Nearly Superparamagnetic Magnetite Particles as Potential Thermal Seeds for Magnetic Hyperthermia

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Abstract. Here, we report the results of the study undertaken to determine relative contributions of Néel and Brownian relaxations on magnetic heat dissipation by investigating the physical, magnetic and heating characteristics of magnetite suspension dispersing particles ranging in average diameter from 10.0 to 15.7 nm. Heating characteristics depended on the primary particle size and the viscosity of the medium. In the case of the sample with average diameter of 12.5 nm, the effective specific absorption rate (ESAR) dropped by 27% (22.9×10⁻⁹ to 16.8×10⁻⁹ Wg⁻¹Oe⁻²Hz⁻¹). In contrast, the decrease of 67% (29.5×10⁻⁹ to 9.7×10⁻⁹ Wg⁻¹Oe⁻²Hz⁻¹) was observed for the sample with average diameter of 15.7 nm. The potential of these particles as thermal seeds was tested by feeding the macrophage with the same and exposing them to an ac magnetic field strength and frequency of 40 Oe and 600 kHz respectively. The uptake of magnetite particles by the macrophage was adequate to raise the temperature of cell suspension by 8°C required for thermal necrosis.

Introduction

Magnetic hyperthermia is a technique that proposes the annihilation of cancer cells through the elevation of cell temperature above 43°C by utilizing the heat dissipated by particles exposed to alternating magnetic field operating at frequencies higher than the frequencies at which the magnetic moment in the particle relaxes either through Néel or Brownian motions [1]. Among various magnetic oxide particles, magnetite (Fe₃O₄) has been considered suitable due to its biocompatibility and easiness to synthesize and particles with different sizes and surface modifications were prepared and their heating efficiencies at various AC magnetic field strength and frequencies have been tested. Though the sizes of particles that could dissipate heat through the Néel and Brownian motion of the magnetic vector has been estimated theoretically [2], contribution of Néel relaxation to heat dissipation in any sample should be determined to generate anticipated heat in in-vivo experiments. Thus in this paper, we have focused our attention to study the specific heat absorption characteristics of magnetite, especially the relative contributions of Néel and Brownian relaxations to heat dissipation, by considering the physical and magnetic properties of samples with two different average...
diameters. Then, we have tested the heating ability of these particles by using the macrophage.

**Heat dissipation mechanism**

Heat dissipation from magnetic particles is caused by the delay in the relaxation of the magnetic vector through either the rotation within the particle (Néel) or the rotation of the particle itself (Brownian), when the particles are exposed to an AC magnetic field with magnetic field reversal times shorter than the magnetic relaxation time of the particle and the amount of heat dissipation is given by the following equation [3].

\[ P = \mu_0 \chi'' f H_{\text{applied}}^2 \]  \hspace{1cm} (1)

\[ \chi'' = \frac{\omega \tau^*}{1 + (\omega \tau^*)^2} \chi_0 \]  \hspace{1cm} (2)

Where \( P \) is the amount of heat dissipated [J/m³s], \( \mu_0 \), vacuum permeability, \( \chi'' \), imaginary part of AC susceptibility, \( f \), the frequency of AC magnetic field, \( H_{\text{applied}} \), AC magnetic field strength, \( \tau^* \) is the effective relaxation time, \( \omega \) is the angular frequency, \( \chi_0 \) is the initial susceptibility.

The \( \chi'' \) in equation (1) refers to the delay in magnetization respect to the applied magnetic field, and is given by equation (2). And \( \tau^* \) is the harmonic mean of Néel and Brownian relaxation, which is given by equation (3).

\[ \tau^* = \frac{\tau_B \tau_N}{\tau_B + \tau_N} \]  \hspace{1cm} (3)

\[ \tau_N = \tau_0 \exp\frac{KV_M}{kT} \]  \hspace{1cm} (4)

\[ \tau_B = \frac{3\eta V_H}{kT} \]  \hspace{1cm} (5)

Where, \( \tau_N \) and \( \tau_B \) are Néel and is Brownian relaxation times, \( \tau_0 = 10^{-9} \) [s], \( K \) is the anisotropy constant, \( V_M \) is the particle volume, \( k \) is Boltzmann constant, \( T \) is the temperature, \( \eta \) is the viscosity of the solvent, \( V_H \) is the hydrodynamic volume of the particle. According to equation (4) and (5), relaxation times \( \tau_N \) and \( \tau_B \) become longer when the particle diameter becomes large. If the measurement time \((1/f)\) is smaller than the \( \tau^* \), delay in relaxation occurs and the magnetic energy is converted to thermal energy. Fig. 1 shows the theoretical amount of heat generated by magnetite particles exposed to an AC magnetic field of 40 Oe and 600 kHz. It should be noted that not only the amount of heat, but also the contribution by Néel and Brownian relaxations are also swayed by the diameter of the magnetite particles. Furthermore, the heat dissipated by Brownian relaxation will be influenced very much by the viscosity of the medium in which these particles are dispersed and the freedom of rotation is either suppressed or arrested in highly viscous media. Consequently, the heat dissipated by these particles will either diminish or seize.
Materials and Methods

3.1 Preparation of magnetite nanoparticle dispersion

Stable water-based magnetite suspensions were supplied by Ferrotec Corporation. The particles were synthesized by coprecipitation method, coated with a surfactant and dispersed in water or oil. The particles in these samples had the ability to relax through both Néel and Brownian relaxations. We fabricated the high viscosity samples by adding polymer in oil or dispersing particles in polyvinyl alcohol hydro-gel. The preparation method of hydro-gel can be found elsewhere [4]. When the particle is located in the hydro-gel, the particle rotation is restricted and the magnetic moment relaxes only through Néel relaxation.

3.2 Physical and magnetic characterization of magnetite nanoparticles

The physical characteristics such as structure and the crystallite size were measured using X-ray diffractometer (XRD, Rigaku Multiflex). The particle morphology was observed by a transmission electron microscope (FE-TEM, Hitachi, HF-2000). The DC/AC magnetic characterization was carried out using the physical properties measuring system (PPMS, Quantum Design). The zero-field-cooled and field-cooled measurements were carried by applying a field of 100 Oe. On the other hand, the AC magnetic susceptibility measurements were performed at frequencies 10, 57, 320, 1788, 9977 Hz in the temperature range between 5 and 265 K.

3.3 Heat evolution experiments

Heat evolution experiments were carried out by introducing the suspensions dispersing 8 wt. % of magnetite in the medium into a test tube and by placing the same at the center of the solenoid coil that generated a magnetic field strength of 40 Oe at 600 kHz. The details of the experimental setup are given elsewhere [5]. The temperature of the magnetic suspension was measured using an optical fiber thermometer. The ESAR values were calculated using the equation given below.

\[
ESAR = \frac{\sum c_i m_i}{m_{Fe_3O_4}} \frac{\Delta T}{\Delta t} \frac{1}{H_{\text{applied}}^2 f}
\]

c: specific heat, \( m_i \): weight of each particle, \( m_{Fe_3O_4} \): weight of magnetite nanoparticle, \( T \): temperature, \( t \): time. In the calculation, \( f \): frequency of applied AC magnetic field, \( H_{\text{applied}} \): strength of applied AC magnetic field. The specific heat of water was assumed 4.2 JK\(^{-1}\)g\(^{-1}\). The specific heat of hydro-gel was estimated to be 2.76 JK\(^{-1}\)g\(^{-1}\) from the differential scanning calorimetry (DSC) measurements. The temperature increment rate (\( \Delta T/\Delta t \)) was evaluated from the temperature-time curve obtained for a measurement time of initial 60 s. The above measurement time was fixed to avoid the melting of the gel at higher temperatures and longer duration.

3.4 In vitro experiments using macrophage

The thermal dissipation potential of the particles that dissipate heat through Néel relaxation was examined by carrying out \textit{in vitro} experiments using macrophage (RAW 264.7). Water dispersed magnetite particles were introduced into culture fluid (D-MEM, Sigma) after filtering and sterilizing the same. Then, the particle concentration was adjusted to 1.0 mg/mL and about 7.5\times10^6 of RAW 264.7 was introduced into the culture media and cultured at 37 °C. After centrifuging the suspension at 1000 G for 5 min, magnetite uptaken cell and free magnetite were separated. The calorimetric analysis using potassium thiocyanate was carried out to
evaluate the Fe\textsuperscript{3+} uptake concentration per cell. On the contrary, the macrophage was exposed to various concentrations of magnetite for different durations to obtain macrophage with different loads of magnetite particles. Then, about $1.5 \times 10^7$ of RAW 264.7 containing culture media with different magnetite uptake concentrations were exposed to the AC magnetic field to measure the temperature rise against time.

**Results and Discussion**

4.1 Physical characteristics of magnetite samples

The XRD profiles of the sample confirmed the presence of Fe\textsubscript{3}O\textsubscript{4} with spinel structure and crystallite sizes estimated from the profiles were as shown in Table 1. The average particle diameters of the samples obtained from measuring 1000 particles from TEM micrograph were also given in Table 1. Representative TEM micrographs of samples A and E are shown in Fig. 2. Both the measurements suggested that the particle diameters of Sample A to E are in the increasing order.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average diameter (nm)</th>
<th>Standard deviation (nm)</th>
<th>Crystallite size (nm)</th>
<th>$T_B$ at 600 kHz (K)</th>
<th>ESAR ($10^{-9}$Wg$^{-1}$Oe$^{-2}$Hz$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Water +PVA\textsuperscript{a} Oil +Polymer</td>
</tr>
<tr>
<td>A</td>
<td>10.0</td>
<td>2.3</td>
<td>8.8</td>
<td>64.1</td>
<td>0.8</td>
</tr>
<tr>
<td>B</td>
<td>11.6</td>
<td>2.7</td>
<td>9.2</td>
<td>140.7</td>
<td>6.7</td>
</tr>
<tr>
<td>C</td>
<td>13.9</td>
<td>3.4</td>
<td>11.0</td>
<td>220.9</td>
<td>16.4</td>
</tr>
<tr>
<td>D</td>
<td>12.5</td>
<td>2.9</td>
<td>13.4</td>
<td>271.4</td>
<td>22.9</td>
</tr>
<tr>
<td>E</td>
<td>15.7</td>
<td>4.4</td>
<td>13.2</td>
<td>–</td>
<td>29.5</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Polyvinyl alcohol.

![Fig. 2 TEM micrographs of the samples. (a) the sample A, and (b) the sample E.](image)

4.2 Magnetic Characteristics of magnetite samples

The imaginary part of initial susceptibility ($\chi''$) as a function of temperature for samples A to E were measured and the results of the samples D and E are given in Fig. 3. The blocking temperature ($T_B$) at the $\chi''$ maximum for the samples were estimated as follows; 30.0 (sample A), 80.0 (sample B), 120.1 (sample C), and 204.9 K (sample D) at the measurement frequency of 10 Hz. Since $\chi''$ value of the sample E continued to increase with increasing temperature, the $T_B$ of the sample E should be higher than R.T. When we increased measuring frequencies, $T_B$ shifted towards higher temperatures. However, the $\chi''$ maximum even at the highest measured frequency of 9977 Hz, was well below the R.T. As the $\chi''$ maximum could be measured only at a maximum frequency of 9977 Hz, we estimated the $T_B$ at the operating frequency of 600 kHz by plotting the inverse of $T_B$ against the logarithm of measurement time (eq. 1) and by extrapolating the straight line fitted for the observed values as shown in Fig. 4.
4.3 Heat dissipation characteristics of magnetite particles

The results of heat dissipation experiments of sample A to E dispersed in water/low viscosity oil and high viscosity oil/hydro-gel are shown in Table 1. The ESAR value of the sample D dispersed in high viscosity oil was $16.8 \times 10^{-9} \text{Wg}^{-1}\text{Oe}^{-2}\text{Hz}^{-1}$, which is about 27% less than the one dispersed in low viscosity oil ($22.9 \times 10^{-9} \text{Wg}^{-1}\text{Oe}^{-2}\text{Hz}^{-1}$). The reduction in heat dissipation was due to the inability to generate heat through particle rotation. Thus the ESAR value could be improved by using particles that have an average $T_B$ less than R.T. and narrow particle size distribution. Similar results were also observed for the sample B. The ESAR of the oil-dispersed sample E was greater than that of the sample D. When we added the polymer in the low viscosity oil, the ESAR of the sample E reduced about 67% and the value was much less than that of the sample D. This suggests that the fraction of the particles that generated heat through Brownian rotation was comparatively large. These observations are also in line with the theoretical calculation (Fig. 1).

The sample which had an average diameter below 13 nm had large fraction of particles relaxing through Néel mechanism. On the other hand, the sample with average diameter larger than 13 nm had large fraction of particles relaxing through Brownian mechanism. Furthermore, these results suggest that it is important to analyze the relative contributions of Néel and Brownian relaxations to heat dissipation to formulate the appropriate strategy for effective in-vivo treatments. Thus, the sample D dispersed in water was considered for the in-vitro experiments using RAW cells to experimentally verify the heating capacity of the magnetite sample.

4.4 Evaluation of the heating potential of magnetite samples using RAW Cell

The magnetite uptake by RAW cell was a function of time and saturated after 24 h. Furthermore, the uptake was also a function of the magnetite particle concentration of the culture media and increased with increased initial concentration. Fig. 5 shows the optical micrographs of (a) RAW cells and (b) cells incubated with magnetite particles. After incubation with magnetite particles, the appearance of RAW cells changed from colorless to black. This clearly indicates the phagocytosis of RAW cells.
Fig. 6 shows the temperature rise of the cell suspension with different concentrations of magnetite uptake. The cell suspension containing magnetite nanoparticles exhibited a temperature rise of 8 °C and above depending on the magnetite particle concentration after an exposure time of 30 min. This suggests that the magnetite uptake concentration was adequate to raise the temperature of targeting tissue above 43 °C and confirms the potential of nearly superparamagnetic particles for effective use in hyperthermia treatment. Since the experiments were carried out at R.T., the temperature of the cell suspension reached was not adequate to thermal necrosis. However, the death of RAW cell was experimentally verified by exposing 1.0 mL of culture fluid containing about 2.5×10⁶ cell/mL in a closed vessel to hot bath maintained at 43 °C for 15 min to 2 h. At the same time, similar sample was cultured at 37 °C for 0~24 h. The cell death rate was determined by using the trypan blue staining test. The RAW cell death rate reached 100 % in samples exposed to 43 °C for 1 h and an elapsed time of 24 h. However, it should be noted that unlike the RAW cells the cancer cells will not easily uptake magnetite unless the surface of these particles are suitably modified to facilitate efficient uptake.

Conclusions

In this study, we have experimentally verified that most of the samples used in MFH studies contain particles that dissipate heat through Néel and Brownian relaxations and the heating characteristics depended on the primary particle size and the viscosity of the medium. The particles with their average diameter of about 12.5 nm exhibited highest dissipation of heat through Néel relaxation of 73 % of the particles. Furthermore, the potential of these particles as thermal seeds was confirmed by carrying out in-vitro experiments using macrophage.

Acknowledgements

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References