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Spatial Organization of Superparamagnetic Iron Oxide Nanoparticles in/on Nano/Microsized Carriers Modulates the Magnetic Resonance Signal

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Abstract

Superparamagnetic iron oxide nanoparticles (SPIONs) are often encapsulated into drug-carrying nano/microsized particles for simultaneous magnetic resonance (MR) imaging and treatment of diseased tissues. Unfortunately, encapsulated SPIONs may have a limited ability to modulate the T_2 -weighted relaxation of water protons, but this insight has not been examined systematically. This study demonstrates that SPIONs immobilized on 200 nm diameter poly(lactic-co-glycolic acid) (PLGA) nanoparticles using Pickering emulsification present 18-fold higher relaxivity than encapsulated SPIONs and 1.5-fold higher relaxivity than free SPIONs. In contrast, the SPIONs

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Supporting Information

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immobilized on 10 μm diameter PLGA particles exhibit a minor increase in MR relaxivity. This interesting finding will significantly impact current efforts to synthesize and assemble advanced MR contrast agents.

Graphical Abstract

INTRODUCTION

Diagnostic probes that carry and transport superparamagnetic iron oxide nanoparticles (SPIONs) have emerged to detect and mark pathologic tissues.^{1,2} Particularly, SPIONs with an average diameter of 5–10 nm enhance the contrast of magnetic resonance (MR) images because of their capability to modulate T_2 -weighted relaxation of water protons and in turn to increase negative contrast of surrounding tissues.^{3,4} SPIONs can also tune T_1 -weighted relaxation of water protons and subsequently increase positive contrast of surrounding tissues.⁵ SPIONs can be further loaded into polymeric nano or micro-sized drug carriers as part of an image-guided molecular therapy.^{6–8}

A common method to prepare SPION-laden particles involves the use of a double emulsion procedure. To encapsulate SPIONs within a polymeric particle with this technique, SPIONs and a water-insoluble polymer are first dissolved into an oil phase. The SPION–polymer solution is then vigorously mixed with an aqueous solution containing a surfactant. After removing the oil phase via evaporation, a SPION-loaded polymeric particle is formed. Despite the ubiquity of this method,^{9,10} these co-encapsulation approaches limit the interaction of SPIONs with water protons of tissue fluids, which might result in reduced magnetic resonance relaxivity. Therefore, it is necessary to study the potential effects of the spatial organization of SPIONs in/on nano- or microcarriers.¹¹

This study demonstrates an advanced method to retain or increase the magnetic resonance relaxivity of SPIONs in drug carriers. We hypothesized that spatially organizing SPIONs on nano- and micro-sized carriers could orchestrate the MR relaxivity of SPIONs and the contrast level of MR images. We examined this hypothesis by immobilizing SPIONs with an average diameter of 10 nm on poly(lactide-*co*-glycolic acid) (PLGA) particles with controlled diameters (i.e., 260 nm or 2 μm) by using the Pickering emulsification process. Unlike a classical double emulsion stabilized by molecular surfactants, the Pickering

emulsion is stabilized by solid nanoparticles, which localize to the interface of the oil and water phases.¹²

Although certain studies have considered the use of SPIONs to stabilize the Pickering emulsion,¹³ no systematic study has been undertaken to compare the differences in MR relaxivity between a SPION-containing particle prepared via a double emulsion and a SPION-coated particle prepared via the Pickering emulsion. In this study, we analyzed the spatial organization of SPIONs on the surface and in the interior of PLGA particles using optical and electron microscopy and surface spectroscopy. The MR relaxivity of SPIONs loaded on or in PLGA nano- and microparticles was determined from MR images of a phantom gel (i.e., an agarose gel loaded with SPIONs).

EXPERIMENTAL SECTION

Synthesis of Iron (Fe)–Oleate Complex.

The Fe-oleate complex was prepared by reacting iron chlorides and sodium oleate. Briefly, 10.8 g of iron chlorides ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 40 mmol, Sigma-Aldrich) and 36.5 g of sodium oleate (120 mmol, TCI) were dissolved in a solvent mixture (80 mL of ethanol, 60 mL of deionized (DI) water, and 140 mL of hexane). The resulting solution was heated to 70 °C and stirred for 4 h. Once the reaction was completed, the upper organic layer containing the Fe-oleate complex was washed three times with 30 mL of DI water in a separatory vessel. After washing, hexane was evaporated to collect the Fe-oleate complex in a waxy solid form.

Synthesis of Oleic Acid-Substituted Superparamagnetic Iron Oxide Nanoparticles (OA-SPIONs).

To prepare highly monodisperse nanoparticles, OA-SPIONs were synthesized via the thermal decomposition of iron oleate, as previously reported.^{14,15} First, 3.6 g of Fe-oleate and 0.9 mL of oleic acid (Sigma-Aldrich, 90%) were dissolved in 25 mL of 1-octadecene (Sigma-Aldrich, 90%). After *thorough vortexing and sonication, the mixture was added to a new three-neck glass flask. Using a temperature controller (CSC32 Omega temp controller, Type J)*, the reaction mixture was then brought from room temperature to 320 °C under reflux. The mixture was held at 320 °C for 30 min, at which point the mixture turned from reddish-brown to black. A constant stream of nitrogen gas was provided to ensure an inert environment. Afterward, the reaction mixture was cooled back to room temperature. SPIONs were precipitated out with approximately 50 mL of ethanol. Using a 40 lb pull force neodymium iron boron (NdFeB) magnet (K&J Magnetics), the SPIONs were removed from the reaction mixture and then redispersed in hexane. The SPIONs in hexane were then precipitated in ethanol again at a 2:1 (ethanol/hexane) volumetric ratio. Afterward, SPIONs were centrifuged at 10 000 rpm (Thermo Scientific Sorvall RC 6 Plus) for at least 10 min. The precipitated SPIONs were redispersed in hexane and then washed at least two more times. Afterward, SPIONs were air-dried and then redispersed in chloroform at approximately 10 mg/mL.

Synthesis of Polyethylenimin-SPIONs (PEI-SPIONs).

PEI-SPIONs were fabricated using a previously reported protocol.¹⁶ To prepare PEI-coated SPIONs, PEI (branched, 25 000 Da, Sigma-Aldrich) was first dissolved in chloroform at 10 mg/mL. The reaction mixture was sonicated for 1–2 min and then placed on a rotating shaking table for at least 48 h. Afterward, about 200 mL of hexane was added to the reaction mixture, which was then precipitated using a 40 lb pull force NdFeB magnet. The precipitated PEI-SPIONs were dried overnight in a chemical hood. Finally, the PEI-SPIONs were dispersed thoroughly in DI water and then washed several times with a centrifugal filter (Millipore Amicon, molecular weight cutoff 100 000 Da) to remove any excess PEI. After thorough washing, the PEI-SPION solution was sonicated for 2–3 min in a bath sonicator (Fisher Scientific) to fully disperse the particles.

Preparation of SPION-Coated Poly(lactic-co-glycolic acid) (PLGA) Micro/Nanoparticles.

First, 10 mL of aqueous PEI-SPIONs (13 mg/mL) was added to the PLGA solution as the oil phase (100 mg of PLGA in 1 mL of methylene chloride (Sigma-Aldrich)) at room temperature. Then, the mixture was emulsified by vortexing for 3 min. Separately, to prepare the PLGA nanoparticles, the mixture was emulsified by sonication for 1 min. The oil phase was evaporated for 20 min using a rotary evaporator. The resulting PLGA micro/nanoparticles were recovered by centrifugation (2000 rpm, 10 min). After centrifugation, the supernatant was discarded, and the precipitate was washed twice with DI water.

Preparation of SPION-Encapsulated PLGA Micro/Nanoparticles.

First, 100 mg of PLGA was added to the 1 mL of OA-SPIONs (13 mg/mL) suspension in chloroform at room temperature. The organic phase was added to 10 mL of DI water, and the mixture was emulsified by vortexing for 3 min. Then, the mixture was added to 50 mL of 1 wt % poly(vinyl alcohol) (PVA, Sigma-Aldrich) solution and stirred for 4 h to evaporate organic solvents. To prepare PLGA nanoparticles, the mixture was emulsified by sonication for 1 min.

Characterization of SPION-Coated or Encapsulated PLGA Nanoparticles.

The size distribution of PLGA nanoparticles was determined using dynamic light scattering (DLS, Malvern Zetasizer Nano ZS). Separately, the nanoparticles were imaged by transmission electron microscopy (TEM) using a JEOL 2100 TEM with an accelerating voltage of 200 kV. Samples were prepared by drying suspensions on holey carbon-coated carbon grids. ImageJ software was subsequently used to determine the size distribution of the SPION particles.

Relaxivity Measurements of SPION-Loaded PLGA Particles.

Prior to MR imaging, the PLGA nano/microparticles loaded with SPIONs on the surface or within the particle were dispersed in an agarose gel (15–20 mg/mL) at different dilution factors. The resulting gels were analyzed with a 3T Siemens Magnetom Trio MR head scanner (Siemens AG) or a ultrahigh field, 14.1T upright, small-bore MRI scanner (Varian). T_2 relaxation time was determined using a spin echo pulse sequence with TR = 1200 ms and

TE varied from 12 to 490 ms. Signal intensity (S) of the phantom gels was measured with ImageJ software and fit to eq 1

$$S(TE) = S_0 \times e^{TE/T_2} + b \quad (1)$$

where S_0 is signal at equilibrium and b is the background noise level. Molar relaxivity (r_2) was then determined by linear regression using eq 2

$$1/T_2 = 1/T_{2, \text{water}} + r_2[\text{Fe}] \quad (2)$$

where $T_{2, \text{water}}$ is the relaxation time of water and $[\text{Fe}]$ is the total iron concentration in mM. To determine iron content, samples were digested in a concentrated nitric acid solution, diluted in DI water, and then analyzed by inductively coupled plasma optical emission spectroscopy (PerkinElmer Optima 2000 DV).

RESULTS AND DISCUSSION

SPIONs were immobilized on PLGA particle surface by using the Pickering emulsification method.¹³ First, the oleic acid-coated SPIONs (OA-SPIONs) were coated with poly(ethylene imine) (PEI). The resulting particles, denoted as PEI-SPIONs, were dispersed in aqueous media and remained stable at room temperature for several days (Figure 1a). According to an analysis of transmission electron microscopy (TEM) images, the average diameter of PEI-SPIONs was approximately 10 nm, same as the unmodified OA-SPIONs (Figures 1b and S1). Iron oxide particles within this size range typically possess a single magnetic domain per particle. As such, these particles possess a high magnetic susceptibility, thus making them ideal for use in vitro imaging and drug delivery applications.^{17,18} Separately, PLGA was dissolved in methylene chloride. Finally, the PEI-SPION suspension and the PLGA-methylene chloride solution were mixed together via vortex mixing (Figure 2a).

The aqueous suspension of the resulting SPION-immobilized microparticles was brown, similar to the pure PEI-SPION suspension. To examine the spatial distribution of SPIONs in the PLGA particles, the suspended particles were incubated with Prussian blue reagent that reacts with SPIONs and in turn generates a dark red color (Figure 2b). According to the optical microscopic images, SPIONs colored in dark red were found exclusively on the surface of PLGA microparticles with an average diameter of 2 μm (Figure 2c).

Due to the presence of SPIONs on the particle surface, the average ζ -potential of the SPION-coated PLGA microparticles was 51 mV, whereas that of pure PLGA particles is -7 mV (Figure S2a). The SPION-tethered PLGA microparticles were dispersed in aqueous media more uniformly than the pure PLGA particles. Moreover, the SPION-immobilized PLGA particles remained stable in aqueous media at least for 20 days, likely because of the increased electrostatic repulsion between particles.

Sonication of the mixture of the PEI-SPION suspension and the PLGA-methylene chloride solution instead of vortex mixing resulted in 200 nm diameter PLGA nanoparticles (Figures 2d and S3). According to TEM and scanning electron microscopy images, SPIONs were localized on the surface of PLGA nanoparticles. An energy-dispersive X-ray spectroscopy

analysis further confirmed the presence of Fe on the PLGA nanoparticle surface (Figure S2b). These nanoparticles were suspended stably in both deionized water and blood-mimicking, serum-supplemented media over 1 month, with no visible sedimentation.

The mass of SPIONs immobilized on the PLGA particles was approximately 95 $\mu\text{g Fe/mg}$ PLGA particles, according to the measurement from inductively coupled plasma spectroscopy. With the mass of SPIONs, the surface coverage with SPIONs (C_{surface}) was calculated from the following equation (eq 3)

$$C_{\text{surface}} = \frac{m_{\text{SPION}} d_{\text{PLGA}}}{4 \rho_{\text{SPION}} d_{\text{SPION}} V_{\text{SPION}}} \quad (3)$$

where m_{SPION} is the mass of SPIONs, V_{PLGA} is the volume of the dispersed PLGA particles, ρ_{SPION} is the density of the SPION, and d_{SPION} and d_{PLGA} are the diameter of the SPION and that of PLGA particles, respectively. The calculated surface coverage was approximately 0.21 for both the SPION-immobilized PLGA nano- and microparticles.

Separately, OA-SPIONs were encapsulated into PLGA microparticles with the double emulsification method. This process was conducted by mixing the aqueous solution of poly(vinyl alcohol) (PVA) with a mixture of PLGA and OA-SPIONs in methylene chloride (Figures 1b–2 and 3a). The aqueous suspension of the resulting particles showed the same white color as an unmodified PLGA particle suspension (Figure 3b).

This particle suspension was further incubated with the Prussian blue reagent to examine the spatial distribution of SPIONs in the PLGA particles. According to optical microscopy images, the PLGA particle interior was colored dark red, which indicated the localization of SPIONs within particles (Figure 3c). The ζ -potential of PLGA particles was -7 mV, similar to that of unmodified PLGA microparticles (Figure S2a). This negative ζ -potential assures the minimal presence of SPIONs on the surface of PLGA particles. In addition, the suspension rapidly formed aggregates.

Sonication of the mixture of PVA in water and PLGA-SPIONs in methylene chloride led to SPION encapsulation within PLGA nanoparticles. According to TEM images, the SPIONs were not found on the PLGA nanoparticle surface. The PLGA shell does not allow electron penetration required for the TEM image, thus shadowing the SPIONs in the particles (Figure 3d). No significant Fe peak of the energy-dispersive X-ray spectroscopy was found on the PLGA particle surface as well (data not shown).

Next, we evaluated the extent by which the immobilization of SPIONs on PLGA particle surfaces enhances the MR relaxivity compared with the encapsulation of SPIONs in the PLGA particles. The MR relaxivity of nanoparticles was quantified by measuring the transverse relaxation times (T_2) at varying Fe concentrations using a 14T MR system and further fitting it to a linear regression curve described in the Experimental Section (Figure S4). The MR relaxivity of SPIONs on the PLGA microparticles was almost 3-fold higher than that of SPIONs encapsulated in the microparticles (Figure 4a).

When SPIONs were localized onto the PLGA surface, decreasing the diameter of PLGA particles from 2 μm to 260 nm led to an almost 6-fold increase of the MR relaxivity (Figure 4b). In contrast, the MR relaxivity of SPIONs encapsulated within the PLGA particles was independent of the particle size. Moreover, the MR relaxivity of SPIONs on the PLGA nanoparticles was 18-fold higher than that of SPIONs encapsulated in PLGA nanoparticles (Figures S5 and 4b). In addition, the SPIONs immobilized on the PLGA nanoparticles displayed a 1.5-fold higher MR relaxivity than the free PEI-SPIONs. Similar trends are noted for free PEI-SPION particles and SPION-coated PLGA nanoparticles tested using a 3T MRI (Figure S6).

According to phantom images, at a low iron concentration of 0.06 mM, the SPIONs immobilized on PLGA nanoparticles resulted in a higher negative contrast against background than those immobilized on PLGA microparticles as well as free PEI-SPIONs (Figure 5). The SPIONs encapsulated in PLGA particles produced an insignificant negative contrast against background regardless of the diameter of PLGA particles. For the SPIONs immobilized on PLGA nanoparticles, the water proton signal was completely dephased at Fe concentration of 0.2 mM, whereas PEI-SPIONs and SPIONs encapsulated in the PLGA nanoparticles showed moderate negative contrast at equivalent and higher Fe concentrations (Figure 6). Phantom gel images taken at different Fe concentrations are presented in Figure S7 for further reference.

In summary, this study demonstrates that the spatial organization of SPIONs in nano-or micro-sized PLGA particles plays an important role in regulating MR relaxivity of SPIONs. Relaxivity depends on the local diffusion of water protons with respect to SPIONs, and organizing SPIONs in a nano-or micro-sized carrier system influences relaxivity accordingly. Our results demonstrated that SPIONs immobilized on PLGA particle surfaces through the Pickering emulsification exhibited higher relaxivity values than SPIONs encapsulated within the PLGA particles as well as free PEI-coated SPIONs. This trend can be elucidated by considering the relative motion of water protons surrounding the SPION cores in each particle system.

Encapsulating SPIONs in the PLGA core likely reduced the exchange between water protons in the exterior environment and the SPIONs, due in part to the hydrophobicity of the PLGA polymer and the density of PLGA used in the emulsion. In addition, the SPIONs encapsulated within the PLGA particles retain an oleic acid coating at their surface, which further prevents water diffusion near the SPION surface. In contrast, the free PEI-coated SPIONs and the SPIONs on the PLGA particles have their oleic acid coating replaced with a hydrophilic PEI polymer, thus improving the degree of water exchange near the SPION surface.¹⁶

Immobilizing SPIONs on the PLGA particle surface also ensures that SPIONs will have increased exposure to the water molecules surrounding the emulsion, thus leading to additional improvements in relaxivity. In the resulting phantom images, SPIONs encapsulated within a particle display the least amount of contrast. PEI-SPIONs display a moderate amount of negative contrast, and SPIONs immobilized on the particle surface produce the most negative contrast. Taken together, SPIONs on the Pickering emulsion

particles produce a significantly higher relaxivity than both the SPIONs within the double emulsion-based particles and the free PEI-SPIONs.

Moreover, our results demonstrate that controlling the size of the Pickering emulsion particles leads to changes in relaxivity. This trend follows previous studies whereby clustering multiple SPIONs together resulted in higher relaxivity compared to individually stabilized particles.¹⁹ Briefly speaking, the magnetic fields associated with individual SPIONs are small enough such that water protons will easily diffuse away from the SPION's field and into a different magnetic environment during relaxation. As SPIONs are clustered together, water protons near the SPIONs will diffuse in a single magnetic environment during relaxation, thus increasing relaxivity up to an optimal point termed the "static dephasing regime". At this point, relaxivity is independent of SPION cluster size.^{19–21}

Beyond this optimum, larger particles will rapidly dephase water protons prior to the application of an echo pulse. In this region (termed the "echo-limited regime"), relaxivity decreases as cluster size increases.²² In our study, SPIONs immobilized on PLGA nanoparticles demonstrate higher relaxivity than SPIONs immobilized on PLGA microparticles. We propose that the SPION-coated PLGA nanoparticles are near the optimum relaxivity associated with the static dephasing regime, and that further increases of the SPION-PLGA carrier size will decrease relaxivity. This trend can be elucidated with further study.

Starting from nanosized Pickering emulsions, we believe that creating ultrasensitive SPION particle systems might be possible with further optimization. Additional increases in relaxivity might be obtained by using high relaxivity zinc-doped SPION cores or by modulating the SPION density on the Pickering emulsion surface.²³ These potential improvements can be confirmed in future studies.

CONCLUSIONS

In conclusion, this study discovers that spatial organization of SPIONS modulated with polymeric carriers plays a significant role in improving the negative contrast of a T_2 -weighted MR image. The SPIONs were localized either on the PLGA particles through the Pickering emulsification or within the PLGA particles through double emulsification. SPIONS tethered to the surface of PLGA particles exhibited a higher MR relaxivity than those encapsulated in the same particles, due to enhanced access of water protons to the SPIONs. The MR relaxivity of SPION was further increased by decreasing the diameter of PLGA particles from 2000 to 260 nm, likely due to the clustering effect of SPIONs. Therefore, the PLGA nanoparticles tethered with SPIONs served to generate a high negative contrast of MR images with the reduced number of SPIONs compared to other conditions. We envisage that the results of this study would be useful for improving diagnostic capability of SPIONs while decreasing the dose.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- (1). Lee JH; Schneider B; Jordan EK; Liu W; Frank JA Synthesis of complexable fluorescent superparamagnetic iron oxide nanoparticles (FL SPIONs) and cell labeling for clinical application. *Adv. Mater* 2008, 20, 2512–2516. [PubMed: 19578472]
- (2). Qiao R; Yang C; Gao M Superparamagnetic iron oxide nanoparticles: from preparations to in vivo MRI applications. *J. Mater. Chem* 2009, 19, 6274–6293.
- (3). Pösel E; Kloust H; Tromsdorf U; Janschel M; Hahn C; Maßlo C; Weller H Relaxivity optimization of a PEGylated iron-oxide-based negative magnetic resonance contrast agent for T₂-weighted spin-echo imaging. *ACS Nano* 2012, 6, 1619–1624. [PubMed: 22276942]
- (4). Paquet C; de Haan HW; Leek DM; Lin H-Y; Xiang B; Tian G; Kell A; Simard B Clusters of superparamagnetic iron oxide nanoparticles encapsulated in a hydrogel: a particle architecture generating a synergistic enhancement of the T₂ relaxation. *ACS Nano* 2011, 5, 3104–3112. [PubMed: 21428441]
- (5). Wei H; Bruns OT; Kaul MG; Hansen EC; Barch M; Wisniowska A; Chen O; Chen Y; Li N; Okada S; et al. Exceedingly small iron oxide nanoparticles as positive MRI contrast agents. *Proc. Natl. Acad. Sci. U.S.A* 2017, 2325–2330. [PubMed: 28193901]
- (6). Yu MK; Jeong YY; Park J; Park S; Kim JW; Min JJ; Kim K; Jon S Drug-loaded superparamagnetic iron oxide nanoparticles for combined cancer imaging and therapy in vivo. *Angew. Chem* 2008, 120, 5442–5445.
- (7). Bourrinet P; Bengele HH; Bonnemain B; Dencausse A; Idee J-M; Jacobs PM; Lewis JM Preclinical safety and pharmacokinetic profile of ferumoxtran-10, an ultrasmall superparamagnetic iron oxide magnetic resonance contrast agent. *Invest. Radiol* 2006, 41, 313–324. [PubMed: 16481915]
- (8). Lv Y; Ding G; Zhai J; Guo Y; Nie G; Xu L A superparamagnetic Fe₃O₄-loaded polymeric nanocarrier for targeted delivery of evodiamine with enhanced antitumor efficacy. *Colloids Surf., B* 2013, 110, 411–418.
- (9). Niu C; Xu Y; An S; Zhang M; Hu Y; Wang L; Peng Q Near-infrared induced phase-shifted ICG/Fe₃O₄ loaded PLGA nanoparticles for photothermal tumor ablation. *Sci. Rep* 2017, 7, No. 5490.
- (10). Yang F; Li Y; Chen Z; Zhang Y; Wu J; Gu N Superparamagnetic iron oxide nanoparticle-embedded encapsulated microbubbles as dual contrast agents of magnetic resonance and ultrasound imaging. *Biomaterials* 2009, 30, 3882–3890. [PubMed: 19395082]
- (11). Veisheh O; Gunn JW; Zhang M Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging. *Adv. Drug Delivery Rev* 2010, 62, 284–304.
- (12). Chevalier Y; Bolzinger M-A Emulsions stabilized with solid nanoparticles: Pickering emulsions. *Colloids Surf., A* 2013, 439, 23–34.
- (13). Zhou J; Qiao X; Binks BP; Sun K; Bai M; Li Y; Liu Y Magnetic Pickering emulsions stabilized by Fe₃O₄ nanoparticles. *Langmuir* 2011, 27, 3308–3316. [PubMed: 21344923]
- (14). Park J; An K; Hwang Y; Park J-G; Noh H-J; Kim J-Y; Park J-H; Hwang N-M; Hyeon T Ultra-large-scale syntheses of monodisperse nanocrystals. *Nat. Mater* 2004, 3, 891. [PubMed: 15568032]
- (15). Sun S; Zeng H; Robinson DB; Raoux S; Rice PM; Wang SX; Li G Monodisperse mfe₂o₄ (m = fe, co, mn) nanoparticles. *J. Am. Chem. Soc* 2004, 126, 273–279. [PubMed: 14709092]

- (16). Duan H; Kuang M; Wang X; Wang YA; Mao H; Nie S Reexamining the effects of particle size and surface chemistry on the magnetic properties of iron oxide nanocrystals: new insights into spin disorder and proton relaxivity. *J. Phys. Chem. C* 2008, 112, 8127–8131.
- (17). Tong S; Hou S; Zheng Z; Zhou J; Bao G Coating optimization of superparamagnetic iron oxide nanoparticles for high T2 relaxivity. *Nano Lett.* 2010, 10, 4607–4613. [PubMed: 20939602]
- (18). Wahajuddin SA Superparamagnetic iron oxide nanoparticles: magnetic nanoplateforms as drug carriers. *Int. J. Nanomed* 2012, 7, 3445.
- (19). Smith CE; Lee J; Seo Y; Clay N; Park J; Shkumatov A; Ernenwein D; Lai M-H; Misra S; Sing CE Worm-Like Superparamagnetic Nanoparticle Clusters for Enhanced Adhesion and Magnetic Resonance Relaxivity. *ACS Appl. Mater. Interfaces* 2017, 9, 1219–1225. [PubMed: 27989109]
- (20). Smith CE; Ernenwein D; Shkumatov A; Clay NE; Lee J; Melhem M; Misra S; Zimmerman SC; Kong H Hydrophilic packaging of iron oxide nanoclusters for highly sensitive imaging. *Biomaterials* 2015, 69, 184–190. [PubMed: 26291408]
- (21). Peng E; Wang F; Xue JM Nanostructured magnetic nanocomposites as MRI contrast agents. *J. Mater. Chem. B* 2015, 3, 2241–2276. [PubMed: 32262055]
- (22). Zanganeh S; Jim Q; Jafari T; Khakpash N; Erfanzadeh M; Spitler R The Evolution of Iron Oxide Nanoparticles for use in Biomedical MRI Applications. *SM J. Clin. Med. Imaging* 2016, 2 (1), 1005.
- (23). Jang J.t; Nah H; Lee JH; Moon SH; Kim MG; Cheon J Critical enhancements of MRI contrast and hyperthermic effects by dopant-controlled magnetic nanoparticles. *Angew. Chem* 2009, 121, 1260–1264.

Figure 1. (a) Schematic description of the preparation of PEI-conjugated SPIONs. (b) TEM images of oleic acid-conjugated SPIONs (b-1) and PEI-conjugated SPIONs (b-2).

Figure 2.

Assembly of PLGA particles with controlled spatial organization of SPIONs. (a) Schematic description of the Pickering emulsification of PLGA particles onto which PEI-conjugated SPIONs are immobilized (SPION-tethered PLGA particles). (b) Optical image of the suspension of SPION-immobilized PLGA microparticles. (c) Bright-field microscopy image of SPION-immobilized PLGA microparticles stained with Prussian blue. (d) TEM images of SPION-tethered PLGA nanoparticles.

Figure 3. Assembly of PLGA particles with controlled spatial organization of SPIONs. (a) Schematic description of the double emulsification to encapsulate oleic acid-conjugated SPIONs into PLGA particles (SPION-encapsulated PLGA particles). (b) Optical image of the suspension of SPION-encapsulated PLGA microparticles. (c) Bright-field microscopy image of the suspension of SPION-encapsulated PLGA microparticles stained with Prussian blue. (d) TEM images of the SPION-encapsulated PLGA nanoparticles.

Figure 4.

Analysis of the effects of spatial organization of SPIONs in PLGA particles on MR relaxivity of SPIONs. (a) SPIONs encapsulated in PLGA microparticles versus those immobilized on PLGA microparticles. (b) SPIONs encapsulated in PLGA nanoparticles versus SPION immobilized on PLGA nanoparticles. In (a) and (b), all error bars represent standard error of the fit parameter and are partially obscured by data markers.

Figure 5. MR phantom images of the agarose gel loaded with SPIONS. Iron concentration was kept at 0.05–0.06 mM. The yellow dotted circles denote the agarose gels.

Figure 6.

MR phantom images of the agarose gel loaded with bare PEI-conjugated SPIONS (Fe concentration = 0.22 mM), SPIONs immobilized on PLGA nanoparticles (Fe concentration = 0.22 mM), and SPIONs encapsulated in PLGA nanoparticles (Fe concentration = 0.45 mM). The yellow dotted circles represent the agarose gels.