BNF and JHU nanoparticles have a single multicrystalline iron oxide core coated with a layer of biocompatible polymer or surfactant. Thus, these MNPs provide a convenient sample set to determine whether the cell-internalised MNPs having different magnetic properties has potential to affect their MRI contrast properties.

Treatment with a cell transfection reagent, poly-D-lysine, provides an established method to modify the surface physicochemical properties of the nanoparticles enabling maximal uptake by cells [49,50]. Even with this modification, differences occur among particles, necessitating ICP-MS quantification of total Fe content to appropriately normalise data for comparison. Further, serial dilutions of each prepared MNP cell stock were measured to provide a robust data set for comparison.

Nanoparticle loading

Representative images of DU-145 cells incubated with nanoparticle formulations and stained with Prussian blue are shown in Figure 3. Nanoparticles appear to be internalised by the cells, with little to no membrane-bound or free MNPs. BNF and JHU MNP formulations appear to have dense intracellular particle distribution, while the Feridex and D-SPIO MNP formulations appear to have much less dense distribution for similar iron concentration.

Clonogenic survival assays were conducted to determine the toxicity of internalised MNPs. To determine the level of toxicity to cells, survival fractions of DU-145 cells loaded at twice the target iron concentration (~ 100 pg/cell) were performed. No significant concentration-dependent toxicity relative to control samples was observed (Figure 4).

Samples loaded with nanoparticle formulations and assessed for iron content with ICP-MS showed a maximum iron concentration in BNF particle loaded cells, indicating this formulation had the highest iron loading efficiency. Decreased loading efficiency was noted with the JHU, Feridex, and D-SPIO formulations, respectively, in part due to the lower iron oxide content per nanoparticle (see Table I). Cells in each group were incubated with the same loading concentration of iron, and control cells were incubated in the medium without MNPs (Figure 5).

Transverse relaxation rates ($R_2 = 1/T_2$) of MNP loaded DU-145 cells demonstrated a linear increase with increased cell numbers (Figure 6A). BNF loaded samples, having the highest loading efficiency, corresponded to the shortest T_2 times and thus increased transverse relaxation rates; whereas Nanomag-D-SPIO loaded samples with the lowest loading efficiency, had the longest T_2 for all cell numbers. When normalised against total Fe concentration (per sample), MR imaging data demonstrated nearly linear increasing R_2 with increasing Fe concentration (Figure 6B). Interestingly, comparable R_2 values were detected for a given Fe concentration for all MNP preparations studied. Nanomag-D-SPIO MNPs generated slightly reduced R_2 values, possibly due to systematic errors in the determination of low iron concentrations in these samples. R_2^* relaxation rate was very short in all measured samples and signal intensities detected in the labelled cell pellets were too low for quantitative analysis at $TE = 1$ ms. Therefore, no quantitative T_2^* data are presented.

Discussion

MR imaging is uniquely suited for *in vivo* visualisation of iron oxide magnetic nanoparticles in target organs because the iron oxide crystal generates strong local inhomogeneities of magnetic field that can be easily detected as hypo-intense areas in T_2 or T_2^* weighted images [23]. SPIOs have also been extensively used in cell tracking MR experiments in which iron-oxide labelled cells are implanted and imaged longitudinally *in vivo* using high resolution MRI [45]. First-in-human studies using endogenous human cells labelled *in vitro* with SPIOs have been recently reported for peripheral blood mononuclear cells [51] and autologous mesenchymal stem cells [52]. This method provides high sensitivity of detection, and a single cell labelled with a single micron-sized magnetic microsphere can be detected [53,54].

Given the considerable potential for biomedical applications in cell sorting and tracking, imaging and hyperthermia, various MNP constructs and formulations have been developed with increasing frequency [54]. Single-purpose MNPs, i.e. superparamagnetic constructs developed only for MRI contrast present a well-studied but limited (and limiting) subset of these materials. For SPIOs, it has been well established that their utility for MRI contrast depends primarily upon cell- or tissue-associated concentration thereby placing a significant burden on their physicochemical properties, which to a large extent, controls the interaction of these particles with biological systems [54].

The more recent recognition that SPIO constructs may fail to realise the full potential of MNPs (i.e. theranostic applications) has motivated development of synthesis techniques to produce MNPs having enhanced magnetic and crystalline anisotropies. These enhanced anisotropies increase the responsiveness of MNPs to magnetic fields, particularly linearly time-varying (or alternating) magnetic fields (AMFs). The focus on these constructs has been directed toward their heating properties (via magnetic hysteresis losses), and less toward their MRI contrast capability. Recent reports suggesting that some of these novel MNP compounds may provide enhanced MRI contrast thus raise the question that established models describing MNP contrast capabilities may necessitate a closer examination [32].

To test this notion we have selected a representative set of MNPs that span both SPIO and high anisotropy samples to determine whether magnetic properties affect the MRI signal, i.e. T_2 relaxivity. Of the chosen samples, three are commercially available and have been extensively characterised. All MNPs chosen for this study have been characterised for their heating properties, a surrogate measure of their anisotropy, at our chosen AMF frequency and amplitude conditions. Other nanoparticle constructs are available, yet this group was selected because extensive structural data and heating data provided a convenient sample set for initial comparative study [6,29,39,40]. Heat generated by MNPs when exposed to AMFs is the result of loss mechanisms that depend upon the frequency and amplitude of the AMF, and upon the total anisotropy energy of the MNPs [6,7]. Measurements of both heating rate and (mass normalised) amplitude-dependent SLP show that each of the particles has a unique combination of both total heating capability and loss power as a function of AMF amplitude. Thus, the set of MNPs selected for this study is appropriate to test the notion whether magnetic properties are potentially capable to modulate or enhance MRI contrast.

Given the dependence of hysteresis on total anisotropy energy [7], measurements of SLP extending through magnetisation saturation (fixed frequency) of particles can provide a convenient relative measure of this energy. Magnetisation saturation of nanoparticles can differ significantly from that of the corresponding bulk material because of the heightened influence of anisotropies and surface effects, which make up a much larger proportion of the sample [6,7]. Typical values of saturation magnetisation for iron oxide nanoparticles (~100 nm) can vary between 0.1 T to 0.2 T [1,6,7,9–11]. SLP measurements potentially provide a qualitative indication of the nature or extent of inter- or intra-particle dipole–dipole interactions [6,7]. In particular, it is useful to note the dependence of SLP on field amplitude, i.e. $SLP(H)$ as the shape or nature of this curve can distinguish between ‘hard’ or ‘soft’ ferrite behaviour, suggesting extent of dipole–dipole interactions that are influenced

by interactions with the external field (H) as recently described [55]. One possible explanation is that a sigmoidal $SLP(H)$ suggests a ‘hard’ magnetic (ferrite) structure, likely due to strong dipole–dipole coupling between particles. Conversely, weaker dipole–dipole interactions give rise to a ‘soft’ response to the external field which is manifest by an exponential $SLP(H)$. These are speculative considerations with regard to the current sample set of particles; however, preliminary results suggest different internal (collective) magnetic structures among the particles [7,30,55], and further study is needed.

Loading MNPs can be problematic, particularly for those constructs (e.g. coating, surface charge, hydrophilicity) that are designed to minimise non-specific interactions with cells. This is a design of MNPs used in many biomedical applications, and is especially important for MNPs used in clinical applications. Commonly used transfecting agents, e.g. poly-D-lysine, are established agents to coat materials and enhance their interactions with cells in order to maximise internalisation. Thus, we have used this material to increase the total amount of MNP uptake by cells, even though such a modification has no direct clinical application. In short, a sufficient intra-cellular (and relatively consistent) MNP concentration is needed to perform comparisons.

Interestingly, from our results it is apparent that the major determinant of MR efficiency of MNPs, as a T_2 contrast agent, is the absolute iron content per cell. Complimentary results demonstrating linear dependence of T_2 -weighted MRI signal from the number of cells loaded with equal amounts of MNP were reported recently for different cell types [54]. The specific magnetic parameters of the iron core such as magnetic anisotropy, residual ferro- or ferrimagnetism and the capacity to generate heat are secondary, and seemingly do not contribute significantly to the resulting T_2 relaxivity. Indeed, it has been shown that the T_2 relaxivity of water protons ($R_2 = 1/T_2$) generated by MNPs in the so-called motional average regime (MAR) is described as:

$$R_2 = \frac{16}{45} \nu \tau_D (\gamma B_{eq})^2, \quad (1)$$

where ν is the volume fraction occupied by MNPs, the diffusion time, τ_D , for MNP size d and water diffusion coefficient D is defined as $\tau_D = d^2/4D$. B_{eq} is the equatorial magnetic field generated by the MNP located in the external magnetic field B_0 , and γ is the gyromagnetic ratio of protons (≈ 42.6 MHz/T).

$$B_{eq} = \frac{\mu_0}{3} M_{sat} \sim 0.16 \text{ T} \quad (2)$$

where μ_0 is the magnetic permeability of vacuum ($4\pi \times 10^{-7}$ Vs/(A m)), and M_{sat} is the saturation magnetisation of MNP [56–58]. Generally, the magnetisation of iron oxide is saturated if the external field B_0 is above ~ 1.5 T. MAR conditions are fulfilled when $\tau_D < 1/\gamma B_{eq}$. R_2 reaches its maximum in the static dephasing regime (SDR) when $\tau_D \approx 1/\gamma B_{eq} \sim 150$ ns and can be written as

$$R_2 = \frac{2\pi}{3\sqrt{3}} \nu \gamma B_{eq}. \quad (3)$$

As long as the radii of different MNPs are within an optimal narrow range, 20–30 nm, the R_2 relaxivity and hence the T_2 contrast of labelled cells in T_2 -weighted MRI is just a function of the total iron content of the samples as has been demonstrated in our experiments. While all our measurements have been performed using a preclinical high-field MR system operating at the B_0 field of 9.4 T, these results are independent of the B_0 strength and should have similar implication for MRI performed at typical clinical fields of 1.5 to 3.0 T.

Conclusions

This study demonstrates that for a range of iron oxide MNP magneto-structures and concentrations internalised by cancer cells, the signal intensity of T_2 -weighted MRI correlates closely with absolute iron concentration associated with the cells. This finding supports models that describe T_2 -shortening by clusters of magnetised spheres as dominated by unrestricted diffusion by water in large spheres when in the dephasing regime. Simultaneously, this study challenges recent claims that some nanoparticle formulations having enhanced magnetic anisotropy for hyperthermia may also display enhanced MRI contrast.

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Figure 1.
Schematic depiction of the experimental design.

Figure 2.

Comparison of amplitude-dependent SLP across peak-to-peak field amplitude spectrum among tested nanoparticle formulations. SLP values for Feridex were obtained from Bordelon et al. [6], and are provided for comparison. Error bars represent standard deviation of the mean calculated from three measurements. AMF frequency was 155 ± 10 kHz.

Figure 3.

Prussian blue stained slides of nanoparticle loaded DU-145 cells. Images were obtained after 24 h incubation with (A) D-SPIO, (B) JHU, (C) Feridex, and (D) BNF.

Figure 4.

Survival fraction of DU-145 cells at 100 pg Fe/cell loading. Cells were incubated 24 h with various nanoparticle formulations.

Figure 5.

Analysis of DU-145 cell-NP loaded iron concentration using ICP-MS. Aliquots ranged from 5×10^5 to 1.1×10^6 cells. Absolute iron concentration was calculated based on known cell number (pg Fe/cell).

Figure 6. T2 relaxation rates (R2) of DU-145 nanoparticle loaded cells as a function of (A) cell number and (B) total iron concentration.

Table I

Physical parameters of MNPs used in the current study.

Name (manufacturer)	Description	MNP concentration (mg/mL)	MNP diameter (nm)/iron type	Iron concentration (mg/mL)	Hydrodynamic diameter (nm)	Measured diameter (nm)/PI	Surface/solvent	SLP (W/g Fe) @ 155 kHz, 16 kA/m
BNF (Micromod)	100 nm core/shell	47	15–20 Fe ₃ O ₄	25.8	100	108/0.033	Starch/H ₂ O	13.5 ± 0.6
Nanonag® D-SPIO (Micromod)	100 nm colloidal SPIOs in dextran	77	10–12 Fe ₃ O ₄	10.0	100	86/0.170	Dextran/H ₂ O	26.1 ± 0.5
Feridex® (Advanced Magnetix)	Colloidal SPIOs in dextran	n/a	4–10 FeO _{1.44}	11.2	120–180	101/0.233	Citrate, dextran/H ₂ O	2.3 ± 0.08
JHU (Johns Hopkins/NanoMaterials)	100 nm core/shell	50	10–20 Fe ₃ O ₄ /Fe ₂ O ₃	13.7	100	82/0.15	Dextran/H ₂ O	71.8 ± 3.8