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Florence Gazeau, Michaël Lévy, Claire Wilhelm. Optimizing magnetic nanoparticles design for nanothermotherapy. *Nanomedicine*, 2008, 3 (6), pp.831-844. 10.2217/17435889.3.6.831 . hal-00348171

HAL Id: hal-00348171

<https://hal.science/hal-00348171v1>

Submitted on 5 Jun 2023

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Optimizing magnetic nanoparticle design for nanothermotherapy

Current developments in nanotechnology offer new tools to the design of nanometric heat-generating 'foci' that can be activated remotely by an external alternating magnetic field. These nanometric heat sources may serve for therapeutic hyperthermia alone or combined with other therapeutic modalities, such as drug delivery or gene therapy. Activable therapeutic tools at the nanoscale fulfill the requirements of future medicine in terms of spatial targeting and temporal control of therapy. The present review discusses fundamental aspects regarding the design of magnetic nanoparticles with optimized properties, by unraveling physical mechanisms that govern heating power in biological media. Towards therapy, achievements and promises of magnetic nanoparticles for cancer-localized hyperthermia, targeting strategies and multivalent functionalities are exposed.

KEYWORDS: cancer, hyperthermia, magnetic nanoparticles, magnetism, targeting

Raising the temperature of tumor tissue is one approach for the treatment of cancers. Hyperthermia is used as adjunctive therapy with established cancer treatments, such as chemotherapy and radiotherapy, to bolster the therapeutic effects of some anticancer drugs or ionizing radiation. Clinical trials started in the 1970s using different strategies, such as the application of different types of radiations (microwaves, radiofrequency and ultrasound), with or without the introduction of activated heat sources.

Thermoablation ($T > 46^\circ\text{C}$) causes tissue necrosis directly, whereas moderate hyperthermia ($41^\circ\text{C} < T < 46^\circ\text{C}$) has various effects both at the cellular level (e.g., on the induction and regulation of apoptosis, signal transduction, multidrug resistance [1] and heat-shock protein (HSP) release [2,3]) and on tissues (e.g., changes in pH and in the perfusion and oxygenation of the tumor microenvironment) [4].

As a general rule, therapeutic research seeks to limit 'bystander' effects by targeting smaller and smaller unit volumes. Historically, heat therapy was applied either to the whole body or to superficial tissues (by contact), whereas far more precise regions are now being targeted with focused radiation beams or with implanted, remotely activated probes. However, these treatments are still limited to readily accessible or relatively superficial targets [5].

Current developments in nanotechnology are making it possible to use nanometric heat-generating 'foci' that can be activated by an external magnetic field. These nanometric heat sources have

decisive advantages over macroscopic implants. Being available in the form of colloidal suspensions, they can, like contrast agents, be injected into the body through a variety of noninvasive routes. Their subsequent distribution within the body can be controlled by various targeting strategies, thereby optimizing their concentration in therapeutic target zones or even specifically within tumor cells. They are small enough to cross biological barriers, meaning that heat can be generated close to the target, be it a tissue, a cell or an organelle. Finally, the heat generated in this way is more homogeneous than that obtained with macroscopic implants; it is also proportional to the local concentration of nanofoci and is limited solely to the site of nanoparticle accumulation.

Among the different nanostructures developed for therapeutic uses, magnetic particles generate heat efficiently when submitted to an alternating magnetic field. The first use of magnetic particles for hyperthermia was reported by Gilchrist in 1957 [6]. Subsequent studies focused on the efficiency of different magnetic particles for inducing sufficient macroscopic hyperthermia to induce tumor regression. In 1993, Jordan compared the different particles available at the time by measuring their absorption efficiency (specific absorption rate [SAR] or specific loss power [SLP]) and identified the mechanisms underlying the transformation of magnetic energy into heat [7]. Nanometric particles proved to be far superior to micrometric particles in terms of heat generation.

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Physical mechanisms underlying hyperthermia mediated by magnetic nanoparticles

A nanometric particle (typically <100 nm) has a single magnetic domain and is therefore equivalent to a simple magnetic dipole. The response of such a dipole to an alternating magnetic field depends on its dynamic behavior, which is governed mainly by thermal fluctuations. The orientation of the magnetic moment fluctuates thermally, through two mechanisms:

Néel fluctuations of the magnetic moment relative to the crystal lattice (internal dynamics) are hindered by ‘magnetic friction’ or, in other words, by the energy of anisotropy that tends to orient the magnetic domain in a given direction relative to the crystal lattice. The time characteristic of Néel fluctuations grows exponentially with the ratio between the energy of anisotropy and the thermal energy kT .

- Brownian fluctuations of the particle itself relative to the medium in which it is placed (external dynamics) are hindered by the viscosity of the medium and by all other phenomena that tend to counter the movement of the particle: the time characteristic of Brownian dynamics increases linearly with the hydrodynamic volume of the particle and with the viscosity of the surrounding medium.

These internal and external sources of ‘friction’ lead to a phase lag between the applied magnetic field and the direction of the magnetic moments, thereby generating thermal losses. One can thus predict the SLP (expressed in watts per gram of magnetic material) for particles with known Néel and Brown relaxation times by using a linear response model [7,8]. The predominant mechanism that governs the heating capacity of a given nanoparticle is the mechanism that induces the most rapid variations of the magnetic moment. The SAR increases with the frequency of the applied magnetic field and is proportional to the square of the field intensity.

Optimizing nanoparticle design for magnetic hyperthermia

The SLP is measured in terms of the rise in temperature per unit time and per gram of magnetic material, multiplied by the calorific capacity of the sample. Jordan’s comparative experiments [7] clearly demonstrated the superiority – in terms of the SLP – of single-domain magnetic particles over multidomain particles. Ma *et al.*

also showed that the SLP of magnetite particles decreases with increasing particle size above 50 nm [9]. Thus, subsequent research focused on nanoparticles (<50 nm) with the best possible SLP. The highest reported SLP values [5] were obtained with maghemite ($\gamma\text{-Fe}_2\text{O}_3$) particles coated with dextran [10] or polyethylene glycol (diameter: 15.3 nm) [11] and with magnetosomes (particles of 20–45 nm surrounded by a biological membrane) isolated from magnetic bacteria (FIGURE 1) [12]. Although the synthesis and surface engineering of iron oxide nanoparticles are known as key parameters for size distribution, magnetic properties, stability and biocompatibility [13], they have not been varied systematically to assess their influence on the heating power of nanoparticles. In particular, the influence of particle coating on the SLP remains to be elucidated.

The different mechanisms of heat generation by single-domain iron oxide nanoparticles (and especially the respective contributions of Néel and Brown relaxation) can be unraveled by independently varying the size distribution of the nanoparticles, the magnetic anisotropy of the material of which they are composed and the viscosity of the surrounding medium [14]. For nanoparticles composed of $\gamma\text{-Fe}_2\text{O}_3$, which are used most commonly for both imaging and therapeutic purposes, the heating capacity depends strongly on the particle size: in an applied magnetic field of given frequency (100 kHz to 1 MHz), the optimal diameter is between 14 and 16 nm. This major influence of size is a signature of the predominant contribution of Néel relaxation to heat generation and comes from the exponential dependency of the Néel time on the nanoparticles’ magnetic volume (and thus their energy of anisotropy). By contrast, the contribution of Brownian dynamics to the SLP is less dependent on particle size and only becomes predominant with larger particles (>17 nm), when the Néel time becomes longer than the Brownian time.

In practice, the heating capacity of a given sample will therefore depend on the correspondence between the particle-size distribution and the size-dependent SLP profile. For example, when the mean diameter of $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles is increased from 5.3 to 16.5 nm, the SAR rises by a factor of 400 (FIGURE 1). Polydispersity generally reduces the SLP because the proportion of particles capable of contributing to heat generation is reduced. The ideal characteristics of a given magnetic material in a given medium can be determined theoretically (FIGURE 2) [14]. In

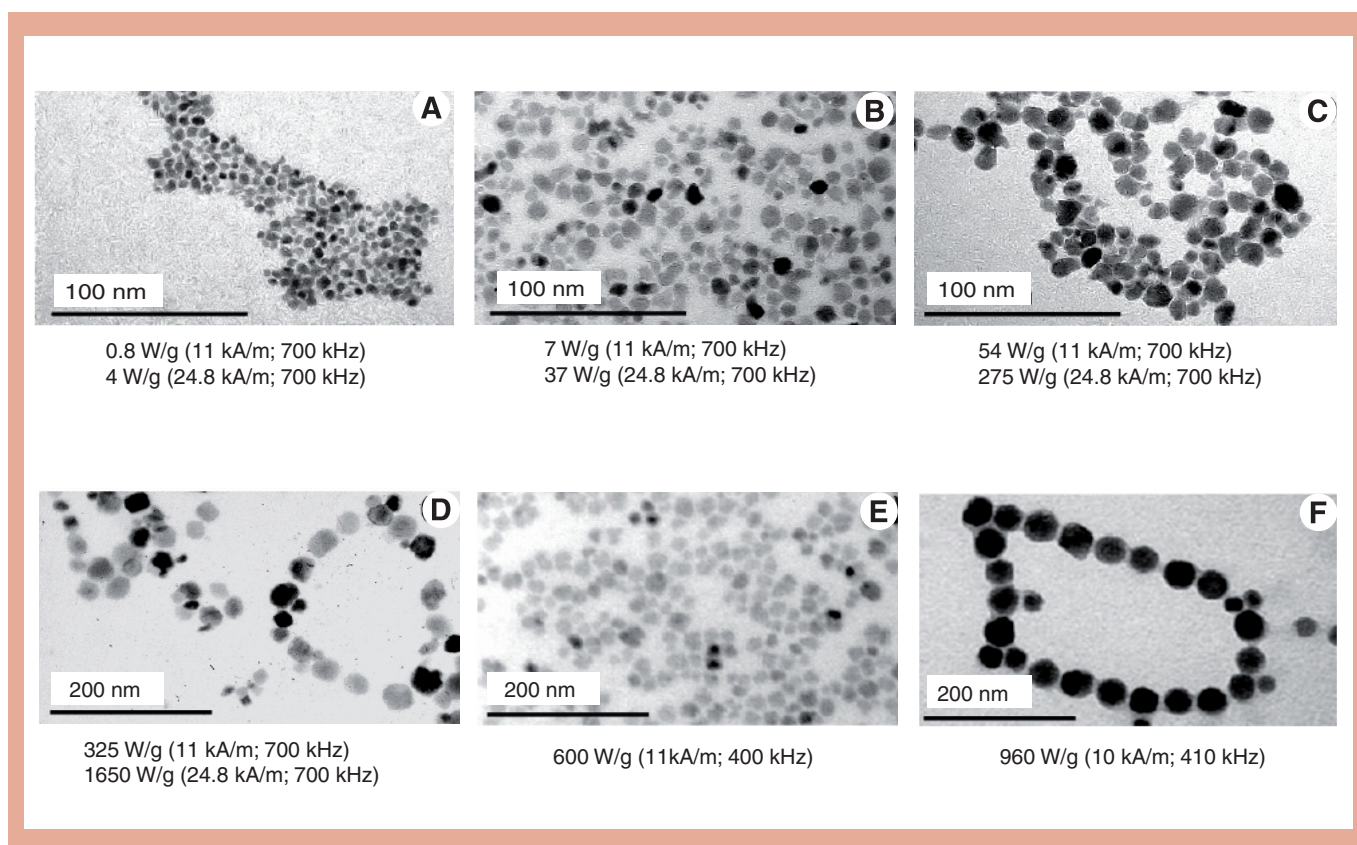


Figure 1. Different maghemite ($\gamma\text{-Fe}_2\text{O}_3$) nanoparticles designed for optimizing heat generation. Their specific-loss power (SLP) is indicated in W per gram of magnetic material for different values of magnetic field amplitude and frequency. **(A–D)** Citrate-coated anionic $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles [14] were sorted in size by successive phase separations: their SLP is increased by three orders of magnitude for log-normal size distributions with a characteristic diameter from 5.3 **(A)** to 16.5 nm **(D)**. **(E)** Polyethylene glycol-coated $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles with a mean diameter of 15.3 nm exhibit high SLP owing to their narrow-size distribution. Adapted with permission from [11]. **(F)** Bacterial magnetosomes synthesized by the magnetotactic bacteria *Magnetospirillum gryphiswaldense*. Magnetosomes consist of nano-sized crystals of $\gamma\text{-Fe}_2\text{O}_3$ (mean core diameter ~ 30 nm) embedded in a biological membrane. They present the largest value of SLP that can be found in the literature. Adapted with permission from [12].

water, most $\gamma\text{-Fe}_2\text{O}_3$ particles are monodisperse and approximately 15 nm in diameter. The size-dependent heating power of $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles has been investigated experimentally [15], showing a non-monotonous peaked behavior in agreement with theoretical predictions for the whole range of particle size (5–50 nm) (FIGURE 3). In particular, beyond the optimal diameter of 16 nm, the SLP decreases with particle size. In parallel, the relative Néel contribution to SLP diminishes in favor of Brownian contribution.

Apart from $\gamma\text{-Fe}_2\text{O}_3$ and magnetite nanoparticles, different magnetic materials have been investigated for their efficiency as heat nanomediators [16,17]. Relevant physical parameters affecting heating power are size- and temperature-dependent magnetization [18] and magnetic anisotropy [14,19] (via the Néel time) of the material.

Materials with high magnetization are needed to produce efficient heat generation. Novel metallic nanoparticles with a pure iron core have been synthesized recently in an attempt to increase

magnetization as compared with iron oxide [20]. However, issues of stability, solubility and biocompatibility arise from the high reactivity of elemental iron in an aqueous environment. The coating of iron core with a biocompatible inert polymer has been successfully proposed to passivate the particle surface and limit particle growth [20].

Besides magnetization, the magnetic anisotropy determines the optimal size and relative contribution of Néel and Brownian mechanisms. For example, for cobalt ferrite, which has a stronger magnetic anisotropy than $\gamma\text{-Fe}_2\text{O}_3$, the diameter associated with the optimal Néel contribution to heating is 7 nm [14]. Above this diameter, such nanoparticles generate heat mainly by Brownian relaxation and are therefore highly sensitive to the viscosity of the medium in which they are placed (FIGURE 2). Thus, the heating capacity of nanoparticles is modified when they are internalized by cells: intracellular confinement of nanoparticles within endosomes [21] hinders

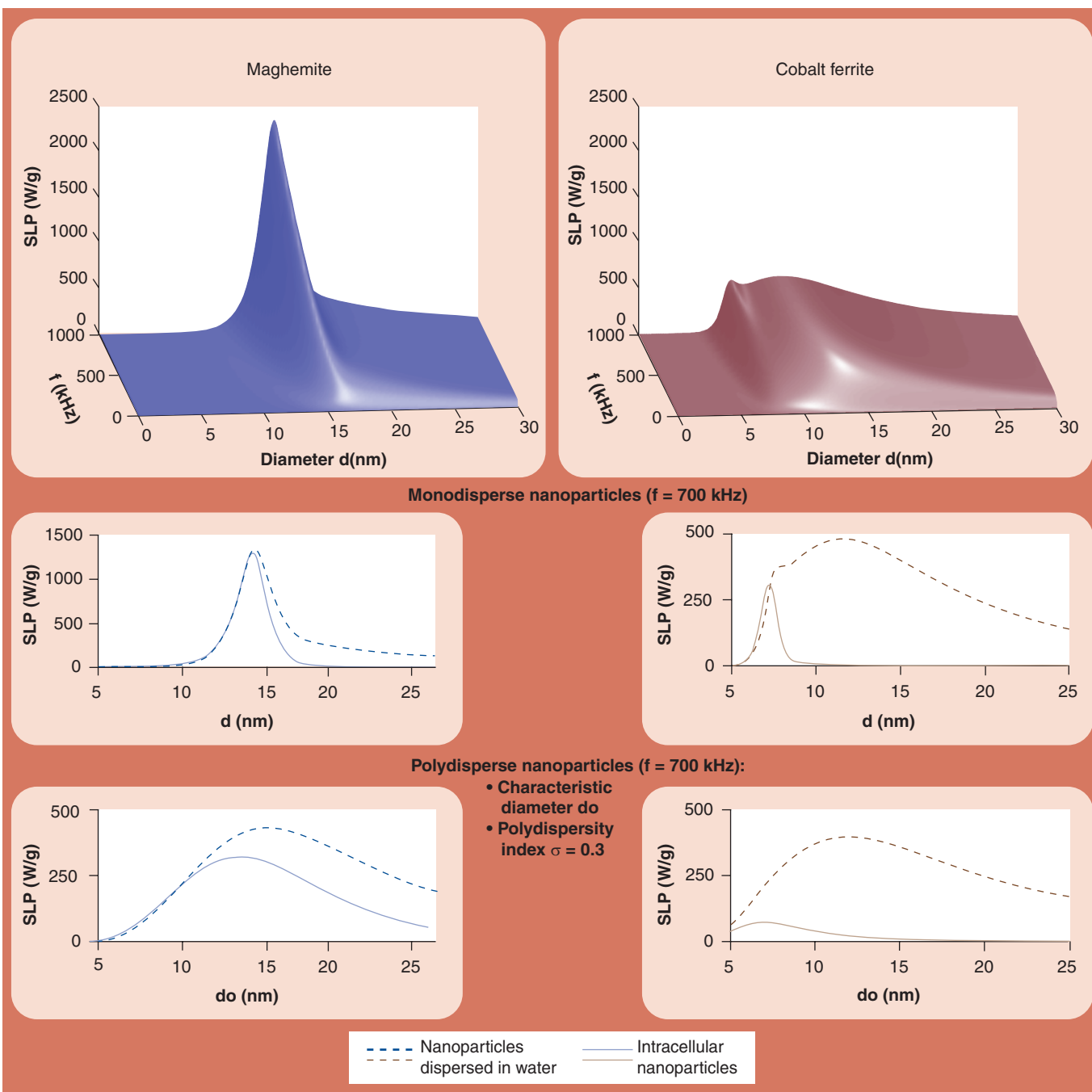


Figure 2. Frequency-dependent heating power is governed by nanoparticle's size, magnetic anisotropy and microenvironment: comparison of maghemite and cobalt ferrite nanoparticles. Top: SLP of monodisperse particles of maghemite ($\gamma\text{-Fe}_2\text{O}_3$) (left) and cobalt ferrite (right) as a function of particle diameter and magnetic field frequency. Particles were dispersed in water. SLP of $\gamma\text{-Fe}_2\text{O}_3$ particles is highly peaked as a function of particle size owing to the dominant contribution of Néel relaxation. By contrast, SLP of cobalt ferrite is mostly due to Brownian relaxation. Middle: SLP as a function of particle diameter for monodisperse particles in two different microenvironments: particles dispersed in water and particles confined within intracellular endosomes. Compared with water, the Brownian contribution to heating is abrogated when particles are internalized in cells. Note that SLP owing to Néel relaxation is maximum for a particle diameter of 14 nm for $\gamma\text{-Fe}_2\text{O}_3$ and of 7 nm for cobalt ferrite. Bottom: SLP as function of characteristic diameter d_0 for polydisperse samples (log-normal distribution with polydispersity index $\sigma = 0.3$). SLP are strongly reduced owing to size dispersion. This effect is much more pronounced for the Néel contribution than for the Brownian contribution. In all graphs, the magnetic field amplitude was $H = 24.8$ kA/m. d : Diameter of monodisperse nanoparticles; d_0 : characteristic diameter for polydisperse nanoparticles with a log normal distribution; SLP: Specific loss power.

the movement of the particles and heat generation is thus solely due to the Néel phenomenon (internal movement of the magnetic moment)

[19,22]. The most suitable particles for intracellular hyperthermia applications therefore generate heat solely through the Néel phenomenon.

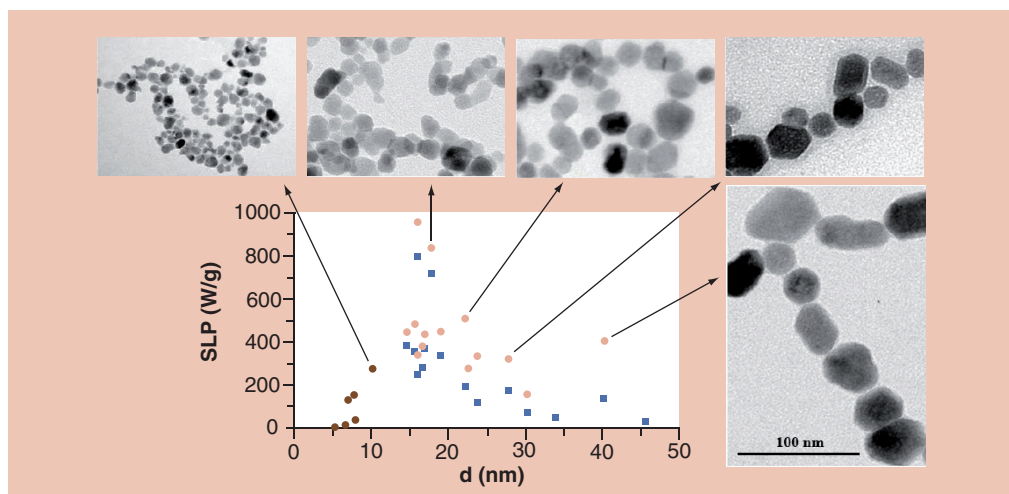


Figure 3. Size-dependent heating power of $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles. SLP of $\gamma\text{-Fe}_2\text{O}_3$ nanoparticles in water (dark gray and light gray circles) and in glycerol (gray squares) as a function of their mean magnetic diameter [15]. Corresponding electron-microscopy pictures are shown for some samples. Magnetic field frequency and amplitude were 700 kHz and 27 kA/m, respectively. Particles with diameter in the range of 5 to 12 nm (dark gray points) were synthesized using the classical coprecipitation Massart's method [75]. A hydrothermal process step was added to synthesize particles with a larger diameter (14–50 nm) showing an acicular shape. In agreement with the theory, SLP shows a non-monotonous behavior, with a maximum SLP for 16 nm magnetic diameter. For particles dispersed in glycerol (with a viscosity 1000-fold higher than water), SLP is slightly reduced owing to vanishing of the Brownian contribution. Because the Néel mechanism dominates the SLP, these biocompatible nanoparticles are well adapted for intracellular hyperthermia [19] after cell internalization [76]. SLP: Specific loss power

In an attempt to regulate thermotherapy temperature and avoid overheating in surrounding normal tissue, materials have been proposed that stop heating above a specified temperature. Indeed ferro and ferromagnetic materials lose their magnetization above Curie temperature (T_C), becoming paramagnetic. Thus, a T_C point near 50°C can be used as an *in vivo* switch to control the temperature rise. For this purpose, nanoparticles composed of substituted ferrite, such as $\text{Mn}_{1-x}\text{Zn}_x\text{Fe}_2\text{O}_4$ [23] or lanthanum manganese perovskite $\text{La}_{1-x}\text{Sr}_x\text{MnO}_3$ [24–26], have been synthesized, showing tunable Curie temperature and, in some cases, self-controlled heating behavior. However, the magnetization value decreased significantly because of a high amount of substitution and therefore SLP values were relatively low.

Hence, there is still a need for research to design biocompatible monodisperse nanoparticles with simultaneously high and self-regulated heating power.

Importantly, the design of nanoparticles for *in vivo* hyperthermia must also address the issues of biokinetics, biodistribution and biodegradation in the body. Specific surface modification of nanoparticles [13] or encapsulation into carriers, such as liposomes [27,28] or biological vesicles [29], are strategies to control the final destination of heat nanomediators. Enhanced blood circulation

and limited uptake by the reticuloendothelial system are requirements for the systemic administration of nanoparticles and potential addressing to the tumor mass. Even after direct injection of nanoparticles in the tumor, preferential and stable interactions with malignant cells are desirable [30]. However, surface engineering of nanoparticles or their confinement into stealthy containers may induce modifications in heating capacity (e.g., by affecting Brownian dynamics or by increasing dipole–dipole interactions) that are worth to be studied.

Antitumoral efficacy of hyperthermia mediated by magnetic nanoparticles

Studies of the antitumoral efficacy of hyperthermia generated by magnetic nanoparticles are being pursued mainly by three teams, working in Japan, Germany and Australia. Most data show very encouraging tumor regression when a concentrated suspension of magnetic nanoparticles is injected directly into the tumor (interstitial hyperthermia). Significant effects have been observed in animal models of breast cancer [31–33], prostate cancer [34–37], brain cancer [38,39], renal cancer [40] and melanoma (FIGURE 4) [41].

Clinical trials are already underway in Berlin for the noninvasive treatment of prostate cancer [42–44] and glioblastoma [45,46], based

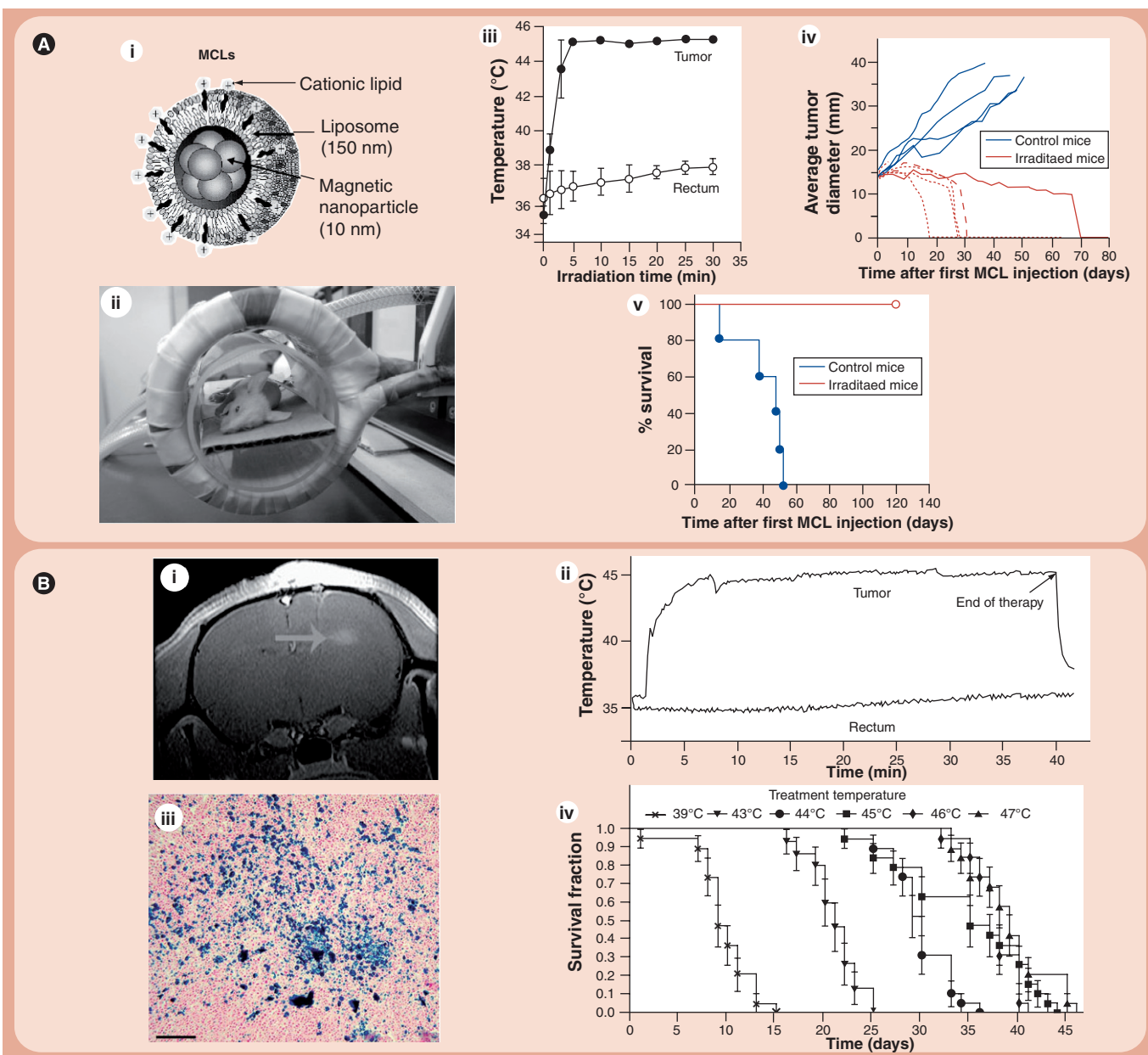


Figure 4. Examples of tumor regression following magnetic hyperthermia. (A) Complete regression of mouse mammary carcinoma by frequent repeated hyperthermia using magnetic cationic liposomes. **(i)** Magnetite nanoparticles were embedded into cationic liposomes (MCLs) and injected directly into 15-mm tumors, which were induced subcutaneously in mice. **(ii)** Alternating magnetic field (AMF) of frequency 118 kHz was generated by a horizontal coil, where the mouse was placed. **(iii)** Temperatures in the rectum and at the surface of the tumor were recorded with an optical fiber probe during AMF exposure. For each irradiation AMF, the tumor was heated to 45°C for 30 min by controlling the AMF intensity. **(iv)** The therapeutic effect of tumor irradiation. MCLs were injected directly into 15-mm diameter tumors (2 mg magnetite). Tumors were irradiated for 30 min, repetitively (one to six rounds). Compared with control (blue lines), the tumor growth was stopped after AMF irradiation (red lines). Each line represent a single tumor ($n = 5$ for both control and treated mice). After repeated hyperthermia, complete regression of the tumor was observed. **(v)** Percentage of survival for the same group of mice. Control mice: full circles; treated mice: open circles. All the treated mice survived for 120 days after treatment, with no regrowth of the tumor. Adapted with permission from [33]. **(B)** Effect of magnetic hyperthermia on rat malignant glioma. **(i)** Tumors were induced by implantation of rat glioma RG-2-cells into the brains of rats and were visualized by MRI (T₁-weighted MR image). **(ii)** Magnetic nanoparticles with two different coatings and sizes were administered in to the tumors by stereotactic injection (20 μ l at 2 mol/l iron concentration). AMF (100 kHz, 0–18 kA/m) was generated using an applicator system (MFH 12-TS[®], MagForce Nanotechnology, Germany). Temperature into the tumor was measured using a fiber optic thermometry device. Although the rectum temperature did not increase by more than 1°C, tumors injected were heated up to 45°C (10°C increase). **(iii)** Prussian blue staining of the tumor after thermotherapy treatment: the injection of the aminosilane-coated particles led to the formation of stable deposit into the tumor: the nanoparticles (in blue) were still distributed throughout the tumor after treatment. **(iv)** Effect of treatment temperature on the survival rate after thermotherapy (one round, 30 min). Intratumoral temperature greater than 43°C significantly increased the lifespan of tumor-bearing rats compared with nontreated controls (survival fraction null at days 8 to 13, data not shown). The gain of survival was correlated with the intratumoral temperature. Adapted with permission from [39]. MCL: magnetic cationic liposomes.

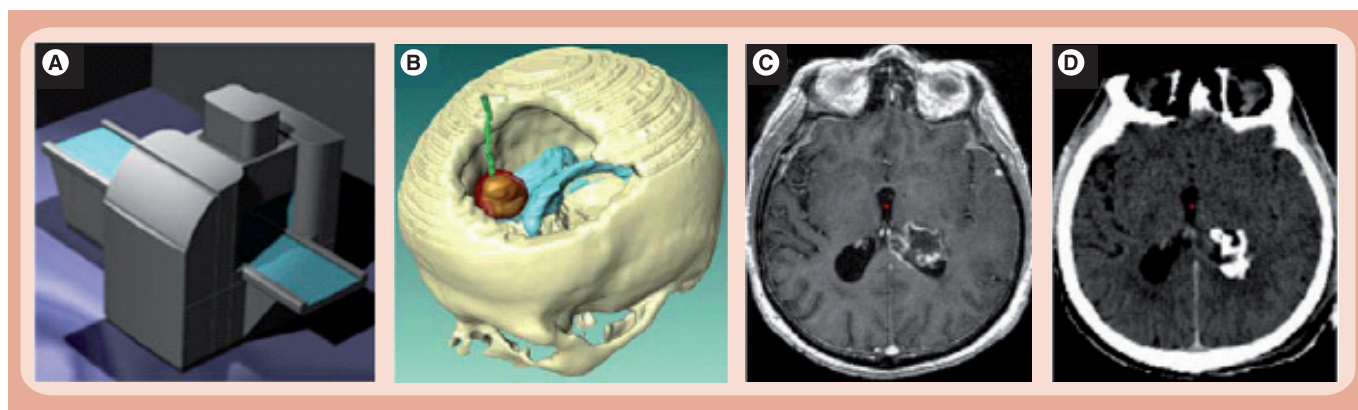


Figure 5. Clinical studies of thermotherapy using magnetic nanoparticles: feasibility. (A) The alternating magnetic field applicator MFH® 300f was developed especially by MagForce Nanotechnologies (Germany) to perform magnetic hyperthermia in humans (100 kHz, 2.5–18.0 kA/m). It can be used for deep heating of any circumscribed tumor within the body. (B–D) Clinical study on patients with glioblastoma multiform. The skull with a frontal glioblastoma was reconstructed in three dimensions (B) after computed tomography (C) and MRI (D), using dedicated software (MagForce NanoPlan®). Based on the 3D reconstruction of the defined tumor volume, target points were defined and magnetic fluid was instilled under neuro-navigational control. To calculate the expected heat distribution within the tumor for each magnetic field strength, the pattern of nanoparticle distribution is measured by computed tomography (D) and matched with preoperatively taken MR images (C). Intratumoral temperature varied from 43.6 to 49.5°C. Nanoparticle deposits were stable over several weeks and the whole procedure was well tolerated at moderate field strength. The efficacy of magnetic hyperthermia for treatment of glioblastoma multiform is being evaluated currently in a Phase II study. Adapted with permission from [45].

on intratumoral injections that are planned and controlled by computed tomography and MRI (FIGURE 5).

This treatment is combined with radiotherapy. The target tissue is exposed repeatedly to a magnetic field (100 kHz, 2.5–18 kA/m) generated by a dedicated machine, enabling deep heating of any circumscribed tumor within the human body. The treatment is well tolerated by the patient (at moderate field strength) but its therapeutic effects have yet to be determined.

The noninvasive thermometry method is desirable to control and regulate temperature distribution because both nanoparticle deposit and thermal regulation of tissue are not homogeneous. In all these studies, the local nanoparticle concentration within the tumor was sufficient to induce a marked temperature rise (up to 57°C), thanks to the affinity of the magnetic complexes for the tumor tissue. On noting that classical dextran-coated nanoparticles tended not to remain within their target tumor, Jordan *et al.* complexed nanoparticles with amino-silane groups that conferred a strong positive charge [30,39]. Likewise, the Japanese group synthesized magnetic liposomes (150 nm) containing positively charged lipids [47]. In both cases, the residual concentration within the tumor was far above that obtained with neutral magnetic complexes. It was also relatively stable over time, permitting repeated thermotherapy sessions without the need for further injections of magnetic material (FIGURE 4).

Induction of antitumoral immunity

Previously discussed antitumor effects of magnetically induced hyperthermia were related to cell necrosis caused by a temperature increase above 46°C (also called thermoablation). However, mild hyperthermia (between 41 and 46°C) may also affect the function of many structural and enzymatic proteins (membranes, cytoskeleton and DNA repair) [1] and cause cell-growth inhibition and cell apoptosis. These effects have been quantified in different studies of tumor cells that have internalized nanoparticles [22,48,49]. Although there is no specified cellular target for hyperthermia, the expression of several genes can be upregulated or downregulated by heat, for example, the family of HSPs. In consequence, heat-dependent immunological reactions may occur *in vivo*. In addition to the observed tumor regression, Nagoya's group in Japan showed that nanoparticle-mediated heat treatment also induced antitumoral immunity [50] (FIGURE 6). Using rats, they implanted a tumor on each femur and then injected magnetic liposomes into one of the two tumors [51]. On exposure to an alternating magnetic field, only the injected tumor heated to more than 45°C. However, both tumors had disappeared 28 days later. Further investigations showed that T lymphocytes (both CD8⁺ and CD4⁺) had invaded both tumors. The hyperthermia induced in one of the tumors had thus led to recognition of tumor antigens by the rat immune system. Subsequent studies [3,52,53] showed the role of HSP70 secreted by heated tumor cells in tumor-antigen presentation. Thus,

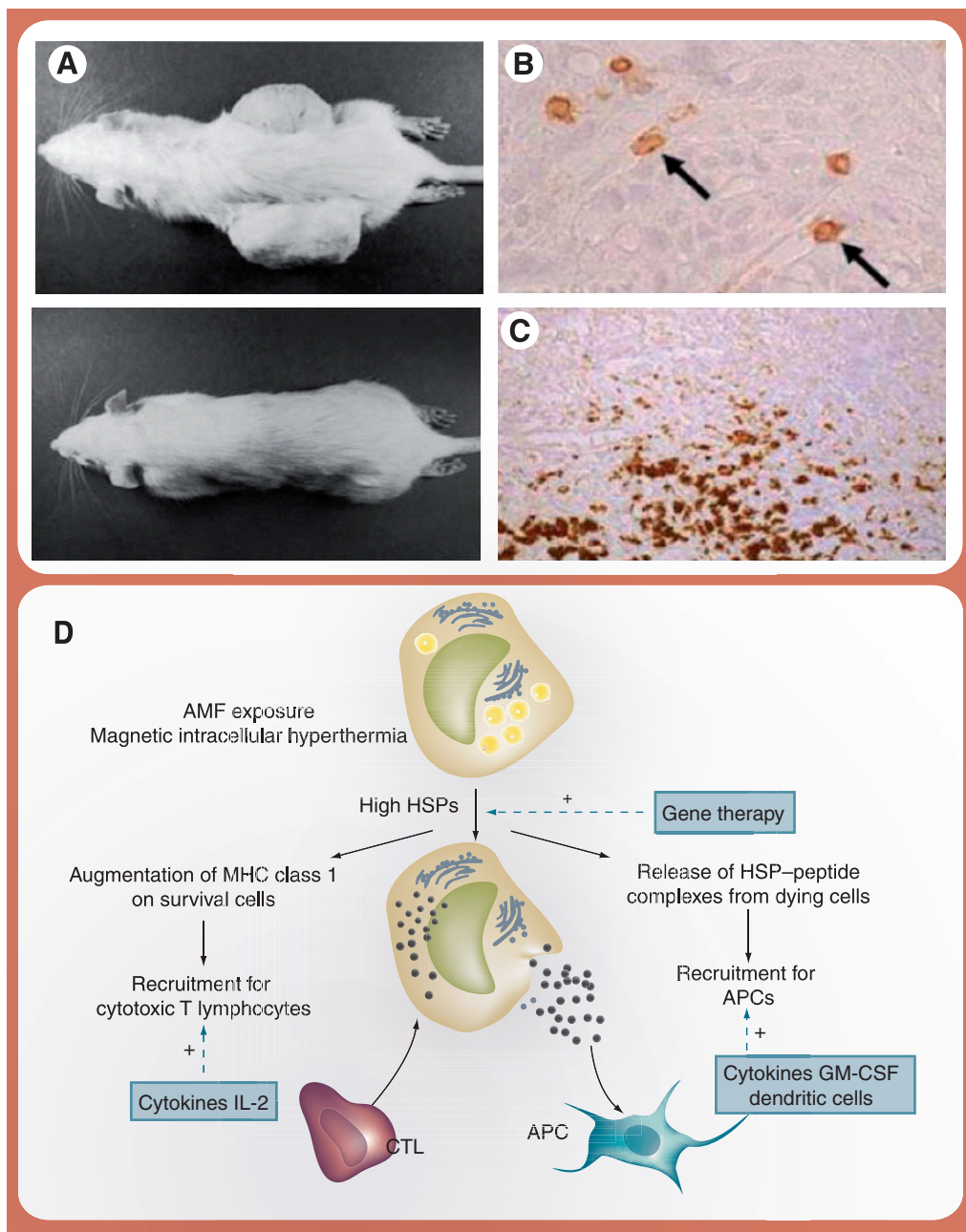


Figure 6. Antitumor immune response induced by hyperthermia with magnetic nanoparticles.

(A) Rats bearing two tumors in the femoral region. Rat glioma cells were first injected into the left side and 9 days after this first transplantation, the same amount of cells were injected into the right side. Top: Rat corresponding to the control group: no hyperthermia treatment. Bottom: Rat on which hyperthermic treatment was performed: magnetic cationic liposomes were injected directly into the left tumor only, 11 days after transplantation.

(B) Immunocytochemical assay revealed CD8-positive and CD4-positive immunocytes around viable cancer tissue after the hyperthermic treatment. **(C)** HSP70 also appeared in the viable area but not in the necrotic area (anti-HSP70 antibody staining) [54]. **(D)** To explain how tumor antigens are recognized by the host immune system, Kobayashi and coworkers proposed HSP-mediated antitumor immunity as a mechanism [3,52]. HSP overexpression and release from cells treated by magnetic hyperthermia could induce the recruitment of cytotoxic T lymphocytes and the activation of antigen-presenting cells. On this basis, different strategies have been proposed to enhance the antitumor immunity induced by magnetic hyperthermia: combination with cytokines [41], heat-inducible TNF- α gene therapy [57], recombinant HSP70 [77], HSP70 gene therapy [56] and dendritic cell therapy [55]. Adapted from [50,54] with the author's permission.

AMF: Alternating magnetic field; APC: Antigen-presenting cell; CTL: Cytotoxic T lymphocyte; GM-CSF: Granulocyte macrophage colony-stimulating factor; HSP: Heat-shock protein.

even moderate (nonlethal) magnetic hyperthermia could induce a form of vaccination against tumor cells and enable both the primary tumor and distant metastases to be eliminated [54]. Immunotherapeutic approaches (FIGURE 6) were subsequently combined with magnetic hyperthermia with the aim of obtaining a synergistic action; these approaches included injections of cytokines [41] or dendritic cells [55] and *HSP70* gene therapy [56]. A strategy based on gene therapy with thermal activation by magnetic liposomes has also been tested, in which expression of TNF- α is induced by a heat-sensitive promoter [57]. In all of these studies, the combination of magnetic hyperthermia with another therapeutic approach (immunotherapy, gene therapy) was more effective than either method used alone.

Towards targeted magnetic hyperthermia

Direct injection of magnetic complexes into solid tumors is only feasible when the tumor is localized and relatively accessible. One major challenge in the development of nanoparticle-mediated hyperthermia is thus to treat poorly accessible primary tumors and metastases. Attempts are being made to attach tumor cell-specific 'address tags' to magnetic complexes (e.g., liposomes and nanoparticles); after systemic injection, the complexes would be guided to their target by a coating ligand, such as an antibody [28,58,59], a peptide [60] or folic acid [61]. Similar research is underway on specific nanoparticles for tumor diagnosis (molecular imaging), although, curiously, there is little overlap between the literature on these two fields. It is particularly remarkable that the same object should have both MRI contrast properties and therapeutic potential. Nonetheless, the concentrations required for therapeutic uses are several orders of magnitude higher than those necessary for MRI diagnosis. Although individual cells can be detected by MRI when labeled with magnetic nanoparticles, Gordon *et al.* postulated [62] that a labeled cell could be heated independently of neighboring unlabeled cells was rejected on both theoretical [63] and experimental [48] grounds. Indeed, because of heat transfer to the surrounding environment, the temperature rise within a labeled cell is negligible in a stationary system. To reach the threshold temperature required for hyperthermia ($T > 43^{\circ}\text{C}$) [63], even with 'ideal' nanoparticles, the minimum volume of labeled cells is estimated to be approximately one cubic millimeter.

Currently, there are only a few reports of tumor hyperthermia after systemic injection of tumor-targeting magnetic nanoparticles (FIGURE 7).

Shinkai *et al.* took an antibody fragment (anti-MN-antigen) that binds to renal carcinoma cells specifically and coupled it to magnetic liposomes [64]. After intra-arterial injection of these immunomagnetic liposomes, the intratumoral concentration was far higher than for neutral liposomes and the tumor temperature increased to 43°C . In the same way, DeNardo *et al.* injected intravenously pegylated nanoparticles coupled to ^{111}I -chimeric L6 monoclonal antibodies [59]. However, to palliate for the low particle concentration within the tumor, the authors had to apply extremely strong magnetic fields (up to 128 kA/m, 153 kHz), which induce nonspecific tissue heating owing to the induced eddy current. Another proposed approach to efficient heating by intravenously injected magnetic nanoparticles is to apply a high-amplitude, confined, pulsed-alternating magnetic field.

Other strategies have been proposed to promote magnetic complex accumulation at tumor sites. One possibility is to use a particular route of injection to obtain the desired spontaneous biodistribution. For example, liver tumors, contrary to healthy liver, are irrigated mainly by the hepatic artery; thus, injection of embolic materials together with magnetic nanoparticles into this artery leads to a concentration of nanofoci within these tumors, causing arterial embolization [40,65–68]. Strategies based on magnetic 'vectorization', using a magnetic force to attract nanocomplexes towards tumors, are other promising approaches [69,70].

Conclusions

The design of optimized magnetic nanoparticles for therapeutic hyperthermia can be guided by a comprehensive understanding of the mechanisms underlying heating capacity. In this review, we describe the respective role of Brownian and Néel relaxations on the dynamical behavior of the magnetic moment of a nanoparticle and their influence on specific loss power. Theoretical dependence of heating power with frequency, particles size and the microenvironment is compared with available experimental data. It appears that advances can be made in the design of heat mediators, by improving material magnetization and size dispersion. The route towards self-regulating heat mediators is open but also requires further improvement to permit sufficient heating.

Most animal and clinical assays using magnetothermotherapy involve direct administration of nanoparticles in the tumor achieving satisfactory antitumoral efficacy while still requiring temperature monitoring and control. However, systemic injection of heat mediators is desirable to target

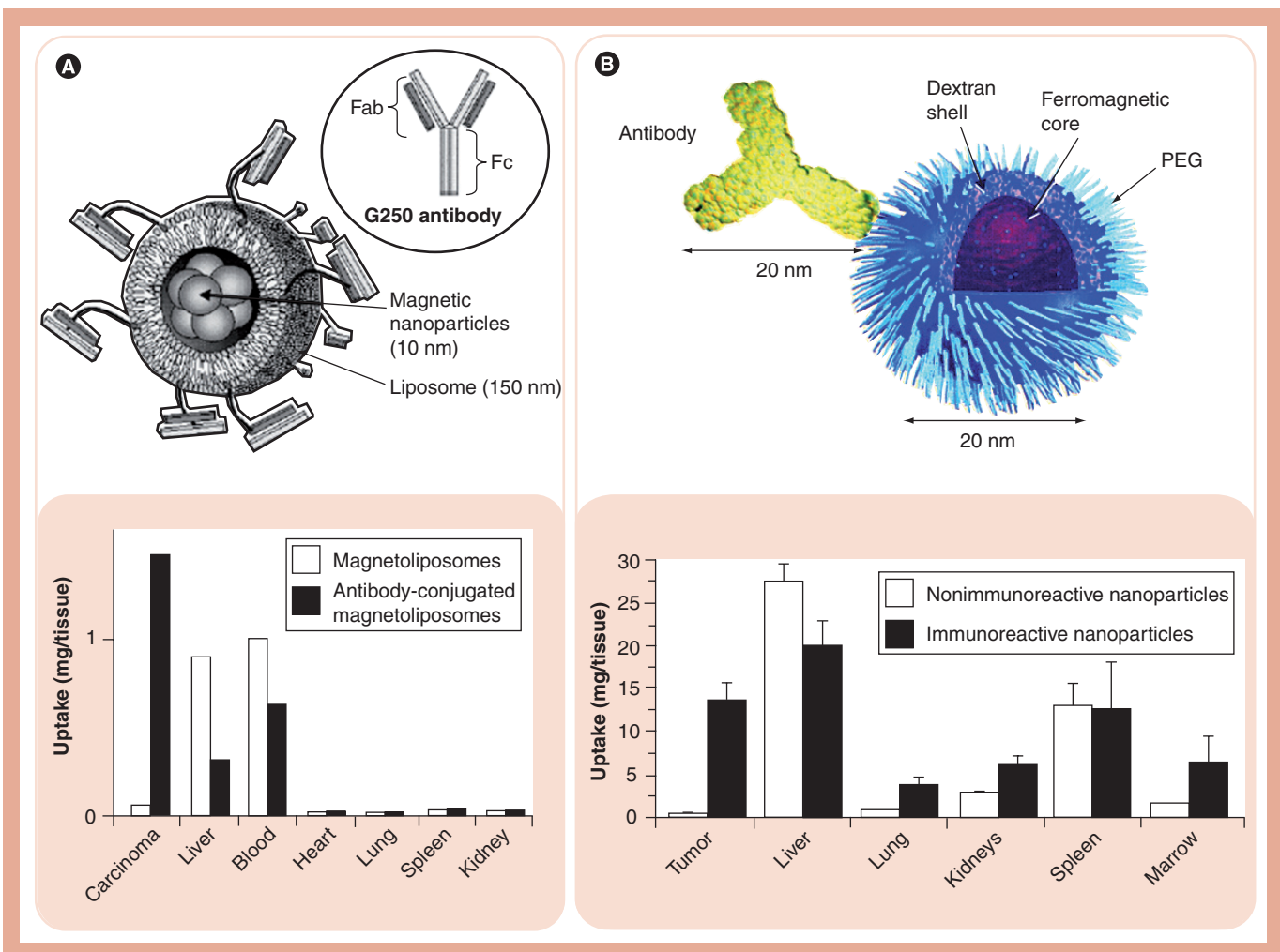


Figure 7. Immunotargeting of magnetic mediators for hyperthermia. (A) Antibody-conjugated magnetoliposomes. Magnetite nanoparticles were encapsulated into a neutral liposome and a G250 Fab' fragment, which binds human antigen MN, was linked to the liposome surface. The delivery of immuno-magnetoliposomes was performed by injection via the heart. Carcinoma consisted of MN antigen-overexpressing mouse renal cells. Biodistribution of immuno-magnetoliposomes (closed columns) and neutral magnetoliposomes (open columns, control group) for carcinomas and various organs 48 h after their intra-arterial injection. Approximately 50% of the total immuno-magnetoliposomes injection accumulated in the carcinoma tissue, which is approximately 27-times more than for the control neutral magnetoliposomes. Adapted with permission from [50]. **(B)** PEG-coated magnetic nanoparticles linked with ¹¹¹I-Chimeric L6 monoclonal antibody. Biodistribution 48 h after intravenous injection of immunoreactive nanoparticles versus nonimmunoreactive nanoparticles. A total of 13.7% of the immunoreactive bioprobes accumulated in the tumor region compared with less than 1% for the control nonimmunoreactive bioprobes. Adapted with permission from [59]. In both studies using immuno-magnetic mediators, many tumors showed regression after AMF irradiation (repeated irradiation in **(A)**, high intensity irradiation in **(B)**) but no tumor had complete regression. Adapted with permission from [59]. PEG: Polyethylene glycol

and treat remote tumors and metastases. In that case, only low concentrations of nanoparticles can be targeted so that improved heating efficiency is once again a key requirement. An indirect effect of magnetic hyperthermia (i.e., induction of antitumor immunity) has been demonstrated and opens new perspectives in cancer immunotherapy.

Future perspective

Current research is seeking to take advantage simultaneously of the multiple properties of magnetic nanoparticles for therapeutic

hyperthermia, tumor targeting by magnetic guidance and/or biological addressing, MRI-based diagnosis and treatment monitoring and self-regulation of therapy. The design of nanoparticles combining these different functionalities is a challenge for researchers, which requires a deep understanding of interconnected physical, chemical and biological behaviors of nanotools. A tight control of size, shape, magnetic and surface properties during synthesis procedures [71,72] should enable optimized characteristics to be 'fitted' for applications.

For example, monodisperse nanoparticles with ideal size and high magnetization could permit a significant increase of heating efficiency, enabling the treatment of disseminated tumors with a low dose of nanoparticles. Hybrid nano-objects [73] combining different materials with interacting physical properties should also provide novel perspectives for noninvasive imaging and controlled therapy.

It is clearly emerging that magnetic hyperthermia is most effective when combined with other therapeutic modalities (e.g., radiotherapy, immunotherapy, chemotherapy and gene therapy). Hyperthermia mediated by magnetic nanoparticles can be used to boost another treatment and/or to activate another therapeutic agent in a controlled manner. Heat-activated local drug release could be of particular interest if suitable nanocontainers may protect and transport both drug and magnetic objects [29,70] and deliver it to the right place

in response to an external magnetic field [74]. Heat-inducible gene therapy could also benefit from remote activation [23].

In conclusion, nanotechnology offers the unique possibility of creating vectors that combine the potential of magnetic properties with other therapeutic agents. The use of magnetic nanoparticles will also facilitate precise spatial and temporal control, which is a prerequisite for a safe and effective treatment.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

Physical mechanisms underlying hyperthermia mediated by magnetic nanoparticles

- The response of a magnetic single domain to an alternating magnetic field depends on its dynamic behavior, which is governed by thermal fluctuations of the magnetic moment inside the crystal lattice (Néel fluctuations) and of the crystal itself in the carrier medium (Brownian fluctuations).

Optimizing nanoparticle design for magnetic hyperthermia

- Heating power of nanoparticles is size dependent with a non-monotonous behavior. Optimal size depends on the field frequency and of material's magnetic properties, principally via the magnetic anisotropy.
- Brownian and Néel contribution to heating power can be distinguished by varying independently the particle size, the magnetic anisotropy of the material (affecting Néel dynamics) and the viscosity of carrier fluid (affecting Brownian dynamics).
- Internalization of magnetic nanoparticles inside intracellular endosomal compartments results in the vanishing of Brownian contribution to heating power.
- Self-regulation of magnetic hyperthermia can be envisaged by synthesizing nanoparticles with Curie temperature of approximately 45–50°C.

Antitumoral efficacy of hyperthermia mediated by magnetic nanoparticles

- Animal models and clinical trials of magnetically induced hyperthermia mainly involve a direct injection of highly concentrated nanoparticles in the tumor, with significant results for tumor regression.

Induction of antitumoral immunity

- Mild hyperthermia induced by magnetic nanoparticles can activate antitumoral immunity, via the expression of heat shock proteins by heated tumor cells.

Towards targeted magnetic hyperthermia

- Biological addressing of magnetic nanoparticles is desirable to treat non-accessible or disseminated tumors. However, the concentration of nanoparticles within tumor sites may be not sufficient for efficient hyperthermia. Basic researches are thus needed to design nanoparticles with superior heating capacity and efficient targeting.
- Combination of several therapeutic modalities within a single nanovector is a promising strategy towards more efficient localized treatment.

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