Comprehensive magnetic resonance characteristics of carbon-encapsulated iron nanoparticles: a new frontier for the core-shell–type contrast agents

Ireneusz P. Grudzinski · Michal Bystrzejewski · Piotr Bogorodzki · Andrzej Cieszanowski · Wojciech Szeszkowski · Magdalena Poplawska · Magdalena Bamburowicz-Klimkowska

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Abstract The development of carbon-encapsulated iron nanoparticles (CEINS) is of considerable interest in many areas of cancer nanotechnology, ranging from basic tumor biology to early detection and treatment of cancer. To meet these challenges, the present study was undertaken to determine the magnetic and relaxometric

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I. P. Grudzinski (✉) · M. Bamburowicz-Klimkowska
Faculty of Pharmacy, Medical University of Warsaw, S. Banacha 1, 02-097 Warsaw, Poland
e-mail: ireneusz.grudzinski@wum.edu.pl

M. Bystrzejewski
Faculty of Chemistry, University of Warsaw, L. Pasteura 1, 02-093 Warsaw, Poland

P. Bogorodzki
Faculty of Electronics and Information Technology, Warsaw University of Technology, Nowowiejska 15/19, 00-665 Warsaw, Poland

A. Cieszanowski
Maria Skłodowska – Curie Memorial Cancer Center and Institute of Oncology, Roentgena 5, 02-781 Warsaw, Poland

W. Szeszkowski
Faculty of Medicine, Medical University of Warsaw, S. Banacha 1a, 02-097 Warsaw, Poland

M. Poplawska
Faculty of Chemistry, Warsaw University of Technology, S. Noakowskiego 3, 00-644 Warsaw, Poland

performance of CEINS used as a new contrast agent for magnetic resonance imaging (MRI) in preclinical phantom models. CEIN samples were synthesized using a carbon arc discharge route, and the as-synthesized nanoparticles were purified and functionalized with surface acidic groups. The presence of various Fe-bearing metallic phases reflecting the net magnetic properties of CEINS was characterized by powder X-ray diffraction (XRD), thermogravimetry (TGA), and vibrating sample magnetometry. The morphological and surface chemistry features were characterized by electron microscopy (transmission electron microscopy [TEM] and scanning electron microscopy [SEM]), Raman spectroscopy, and Fourier transform-infrared (FT-IR) spectroscopy. The textural properties of CEINS, including porosity, surface total charge density, and zeta potentials, were also measured. The as-synthesized different CEIN samples were finally examined as a potent MRI contrast drug candidate. Magnetic resonance relaxation measurements were performed in bovine gelatin-based phantom models by using a 1.5-T MRI scanner equipped with a standard radiofrequency “birdcage” type head coil. To obtain data, T1- and T2-weighted MR images were acquired using the inversion recovery spin echo (SE) and the SE protocol with multiple time of echo (TE), respectively. Chemical characterization showed similarity in morphology and textural properties between as-synthesized CEINS, purified CEINS, and CEINS functionalized with acidic groups. The as-synthesized CEINS had significantly higher Fe content and higher saturation magnetization. The analysis of the

relaxometric properties of CEINS revealed that all the CEIN samples decreased T2 relaxation times on the T2-weighted images. The relaxation rate ($1/T_2$) showed some differences between the as-synthesized, purified, and surface-functionalized CEINS containing surface carboxylic groups. Both the as-synthesized and purified CEINS slightly decreased the T1 relaxation times, which was evident through increase in the relaxation rates ($1/T_1$). This study concludes that CEINS may represent a novel “core-shell”-type negative contrast drug candidate for MRI. It should be emphasized that all the studied CEIN samples have acceptable r_2 relaxivities at the field strength of 1.5 T where most of the MRI systems operate in clinical radiology.

Keywords Core-shell-type carbon-iron nanoparticles · Surface acidic functionalization · Magnetic resonance imaging · Relaxometric measurements · Magnetic measurements · Nanocomposites · Nanomedicine

Introduction

There have been an increasing number of investigations on various aspects of magnetic nanomaterials in the past two decades (Jasmin de Souza et al. 2017; Lee et al. 2013; Pankhurst et al. 2009; Shubayev et al. 2009; Sun et al. 2008; Vallabani et al. 2019; Wang et al. 2001). Among the different magnetic nanoparticles studied to date, iron oxide nanoparticles have received much attention as contrast agents for use in magnetic resonance imaging (MRI) (Chung et al. 2011; Maurea et al. 2014; Vasanawala et al. 2016; Wang 2011; Wang and Idée 2017). According to the hydrodynamic size of iron oxide nanoparticles, these iron species are classified into three categories of particles: micro-size iron oxide (MPIO; > 1000 nm), superparamagnetic iron oxide (SPIO; 60–180 nm), and ultra-small superparamagnetic iron oxide (USPIO; < 50 nm). Subsets of USPIO are also identified as very small nanoparticles of iron oxide (VSOP; 7–9 nm) and monocrystalline iron oxide nanoparticles (MION; 10–30 nm). Most of the iron oxide nanoparticles used as contrast agents in MRI are composed of both Fe(II) and/or Fe(III) cations and O_2^{2-} and/or OH^- anions forming a group of oxides, hydroxides, and oxy-hydroxides (Laurent et al. 2008). In the presence of an external magnetic field, these magnetic particles tend to align with the field and exhibit net magnetization in the direction of the magnetic field (Di Marco

et al. 2007). It should be emphasized that a major disadvantage of iron oxide nanoparticles is related to their relatively moderate magnetic performance (e.g., in comparison to bare Fe nanoparticles), which is considered to be an important parameter in molecular MRI. To elucidate this in a better way, one may compare the saturation magnetization of the magnetite (Fe_3O_4) and alpha iron (Fe) nanoparticles. The maximum achievable magnetization for the first and second phases is estimated to be ca. 80 and 224 emu/g (both values refer to room temperature), respectively (Pankhurst et al. 2009; Tartj 2006). In other words, one can easily predict that the mitigation of signal intensity observed on postcontrast T2- and T2*-weighted MR images can be achieved at the lower concentration, when pure Fe nanoparticles are used to obtain data. Hence, the nanoparticles comprising pure iron (Fe) phases have substantially better magnetic characteristics for application in cancer diagnostics based on molecular MRI. It should be noted that magnetic nanoparticles composed of different bare metals (i.e., Fe, Co, Ni, or their alloys) have been found to have some limited resistance properties to oxidation and agglomeration/aggregation processes (El-Gendy et al. 2009). These drawbacks can be substantially overcome by encapsulation of pure metallic nanoparticles in carbon layers, leading to production of the so-called carbon-encapsulated iron nanoparticles (CEINS) (Bystrzejewski et al. 2007). Such heterogeneous nanoparticles are composed of the metallic core (generally of the spherical shape), which is covered by few layers of graphene coating, leading to the “core-shell”-type magnetic nanostructures (Bystrzejewski et al. 2007). The carbon coating is mainly formulated of highly crystalline and curved graphene monolayers, which protect the core against any oxidation processes, preserve its specific magnetic properties, and further endow the encapsulated metal nanoparticles with the features of biocompatibility and stability in many organic and inorganic media, including animal and human fluids (Bystrzejewski et al. 2011). The encapsulation of iron nanoparticles in graphene-like shells was found to be a well-accepted method to increase their stability and preserve specific magnetic properties. The carbon coating is also considered as the optimal encapsulating material because it is light and tightly covers the magnetic core in nanoparticles (Bystrzejewski et al. 2011). Our previous studies showed that CEINS have a high corrosion resistance (Bystrzejewski et al. 2006) and are readily susceptible to various functionalization

processes (Kasprzak et al. 2015). It should also be noted that the mean diameter distribution of CEINS can be controlled by changing a very simple macroscopic parameter, namely grain size of the starting Fe powder used in synthesis processes or the chemical composition of the anode (Bystrzejewski et al. 2013). This phenomenon opens up new possibilities to control the magnetic characteristic of CEINS, leading to increase in the magnetic performance. More recently, studies have shown that CEINS can be precisely functionalized with a large number of functional groups that can be successfully linked with some biological molecules (Kasprzak et al. 2016; Poplawska et al. 2014). Interestingly, carbon-encapsulated iron oxide nanoparticles (Fe_3O_4) and carbon-coated gadolinium particles have been also tested as novel carbon-shell-type contrast drug candidates in MRI (Atabaev et al. 2015; Bae et al. 2012). Some gadolinium Gd(III) chelates such as PrimovistTM, OmniscanTM, and MultiHanceTM as well as Fe_3O_4 nanoparticles coated with polyethylene glycol, such as FeridexTM (SPIO), ResovistTM (SPIO), and CombidexTM (USPIO), are already available for clinical trials as positive (gadolinium) and negative (iron oxide) MRI contrast agents. Because carbon-based shell containing few layers of graphene is now recognized as a novel platform for both drug and gene delivery systems (DGDS) in nanomedicine (Bamburowicz-Klimkowska et al. 2019), the CEINS nanomaterials composed of the iron (Fe) core covered with carbon shells are now expected to be the perfect targeting magnetic core-shell-type negative contrast candidate when decorated with surface ligands in MRI. This feature has opened up some new exciting avenues for bioengineering carbon-coated magnetic nanomaterials as potent theranostic agents in preclinical MRI studies (Bae et al. 2012; Lee et al. 2014; Khramtsov et al. 2019; Park et al. 2011).

In the present study, we report the comprehensive and systematic characteristics of carbon-encapsulated iron nanoparticles examined as a potent contrast drug candidate for MRI by testing in the phantom gelatin models (in vitro). The CEINS were synthesized using a carbon arc discharge route, and the as-synthesized CEINS were purified and functionalized with acidic groups, including carboxylic moieties. The size and hydrodynamic properties of the CEIN samples were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and dynamic light scattering (DLS). Further structural details were evaluated from powder X-ray diffraction (XRD),

thermogravimetry (TGA), Raman spectroscopy, and Fourier transform-infrared (FT-IR) spectroscopy. The global surface charge density and zeta potential measurements were also performed to obtain data. The magnetic properties of CEINS were measured using a vibrating sample magnetometer. Finally, the spin-lattice relaxation (T1) and spin-spin (T2) processes were examined in the aqueous gelatin media of CEINS by using a magnetic resonance scanner operating at 1.5 T. Our results revealed that CEINS can function as a new T2 (negative) contrast agent in MRI.

Materials and methods

Synthesis and surface modifications

CEINS were synthesized by a carbon arc discharge route. The detailed procedure is described elsewhere (Borysiuk et al. 2008; Bystrzejewski et al. 2005). The synthesis method is based on the simultaneous sublimation of a heterogeneous anode containing iron (Fe) and graphite (C) powders. The anode material is transformed to the vapor phase congruently due to the very high temperature of the carbon arc (5000–6000 K). Next, the as-formed metal-carbon gas undergoes rapid cooling, which leads to the nucleation of Fe-C vapor and subsequent solidification to form iron nanoparticles encapsulated in few graphene monolayers. Briefly, the carbon arc discharge was ignited between the graphite cathode and the graphite anode, which was doped with Fe (45% wt.). The discharge was maintained at the current of 70 A under Ar-H₂ atmosphere (50:50 vol.%) at the total pressure of 60 kPa. The as-obtained raw product contained CEINS, and nonencapsulated iron particles were designated as Fe@C/Fe. A fraction of the as-synthesized Fe@C/Fe unpurified sample was further subjected to a purification process to remove the nonencapsulated Fe particles (Fe). The purification procedure included 24 h refluxing in 3 M HCl, washing in excess of water and ethanol, and drying in air at 70 °C. The as-purified product was designated as Fe@C. The raw CEINS were also chemically oxidized to introduce surface acidic groups, mainly carboxylic, which are covalently bound to carbon coatings. The corresponding product was designated as Fe@C-COOH. In a typical run, 2 g of the raw Fe@C/Fe product was suspended in a mixture containing 80 ml of concentrated H₂SO₄ and 26 ml of concentrated HNO₃. The sample was sonicated

in a water bath at 25–30 °C for 3 h (80 W). Then, the suspension was diluted by 1 l of distilled water and allowed to cool down for 2 h. The suspended particles were recovered on a membrane filter under reduced pressure and washed with excess of water and ethanol. All reagents used in the CEINS synthesis were obtained from Sigma-Aldrich and used without further purification.

Physicochemical characterizations

Morphological details of CEINS were studied by TEM (Zeiss Libra 120 operated at 120 kV). The diameter distribution was obtained by analyzing at least 300 objects on TEM images. The qualitative phase composition was evaluated by powder XRD (Bruker D8) using Cu K- α radiation in a 2θ range between 10 and 70 with a step of 0.02. Raman spectra were acquired using a dispersive spectrometer (Jobin Ivon T-64000) equipped with a 515-nm excitation laser with the spectral resolution of 4 s cm^{-1} . FT-IR spectra were recorded in a transmission mode with a Perkin Elmer System 2000 spectrophotometer. The amount of the surface acidic groups in CEINS was also determined by Boehm titration (Boehm 1994). Magnetic measurements were carried out at 25 °C using a vibrating sample magnetometer (Lake Shore 668). The measured magnetization referred to the total mass of the investigated sample. The Fe content was evaluated by TGA using a TA Q-50 instrument. The TGA measurements were carried out under N₂-O₂ (60/40) atmosphere at the constant heating rate of 10 °C $\cdot$ min⁻¹. The hydrodynamic diameter and zeta potential were measured using the Malvern Zetasizer instrument.

Phantom relaxometry measurements

In the present study, a purified (Millipore) water solution of carboxymethyl cellulose (CMC; Sigma-Aldrich) at the concentration of 0.1 mg/ml (viscosity 0.957 mPa s) was used as a stabilizing agent to increase the sedimentation stability of water suspensions of CEINS. To obtain data, the sedimentation studies of CEINS in different CMC-water media were described elsewhere in details (Grudzinski et al. 2013). In the phantom relaxometric studies, the CMC-water media of CEINS were embedded in the bovine gelatin type B (Sigma-Aldrich) and placed into a plastic centrifugation tube (16 mm in diameter, 100 mm long) to perform MRI

measurements. Each phantom was filled with 5.0% gelatin type B containing respective CEINS at the final concentration ranges of 0.0001, 0.001, 0.01, 0.1, 0.5, 1, 5, 10, 25, and 50 $\mu\text{g}/\text{mL}$. Water and gelatin B media without CEINS served as controls. For preparing phantom gels, the gel suspension of 5.0% (w/v) low-melting-point gelatin gel type B was preliminarily blended in pure water and heated first in a thermostat bath up to 65 °C. The contents were blended in a Vortex mixer and then cooled down to a bath temperature of between 43 and 40 °C just before the addition of CEINS. The suspensions of CEINS were prepared in the aqueous solution of CMC (0.1 mg/ml). The prepared gel solution was mixed thoroughly with vigorous shaking with different CEINS suspensions at 40 °C to prevent gelation while mixing and poured in plastic centrifuge tubes. The resultant mixture was quickly cooled down to prevent CEINS sedimentation. The plastic tubes containing CEINS were finally placed in a plastic rack and used for MRI measurements. Additionally, the gelatin phantoms containing CEINS were also studied by DLS (Malvern Zetasizer, 173 deg scattering angle) to evaluate the mean hydrodynamic diameter. The as-prepared phantom samples were melted and immediately transferred to polystyrene cuvettes for MRI studies.

All the MRI experiments were performed using a 1.5-T system (Magnetom Avanto, Siemens Medical Solutions, Erlangen, Germany), with explorer gradients (maximum gradient of 40, 40, and 45 mT/m along the x, y, and z axes, respectively, and slew rate of 200 mT m⁻¹ ms⁻¹) and a 32-multichannel system. The standard “birdcage” type radiofrequency (RF) coil (Siemens) was used throughout the experiments. The phantom gel aliquots were kept inside the coil and placed within the MRI (1.5 T) scanner for imaging. The temperature was controlled at 20 °C in the magnet room of the scanner. The experimental setup is shown in Suppl. Fig. S1.

For T1 measurements, T1-weighted images were acquired using the inversion recovery spin echo (SE) sequence with different inversion times (TI 50, 100, 200, 400, 800, 1200, 1600, and 2400 ms). The remaining spatial resolution parameters were acquired as follows: TR (3500 ms), TE (14 ms), echo spacing (14.1 ms), voxel size (1.1 $\times$ 1.1 $\times$ 2.0 mm), field of view (140 mm), flip angle (150 deg), slice thickness (2.0 mm), and averages (3).

For T2 measurements, T2-weighted images were acquired using the SE sequence with TR (3570 ms)

and variable echo times (TE 22.0, 44.0, 66.0, 88.0, 110.0, 132.0, 154.0, 176.0, 198.0, 220.0, 242.0, 264.0, 286.0, 308.0, 330.0, and 352.0 ms). The remaining spatial resolution parameters were acquired as follows: acquisition matrix of 256×256 , voxel size ($1.4 \times 1.1 \times 5.0$ mm), field of view (270 mm), slice thickness (5 mm), flip angle (180 deg), and averages (1).

After acquiring the MR images, the mean signal intensity (INS) was measured within the manually drawn equal-sized regions of interest (ROI) for each tested sample. The relaxation times were calculated by mono-exponential curve fitting of the signal intensity vs. time (TI or TE) using the MATLAB software. The following equation was used to fit the curves:

$$S(T_I) = S_0 \cdot \left| 1 - 2e^{\frac{-T_I}{T_1}} + e^{\frac{-T_R}{T_1}} \right| \quad (1)$$

where $S(T_I)$ is the signal intensity with inversion pulse of duration T_I applied, T_R is the repetition time, S_0 is the proton density, and T_1 is the longitudinal relaxation time. The absolute values account for the fact that the signal intensity in data is represented by the unsigned integer. T_1 was calculated numerically with the nonlinear regression model using the Levenberg-Marquardt algorithm (LMA) to fit the model curve to the experimental data.

For the signal intensity function of T2 relaxation, the following equation was used to determine T2 relaxation times:

$$M(T_E) = M_0 e^{\frac{-T_E}{T_2}} \quad (2)$$

where T_E is the time of echo, T_2 is the transversal relaxation time, and M_0 is the proton density.

T1 and T2 relaxation times were calculated for all the studied CEIN samples with different iron concentrations in the gelatin phantom. According to the relaxation theory (Dayie et al. 1996; Roch et al. 1999), the relaxation rates ($1/T_1$, $1/T_2$) are linearly dependent on Fe concentration. Therefore, the relaxivities (r_1 and r_2) were calculated as the slope of the respective plot of the $1/T_1$ and $1/T_2$ rates vs. the Fe content in different gelatin phantoms of CEINS to compare the contrast-enhancing effect of the raw (Fe@C/Fe), purified (Fe@C), and surface-functionalized (Fe@C-COOH) CEINS. A coefficient of determination (R-squared) was calculated to assess the goodness of each curve fitted.

Results

Physicochemical characterizations

Figure 1 shows the representative SEM images of the studied CEINSs. The as-obtained raw (Fe@C/Fe), purified (Fe@C), and surface-functionalized (Fe@C-COOH) samples nearly have the same morphology. They are composed of spheroidal nanoparticles with diameters below 100 nm. The nanoparticles in the solid state form fractal agglomerates. Further details can be obtained from TEM images (Fig. 2). The TEM inspection reveals the core-shell structure of the studied CEIN samples. The as-synthesized sample (Fe@C/Fe) contains two types of nanoparticles: CEINS with well-developed carbon coatings and some iron particles that are not fully encapsulated or encapsulated in noncontinuous carbon shells. The purified (Fe@C) and surface-functionalized (Fe@C-COOH) material also contain two types of nanostructures. The first type, similar to Fe@C/Fe, includes CEINS. The nanoparticles have a typical core-shell structure, i.e., the metallic (iron) cores are covered by carbon coatings. The diameter of individual pristine CEINS varies between 5 and 100 nm. Importantly, the greatest fraction of the sample has the size ranging between 10 and 50 nm (Fig. 2).

The water-CMC suspensions of Fe@C/Fe, Fe@C, and Fe@C-COOH nanoparticles were subjected to DLS and zeta potential measurements. The hydrodynamic diameter range and mean hydrodynamic diameter are listed in Table 1. The diameter range covers relatively broad limits and exceeds the size of pristine nanoparticles evaluated from TEM images (Fig. 2). The same conclusion applies to the mean hydrodynamic diameter, which is ca. 10 times larger than that of the pristine CEINS. This finding clearly demonstrates that the individual nanoparticles exist in an agglomerated form in the water-CMC suspension. Interestingly, the largest mean hydrodynamic diameter is found for the raw Fe@C/Fe sample. This is an unexpected result, because this sample is highly hydrophobic as it contains both uncoated Fe nanoparticles and nonfunctionalized carbon encapsulates. Surface-functionalized Fe@C-COOH have more hydrophilic characteristic and form (with the aid of CMC surfactant) smaller agglomerates. The zeta potential values of CEINS suspensions are between -24.7 and -25.4 mV. This finding shows that the obtained suspensions have a fair stability in the experimental

media. Therefore, the bovine gelatin type B phantoms of CEIN samples were prepared for MRI measurements.

The qualitative analysis of the magnetic core using an X-ray microprobe (EDX) indicates the presence of iron and carbon. Some Cu signals are derived from the microscopic mesh, and traces of oxygen are also evidenced, probably as an air pollutant (Fig. 3). It should be noted that the carbon shell of CEINS nanoparticles is

composed of well-crystallized graphene layers, ensuring the tightness of the encapsulated iron nanoparticles (please see the insert in Fig. 3).

The phase composition of the metallic core was studied by powder XRD. The diffractograms are shown in Fig. 4. The samples have nearly the same qualitative

Fig. 1 Representative SEM images of Fe@C/Fe (a), Fe@C (b), and Fe@C-COOH (c) nanoparticles

Fig. 2 Representative TEM images of Fe@C/Fe (a), Fe@C (b), and Fe@C-COOH (c) nanoparticles

Table 1 Diameter, hydrodynamic diameter, and zeta potential of water-CMC suspensions of CEINS

CEINS	Diameter range (nm)	Mean diameter (nm)	Hydrodynamic diameter range (nm)	Mean hydrodynamic diameter range (nm)	Zeta potential (mV)
Fe@C/Fe	5–100	56	520–900	710	− 25.4
Fe@C	5–100	52	270–460	340	− 24.7
Fe@C-COOH	5–100	46	190–1280	420	− 25.3

phase composition. Two Fe-containing crystalline phases were found in the studied CEIN samples, i.e., bcc Fe and solid solution of C in fcc Fe. Additionally, the samples contain some amounts of iron carbide (Fe_3C). The reflections ascribed to this phase are of the lowest intensity and are unlabeled in Fig. 4. The fcc Fe-C (111), bcc Fe (110), bcc Fe (200), and C (002) crystal planes in the XRD patterns were matched with those of the ferrite and graphite crystalline structures (fcc Fe-C 41506-ICSD, bcc Fe 52258-ICSD, and C (graphite) 76767-ICSD).

Figure 5 shows the Raman spectra of raw (Fe@C/Fe), purified (Fe@C), and functionalized with acidic groups (Fe@C-COOH) nanoparticles. The bands G and D are clearly evidenced. The ratio of G/D in the raw and purified samples is 1.89 ± 0.10 . The oxidized nanoparticles have a lower value for this parameter (1.56 ± 0.07). The decrease in the H/D ratio is due to

the introduction of carboxyl groups and the amorphous graphene planes in the nanoparticles.

The surface functionalization of CEINS was confirmed by FT-IR spectra and quantitative evaluation of surface functional acidic groups by Boehm titration (Fig. 6). The FT-IR spectra of Fe@C-COOH have several bands, which are absent in the raw Fe@C/Fe and purified Fe@C samples. The band located at 1710 cm^{-1} is due to the C=O vibration in carboxylic groups. The strong feature at 1580 cm^{-1} is typical for the C=C stretching vibration. For the Fe@C material, this band is very weak and is upshifted to 1620 cm^{-1} , probably due to more symmetric structure of carbon sheets in the nonfunctionalized carbon encapsulates. The broad band at 1180 cm^{-1} corresponds to the C–O vibrations. These results undoubtedly prove that the surface functional groups were successfully introduced on the carbon surface. The presence of surface functional acidic groups

Fig. 3 Energy-dispersive X-ray spectroscopy of the Fe@C sample and representative TEM image of this sample (insert)

Fig. 4 Powder XRD patterns of Fe@C/Fe, Fe@C, and Fe@C-COOH nanoparticles

was also confirmed by Boehm titration. The raw (Fe@C/Fe) and purified (Fe@C) nanoparticles have very low content of surface carboxylic groups (below 0.02 mmol/g). The surface-functionalized samples (Fe@C-COOH) have substantially higher surface carboxylic groups (up to 0.53 mmol/g). The relative fractions of various other functional acidic groups (e.g., phenolic and lactonic) have also been found on a similar level for the surface-modified CEINS (ca. 0.3 and 0.28 mmol/g, respectively). The raw (Fe@C/Fe) and purified (Fe@C) samples

have very low content of the total surface acidic groups such as carboxylic, phenolic, and lactonic (below 0.2 mmol/g). The surface-functionalized CEINS have substantially higher total acidity at the surface (ca. 1.11 mmol/g).

Magnetic measurements

The magnetic hysteresis loops are shown in Fig. 7. All the studied CEIN samples have typical ferromagnetic characteristics with a relatively low coercive field. The

Fig. 5 Raman spectroscopy of Fe@C/Fe, Fe@C, and Fe@C-COOH nanoparticles

Fig. 6 FT-IR spectroscopy of Fe@C and Fe@C-COOH nanoparticles

values of coercive field are 57, 214, and 301 Oe for Fe@C/Fe, Fe@C, and Fe@C-COOH, respectively. The difference between the coercive field for the as-synthesized (Fe@C/Fe) and two other CEIN samples (Fe@C and Fe@C-COOH) may be related to different size distribution and plausibly larger content of superparamagnetic nanoparticles in the as-synthesized material. The saturation magnetization (M_s) is also

different for all the studied samples. The as-synthesized sample has the highest magnetization (107 emu/g), while the M_s values for purified and surface-functionalized CEINS are 63 and 53 emu/g, respectively. The reduction of saturation magnetization is caused by irreversible dissolution of incompletely encapsulated Fe particles during the purification and surface functionalization process. The samples with

Fig. 7 Magnetic hysteresis loops of Fe@C/Fe, Fe@C, and Fe@C-COOH nanoparticles

higher Fe content have higher saturation magnetization. In fact, there is a clear correlation between the M_s values and Fe content evaluated from TGA measurements (the higher the Fe content, the higher is the M_s value).

Phantom relaxometry measurements

The relaxation study of the CEIN samples was performed at the magnetic field of 1.5 T using a typical clinical scanner (the experimental setup is shown in Suppl. Fig. S1). In the present study, T1-weighted images were acquired using the inversion recovery SE sequence with eight inversion times (TI 50, 100, 200, 400, 800, 1200, 1600, and 2400 ms). For the T2-weighted images, the SE sequence with 16 variable echo times (TE 22, 44, 66, 88, 110, 132, 154,

176, 198, 220, 242, 264, 286, 308, 330, and 352 ms) was acquired.

The signal intensity versus the time of inversion for different concentrations of CEINS is shown in Fig. 8. The curves clearly evidence the returning process to the equilibrium magnetization after inversion in absolute values at the field strength of 1.5 T. The longitudinal magnetization almost returns to the point of origin. This effect, however, is not observed at two higher concentrations of 25 and 50 $\mu\text{g/ml}$ for both the as-synthesized (Fe@C/Fe) and purified (Fe@C) CEIN samples, respectively. As shown in Fig. 8 (top and middle), the signal intensities of the CEIN samples are completely diminished. This effect is not observed for the Fe@C-COOH sample (Fig. 8 bottom). The representative T1-weighted images are presented in Fig. 9.

Fig. 8 Signal intensity versus the time of inversion for different concentrations of Fe@C/Fe (top), Fe@C (middle), and Fe@C-COOH (bottom) nanoparticles

The as-synthesized raw and purified nanoparticles slightly decrease the T1 time at higher concentrations, i.e., only at 25 and 50 $\mu\text{g/ml}$, but this effect is not observed for the functionalized CEINS containing the surface carboxylic groups (Suppl. Fig. S2A). The relaxation rates ($r1 = 1/T1$) calculated for the studied CEIN samples show a quite opposite direction (Suppl. Fig. S2B). This is also confirmed in expressing the $1/T1$ values versus iron (Fe) concentrations in different CEIN samples. As shown in Fig. 10, the $r1 = 1/T1$ values exhibit a linear dependence on Fe concentration in the phantom gelatin media. In the inversion recovery T1 studies, the fit (R-squared) is found to be good for the Fe@C/Fe ($R^2 = 0.9626$, $p < 0.05$), Fe@C ($R^2 = 0.9122$, $p < 0.05$), and Fe@C-COOH ($R^2 = 0.9628$, $p < 0.05$) samples measured at the magnetic field strength of 1.5 T; hence, the $r1$ relaxivity value is also determined accurately (please see the left panel of Fig. 10). The $r1$ relaxivities are calculated as the slope of the plot of $1/T1$ vs. iron concentration to compare the contrast-enhancing effect due to purification and surface functionalization processes for the as-synthesized CEINS. The $r1$ relaxivity values are shown in Table 2. It is interesting to note that the raw (Fe@C/Fe) and purified (Fe@C) CEINS have a higher contrast-enhancing effect than the surface-functionalized (Fe@C-COOH) samples (Table 2).

The T2 relaxation curves of CEINS studied in the phantom gelatin media with different iron concentrations are shown in Fig. 11. The representative T2-weighted images are presented in Fig. 12. The signal intensity expressed as arbitrary units for different echo times at the field strength of 1.5 T is steeply decreased with increasing CEINS concentration, but remains roughly unchanged at two higher concentration doses (25 and 50 $\mu\text{g/ml}$), thereby achieving the minimal value as compared to that of the background intensities (Fig. 11). In general, a similar profile of signal intensity is observed for both the naked (raw and purified CEINS) and surface-functionalized encapsulates containing acidic groups (Fe@C-COOH). This behavior was also confirmed on T2 relaxation times (Suppl. Figs. S3A, S3B). At the magnetic field strength of 1.5 T, the relationship between the $1/T2$ and Fe content is nearly linear for all carbon-encapsulated magnetic nanoparticles studied here, thus achieving the R-squared values of 0.9711 ($p < 0.05$), 0.9585 ($p < 0.05$), and 0.9539 ($p < 0.05$) for Fe@C/Fe, Fe@C, and Fe@C-COOH, respectively (Fig. 10, right panel). The $r2$ relaxivities, calculated

from the slopes $1/T2$ vs. Fe concentration, obtained from different CEIN samples are shown in Table 2. This study evidences that the as-synthesized raw Fe@C/Fe sample has the highest $r2/r1$ ratio among all the studied CEIN samples.

Discussion

The major aim of this study was to perform the comprehensive magnetic characterization of CEINS used as a new contrast drug candidate for MRI. We synthesized three different batches of CEINS by using the arc plasma method and examined the as-synthesized raw (Fe@C/Fe), purified (Fe@C), and surface-functionalized nanoparticles mainly containing carboxylic groups (Fe@C-

Fig. 9 Representative T1-weighted images of the gelatin phantoms composed of CEINS. T1-weighted images were acquired using the inversion recovery SE sequence with different inversion times (TI 50, 100, 200, 400, 800, 1200, 1600, and 2400 ms). The remaining spatial resolution parameters were acquired as follows: TR (3500 ms), TE (14 ms), echo spacing (14.1 ms), voxel size ($1.1 \times 1.1 \times 2.0$ mm), field of view (140 mm), flip angle (150 deg), slice thickness (2.0 mm), and averages (3)

COOH). The study showed that the as-obtained CEINS have similar morphological features. They were composed of mainly spheroidal nanoparticles with a typical core-shell structure containing different amounts of iron phases. All CEINS samples had typical ferromagnetic characteristics with a relatively low coercive field. We also observed some differences between the coercive field obtained for the as-synthesized raw nanomaterials and two other CEIN samples; these differences were mainly related to different size distribution and plausibly larger superparamagnetic content in the as-synthesized raw material. It should be noted that saturation

Table 2 The calculated value of the r_1 and r_2 relaxivities of CEINS studied at 1.5 T in the gelatin B phantoms. Data are expressed as mean $\pm$ SD ($n = 6$)

CEINS	r_1 ($\text{mM}^{-1} \text{s}^{-1}$) [inversion recovery SE]	r_2 ($\text{mM}^{-1} \text{s}^{-1}$) [16-echo SE]	r_2/r_1
Fe@C/Fe	1.72 ± 0.12	82.59 ± 5.04	48.0
Fe@C	1.97 ± 0.22	76.08 ± 5.68	38.6
Fe@C-COOH	2.10 ± 0.14	69.81 ± 7.50	33.2

magnetization was also quite different for all the CEIN samples. Among the examined CEINS, the as-

Fig. 10 Plots of $1/T_1$ and $1/T_2$ versus iron concentration in the raw (Fe@C/Fe), purified (Fe@C), and surface-functionalized (Fe@C-COOH) CEINS

synthesized raw material was found to have the highest magnetization on magnetic hysteresis loops.

In the present study, the effects of carbon-encapsulated iron nanoparticles on the spin-lattice (T1) and spin-spin (T2) relaxation processes were studied using the phantom water-gelatin models (in vitro). The experiments were performed at the low magnetic field

strength of 1.5 T by using an MRI clinical scanner equipped with the standard RF “birdcage” type head coil. In the experiments, both the longitudinal (T1) and transversal (T2) relaxation times and the r_1 and r_2 relaxivities were examined due to different iron phase composition and surface features of the studied CEIN samples. To obtain data, T1- and T2-weighted MR

Fig. 11 Signal intensity versus the time of echo for different concentrations of Fe@C/Fe (top), Fe@C (middle), and Fe@C-COOH (bottom) nanoparticles

images were acquired using the inversion recovery SE and the SE with multiple TE, respectively. The present data showed that the raw and purified CEIN samples had significantly lower r_1 relaxivities than the surface-functionalized CEIN samples. The as-synthesized raw Fe@C/Fe samples were noted to have the surface charge density (C/m^2) nearly equal to zero due to the absence of any surface acidic groups (Grudzinski et al. 2013). In contrast, the carbon surface density of the Fe@C ($0.20 C/m^2$) and Fe@C-COOH ($1.12 C/m^2$) samples were found to have some acidic sites implicated by the point of zero charge values, which were definitely lower than 7.0 in the CMC media (Grudzinski et al. 2013). Therefore, it seems reasonable that the exchange rate of hydrated protons was more pronounced in those CEINS where the protons are available in the surface acidic groups. As such, the proton exchange in the surface acidic groups, mainly carboxylic ($-COOH$), may play a significant role in affecting the r_1 relaxivity in the examined CEIN samples. Note that different surface coatings can largely affect T1 relaxation times due to the inner, intermediate, and outer sphere relaxivities in nanoparticle-based MRI (Caravan et al. 2009; Roch et al. 2005). More detailed mathematical approaches and discussions on these processes have been recently presented (Zhang et al. 2018).

The effect of CEINS on the T2 spin-spin relaxation is a considerably different process from the abovementioned T1 spin-lattice relaxation. T2 relaxation occurs through three major mechanisms, which are related to (i) Curie spin relaxation, which originates from the dipolar interaction between water protons and a large static magnetic moment arising from electrons, (ii) dipole-dipole coupling between the metal ions and the water hydrogen nuclei, and (iii) scalar or contact relaxation processes (Caravan et al. 2001; Krishnan 2010). Our present study showed that the r_2 relaxivity, as calculated from $1/T_2$ vs. iron concentration, resulted in the highest values for the Fe@C/Fe sample among the studied CEIN samples. This is a direct consequence of magnetic properties of this sample. In fact, the M_s value for the Fe@C/Fe material significantly exceeded those of the two other studied samples, i.e., Fe@C and Fe@C-COOH. This observation is also in agreement with the chemical composition of the studied CEINS, i.e., the Fe content changes in the following order: Fe@C/Fe > Fe@C > Fe@C-COOH (Grudzinski et al. 2013). There was also a clear correlation between the M_s values and Fe content evaluated from TGA measurements in our study (the higher

the Fe content, the higher was the M_s value). In general, Fe@C/Fe made of high M_s materials can more efficiently induce field inhomogeneity and can plausibly influence a greater volume of water. Therefore, it can be hypothesized that the T2 relaxation is mostly dominated by scalar or contact relaxation processes in these core-shell-type nanoparticles. More recently, the coating thickness was shown to influence the relaxivities of iron oxide nanoparticles (LE et al. 2017). The authors demonstrated that the thinner the stabilizing layer is, the larger is the r_2 relaxivity. For CEINS, the carbon coating, which is composed of few graphene layers, has the same thickness (ca. 3–4 nm) in all CEIN samples (please see insert in Fig. 3); thus, it cannot be concluded that the carbon surface diameter would strongly affect the r_2 relaxivity in the studied CEINS. Other recent studies showed that the r_2

Fig. 12 Representative T2-weighted images of gelatin phantoms composed of CEINS. T2-weighted images were acquired using the SE sequence with TR (3570 ms) and variable echo times (TE: 22, 44, 66, 88, 110, 132, 154, 176, 198, 220, 242, 264, 286, 308, 330, and 352 ms). The remaining spatial resolution parameters were as follows: acquisition matrix of 256×256 , voxel size ($1.4 \times 1.1 \times 5.0$ mm), field of view (270 mm), slice thickness (5 mm), flip angle (180 deg), and averages (1)

relaxation rates are sensitive to some aggregation states, hydrodynamic size, and morphology of iron oxide nanoparticles (Ebert et al. 2015; Joos et al. 2017; Ta et al. 2017). Among the CEIN samples studied here, the raw nanoparticles were found to have the highest potential to undergo agglomeration and aggregation processes as compared to purified and surface-functionalized CEINS. This was also confirmed in the present study on the hydrodynamic size of CEINS measured with DLS performed on the water-CMC suspensions of Fe@C/Fe, Fe@C, and Fe@C-COOH samples (Table 1) and the gelatin type B phantoms of these nanoparticles (Suppl. Fig. S4A–D). Comparing the results obtained for T1/T2 relaxivity properties, it can be noted that carbon-encapsulated magnetic nanoparticles result in a higher ratio (r_2/r_1) for the as-synthesized and purified samples than those calculated for the surface-functionalized CEINS containing some carboxylic groups. Note that the r_2/r_1 ratio for CEINS, ranging from 33.2 to 48.0, was higher than those measured at the same magnetic field strength for iron oxide nanoparticles in human fluids such as serum and whole blood, phosphate-buffered saline, and water (Ahmad et al. 2018; Jarrett et al. 2007; Knobloch et al. 2018). Because the r_1 and r_2 relaxivities are magnetic field-dependent, in general, the r_1 value decreases with the increasing magnetic field, but the r_2 value usually increases; thus, the application of 1.5 T seems to be reasonable because most of the MRI clinical scanners used worldwide operate in this field. Therefore, CEINS could be considered as an excellent negative contrast agent in the low magnetic field (1.5 T). Because purification and surface decoration processes are further required in CEINS to obtain nanoparticles that can recognize some specific molecular targets in animal and human tissues, one should be aware that such surface processes may affect the relaxivity of the final contrast drug candidate. Therefore, more detailed studies are required to elucidate the magnetic relaxation effects of CEINS decorated with different chemical and biological ligands. A more through approach on relaxation studies is also needed for CEINS studied in both cellular and preclinical animal models.

Conclusion

We synthesized CEINS by using a carbon arc discharge route and revealed their physicochemical properties and MRI contrast abilities at the field strength of 1.5 T. The

experiments presented here showed that CEINS demonstrate strong transverse relaxivities as a potent high-efficacy T2 (negative) contrast agent. Furthermore, the r_2/r_1 ratio for the studied CEINS samples was much higher than that of other reported iron oxide nanoparticles examined at the same magnetic field strength. The results showed that the hydrodynamic size, chemical composition, and surface characteristic of CEINS play a significant role in reflecting the relaxivity performance of these nanoparticles. The relevance of this study could be the level of its potent applicability because CEINS as a T2 contrast agent for MRI could open a new frontier for the core-shell (iron-carbon)-type theranostics.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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References

- Ahmad A, Bae H, Rhee I (2018) Highly stable silica-coated manganese ferrite nanoparticles as high-efficacy T2 contrast agents for magnetic resonance imaging. *AIP Adv* 8:055019
- Atabaev TS, Lee JH, Han DW, Kim HK, Hwang YH (2015) Fabrication of carbon coated gadolinia particles for dual-mode magnetic resonance and fluorescence imaging. *J Adv Ceram* 4(2):118–122
- Bae H, Ahmad T, Rhee I, Chang Y, Jin SU, Hong S (2012) Carbon-coated iron oxide nanoparticles as contrast agents in magnetic resonance imaging. *Nanoscale Res Lett* 7:44

- Bamburowicz-Klimkowska M, Poplawska M, Grudzinski IP (2019) Nanocomposites as biomolecules delivery agents in nanomedicine. *J Nanobiotechnol* 17:48
- Boehm HP (1994) Some aspects of the surface chemistry of carbon blacks and other carbons. *Carbon* 32(5):759–769
- Borysiuk J, Grabias A SJ, Bystrzejewski M, Twardowski A, Lange H (2008) Structure and magnetic properties of carbon encapsulated Fe nanoparticles obtained by arc plasma and combustion synthesis. *Carbon* 46:1693–1701
- Bystrzejewski M, Huczko A, Lange H (2005) Arc plasma route to carbon-encapsulated magnetic nanoparticles for biomedical applications. *Sens Actuators B-Chem* 109:81–85
- Bystrzejewski M, Cudziło S, Huczko A, Lange H (2006) Thermal stability of carbon-encapsulated Fe–Nd–B nanoparticles. *J Alloys Compd* 423:74–76
- Bystrzejewski M, Cudziło S, Huczko A, Lange H, Soucy G, Cota-Sanchez G, Kaszuwara W (2007) Carbon encapsulated magnetic nanoparticles for biomedical applications: thermal stability studies. *Biomol Eng* 24(5):555–558
- Bystrzejewski M, Karoly Z, Szepvolgyi J, Huczko A, Lange H (2011) Continuous synthesis of controlled size carbon-encapsulated iron nanoparticles. *Mater Res Bull* 46:2408–2417
- Bystrzejewski M, Łabędź O, Kaszuwara W, Huczko A, Lange H (2013) Controlling the diameter and magnetic properties of carbon-encapsulated iron nanoparticles produced by carbon arc discharge. *Powder Technol* 246:7–15
- Caravan P, Greenfield MT, JWM B (2001) Molecular factors that determine curie spin relaxation in dysprosium complexes. *Magn Reson Med* 46:917–922
- Caravan P, Farrar CT, Frullano L, Uppal R (2009) Influence of molecular parameters and increasing magnetic field strength on relaxivity of gadolinium- and manganese-based T1 contrast agents. *Contrast Media Mol Imaging* 4:89–100
- Chung J, Yu JS, Kim DJ, Chung JJ, Kim JH, Kim KW (2011) Hypervascular hepatocellular carcinoma in the cirrhotic liver: diffusion-weighted imaging versus superparamagnetic iron oxide-enhanced MRI. *Magn Reson Imaging* 29:1235–1243
- Dayie KT, Wagner G, Lefèvre JF (1996) Theory and practice of nuclear spin relaxation in proteins. *Annu Rev Phys Chem* 47:243–282
- Di Marco M, Sadun C, Port M, Guilbert I, Couvreur P, Dubernet C (2007) Physicochemical characterization of ultrasmall superparamagnetic iron oxide particles (USPIO) for biomedical application as MRI contrast agents. *Int J Nanomedicine* 2(4):609–622
- Ebert S, Bannwarth MB, Musyanovych A, Landfester K, Münnemann K (2015) How morphology influences relaxivity – comparative study of superparamagnetic iron oxide–polymer hybrid nanostructures. *Contrast Media Mol Imaging* 10:456–464
- El-Gendy AA, EMM I, Khavrus VO, Krupskaya Y, Hampel S, Leonhardt A, Klingeler BR (2009) The synthesis of carbon coated Fe, Co and Ni nanoparticles and an examination of their magnetic properties. *Carbon* 47(12):2821–2828
- Grudzinski IP, Bystrzejewski M, Cywinska MA, Kosmider A, Poplawska M, Cieszanowski A, Ostrowska A (2013) Cytotoxicity evaluation of carbon-encapsulated iron nanoparticles in melanoma cells and dermal fibroblasts. *J Nanopart Res* 15:1835
- Jarrett BR, Frendo M, Vogan J, Louie AY (2007) Size controlled synthesis of dextran sulfate coated iron oxide nanoparticles for magnetic resonance imaging. *Nanotechnology* 18:035603
- Jasmin de Souza GT, Louzada RA, Rosado-de-Castro PH, Mendez-Otero R, Campos de Carvalho AC (2017) Tracking stem cells with superparamagnetic iron oxide nanoparticles: perspectives and considerations. *Int J Nanomedicine* 12:779–793
- Joos A, Löwa N, Wiekhorst F, Gleich B, Haase A (2017) Size-dependent MR relaxivities of magnetic nanoparticles. *J Magn Magn Mater* 427:122–126
- Kasprzak A, Poplawska M, Bystrzejewski M, Łabędź O, Grudzinski IP (2015) Conjugation of polyethylenimine and its derivatives to carbon-encapsulated iron nanoparticles. *RCS Adv* 5:85556
- Kasprzak A, Poplawska M, Bystrzejewski M, Grudzinski IP (2016) Sulfhydrylated graphene-encapsulated iron nanoparticles directly aminated with polyethylenimine: a novel magnetic nanopatform for bioconjugation of gamma globulins and polyclonal antibodies. *J Mater Chem B* 4:5593–5607
- Khamrtsov P, Kropaneva M, Byzov I, Minin A, Mysik A, Timganova V, Bochkova M, Uimin M, Zamorina S, Yermakov A, Rayev M (2019) Conjugation of carbon coated-iron nanoparticles with biomolecules for NMR-based assay. *Colloids Surf B: Biointerfaces* 176:256–264
- Knobloch G, Colgan T, Wiens CN, Wang X, Schubert S, Hernando D, Sharma SD, Reeder S (2018) Relaxivity of ferumoxytol at 1.5T and 3.0 T. *Investig Radiol* 53(5):257–263
- Krishnan KM (2010) Biomedical nanomagnetism: a spin through possibilities in imaging, diagnostics, and therapy. *IEEE Trans Magn* 46(7):2523–2558
- Laurent S, Forge D, Port M, Roch A, Robic C, Vander Elst L, Muller RN (2008) Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations and biological applications. *Chem Rev* 108(6):2064–2110
- LE LC, Nitin N, Zurkiya O, Caruntu D, O'Connor CJ, Hu X, Bao G (2017) Coating thickness of magnetic iron oxide nanoparticles affects r2 relaxivity. *J Magn Reson Imaging* 26:1634–1641
- Lee KJ, An JH, Chun JR, Chung KH, Park WY, Shin JS, Kim DH, Bahk YY (2013) In vitro analysis of the anti-cancer activity of mitoxantrone loaded on magnetic nanoparticles. *J Biomed Nanotechnol* 9(6):1071–1075
- Lee HJ, Sanetuntikul J, Choi ES, Lee BR, Kim JH, Kim E, Shanmugam S (2014) Photothermal cancer therapy using graphitic carbon-coated magnetic particles prepared by one-pot synthesis. *Int J Nanomedicine* 10:271–282
- Maurea S, Mainenti PP, Tambasco A, Imbriaco M, Mollica C, Laccetti E, Camera L, Liuzzi R, Salvatore M (2014) Diagnostic accuracy of MR imaging to identify and characterize focal liver lesions: comparison between gadolinium and superparamagnetic iron oxide contrast media. *Quant Imaging Med Surg* 4:181–189
- Pankhurst QA, NKT T, Jones SK, Dobson J (2009) Progress in applications of magnetic nanoparticles in biomedicine. *J Phys D Appl Phys* 42:224001
- Park JH, Jung J, Subramaniam P, Shah BP, Kim C, Lee JK, Cho JH, Ch L, Lee KB (2011) Graphite-coated magnetic

- nanoparticles as multimodal imaging probes and cooperative therapeutic agents for tumor cells. *Small* 7(12):1647–1652
- Poplawska M, Bystrejewski M, Grudzinski IP, Cywinska MA, Ostapko J, Cieszanowski A (2014) Immobilization of gamma globulins and polyclonal antibodies of class IgG onto carbon-encapsulated iron nanoparticles functionalized with various surface linkers. *Carbon* 74:180–194
- Roch A, Muller RN, Gillis P (1999) Theory of proton relaxation induced by superparamagnetic particles. *J Chem Phys* 110(11):5403–5411
- Roch A, Gossuin Y, Muller RN, Gillis P (2005) Superparamagnetic colloid suspensions: water magnetic relaxation and clustering. *J Magn Magn Mater* 293:532–539
- Shubayev VJ, Pisanic TR, Jin S (2009) Magnetic nanoparticles for theragnostics. *Adv Drug Deliv Rev* 61(6):467–477
- Sun C, JSH L, Zngang M (2008) Magnetic nanoparticles in MR imaging and drug delivery. *Adv Drug Deliv Rev* 60(11):1252–1265
- Ta HT, Li Z, Wu Y, Cowin G, Zhang S, Yago A, Whittaker AK, Xu ZP (2017) Effects of magnetic field strength and particle aggregation on relaxivity of ultra-small dual contrast iron oxide nanoparticles. *Mater Res Express* 4(11):116105
- Tartj P (2006) Nanomagnetism from fundamental physics to biomedicine. *Curr Nanosci* 2:43–53
- Vallabani NVS, Singh S, Karakoti AS (2019) Magnetic nanoparticles: current trends and future aspects in diagnostics and nanomedicine. *Curr Drug Metab* 20(6):457–472
- Vasanawala SS, Nguyen KL, Hope MD, Bridges MD, Hope TA, Reeder SB, Bashir MR (2016) Safety and technique of ferumoxytol administration for MRI. *Magn Reson Med* 75:2107–2111
- Wang YXJ (2011) Superparamagnetic iron oxide based MRI contrast agents: current status of clinical application. *Quant Imaging Med Surg* 1(1):35–40
- Wang YXJ, Idée JM (2017) A comprehensive literatures update of clinical researches of superparamagnetic resonance iron oxide nanoparticles for magnetic resonance imaging. *Quant Imaging Med Surg* 7(1):88–122
- Wang YXJ, Hussain SM, Krestin GP (2001) Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging. *Eur Radiol* 11(11):2319–2331
- Zhang W, Liu L, Chen H, Hu K, Delahunty I, Gao S, Xie J (2018) Surface impact on nanoparticle-based magnetic resonance imaging contrast agents. *Theranostics* 8(9):2521–2548

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