Magnetic nanoparticle-loaded polymer nanospheres as magnetic hyperthermia agents†

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Uniform magnetic nanoparticle-loaded polymer nanospheres with different loading contents of manganese ferrite nanoparticles were successfully synthesized using a flexible emulsion process. The MnFe₂O₄-loaded polymer nanospheres displayed an excellent dispersibility in both water and phosphate buffer saline. The effect of loading ratio and size of MnFe₂O₄ nanoparticles within the nanospheres on the specific absorption rate (SAR) under an alternating magnetic field was investigated. Our results indicate that a large size (here 18 nm) and a low loading ratio are preferable for a high SAR. For a smaller particle size (6 nm), the low loading ratio did not result in an enhancement of the SAR value, while a very low SAR value is expected for 6 nm. In addition, the SAR of low-content MnFe₂O₄ (18 nm)-loaded polymer nanospheres in the agarose gel which is simulated for in vivo environment is the highest among the samples and does not change substantially in physiological environments. This differs largely from the behaviour of singly dispersed nanoparticles. Our results have paved the way for the design of MnFe₂O₄-loaded polymer nanospheres as magnetic hyperthermia agents for in vivo bio-applications.

Introduction†

Magnetic hyperthermia is a promising therapeutic concept for malignant tumor treatment by converting electromagnetic energy into heat using magnetic nano-mediators. This is based on the evidence that cancer cells are more sensitive than normal cells to temperatures higher than 41 °C. By engineering of magnetic nanoparticles, the magnetic hyperthermia effect can be utilized for controlled delivery of drugs or combined with magnetic resonance imaging (MRI) for in situ assessment of therapeutic efficacy at the cellular and molecular levels. These make it a promising modality for cancer diagnosis and treatment in clinical oncology. Superparamagnetic nanoparticles are widely used in hyperthermia investigations. However, small sized superparamagnetic nanoparticles have their limitation on these multi-modality bio-related applications, while large size nanoparticles are desired for many biomedical applications. For example, the nanocarriers loaded with therapeutic drugs often have an optimum size range. This favourable size range is about 100–200 nm in diameter in order to enhance permeability and retention (EPR) of nanocarriers due to the leaky vasculature of the tumour microenvironment and to avoid the human complement system, macrophage uptake, splenic as well as liver filtration. Feng et al. also reported that larger nanoparticles acquire much better properties for cellular uptake. Béalle et al. reported that the SAR for 200 nm ultra magnetic liposome (UML) particles, in which magnetic nanoparticles are encapsulated, is remarkably higher than that of smaller sized magnetic nanoparticles. Recently, magnetic nanoparticle-loaded polymer nanospheres (MLPNs), at the range of this size, have attracted significant attention. Off-resonance MRI imaging by using those MLPNs has been reported. However, the property of hyperthermia mediated by MLPNs is not well understood. It is very interesting to study if those MLPNs can be used as magnetic hyperthermia agents and how the loading ratio and particle size affect the hyperthermia performance.

Previous studies of hyperthermia agents mainly focused on designing and optimizing the size, composition and surface coating of magnetic nanoparticles to maximize the specific

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absorption rate (SAR) of the magnetic hyperthermia agent. A high SAR has been reported for magnetic nanoparticles with a high value of magnetization. However, one of the critical challenges in future clinical applications of magnetic hyperthermia is to address the issues of biokinetics, biodistribution and biodegradation of hyperthermia agents in the body and at the same time retain a high SAR. For example, preferential interactions with malignant cells are always desirable for nanoparticles injected into the tumors. In addition, aggregation of some degree, which could strongly affect the hyperthermia performance, is a common existence state for nanoparticles in a biosystem when attached to the surface or internalized into malignant cells. As illustrated by Fortin et al., the intracellular nanoparticles generated less heat than those dispersed in water, which is attributed to the abrogation of the Brownian contribution to heat generation when the particles were confined within intracellular vesicles. Therefore, free-standing magnetic nanoparticles may suffer significant heat loss in tissue and cellular environments in the SAR compared to that in water solution due to remarkably reduced Brownian relaxation loss. In this context, MLPNs may provide a model system to study partial aggregation and offer a viable solution to overcome the drawbacks of small magnetic nanoparticles and meet the above demands for a non-changing SAR in a physiological environment.

In the present study, we construct uniform MnFe₂O₄-loaded polymer nanospheres as hyperthermia agents. The MnFe₂O₄ nanoparticles are embedded within the amphiphilic copolymer (PBMA-g-C₁₂) through a previously established mini-emulsion method. By varying the concentration of MnFe₂O₄ nanoparticles in the mini-emulsion system, the amounts of magnetic nanoparticles encapsulated in the spherical matrix could be controlled. The crystal structure, morphology and magnetic properties of all the samples as well as the colloidal stability of dispersions are characterized. The dependence of the measured SAR on different loading ratios and varied sizes of loaded magnetic nanoparticles for MLPNs is systematically investigated. In order to demonstrate the potential of MLPNs as ideal mediators for magnetic hyperthermia in biological applications, MLPNs are dispersed within the agarose gel, which served as a tissue-equivalent phantom. The inductive heating properties in agarose gel phantom systems are also systematically studied. The goal of this study is to gain insight into the effect of loading ratio and the interplay of different sized nanoparticles in MLPNs on the SAR as well as provide a general approach to optimize MLPNs for retaining high performance hyperthermia treatment in a physiological simulated environment for future clinical applications.

**Experimental section**

**Chemicals**

Poly(isobutylene-alt-maleic anhydride) (PBMA; Mₜₐₐ = 6000, 85%), 1-dodecylamine (98%), poly(vinyl alcohol) (PVA; average Mₜₐₐ = 31 000–50 000, 87–89% hydrolyzed), 1,2-hexadecanediol (70%), manganese(acac)₂, benzyl ether (99%), and tetrahydrofuran (THF; 99.9%) were obtained from Aldrich. Iron(acac)₃, oleic acid, and oleylamine (70%) were purchased from Fluka. Hexane (J.T. Baker, 99.0%) and chloroform (Fisher Scientific; 99.99%) were used as received.

**Synthesis of PBMA-g-C₁₂**

To synthesize the amphiphilic copolymer, the pre-polymer PBMA was grafted with 1-dodecylamine (C₁₂) as reported previously. Briefly, in a cap-sealed conical flask, PBMA (1 g, 6.5 mmol anhydride) and 1-dodecylamine (0.9 g, 75 mol% anhydride) were mixed in THF (15 mL). The reaction flask was then heated to 50 °C and the reaction mixture turned to a clear yellow solution. The mixture was incubated overnight at 50 °C to ensure a complete reaction. The final product was extracted by solvent evaporation and then dried in vacuo at room temperature for 2 days to obtain yellow powder.

**Synthesis of MnFe₂O₄ nanoparticles**

The synthesis of MnFe₂O₄ nanoparticles followed closely to the procedures as reported in our previous work with slight modifications. Mono-dispersed MnFe₂O₄ nanoparticles (average particle diameter: 6 nm) were synthesized in a one-pot reaction by the following formulation: Fe(acac)₃ (8 mmol), Mn(acac)₂ (4 mmol), oleic acid (40 mmol), and benzyl ether (50 mL) were charged into a three-neck round bottom flask. The temperature was slowly raised from room temperature to 165 °C and maintained isothermally for 30 min. The reaction flask was then quickly heated to 280 °C. Finally, the reaction mixture was refluxed at 280 °C for 30 min to allow the nanocrystals to grow. The resulting black mixture was cooled and washed by repeated dispersion and precipitation using hexane and ethanol, respectively. The final product was dispersed completely in chloroform (50 mg mL⁻¹) and the resultant ferrofluid was stored in a sealed glass vial.

18 nm MnFe₂O₄ nanoparticles were synthesized by the following formulation: Fe(acac)₃ (8 mmol), Mn(acac)₂ (4 mmol), oleic acid (28 mmol), and benzyl ether (35 mL) were charged into a three-neck round bottom flask. The temperature was slowly raised from room temperature to 165 °C and maintained isothermally for 30 min. The reaction flask was then quickly heated to 280 °C. Finally, the reaction mixture was refluxed at 280 °C for 30 min to allow the nanocrystals to grow. The resulting black mixture was cooled to room temperature. Following the purification and extraction procedures described in the synthesis of 6 nm MnFe₂O₄ nanoparticles, a black-brown chloroform dispersion of 18 nm MnFe₂O₄ nanoparticles was produced.

**Synthesis of MLPNs with different loading ratios of MnFe₂O₄ nanoparticles**

The formation of MLPNs was achieved by the combination of mini-emulsion and solvent evaporation techniques as reported previously. Individual stock solutions of PBMA-g-C₁₂ and magnetic nanoparticles were first dissolved in chloroform and the organic phase was prepared by mixing the two solutions with different ratios. To make a mini-emulsion, the organic...
phase was poured into the aqueous phase (10× vol excess, 1 wt % PVA as stabilizer) and the emulsion can be formed by using an ultrasonic homogenizer (SONICS VCV 130 PB) at 20 kHz frequency and 60% amplitude for 5 min. The emulsion was then heated at 50 °C in an open glass beaker under rapid stirring to allow chloroform evaporation. All the samples reported here were prepared with a fixed amount of polymer stock solution (0.5 mL, 20 mg mL\(^{-1}\)), while varying the amount of magnetic nanoparticles (10 mg for high loading and 1 mg for low loading). The as-prepared colloids firstly underwent centrifugation at 8000 rpm for 10 min to remove unusually large aggregates. The colloids were then purified by centrifugation at 8000 rpm for 10 min for complete separation of MLPNs, which were then redispersed in water–methanol (50 : 50). Purification was repeated at least twice before the MLPNs were finally redispersed in water or appropriate aqueous medium.

### Phase transfer of hydrophobic MnFe\(_2\)O\(_4\) nanoparticles

Monodispersed hydrophilic magnetic nanoparticles were prepared as control by using the same polymer. To achieve this, a large surfactant/nanoparticle ratio was used to avoid the aggregation of the magnetic nanoparticles. In a typical process, MnFe\(_2\)O\(_4\) nanoparticles (5 mg) and PBMA-g-C\(_{12}\) (25 mg) were dispersed in chloroform (1 mL). The solvent was evaporated and then dried in vacuo for 2 days to obtain a completely dried MnFe\(_2\)O\(_4\) nanoparticle/polymer composite film. The film could be re-dissolved in water (5 mL) through the coordination of the amphiphilic copolymer around the hydrophobic MnFe\(_2\)O\(_4\) nanoparticles to form stable colloids. To attain complete dispersion of the MnFe\(_2\)O\(_4\) nanoparticles, 1 mL of aqueous sodium hydroxide (0.1 M) was added. Sodium hydroxide acted as an effective hydrolyzing agent to solubilize the excess surfactants and break up any aggregation caused by excess surfactants. The product was a clear colloid solution of MnFe\(_2\)O\(_4\) nanoparticles. The particles were purified by dialyzing against Millipore water for 3 days in a dialysis sac (molecular weight cut-off = 12 kDa).

### Characterizations

Magnetic properties of MLPNs and magnetic nanoparticles were characterized using a LakeShore Model 7407 vibrating sample magnetometer (VSM) at room temperature and a superconducting quantum interference device (SQUID) magnetometer at temperatures ranging from 5 K to 300 K. The loading ratio of magnetic nanoparticles within MLPNs was determined using a thermogravimetric analysis (TGA) SDTQ600 instrument. The crystal phase was verified by X-ray diffraction (XRD) patterns recorded on a powder diffractometer (Bruker D8 Advanced Diffractometer System) with a Cu K\(_z\) (1.5418 Å) source. The morphology of MLPNs was observed under a scanning electron microscope (SEM) (Zeiss Supra 40) and a transmission electron microscope (TEM) (JEOL 100CX). Fe and Mn concentrations of hyperthermia samples were determined by inductively coupled plasma (ICP) analysis using a Perkin-Elmer Dualview Optima 5300 DV ICP-OES system. Dynamic light scattering (DLS) measurements were performed in a Malvern Zetasizer Nano-ZS device to determine the hydrodynamic size of samples in a colloidal suspension. Samples were equilibrated at 37 °C for 1 min before measurement and 5 sets of measurements were taken.

### In vitro hyperthermia

MLPNs and MnFe\(_2\)O\(_4\) nanoparticles were dispersed in water or in 5% agarose gel. Plastic bottles containing 1 mL samples with concentration of 0.3 mg mL\(^{-1}\) MnFe\(_2\)O\(_4\) and thermally insulated with ceramic wool were placed within a six-loop water-cooled copper coil driven by an Inductelec A.C. generator (Sheffield, UK). The applied frequency was 435 kHz and the heating behaviour of the samples was studied at a field strength (H\(_{0}\)) of 4 kA m\(^{-1}\). The ambient temperature was around 30 °C. A LuxtronMD600 fiber optic thermometry unit connected to a computer was used to measure the samples’ temperature. The specific absorption rate (SAR) of samples was calculated from the following equation:

$$\text{SAR} = \frac{C \frac{\Delta T}{\Delta t} \frac{1}{m_{\text{Fe}} + m_{\text{Mn}}}}{\text{C}}$$

where C is the specific heat of the medium (C\(_{\text{water}}\) = 4.18 J g\(^{-1}\) °C\(^{-1}\), C\(_{\text{gel}}\) = 4.18 J g\(^{-1}\) °C\(^{-1}\)), \(\frac{\Delta T}{\Delta t}\) is the maximum slope of the time-dependent temperature curve and \(m_{\text{Fe}}\) + \(m_{\text{Mn}}\) is the weight fraction of the magnetic element (i.e. Fe and Mn) in the sample.

### Cytotoxicity assay

NIH3T3 cells were routinely cultured in DMEM (Dulbecco’s Modified Eagle Medium) supplemented with 10% fetal calf serum in a 5% CO\(_2\) atmosphere at 37 °C. Cells in exponential phase were seeded into a 96-well plate (TPP) at a concentration of 8000 cells per well and were allowed to grow for another 24 h. 20 μL magnetic suspensions with various MnFe\(_2\)O\(_4\) concentrations (0.1 to 200 μg mL\(^{-1}\)) were added to each well. After 24 h co-incubation, CCK-8 (10 μL) was added to each well and the 96-well plate was further incubated for 4 h before the absorbance readings, which were conducted at 450 nm using a FluoStar Optima microplate reader.

### Statistical analysis

The One-way ANOVA with Tukey’s post-hoc test was used to analyze the difference between three groups, using Graph Pad Prism Version 5.0 (GraphPad Software). Data are reported as mean values ± SEM. A P value of less than 0.05 was considered statistically significant.

### Results and discussion

**Synthesis and characterizations of MLPNs**

MnFe\(_2\)O\(_4\) nanoparticles (NPs) with different sizes were first synthesized using Mn(acac)\(_2\) and Fe(acac)\(_3\) as metal precursors.\(^{39}\) In this work, we first choose MnFe\(_2\)O\(_4\) NPs instead of widely used Fe\(_3\)O\(_4\) nanoparticles because of their higher magnetization (approximate magnetic spins of 5 μ\(_B\) per MnFe\(_2\)O\(_4\) formula while Fe\(_3\)O\(_4\) has magnetic spins of 4 μ\(_B\) per
chemical formula) and reduced magnetocrystalline anisotropy,\textsuperscript{35} which are favorable for higher susceptibility and therefore for enhanced magnetic hyperthermia performance. The as-synthesized MnFe\textsubscript{2}O\textsubscript{4} NPs were completely dispersed in hexane or other non-polar solvents to form a perfect ferrofluid. TEM images in Fig. 1a and b clearly show the MnFe\textsubscript{2}O\textsubscript{4} NPs with a mean size of 6.0 ± 1.1 nm and 18.0 ± 0.9 nm, respectively. The particles are highly uniform in morphology and size. It should be noted that the octahedron geometry of 18 nm MnFe\textsubscript{2}O\textsubscript{4} NPs is transformed into a nearly spherical shape when the particle size decreases to 6 nm due to minimization of surface energy.

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The crystal structure of the MnFe\textsubscript{2}O\textsubscript{4} NPs was identified by using XRD (Fig.1c). All diffraction peaks can be exclusively indexed as cubic spinel MnFe\textsubscript{2}O\textsubscript{4} (JCPDS no. 10-0319), indicating the high crystallinity. As compared to 18 nm MnFe\textsubscript{2}O\textsubscript{4} NPs, the broadening of XRD peaks is observed for 6 nm MnFe\textsubscript{2}O\textsubscript{4} NPs due to the finite size effect. The mean crystallite size estimated on the basis of the most intense peak of the XRD pattern using the Scherrer equation is about 5.58 nm and 17.03 nm for 6 nm and 18 nm MnFe\textsubscript{2}O\textsubscript{4} NPs, which is consistent with the observation under a TEM.

Scheme 1 illustrates the formation of MLPNs via the mini-emulsion technique. In this process, the polymer PBMA-g-C\textsubscript{12} and MnFe\textsubscript{2}O\textsubscript{4} NPs condense into a tight spherical cluster with the evaporation of the organic solvent. The MnFe\textsubscript{2}O\textsubscript{4} NPs are bound together primarily via strong interdigitated hydrophobic interactions between the tight hydrophobic brushes of PBMA-g-C\textsubscript{12} and the oleic acid ligands of MnFe\textsubscript{2}O\textsubscript{4} NPs. By varying the ratios of MnFe\textsubscript{2}O\textsubscript{4} NPs in the mini-emulsion system, controlled amounts of MnFe\textsubscript{2}O\textsubscript{4} NPs could be loaded in the matrix. Herein, according to their varied MnFe\textsubscript{2}O\textsubscript{4} NPs’ content and size, MLPNs are labeled as MLPNs 1 (high-content (6 nm)), MLPNs 2 (low-content (6 nm)), MLPNs 3 (high-content (18 nm)), and MLPNs 4 (low-content (18 nm)).

Scheme 1  Schematic diagram illustrating the formation of water dispersed MLPNs with different contents through the oil-in-water mini emulsion/solvent evaporation process.

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Fig. 2  (a) SEM image and (e) TEM image of MLPNs 1 (high-content (6 nm)); (b) SEM image and (f) TEM image of MLPNs 2 (low-content (6 nm)); (c) SEM image and (g) TEM image of MLPNs 3 (high-content (18 nm)); (d) SEM image and (h) TEM image of MLPNs 4 (low-content (18 nm)).
SEM images in Fig. 2a–d show that monodispersed spherical MLPNs with excellent size uniformity are achieved. Regardless of the loading amount and the size of MnFe₂O₄ NPs loaded, all the MLPNs show an average size of around 80 nm by controlling the emulsion conditions. More importantly, MnFe₂O₄ NP agglomeration is prevented as the particles are dispersed uniformly throughout the entire matrix. As confirmed by the TEM images, both 6 nm and 18 nm MnFe₂O₄ NPs are dispersed and embedded within the spherical matrix. Estimations of intra-particle distance \( d_{\text{sep}} \) (see ESI S1† for details of calculation) of MnFe₂O₄ NPs within MLPNs were calculated using the TGA data (in Fig. 3a and 4a) and the results are shown in Table 1. The \( d_{\text{sep}} \) values from Table 1 agreed well with those that are observed in the TEM images, as it could be seen that \( d_{\text{sep}} \) of the high-content MLPNs is much lower than that of the low-content ones. Moreover, regardless of the size of the loaded MnFe₂O₄ NPs, similar \( d_{\text{sep}} \) values are noticed for both MLPNs corresponding to high-content and low-content.

The weight loss below 200 °C, as illustrated in Fig. 3a and 4a, is attributed to the evaporation of the absorbed water. The weight loss at around 200 °C and 600 °C of the MnFe₂O₄ NPs and MLPNs may account for the mass loss of oleic acid and PBMA-g-C₁₂ on the sample surface. It is obvious to note that the high-content of nanoparticles in MLPNs (MLPNs 1 and MLPNs 3) would result in less mass loss due to lower polymer mass ratios within the MLPNs. The observations prove that the nanoparticle-content within the MLPNs can be controlled by simply varying the loaded MnFe₂O₄ NPs during the emulsion process. Fig. 3a and 4a also indicate that weight loss is higher for the MLPNs loaded with 6 nm MnFe₂O₄ NPs than those loaded with 18 nm MnFe₂O₄ NPs. Estimations of the volume fraction \( V_{\text{MNP}}/V_{\text{MNC}} \) of MnFe₂O₄ NPs inside a MLPN (see ESI S1† for details of calculation) were calculated using the TGA data (in Fig. 3a and 4a) and the results are shown in Table 1. It could be seen that \( V_{\text{MNP}}/V_{\text{MNC}} \) of the high-content MLPNs is much higher than that of the low-content ones. Moreover, \( V_{\text{MNP}}/V_{\text{MNC}} \) is higher for the MLPNs loaded with 18 nm MnFe₂O₄ NPs than that of the MLPNs loaded with 6 nm MnFe₂O₄ NPs. This can be explained by the larger surface to volume ratio for the 6 nm sized samples.

The magnetic properties of MLPNs and MnFe₂O₄ NPs were evaluated by field-dependent magnetization \( M-H \) measurements at 300 K using a VSM. As shown in Fig.3b, MLPNs 1 and MLPNs 2 show the superparamagnetic behavior similar to that of 6 nm MnFe₂O₄ NPs despite the large size of 80 nm for MLPNs. The saturation magnetization, \( M_s \) (in emu per gram), dropped down from 49 to 11 emu g⁻¹ (for MLPNs 1) and to 5 emu g⁻¹ (for MLPNs 2). Similarly, for 18 nm MLPNs as observed from Fig.4b, \( M_s \) dramatically decreases after mini-emulsion and further decreases with fewer magnetic nanoparticles loaded. The decreased \( M_s \) (magnetic moment per weight unit) attributes to the decreased effective weight fraction of the magnetic core. The dependence of \( M_s \) on the loading ratio is plotted against MnFe₂O₄ NP loading in Fig. S2.† The results show a linear relationship with an \( R^2 \) value of 0.99 for both 6 and 18 nm particles. The MLPNs loaded with 18 nm MnFe₂O₄ NPs also show no hysteresis. The temperature-dependent field cooling (FC) and zero-field cooling (ZFC) magnetization are further measured for all the samples by using a superconducting quantum interference device (SQUID) with the temperature ranging from 5 K to 300 K. The ferromagnetic-superparamagnetic transition temperature, \( T_B \), is taken as the point of divergence between the FC and ZFC curves, below which the particles are no longer superparamagnetic. In the

![Fig. 3](image1)

![Fig. 4](image2)

**Fig. 3** (a) TGA profiles. (b) VSM for 6 nm MnFe₂O₄ NPs, MLPNs 1 (high-content (6 nm)) and MLPNs 2 (low-content (6 nm)); (c) and (d) ZFC and FC profiles of 6 nm MnFe₂O₄ NPs, MLPNs 1 (high-content (6 nm)) and MLPNs 2 (low-content (6 nm)) measured using SQUID. For the sake of presentation, we have normalized the magnetization data with respect to the value at the maximum of ZFC magnetization, \( M (T_B) \), for individual samples.
hydrodynamic size, inter-particle distance \( (d_{sep}) \), saturation magnetization \( (M_s) \), volume fraction \( (V_{MNP}/V_{MNC}) \) calculated theoretical SAR and experimental SAR for all samples

<table>
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<tr>
<th>Hydrodynamic size (nm)</th>
<th>( d_{sep} ) (nm)</th>
<th>( M_s ) (emu g(^{-1}))</th>
<th>( V_{MNP}/V_{MNC} )</th>
<th>Calculated SAR</th>
<th>Experimental SAR (W g(^{-1}))</th>
</tr>
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<tbody>
<tr>
<td>6 nm MnFe(_2)O(_4) NPs</td>
<td>13</td>
<td>—</td>
<td>49</td>
<td>—</td>
<td>83</td>
</tr>
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<td>MLPNs 1</td>
<td>100</td>
<td>9.2</td>
<td>11</td>
<td>0.04</td>
<td>27</td>
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<tr>
<td>MLPNs 2</td>
<td>105</td>
<td>14.6</td>
<td>5</td>
<td>0.02</td>
<td>7</td>
</tr>
<tr>
<td>18 nm MnFe(_2)O(_4) NPs</td>
<td>28</td>
<td>—</td>
<td>83</td>
<td>—</td>
<td>580</td>
</tr>
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<td>MLPNs 3</td>
<td>101</td>
<td>8.6</td>
<td>32</td>
<td>0.24</td>
<td>43</td>
</tr>
<tr>
<td>MLPNs 4</td>
<td>106</td>
<td>15.2</td>
<td>19</td>
<td>0.12</td>
<td>102</td>
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</tbody>
</table>

ZFC/FC curves in Fig. 3 for 6 nm samples, both MLPNs samples (MLPNs 1 and 2) [MnFe\(_2\)O\(_4\) NPs embedded in the polymer matrix] have a blocking temperature of approximately 25 K \( (T_B \approx 25\,K) \). This value corresponds well to a small number of MnFe\(_2\)O\(_4\) NPs (6 nm) with low magnetocrystalline anisotropy. In contrast, the bare MnFe\(_2\)O\(_4\) NPs possessed a much higher blocking temperature of \( T_B = \sim 183\,K \). The possible reason is that the severe agglomeration after drying led to enhancement due to strong particulate interactions. As illustrated in Fig. 4c, the \( T_B \) values of MnFe\(_2\)O\(_4\) NPs, MLPNs 3 (high-content) and MLPNs 4 (low-content) are determined to be approximately 300 K, 148 K and 95 K, respectively. The high blocking temperature of 300 K of dried magnetic particles also indicated a strong particulate interaction. The depression of \( T_B \) when the MnFe\(_2\)O\(_4\) NPs are encapsulated into the polymeric nanospheres to form MLPNs provided evidence that the dipole–dipole interaction between the MnFe\(_2\)O\(_4\) NPs is reduced. \( T_B \) decreased even more when the loading density of MnFe\(_2\)O\(_4\) NPs is reduced. The results suggest that the polymer matrix could be seen here as being effective at isolating magnetic particles, thus reducing the magnetic interactions.

Since the dispersibility and colloidal stability of magnetic samples play an important role in magnetic hyperthermia treatment, the stability of MLPNs was examined using DLS. Fig. 5a and b show the hydrodynamic size of 6 nm and 18 nm samples dispersed in water, respectively. The average sizes of the samples are summarized in Table 1. The measured hydrodynamic size in aqueous solution is larger than that of TEM observations. Such a difference obtained by DLS and TEM is commonly observed.\(^{36}\) The larger colloidal particle size in the aqueous dispersion than in the dry state or in the non-polar organic dispersion is essentially due to the highly hydrated ligand and surface molecule layer on the nanoparticles. All the polar functional groups are capable of forming hydrogen bonds with the solvent water molecules or being solvated by water molecules through polar interaction causing extensive hydration of the nanoparticles. Thus, the average diameters of the samples obtained by the DLS measurements are higher than those obtained by the TEM measurements. DLS results show that MLPNs are homogeneously dispersed. The hydrodynamic sizes of 6 nm and 18 nm MnFe\(_2\)O\(_4\) NPs are 13 nm and 28 nm, respectively. For all the MLPNs, the hydrodynamic size is close to 100 nm and no obvious difference despite the varied loaded content and size. The colloidal stability of MLPNs is further tested in PBS solution. No observable aggregation and change in size in PBS is observed. The results from stability studies indicate that all the MLPNs exhibit excellent colloidal stability in both water and PBS (Fig. 5c–f) after 20 days of incubation at 37 °C. Such excellent stability of MLPN dispersions makes them suitable for biomedical applications.

**Magnetic hyperthermia**

In order to assess the efficacy of MLPNs as hyperthermia mediators, magnetic heating characterization is carried out using an induction heating system. Fig. 6a shows the experimental setup for calorimetric measurements. As shown in Fig. 6b, 6 nm MnFe\(_2\)O\(_4\) NPs or MLPNs reveal a rather slower temperature rising profile. Though 6 nm MnFe\(_2\)O\(_4\) NPs possess the best inductive heating characteristics among the three samples under investigation, within the observed time period of...
where $V$ is the volume of the spherical nanoparticle, $k_B$ is the Boltzmann constant, $T_B$ is the blocking temperature, $T$ is the temperature and $\eta$ is the medium viscosity. As for a finite-size sample system, Brownian and Néel processes take place in parallel, and the effective relaxation time $\tau$ is thus given by

$$\frac{1}{\tau} = \frac{1}{\tau_N} + \frac{1}{\tau_B} \quad (4)$$

Generally, for a certain magnetic nanoparticle, the measured SAR depends on the parameters of magnetic nanoparticles (size, shape, crystal structure, saturation magnetization, and magnetic susceptibility) as well as field strength ($H$) and frequency ($f$) of the AMF, according to eqn (1) (ref. 38)

$$\text{SAR} = \frac{8\pi^3 \mu_0^2 M_s^2 f^2 \gamma T}{3pk_B(1 + 2\pi f/\tau)^2} \quad (5)$$

where SAR is expressed as W g$^{-1}$ of magnetic elements, $\mu_0 = 4\pi \times 10^{-7}$ N A$^{-1}$ is the permeability of free space, $H_s$ (A m$^{-1}$) is the magnetic field, $\rho$ is the density of the magnetic nanoparticles, $f$ is the field frequency, and $M_s$ is the saturation magnetization of the magnetic nanoparticles.

The measured SAR values of samples are summarized in Table 1. As can be seen in Table 1, the 18 nm samples consistently exhibit a higher SAR than the 6 nm samples with the highest SAR of 580 W g$^{-1}$ by 18 nm MnFe$_2$O$_4$ NPs and 332 W g$^{-1}$ for MLPNs 4 (low-content (18 nm)). In addition, the SAR value for MLPNs 4 is around 2.25-fold higher than that of MLPNs 3 (low-content (18 nm)), although the latter exhibits larger $M_s$.

From eqn (5), the samples with high $M_s$ will lead to a high SAR at a certain external AMF. As $M_s$ decreases with decreasing size, the 6 nm samples with smaller $M_s$ values are expected to exhibit a lower SAR. This is consistent with our observation that both 6 nm MnFe$_2$O$_4$ NPs and 6 nm MnFe$_2$O$_4$-loaded MLPNs show a smaller SAR as compared to the 18 nm samples. Moreover, for 6 nm MLPNs, the SAR increased with increasing loading ratio, the SAR for MLPNs 1 is 7 W g$^{-1}$, a little higher than that of MLPNs 2, which may also be due to a higher $M_s$ for MLPNs 1.

An interesting observation has been found about the SAR for 18 nm MnFe$_2$O$_4$-loaded MLPNs. The SAR for MLPNs 4 (low-content (18 nm)) is 330 W g$^{-1}$ and almost 2.25-fold higher than that of MLPNs 3 (high-content (18 nm)), although the latter exhibits larger $M_s$. The high SAR of low-content MLPNs may be attributed to a relatively lower magnetic nanoparticle-interaction due to a large amount of polymer fully isolating the MnFe$_2$O$_4$ NPs, which is confirmed by $d_{sep}$ and $T_B$ data. High-content MLPNs possessing stronger magnetic particle-interactions may result in the decrease of contribution of both Néel and Brownian relaxation losses on heat, which leads to a lower SAR.\(^\text{19}\)

To confirm our results, we calculate the theoretical SAR of all samples by using eqn (2)-(5), as presented in Table 1. It can be seen that the trend of SAR for 6 nm MLPNs and 18 nm MLPNs is different, but both are the same as the experimental results. For 6 nm samples, the SAR is proportional to $M_s$, a higher SAR is attributed to a higher $M_s$. For 18 nm MLPNs, the calculated theoretical SAR for MLPNs 4 is 11-fold higher than that of MLPNs 3.

800 s, only a 5 °C temperature increase is noticed. However, larger MnFe$_2$O$_4$ NPs may have a better inductive heating property than the smaller ones. As demonstrated in Fig.6c, 18 nm MnFe$_2$O$_4$ NPs, whether free or loaded within MLPNs, may induce a more remarkable temperature increase under the same AMF parameters. As shown in Fig.6d, the raised temperature within the hyperthermia time period of 800 s is approximately 23 °C for 18 nm MnFe$_2$O$_4$ NPs, 7 °C for MLPNs 3 (high-content (18 nm)) and 15 °C for MLPNs 4 (low-content (18 nm)). We can conclude that bare MnFe$_2$O$_4$ NPs have better inductive heating property than those when formulated in MLPNs. It is also worth noting that MLPNs with low-content have better heating property than the high-content ones.

In the finite-size nanoparticle system, the heat generated by magnetic fluids under an AMF is mainly attributed to two contributions: hysteresis loss and Néel–Brownian relaxation losses.\(^\text{27}\) Because the magnetic field is relatively low, there is no remanence of these samples. Herein we will just consider the contribution of Néel and Brownian relaxation on heat. Heat generation through Néel relaxation is due to rapidly occurring changes in the direction of magnetic moments relative to the crystal lattice (internal dynamics). This is hindered by anisotropy energy that tends to orient the magnetic domain in a given direction relative to the crystal lattice. Brownian relaxation is attributed to physical rotation of particles within a medium in which they are placed (external dynamics) and is hindered by the viscosity that tends to counter the movement of particles in the medium. Their characteristic relaxation times are given below:

$$\tau_N = \tau_0 \exp \frac{25T_B}{T} \quad (2)$$

$$\tau_B = \frac{3\eta V}{k_B} \quad (3)$$

Fig. 6 (a) Experimental setup for calorimetric measurements; time-dependent heating profiles of 1 mL (b) 6 nm and (c) 18 nm MnFe$_2$O$_4$ related samples with the same concentration (0.3 mg mL$^{-1}$ MnFe$_2$O$_4$) on exposure to 4 kA m$^{-1}$ alternating current field at 435 kHz frequency; (d) the increase of temperature before and after hyperthermia within 800 s for all the samples.
To better understand the inductive heating property of the MnFe$_2$O$_4$ NPs and MLPNs in a physiological environment, 18 nm MnFe$_2$O$_4$ NPs and MnFe$_2$O$_4$-loaded MLPNs were dispersed in 5% agarose gel, which is used to simulate the *in vivo* environment of animal tissue phantoms. Fig. 7 compares the SAR of 18 nm MnFe$_2$O$_4$ NPs and MLPNs with different loading contents in 5% agarose gel. Previous experimental evidence has shown that the Brownian loss could contribute to ~53% of total heating capacity for a finite sized magnetite nanoparticle dispersion, which is similar to our results with 18 nm MnFe$_2$O$_4$ NPs after being dispersed in the agarose gel because the agarose gel fixation prevented any Brown relaxation loss. It is further confirmed by the evidence that the SAR for MLPNs 4 (low-content (18 nm)) does not change after being dispersed in the agarose gel. In other words, the heating capacity of low-content MLPNs will not be influenced by physiological environments. We can think that the magnetic loss of MLPNs may be mainly attributed to Néel loss. When MnFe$_2$O$_4$ NPs are embedded in a polymer matrix with a low loading, the high viscosity of the polymer matrix does not allow Brownian motion of MnFe$_2$O$_4$ NPs inside the polymer matrix. The MLPNs have a much larger hydrodynamic size (over 100 nm, as shown in Table 1). We should not expect fast Brownian relaxation of such big spheres. Therefore, the SAR of MLPNs in the agarose gel is similar to that of MLPNs in water, as the Néel contribution is the dominant mechanism.

Overall, the heating capacity of low-content MnFe$_2$O$_4$-loaded MLPNs will not be influenced substantially by physiological environments. We can think that the magnetic loss of MnFe$_2$O$_4$-loaded MLPNs is mainly attributed to Néel loss. Though further *in vitro* studies are necessary, currently available results have demonstrated that the low-content MnFe$_2$O$_4$ (18 nm)-loaded MLPN dispersion could exhibit better heating capacity in physiological environments.

**In vitro cell cytotoxicity assay**

Since the hyperthermia agents would eventually be applied *in vivo* or in clinical, it is very important to evaluate the biocompatibility for the potential hyperthermia agents. The cell viabilities are determined after a 24 h co-incubation with fibroblast NIH3T3 cells. From Fig. 8, we can see that 18 nm MnFe$_2$O$_4$ NPs induce a cytotoxic effect in NIH3T3 cells at a concentration of 200 µg mL$^{-1}$ MnFe$_2$O$_4$. It can also be seen that MLPNs do not induce a notable cytotoxic effect within a concentration of 0.1 µg mL$^{-1}$ to 200 µg mL$^{-1}$ MnFe$_2$O$_4$. This means that MLPNs clearly exhibit higher biocompatibility than bare MnFe$_2$O$_4$ NPs, especially at high concentration. These results suggest that the MLPNs are excellent candidates for various biomedical applications such as hyperthermia treatment.

**Conclusions**

In summary, the uniform MLPNs with a controlled loading ratio and loaded MnFe$_2$O$_4$ NPs of different sizes were synthesized. The effects of MLPNs with varied loading ratios and sizes on the SAR have been studied under an AMF (4 kA m$^{-1}$; 435 kHz). The results indicate that MLPNs loaded with large sized MnFe$_2$O$_4$ NPs have a better inductive heating property than the ones with small sized MnFe$_2$O$_4$ NPs because of the increased magnetic core size and larger $M_s$. Moreover, the loading ratios of MLPNs play an important role in the obtained SAR. The effect of loading content on the SAR is different between small-size and large-size MnFe$_2$O$_4$ nanoparticles loaded. For 6 nm loaded MLPNs, the SAR is increased as $M_s$ increased. But for 18 nm loaded MLPNs, low-content MLPNs show a higher SAR than the high-content MLPNs. In particular, the SAR for low-content (18 nm) MLPNs is almost 2.25-fold higher than that of the high-content one, although the latter has an $M_s$ 2-fold larger than its low-content counterpart. This is quite different from the design of MLPNs for MRI contrast agents while large $M_s$ is favoured to high $T_2$ relativity. This high SAR may be ascribed to a relatively lower magnetic nanoparticle-interaction in low-content MLPNs due to a large amount of polymer fully isolating the larger MnFe$_2$O$_4$ NPs. This is confirmed from $d_{sep}$ and $T_B$ data, which suggest that the polymer matrix could be seen as being effective at isolating magnetic particles and reducing the magnetic interactions.

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**Fig. 7** The comparison of the SAR value for 18 nm samples dispersed in 5% agarose gel and in water.

**Fig. 8** Cell viability data of 18 nm MnFe$_2$O$_4$ NPs and MLPNs with different loading contents obtained from cultured NIH3T3 cells using standard CCK-8 colorimetric assays. Error bars = SEM; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. 

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interactions. The theoretical calculation indicates that the trend of SAR is the same as the experimental results. In addition, the SAR of low-content (18 nm) MLPNs in the agarose gel is the highest among the samples and does not change substantially in physiological environments, which is crucial for medical translation from bench to bed. In vitro cytotoxicity tests further demonstrate the good biocompatibility of the obtained MLPNs. The findings obtained in this work provide a profound and meaningful understanding of the use of MLPNs for magnetic hyperthermia treatment and pave the way for using such kind of MLPNs for future clinical applications.

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Notes and references