Research articles

Heating efficiency of magnetic nanoparticles decreases with gradual immobilization in hydrogels

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ABSTRACT

Magnetic nanoparticles (MNP) applied in magnetic fluid hyperthermia cancer treatment interact with cancerous tissue in various ways. The impact of these interactions on MNP heating efficiency is hard to quantify and strongly depends on the MNP mobility inside the cancerous environment. There, this MNP mobility is inhibited by cell attachment and internalization. In this study, we model this impact and analyze the MNP heating under gradual immobilization of MNP in acrylamide hydrogels with tailored mesh size. Our results confirm a clear particle heating dependency on the state of immobilization of MNP. This state is related to the mean mesh size of the respective hydrogel. From this, the contributions of Brownian and Néel relaxation mechanisms to the overall particle heating are estimated. In fact, the heating efficiency decreases by up to 35% for the highest immobilization state of MNP. This result is discussed in the context of the field-dependent Brownian and Néel relaxation time, showing that the former significantly contributes to the heating efficiency even for small particles under the field parameters employed in this study.

1. Introduction

Magnetic Nanoparticles (MNP) generate heat when subjected to an alternating magnetic field (AMF) [1,2]. Such heat can be locally delivered at a target site, e. g. a tumor, and be used therapeutically for hyperthermic tumor destruction [3,4] or as an adjunct in chemotherapy [5,6] or radiation therapy [7,8]. The local delivery of heat via MNP is known as magnetic fluid hyperthermia (MFH) and has been successfully applied to glioblastoma brain tumors and prostate tumors in clinical trials within the last decade [7,9]. Generally, cell death is induced at temperatures higher than 43 °C for treatment durations longer than 10 min [2,10]. Usually, solutions containing freely dispersed MNP easily develop such high temperatures when they are exposed to an AMF. In this case, both the Néel and the Brownian relaxation mechanisms contribute to the overall heating of the MNP solution. However, MNP are immobilized in a tumor environment as they inevitably interact with cells, attach and agglomerate at the cell membrane from where they are internalized into lysosomes [11,12]. Such intracellular MNP generate much less heat compared to MNP freely dispersed in water [13] or cell medium [14]. Concerning this issue, it was recently shown

that the inhibition of physical Brownian particle rotation upon MNP immobilization is the dominating factor for the observed decrease in heating [15].

To study the effect of particle immobilization in human tissue, hydrogels offer versatile possibilities to design tissue-equivalent phantoms due to their biocompatibility, mechanical properties and especially their tunable mesh sizes [16,17]. The incorporation of MNP into hydrogels leads to magnetically manipulable hybrid structures, so called ferrohydrogels, with tunable properties depending on the crosslinking density and softness of the hydrogel on the one hand, and on the shape and magnetic properties of the MNP on the other hand. These ferrohydrogels offer promising possibilities in materials analysis and in biomedical applications: Hydrogel-entrapped MNP can be used as magneto-mechanical probes to non-invasively analyze the mechanic properties of hydrogel networks [18]. Further, the deformation of ferrohydrogels in magnetic field gradients has been exploited to design remote controlled drug and cell delivery systems [19]. Due to their tunable heating properties, ferrohydrogels were also used for MFH in tumor therapy [20] and thermosensitive drug delivery [21].

In the present study, we investigate the effect of gradual

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immobilization of MNP on their heating efficiency. Therefore, poly-acrylamide ferrohydrogels with tailored mesh sizes in the range of the hydrodynamic size of the MNP are synthesized. The MNP are incorporated inside these gels. The mean mesh size of the hydrogels is determined from rheological measurements. The MNP characterization is performed with transmission electron microscopy (TEM), dynamic light scattering (DLS) and SQUID magnetometry. From the heating measurements we estimate the contribution of Brownian particle relaxation to MNP heating and discuss its dependence on the field parameters employed in this study.

2. Material and methods

2.1. Particle synthesis and characterization

Iron-oxide MNP were synthesized via alkaline co-precipitation of $FeCl_2$ and $FeCl_3$ salts in NH_3 aq. (20%), according to the procedure of Massart [22], and coated with sodium citrate, as previously reported in [23].

Transmission electron microscopy (TEM) characterization of MNP samples was conducted with a Zeiss LEO 906 TEM (Carl Zeiss GmbH, Oberkochen, Germany) operated at 60 kV. Before each measurement, 1 μL sample was dried on Cu/Rh-150 maxtaform-grids (Electron Microscopy Sciences, Hatfield, PA, USA) for one hour. Particle core diameters (d_C) were determined from TEM images by manual measurement employing the software PaintNET [24]. The resulting values were fitted with the cumulative distribution function (CDF) of the log-normal distribution probability density function (s. Appendix A).

The MNP hydrodynamic size was characterized by dynamic light scattering (DLS) with a Zetasizer Nano S (Malvern Instruments Ltd., Worcestershire, UK). For that, 500 μL MNP solution diluted to an iron concentration of 1.2 μg(Fe)/mL was measured at 293.15 K at a back-scattering angle of 173° and the procedure was repeated in triplicate The average hydrodynamic diameter was determined from fitting the log-normal distribution probability density function (PDF) (s. Appendix A) to the intensity-weighted MNP hydrodynamic size distribution.

For the MNP magnetic characterization, superconducting quantum interference device (SQUID) magnetometry was carried out with a Magnetic Properties Measurement System (MPMS; Quantum Design Inc., San Diego, CA, USA) operated at 295 K. For this, 60 μL MNP samples containing 6.5 wt-% mannitol were freeze-dried in 100 μL polycarbonate capsules (Quantum Design Inc.). The magnetization, $M(H)$, was measured varying the magnetic field from -4000 kA·m^{-1} to 4000 kA·m^{-1}. The resulting $M(H)$ data was normalized to the absolute amount of iron per sample and fitted with the Langevin function $M(H) = M_S(\coth(z) - 1/z)$, with $z = (\xi - \xi_0)/s$, $\xi = \mu_0 M_S V_C H/(k_B T)$, $V_C = \pi/6 \cdot d_C^3$ the MNP core volume and $\mu_0 = 4\pi \cdot 10^{-7} \text{ VsA}^{-1}\text{m}^{-1}$ the vacuum magnetic permeability. Furthermore, zero-field-cooled field-cooled (ZFC-FC) measurements were performed on the same batch of particles varying the temperature from 5 K to 295 K at constant magnetic field of 0.8 kA·m^{-1}. The ZFC-FC curves were normalized to the saturation magnetization M_S derived from fitting the Langevin function to the $M(H)$ data.

2.2. Hydrogel synthesis and characterization

Acrylamide (Aam, ≥99%), N,N′-methylenebisacrylamide (BIS, 99%), N,N,N′,N′-tetramethylethylene-diamine (TEMED, 99%) and ammonium persulfate (APS, ≥98%) were purchased from Sigma-Aldrich.

For the preparation of hydrogels, Aam and the crosslinker BIS were dissolved in deionized water (DI-H_2O) at a volume fraction of $\nu_{pol} = 0.08$ =$((m_{Aam} + m_{BIS})/\rho_{PAam})/(m_{tot}/\rho_{H2O})$, with $\rho_{PAam} = 1.3 \text{ g·cm}^{-3}$ and $\rho_{H2O} = 1.0 \text{ g·cm}^{-3}$ the density of polyacrylamide and water, respectively, and m_{Aam}, m_{BIS} and m_{tot} denoting the masses of Aam, BIS and the total solution mass. The crosslinking degree for different hydrogels was adjusted by variation of the crosslinker mole fraction

$\alpha = n_{BIS}/(n_{BIS} + n_{Aam})$ between $\alpha = 0$ and $\alpha = 0.005$ (s. Supplementary Material S3 for further details). The free radical polymerization was initialized by adding APS with a mass fraction of $\mu_m = 0.002$ and TEMED to the polymer solution. After mixing all components in a glass vial, the mixture was transferred to a cylindrical Teflon mold (diameter $d = 40$ mm, volume $V = 2.6$ mL) and was kept for polymerization at room temperature for at least 1 h. Then, the resulting gel was carefully removed from the mold.

Rheological measurements were performed using an AR-G2 rheometer (TA Instruments, New Castle, DE, USA) with a plate-plate geometry ($d = 40$ mm). A solvent trap filled with DI-H_2O was used to adjust the water vapor pressure and prevent solvent evaporation from the hydrogel. Strain sweep experiments were performed at a frequency of 1 Hz in the strain regime $0.0002 \leq \gamma \leq 0.02$. Frequency sweep experiments in the range of 0.1–100 Hz were carried out in the linear viscoelastic regime at a constant strain of 0.002. From these measurements, the frequency dependent storage modulus, G', and loss modulus, G'', of the hydrogels are determined.

Vibrating sample magnetometry (VSM) measurements were conducted on ferrohydrogel samples employing a EV 7 vibrating sample magnetometer (MicroSense, Lowell, MA, USA) operated at $T = 300$ K with a magnetic field ranging from -1430 kA·m^{-1} to 1430 kA·m^{-1}. Ferrohydrogel samples were prepared as described above in cylindrical teflon moulds with a volume of approximately 55 μL with $\nu_{pol} = 0.08$ and various crosslinker mole fractions ranging from $\alpha = 0.0005$ to $\alpha = 0.005$. The MNPs were added as suspension in water (partially replacing water added for gel synthesis) to achieve a final MNP concentration of 300 μg(Fe)/mL. As a reference sample a dispersion of MNP in water was measured.

TEM images of freeze-fractured, freeze-dried ferrohydrogel samples were acquired with a Zeiss LEO 912 Omega (Carl Zeiss). For that, 55 μL ferrohydrogel ($\nu_{pol} = 0.08$, $\alpha = 0.00075$) with an iron concentration of 300 μg(Fe)/mL was frozen in liquid nitrogen, finely ground and placed onto a copper grid with a carbon-hole film (Quantifoil® Multi A, Quantifoil Micro Tools GmbH, Großlöbichau, Germany). The ferrohydrogel on the copper grid was freeze-dried in a VTR 5036 vacuum drying oven (Heraeus Holding GmbH, Hanau, Germany) overnight.

2.3. Calorimetric particle heating measurements

Calorimetric particle heating measurements were carried out with a custom-built hyperthermia setup (Trumpf Hüttinger, Freiburg, Germany), consisting of a DC generator, an AC-resonant oscillator and a water-cooled copper coil. The field amplitude, H_0, and the frequency, f, were set to $H_0 = 40 \text{ kA·m}^{-1}$ and $f = 270$ kHz. 1 mL samples were prepared in 4 mL glass vials, which were wrapped in 1 mm thick Styrofoam to reduce heat losses and placed in the 7-turn coil (s. Supplementary Materials S1 for further details on the setup). Samples were exposed to the alternating magnetic field for 30 min starting at an initial temperature of $T_0 = 308.15$ K (35 °C). During exposure, the sample temperature was recorded with a fiber-optic thermometer Luxtron 812 (LumaSense Inc., Santa Barbara, CA, USA). Sample heating curves are shown in the Supplementary Material S2. The measured temperature-time data, $T(t)$, were fitted with the Box-Lucas function

$$T(t) = T_{rise} \cdot (1 - \exp(-\frac{t}{\tau})) + T_0 \tag{1}$$

from which the specific absorption rate (SAR) value was calculated according to [25]:

$$\text{SAR} = \frac{c}{\rho} \cdot \frac{dT}{dt}|_{t \to 0} \tag{2}$$

with the specific heat capacity of water, $c = 4.187 \text{ J (g·K)}^{-1}$, the MNP weight fraction, ρ, and $dT/dt|(t \to 0) = \frac{T_{rise}}{\tau}$, derived from the Box-Lucas fitting parameters.

Ferrohydrogel samples were prepared as described above (cf.