Published in final edited form as:

Biomaterials. 2010 August ; 31(22): 5842–5848. doi:10.1016/j.biomaterials.2010.03.072.

The Magnetophoretic Mobility and Superparamagnetism of Core-Shell Iron Oxide Nanoparticles with Dual Targeting and Imaging Functionality

Faquan Yu^{a,b}, Lei Zhang^{a,c}, Yongzhuo Huang^a, Kai Sun^d, Allan E. David^a, and Victor C. Yang^{a,c,*}

^aDepartment of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109, USA

^bKey Laboratory for Green Chemical Process of Ministry of Education, Wuhan Institute of Technology, Wuhan 430073, China

^cSchool of Chemical Engineering, Tianjin University, Tianjin 300072, China

^dDepartment of Materials Science and Engineering, University of Michigan, Ann Arbor, Michigan 48109, USA

Abstract

With the goal to achieve highly efficacious MRI-monitored magnetic targeting, a novel drug carrier with dual nature of superior magnetophoretic mobility and superparamagnetism was synthesized. This carrier was specially designed in a core-shell structure. The core was achieved by utilizing a strategy of self-assembly of oppositely charged ultrafine superparamagnetic iron oxide nanoparticles previously prepared. The final particles were formed by coating such cores with carboxymethyldextran (CMD) polymer. By exclusion of non-magnetic materials from the interior part of the particles, this structure maximized the amount of magnetic material and thus yielded a superior magnetophoretic mobility. Such a strategy avoids the challenge of superparamagnetism loss, which would be caused by cores exceeding a critical domain size. Coating the self-assembled core enables the magnetic carrier to be stable upon usage and storage and to be readily linked with drug molecules for therapeutic applications. *In vitro* characterization showed that these nanoparticles displayed a 3- to 4-fold enhancement in magnetophoretic mobility, and a markedly improved stability when stored in 50% serum as a comparison of conventional iron oxide-based magnetic nanoparticles. Preliminary *in vivo* studies revealed that the nanoparticles also function well as a contrast enhancer for MR imaging of brain glioma. This technology could lead to the development of a new paradigm of magnetic carriers that meet with the needs of various clinical applications.

Keywords

Magnetophoresis; Superparamagnetism; Core-shell structure; MRI; Iron Oxide

© 2010 Elsevier Ltd. All rights reserved.

*Correspondence and reprint requests should be addressed to: Victor C. Yang, Ph.D., Albert B. Prescott Professor of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, 428 Church Street, Ann Arbor, Michigan 48109-1065, Tel: (734) 764-4273; Fax: (734) 763-9772, vcyang@umich.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

The application of magnetic nanoparticles as drug carrier for brain tumor targeting has recently drawn widespread attention because of the potential to achieve site-specific targeting with the aid of an external magnetic field and the ability to non-invasively monitor nanoparticle accumulation with magnetic resonance imaging (MRI). Monocrystalline iron oxide nanoparticles (MION), which are nontoxic and exhibit superparamagnetic behavior, are widely employed in these fields of research. It has been demonstrated that nanoparticles can penetrate through the leaky capillary endothelium of tumors, and accumulate in the lesion via the enhanced permeability and retention (EPR) effect [1,2,3]. The feasibility of MION delivery to brain tumors after intravenous administration has already been reported [4,5]. Additionally, our previous work has shown that active magnetic targeting, in concert with EPR retention, greatly enhances the exposure of tumors to magnetic nanoparticles compared to the passive EPR accumulation alone [6]. Another attractive feature of iron oxide-based magnetic nanoparticles is their strong enhancement of proton spin-spin (T_2/T_2^*) relaxation. The resulting reduction in signal intensity due to accumulation of these nanoparticles allows for the effect of drug therapy on a tumor to be non-invasively visualized via MRI.

In order to utilize the dual functions of magnetic targeting and MRI, the properties of the iron oxide nanoparticles must be optimized. In order to avoid aggregation during clinical application, the magnetic nanoparticles must possess superparamagnetic properties which is achieved only when the domain size for iron oxide is below 15 nm [7–10]. However, it has also been demonstrated that the magnetic response force, to externally applied magnetic fields, becomes weak with reduction in size. Studies have shown that saturation magnetization decreases linearly with decreasing crystallite size [11]. Although the small, superparamagnetic nanoparticles retain MRI functionality [12], they cannot be retained under physiological flow conditions (e.g. in blood), for magnetic targeting, with clinically acceptable magnetic field gradients [13]. The efficiency of magnetic targeting depends primarily on the magnetophoretic mobility, a parameter that can be enhanced only by increasing the size of magnetic materials. Therefore, in order to simultaneously fulfill both functions, the nanoparticles must be enhanced in size to improve targeting while also kept small to maintain its superparamagnetism.

Unfortunately, most attempts toward achieving the dual function of MRI and targeting failed to recognize this contradiction. As noted, current superparamagnetic nanoparticles are composed with MION in a polymer matrix [14]. The polymer on the surface is essential for attaining biocompatibility, or for conjugation with biological ligands or drug molecules. However, the polymer inside the particle does not provide any important function but rather lowers the MION content, thus reducing the magnetophoretic mobility. In addition, in order to take full advantage of the EPR effect for passive tumor targeting, a particulate-based drug carrier has to be designed within the size range of 100–300 nm; particles larger than 300 nm would be subjected to hepatic elimination or clearance by the reticuloendothelial system (RES), whereas particles smaller than 10 nm are eliminated by renal filtration [15].

To the best of our knowledge, there is no report toward achieving the high magnetophoretic mobility without losing superparamagnetism. Presented herein is a facile method to synthesize core-shell structured nanoparticles, with desired size (100–200 nm), that consisted of a large core and a thin shell. The large core was synthesized by the self-assembly of oppositely charged MIONs. While the intrinsic superparamagnetic behavior of MION was retained, the larger core provided superior magnetophoretic mobility.

2. Materials and methods

2.1 Chemicals

All materials were purchased and used as received without further treatment. Chemicals used include sodium citrate dihydrate (Fisher Scientific), glycine (Aldrich), ferrous chloride tetrahydrate (Fluka), iron chloride hexahydrate (Sigma-Aldrich), carboxymethyl dextran (CMD, Mr:14,400Da, Fluka).

2.2 Preparation of iron oxide nanoparticles

2.2.1 Preparation of MION—MION was synthesized according to a modified procedure of Kim et al [16]. Briefly, a solution containing 0.76 mol/L of ferric chloride and 0.4 mol/L of ferrous chloride (molar ratio of ferric to ferrous was approximately 2:1) was prepared at pH 1.7 under N₂ protection. The iron solution was then added drop-wise into a 1.5M NaOH solution under vigorous mechanical stirring. The reaction mixture was gradually heated (1°C/min) to 78°C and held at this temperature for one hour under stirring and N₂ protection. After separation of the supernatant using a permanent magnet, the wet sol was treated with 0.01M HCl and then sonicated for 1 hour. The acidified colloidal suspension of MION was filtered through 0.45 µm and then 0.22 µm membranes, followed by concentration to a suspension containing 0.7 mg Fe/ml using a Millipore (USA) ultrafiltration unit.

2.2.2 Preparation of negatively-charged (nMION) and positively-charged MION (pMION)—To 200 ml of 1 mg/ml sodium citrate, 200 ml of 0.7 mg Fe/ml iron oxide nanoparticles were added under stirring. The mixture was sonicated for 20 min and then further stirred for 2 hours. After ultrafiltration to remove free sodium citrate, the concentration was adjusted to 0.35 mg Fe/ml. The resulting nanoparticles were negatively-charged (nMION). An analogous procedure was used to prepare glycine-modified, positively-charged nanoparticles (pMION).

2.2.3 CMD coated nanoparticles—To obtain self-assembled magnetic cores, an aqueous suspension of nMION was added to aqueous pMION under ultrasonication, see Figure 1. An excess amount of pMION was used during the self-assembly process to obtain cores with net positive charges. These positively-charged large MION cores were then added into the same volume of CMD aqueous solution under mechanical stirring. The final CMD-coated nanoparticles were then separated from the free CMD polymer using magnetic separation.

2.3 Transmission electron microscopic (TEM) images

Transmission electron microscopy (TEM) was conducted using a JEOL 3011 high-resolution electron microscope (JEOL Tokyo, Japan) operated at an accelerated voltage of 300 kV. Samples were prepared by applying diluted particle suspensions onto formvar film-coated copper grids (01813-F, Ted Pella, Inc, USA), followed by drying the grids at room temperature. Bright field (STEM-BF) imaging and X-ray energy dispersive spectroscopy (EDS) measurements were carried out on samples being placed on a carbon network coated copper grid, using a JEOL-2010F analytical electron microscope (AEM) operated in STEM mode at 200 kV. The lens conditions were set to define a probe size of 0.5 nm, and EDAX Genesis software was employed for EDS data collection and analysis.

2.4 Magnetization measurement

Superparamagnetic properties were assessed at 25 °C using a superconducting quantum interference device (SQUID) (Quantum Design Inc., San Diego, CA, USA). Iron content of nanoparticles was measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES) on an Optima 2000 DV instrument (Perkin-Elmer, Inc., Boston, MA, USA).

Samples were spiked with yttrium internal standard and calibrated with water dilutions of an iron standard (GFS Chemicals, Columbus, OH).

2.5 DLS size and zeta potential measurement

Particle size and zeta potential were measured on a NICOMP 380 ZLS dynamic light scattering (DLS) instrument (PSS, Santa Barbara, CA, USA) using a HeNe laser at 632 nm as the incident light. For size measurements, the volume-weighted distribution was obtained.

2.6 Thermogravimetric analysis (TGA)

A thermogravimetric analyzer (TGA; TA Instruments Q50, New Castle, DE, USA), calibrated with nickel and alumel standards, was employed to determine the content of polymer coating of the nanoparticles. Samples were analyzed in a nitrogen atmosphere with a heating rate of 20 °C/min.

2.7 Fourier transform infrared spectroscopy (FTIR)

FTIR was performed on lyophilized samples after compression into ~1 mm thick discs containing spectroscopic grade potassium bromide.

2.8 Magnetophoretic mobility

Magnetophoretic mobility of the nanoparticles was measured by monitoring the change of nanoparticle concentration in water with time using a UV/Vis spectrophotometer (Beckman Du® 650 Spectrophotometer, USA) (as illustrated in Fig s1, Supporting Information). Briefly, nanoparticles were dispersed into a water solution and the suspension then placed into a 1cm×1cm quartz cuvette fixed with a sample holder. A permanent magnet (DynaL MPC®-L, Invitrogen) was placed along the side of the sample holder. The absorbance was monitored at a wavelength of 360 nm at which the absorbance was found to be linearly proportional to the iron content determined by ICP-OES. The magnetic field strength was measured (shown in Fig s2 in Supporting Information) using a 3-axis Hall Teslameter (THM 7025, GMW Associates). The gap between the magnet and nearest side of the cuvette was ~5 mm - indicating the sample was located 5 to 15 mm from the magnet. All test samples were prepared at the same initial nanoparticle concentration.

2.9 Stability assessment

Stability was assessed by dispersing the magnetic nanoparticle samples into a 0.1 M PBS buffer (pH 7.4) containing 50% calf serum. The suspensions were then placed into a 1cm×1cm quartz cuvette held by a sample holder, and the turbidity change monitored with time at 370 nm using a Beckman Du® 650 Spectrophotometer equipped with a thermostat at 37°C.

2.10 In vivo magnetic resonance images (MRI)

Magnetic targeting and MRI were performed according to our previously published procedure [6]. Intracerebral 9L tumors were induced in male Fisher 344 rats (~150 g) using our previously established protocol [6]. After the tumor size reached to ~70 µl, monitored and estimated by MRI, the animal was injected intravenously with the nanoparticle suspension at a dose of 12 mg Fe/kg. For magnetic targeting, the head of the animal was positioned between the poles of an electromagnet. The magnetic field density was adjusted to 0 T for control animals (i.e. without magnetic targeting) or 0.4 T for targeted animals for 30 min. MRI was conducted using a 12-cm horizontal-bore, 7 Tesla Unity Inova imaging system (Varian, Palo Alto, CA), and T₂-weighted MRI images were acquired.

3. Results

MION were first prepared using our modified protocol [17], based on Kim's method [16]. TEM images (Figure 2) showed that the average size of MION was 9 ± 1.9 nm, whereas their average hydrodynamic size was determined to be 16.7 nm by the DLS method. In HCl medium at pH 4.0, these bare MION displayed a positive zeta potential of +29.7 mV, presumably originated from the superficial hydroxyl groups that were protonated in the acidic medium [18–20]. Treating these bare MION with either sodium citrate or glycine yielded negatively- or positively-charged nanoparticles (nMION, pMION), with zeta potentials being changed to -19.4 mV and +34.4 mV, respectively. Both nMION and pMION displayed a new peak at 1402cm^{-1} in FTIR, compared with the original un-modified or bare MION (Fig s3 in “Supporting Information”). This new peak was assigned to the symmetric stretching band of two-oxygen coordination of the carboxylate group with iron atom [21], providing evidence that a polar covalent bond of Fe-O-C (the C in the COO^- group of citrate or glycine) linkage was formed [22]. Self-assembly of nMION and pMION via electrostatic interaction would occur to yield a large-size magnetic core, which would carry a positive surface charge when an excess amount of pMION is applied (Figure 1). The carboxylate group carrying polymer, carboxymethyldextran (CMD), would then be readily coated onto the large MION cores to produce magnetic nanoparticles with the desired core-shell structure.

Figure 3a revealed that after coating the aforementioned self-assembled, large-size MION core by CMD, the zeta potential of the coated nanoparticles sharply switched to a negative value, apparently due to the presence of free carboxylate groups from CMD on the particle surface. Importantly, both the sizes of the MION core and the CMD-coated nanoparticles could be readily regulated by altering the mass ratio between the pMION and nMION in the self-assembly process (Figure 3b). A series of CMD-coated magnetic nanoparticles with average hydrodynamic sizes ranging from about 40 to 280 nm were successfully synthesized using this procedure.

The core-shell structure of CMD-coated particles was verified by utilizing a variety of methods including scanning transmission electron microscopy (STEM), X-ray energy dispersive spectroscopy (EDS) scan, and thermogravimetric analysis (TGA). All of the analyses were performed on nanoparticles of approximately 100 nm in size (termed CMD-coated100), and data were compared with two existing commercial products, fluidMAG-CMX (CMD-coated, 100 nm) and fluidMAG-D (starch-coated, 100 nm) supplied generously by the Chemicell Company.

Results from the bright field images (STEM-BF) clearly demonstrated a shell-like structure of the polymer coating on these nanoparticles (Figure 4). Thickness of the polymer shell for CMD-coated100 was estimated to be about 2.7 nm. To further verify the core-shell structure in the synthesized nanoparticles, we sought to confirm that no polymer exists within the particle interior. Hence, element analyses of both carbon and iron contents was carried out by using EDS linescan spectroscopy along a linear path through the particle. In principle, if the nanoparticle is comprised of a core-shell structure and the core composed purely of iron oxide, the carbon content would not vary, while the iron content increases, along the radius toward the particle center. On the other hand, if polymer was somehow incorporated into the interior part of the particles, both carbon and iron content should increase gradually toward the particle center.

As shown in Figure 4a, the iron content (red curve) of CMD-coated100 increased gradually along the line toward the center. It is consistent with pixel brightness observed from the STEM-BF images. Take area-B in Figure 4a as an example, it was relatively darker in the images, indicating the presence of high iron content. Conversely, the carbon content (blue curve in Fig

4a) remained nearly constant and did not exhibit significant difference at area-B compared with those close to the two edges (i.e. at the 25- and 100-nm positions on the scan line) of the nanoparticles. It is suggested that the CMD polymer resided primarily on the particle surface; otherwise the change in carbon content should be in accordance to that in iron content. Notably, although there was a slight inconsistency in carbon and iron ratio (i.e. increase in carbon content but low iron content) in area-A (~25 nm from the particle surface), this was believed to result from the rough and irregular morphology at the particle surface, such as existence of basins, which entrapped more polymer, thereby causing an increased carbon content.

In contrast, fluidMAG-CMX displayed a distinctive pattern concerning the content ratio between carbon and iron along the EDS linescan. As seen in Figure 4b, both carbon and iron contents increased along the line toward the particle center, suggesting that the polymer in fluidMAG-CMX was significantly incorporated into the interior part of the particles.

Figure 5 revealed the TGA results for these magnetic nanoparticles. A trivial weight loss of 1.6%, originating from thermal removal of the citrate and glycine coating, was observed from the MION core formed by self-assembly of nMION and pMION. CMD-coated100 sustained a noteworthy weight loss of 4.8%, due to the additional CMD coating the particle. In sharp contrast, both fluidMAG-CMX and fluidMAG-D underwent remarkable weight losses of 14.3% and 15.8%, respectively. As calculated, CMD-coated100 consisted of only 30% of the polymer content of fluidMAG-CMX (4.8% versus 14.3%, respectively) after normalization by iron content. Since the shell coating contains almost the same polymer content, the significantly low polymer content in CMD-coated100 further confirmed the applicability of the described method in producing a shell-core type of nanoparticles. The dearth of polymer matrix within the interior part of particles would presumably enhance the mobility of the nanoparticles under the influence of a magnetic field.

The SQUID results, shown in Figure 6, demonstrated that both MION and CMD-coated100 exhibited superparamagnetic properties, without remnant hysteresis loop observed in the magnetization/demagnetization curves. The saturation magnetism for CMD-coated100 was estimated to be 69.4 emu/g Fe, slightly lower than the value of 73.3 emu/g Fe obtained for the MION.

Figure 7 clearly demonstrated that CMD-coated100 moved significantly faster, under a magnetic force, than either of the two commercial products. The magnetophoretic mobility was quantitatively compared by using the migration half-time ($t_{1/2}$) as the indicator, which was defined as the time required to reduce the turbidity by 50%. The $t_{1/2}$ of CMD-coated100 was about 40 min, which was an approximately 3- to 4-fold enhancement of the mobility compared to the values of 125 min and 155 min for fluidMAG-CMX and fluidMAG-D, respectively.

To examine the utility of CMD-coated100 as a MRI contrast enhancer for monitoring brain tumors, preliminary proof-of-principle animal studies were conducted on rats harboring brain glioma according to a previously established protocol [6]. As a control, the rat was treated with the same dose of CMD-coated100 without targeting via the magnetic force. As seen in Figure 8, the post-injection image of the non-targeted control animal (Fig 8a) displayed a very slight signal reduction within the glioma lesion. In contrast, GE images of the targeted animal acquired 30 min post-injection (Fig 8b) exhibited a region of pronounced hypointensity, implicating the accumulation of magnetic nanoparticles inside the tumor.

To assess stability, the above three magnetic nanoparticles (CMD-coated100, fluidMAG-CMX, and fluidMAG-D) were exposed to a simulated body fluid containing 50% (v/v) calf serum in 0.1 M PBS (pH 7.4) and the turbidity monitored at 370 nm as an indication of particle aggregation. As seen in Figure 9, the turbidity of fluidMAG-D reached a summit at the 40-min mark and then dropped rapidly thereafter. This drop was due to the occurrence of precipitation

of the large aggregates. The precipitate could be visually detected after 1.5 hours of exposure to the simulated body fluid. In contrast, the turbidity of both CMD-coated100 and fluidMAG-CMX increased slowly and then remained at a plateau till the 12-hour mark after exposure to the medium. In addition, no precipitate was ever visually observed during this 12-hour period of incubation.

4. Discussion

As known, superparamagnetic properties of iron oxide particles can be achieved only when the particle size is below a critical domain size [7–10]. On the other hand, the magnetophoretic mobility, which benefits the targeting efficiency when the particles are utilized as a vehicle for drug delivery, increases with the size. The divergent requirements in structures for dual fundamental properties are obvious, but not realized. Rarely have magnetophoresis and its effect on targeting efficiency been addressed in the literature. This research integrated the divergent requirements into a structure designed toward achieving the dual natures.

A core-shell structure is a state-of-the-art design for this purpose. This structure provides the highest iron oxide content, considering that the coating polymer shell is necessary for further conjugation of drug, for biocompatibility, and for stability. Any polymer located inside the particles only occupies space that could otherwise hold more iron oxide. Preventing the presence of polymer within the particle interior and insuring a tightly packed iron oxide core is the key to improving mobility, as illustrated in the currently developed strategy.

While a large core is essential for quick magnetophoretic mobility, the superparamagnetism will be lost if the single-crystal domain exceeds a critical size. Therefore, the strategy was designed to generate a core by way of self-assembly of small size superparamagnetic particles.

TEM, EDS linescan, and TGA have provided proof of the core-shell structure. Interestingly, the TGA results were found to be highly consistent with the EDS spectra. Based on the peak areas of carbon and iron elements in EDS results, the C/Fe ratios were estimated to be 0.050 and 0.158 for CMD-coated100 and fluidMAG-CMX, respectively (see Fig s4-C in “Supporting Information”). In agreement with the TGA findings, comparison of these two numbers yielded a 31% ratio of the carbon content or, in other words, of the polymer content between CMD-coated100 and fluidMAG-CMX. This is almost identical to the ratio of 30.2% obtained earlier from the TGA results (see “Supporting Information”). This agreement between TGA results with X-ray energy dispersive spectroscopy (EDS) proved that both these analyses were reliable.

What is more, the polymer content of CMD-coated100 could also be calculated as 4.2 wt.% with assumption of a shell-core structure (see “Supporting Information”). This value is very close to the experimental weight loss of 4.8% measured by TGA. The slightly higher value obtained experimentally could be a result of the presence of non-spherical or irregular morphology of the actual nanoparticles, rendering the surface area larger and subsequently the CMD content higher.

Instead of a polymer, small molecules such as citrate or glycine were adopted to create surface charges on MION for production of nMION and pMION [17,23,24,25]. These charged nanoparticles remained under the size threshold essential for retaining superparamagnetic behaviors.

The self-assembly technology was carefully optimized. Low concentrations of nMION or pMION were employed, for instance, to lower the probability of the occurrence of precipitation. Ultrasonication is also important to maintaining uniform size as it assists the dispersion and homogenization of freshly added nMION. Addition order of nMION into pMION was specifically taken as well. Since we wanted to produce a core of net positive charge, if pMION

were added to nMION, the net charge in the system would have to cross from negative to positive, or pass the charge balance point, at which point it would precipitate.

The size of both cores and coated particles could be readily regulated by simply varying the ratio of pMION to nMION (Fig 3b). When adding nMION into pMION, positively charged pMION self-assemble around nMION via electrostatic interaction, resulting in size increase. As more nMION is added, larger-sized, positively-charged particles will assemble around the nMION further and result in sharp size increase. An obvious inflection point was observed in the size variation curve (Fig 3b). With the further increase in the ratio of nMION to pMION, the net positive charge in the system would decrease and not be strong enough to stabilize the large particles. Ultimately, precipitation occurred. Thus this technique provided a convenient route for the synthesis of magnetic nanoparticles of controlled size.

CMD, a biocompatible polymer that has already been used widely as a matrix material to synthesize magnetic nanoparticles, was chosen as the coating material. Another advantage of the CMD coating lies in the presence of abundant carboxylate groups on the polymer that provide functional anchors for subsequent conjugation with drug molecules or biological agents.

It is also important to determine the effect of self-assembly and the coating process on magnetic properties. For a superparamagnetic material, the demagnetization curve normally follows the magnetization profile closely with no remnant magnetization being observed [26]; an essential property in achieving MRI and magnetic-mediated tumor targeting. This observation in Figure 6 indicated that all the processes involved in the synthesis, including MION surface modifications, self-assembly of the $\pm$ MION, and polymer coating did not alter the superparamagnetic behavior of the produced magnetic nanoparticles.

The magnetophoretic mobility of these nanoparticles was comparatively assessed by an innovative method designed here. The turbidity of the suspension was monitored using a spectrophotometer at the wavelength of 360 nm (see Fig s1 and s2 in “Supporting Information”). The quicker the nanoparticles migrated under the influence of the magnetic field, the faster the turbidity decreased. The three test nanoparticles in Figure 7 all possessed similar hydrodynamic sizes. Therefore, the superior mobility of CMD-coated100 was attributed primarily to the core-shell structure. As previously discussed, the specifically designed synthesis strategy in producing core-shell nanoparticles would minimize polymer embedding within the interior part of the particles, and consequently maximize the response of these particles toward the magnetic field.

The animal studies were performed following the protocols approved by University Committee on Use and Care of Animals (UCUCA) of University of Michigan, USA. These findings suggested that CMD-coated100 could be employed as a MRI-visible drug carrier to potentially deliver therapeutics to the region of a brain tumor lesion. Further extensive animal investigation utilizing drug-attached CMD-coated100 to achieve MRI-synchronized drug therapy in treating a brain tumor is currently underway in our laboratory.

One of the governing factors concerning the possibility for future clinical applications of magnetic nanoparticles lies in their stability upon usage and storage, because these particles have a strong self-aggregation tendency in medium caused by their nano-scale nature and magnetic property. The CMD-coated100 exhibited superior stability. It was noted that CMD-coated100 and fluidMAG-CMX were made by the same coating material, negatively charged carboxymethyldextran. Hence, the charge repulsion could be the mechanism responsible for the enhanced stability of these two types of magnetic nanoparticles. On the other hand, fluidMAG-D was reported to be manufactured by starch coating, which lacked charges for repulsion thereby leading to the demise in stability.

5. Conclusion

For the purpose of achieving the dual functions of MRI and magnetic targeting, a strategy was developed to synthesize CMD-coated, iron oxide based core-shell magnetic nanoparticles with controlled sizes. Characterization demonstrated that nanoparticles thus synthesized possessed a shell-core structure consisting of a thin polymer shell coating on a self-assembled magnetic core made of agglomeration of superparamagnetic, monocrystalline iron oxide nanoparticles (MION). Because of dearth of polymer material in the interior part of the particles, CMD-coated100 displayed a 3- to 4-fold enhancement in magnetophoretic mobility over the widely used commercial products fluidMAG-CMX and fluidMAG-D. Preliminary *in vivo* investigation also confirmed the suitability of utilizing these nanoparticles in yielding distinctive MR imaging of the brain tumor in a rat model. In addition, these nanoparticles exhibited a remarkable stability in simulated body fluid. Overall, CMD-coated100 could potentially be employed as a drug carrier to achieve a MRI-synchronized magnetic tumor targeting and therapy. Further research toward this direction is currently in progress in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by NIH R01 Grants CA114612 and NS066945. This research was also partially sponsored by Grant R31-2008-000-10103-01 from the World Class University (WCU) project of the MEST and NRF of South Korea. Victor C. Yang is currently a Participating Faculty in the Department of Molecular Medicine and Biopharmaceutical Sciences, College of Medicine & College of Pharmacy, Seoul National University, South Korea. In addition, the support from Hubei Natural Science Fund for Distinguished Young Scholars (2005ABB031), Hubei, China, of which Dr. Faquan Yu was a recipient, is also acknowledged.

References

1. Maeda H, Bharate GY, Daruwala J. Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect. *Eur J Pharm Biopharm* 2009;71(3):409–419. [PubMed: 19070661]
2. Chandran SS, Banerjee SR, Mease RC, Pomper MG, Denmeade SR. Characterization of a targeted nanoparticle functionalized with a urea-based inhibitor of prostate-specific membrane antigen (PSMA). *Cancer Biol Ther* 2008;7(6):974–982. [PubMed: 18698158]
3. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Control Release* 2000;65(1–2):271–284. [PubMed: 10699287]
4. Tina I, Lee J. Current state and future applications of active targeting in malignancies using superparamagnetic iron oxide nanoparticles. *Cancer Biomark: Sectoin A of Disease Markers* 2009;5(2):99–107.
5. Reddy GR, Bhojani MS, McConville P, Moody J, Moffat BA, Hall DE, et al. Vascular targeted nanoparticles for imaging and treatment of brain tumors. *Clin Cancer Res* 2006;12(22):6677–6686. [PubMed: 17121886]
6. Chertok B, Moffat BA, David AE, Yu F, Bergemann C, Ross BD, et al. Iron oxide nanoparticles as a drug delivery vehicle for MRI monitored magnetic targeting of brain tumors. *Biomaterials* 2008;29(4):487–496. [PubMed: 17964647]
7. Jeong U, Teng X, Wang Y, Yang H, Xia Y. Superparamagnetic colloids: controlled synthesis and niche applications. *Adv Mater* 2007;19(1):33–60.
8. Tartaj P, Morales MP, Veintemillas-Verdaguer S, Gonzalez-Carreno T, Serna CJ. The preparation of magnetic nanoparticles for applications in biomedicine. *J Phys D: Appl Phys* 2003;36:182–197.
9. Frenkel J, Doefman J. Spontaneous and induced magnetization in ferromagnetic bodies. *Nature* 1930;126:274–275.

10. Sun C, Lee JSH, Zhang M. Magnetic nanoparticles in MR imaging and drug delivery. *Adv Drug Deliv Rev* 2008;60(11):1252–1265. [PubMed: 18558452]
11. Morales MP, Andres-Verges M, Veintemillas-Verdaguer S, Montero MI, Serna CJ. Structural effects on the magnetic properties of γ -Fe₂O₃ nanoparticles. *J Magn Magn Mater* 1999;203(1–3):146–168.
12. Josephson L, Lewis J, Jacobs P, Hahn PF, Stark DD. The effects of iron oxides on proton relaxivity. *Magn Reson Imaging* 1988;6(6):647–653. [PubMed: 2850434]
13. Bean CP, Livingston JD. Superparamagnetism. *J Appl Phys Suppl* 1959;30(4):120–129.
14. Neuberger T, Schöpf B, Hofmann H, Hofmann M, von Rechenberg B. Superparamagnetic nanoparticles for biomedical applications: possibilities and limitations of a new drug delivery system. *J Magn Magn Mater* 2005;293(1):483–496.
15. Longmire M, Choyke PL, Kobayashi H. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. *Nanomed* 2008;3(5):703–717.
16. Kim DK, Zhang Y, Voit W, Rao KV, Muhammed M. Synthesis and characterization of surfactant-coated superparamagnetic monodispersed iron oxide nanoparticles. *J Magn Magn Mater* 2001;225(1–2):30–36.
17. Yu F, Yang VC. Size-tunable synthesis of stable superparamagnetic iron oxide nanoparticles for potential biomedical applications. *J Biomed Mater Res A* 2010;92(4):1468–1475. [PubMed: 19402138]
18. Fauconnier N, Bée A, Roger J, Pons JN. Synthesis of aqueous magnetic liquids by surface complexation of maghemite nanoparticles. *J Mol Liq* 1999;83:233–242.
19. Campos AFC, Cotta TAPG, Tourinho FA, Depeyrot J. Speciation diagrams as a useful tool to improve nanoparticle surface–ligand complexation for biocompatible magnetic colloids. *Progr Colloid Polym Sci* 2004;126:86–89.
20. Hingston FJ, Atkinson RJ, Posner AM, Quirk JP. Specific adsorption of anions. *Nature* 1967;215:1459–1461.
21. Yu S, Chow GM. Carboxyl group (–CO₂H) functionalized ferrimagnetic iron oxide nanoparticles for potential bio-application. *J Mater Chem* 2004;14(8):2781–2786.
22. Harris LA, Goff JD, Carmichael AY, Riffle JS, Harburn JJ, St. Pierre TG, et al. Magnetite nanoparticle dispersions stabilized with triblock copolymers. *Chem Mater* 2003;15(6):1367–1377.
23. Fauconnier N, Bee A, Massart R, Dardoize F. Direct determination of organic acids in a ferrofluid (γ -Fe₂O₃) by high-performance liquid chromatography. *J Liq Chromatogr R T* 1996;19:783–797.
24. Laaksonen P, Kivioja J, Paananen A, Kainlahti M, Kontturi K, Ahopelto J, et al. Selective nanopatterning using citrate-stabilized Au nanoparticles and cysteine-modified amphiphilic protein. *Langmuir* 2009;25(9):5185–5192. [PubMed: 19253945]
25. Yu F, Huang Y, Cole AJ, Yang VC. The artificial peroxidase activity of magnetic iron oxide nanoparticles and its application to glucose detection. *Biomaterials* 2009;30(27):4716–4722. [PubMed: 19515418]
26. Kim T, Shima M. Reduced magnetization in magnetic oxide nanoparticles. *J Appl Phys* 2007;101(9):09M516–09M516-3.

Figure 1. Schematic illustration of the synthesis of “core-shell” nanoparticles with dual nature of superior magnetophoretic mobility and superparamagnetism.

Figure 2.
TEM image of MION for the generation of nMION and pMION.

Figure 3.

Changes on (a) zeta potentials; and (b) sizes of the assembled MION cores as well as of the CMD-coated core-shell nanoparticles. In (a), MION cores were positively charged when an excess amount of pMION was used (▲). After coating these MION cores with CMD, however, zeta potentials immediately turned into negative values (■). In (b), both the sizes of the magnetic cores (▲) and of the CMD-coated nanoparticles (■) increased by increasing the ratio of nMION over pMION.

Figure 4. STEM-BF images of (a) CMD-coated100 and (b) fluidMAG-CMX. Changes of carbon (blue curve) and iron (red) contents were determined via elemental analysis by EDS linescan along the black line path. The x-axis corresponded to the linescan position whereas y-axis represented the relative abundance of carbon and iron elements.

Figure 5. Thermogravimetric analysis (TGA) results of magnetic nanoparticles including the self-assembled nMION/pMION core, CMD-coated100, fluidMAG-CMX, and fluidMAG-D.

Figure 6. Superparamagnetic properties measured by SQUID. Both MION and CMD-coated100 particles displayed superparamagnetic behavior in that magnetization and demagnetization curves completely overlapped. In addition, both particles also demonstrated almost identical magnetic susceptibility and saturation magnetization, suggesting that the applied synthesis strategy did not alter the magnetic properties.

Figure 7.

Magnetophoretic mobility of the magnetic nanoparticles. The $t_{1/2}$ values, which was defined as the time required to reduce the turbidity of the nanoparticle suspension by 50%, for CMD-coated100 (bottom curve), fluidMAG-CMX (middle curve), and fluidMAG-D (top curve), were estimated to be 40min, 125min and 155 min, respectively. All of the nanoparticles possessed similar hydrodynamic sizes (~100 nm).

(a)

(b)

Figure 8. MRI images of the brain tumors in (a) control rat without magnetic targeting; and (b) experimental rat with magnetic targeting for 30 min after tail-vein injection of CMD-coated100.

Figure 9. Stability of magnetic nanoparticles in a simulated body fluid containing 50% (v/v) calf serum in pH7.4 PBS. Absorbance at 370 nm was used to monitor the stability of these test nanoparticles.