Abstract

Superparamagnetic hyperthermia, obtained by increasing the temperature to 42–43 °C in tumor tissue where magnetic nanoparticles are found, as a result of superparamagnetic relaxation, following the application of an external alternating magnetic field at a frequency of hundreds of kilohertz, is an alternative method that is noninvasive and apparently lacking toxicity and that has real potential as a cancer therapy. However, magnetic nanoparticles used as thermal mediators play a very important role in the efficacy of the method in the irreversible destruction of tumors. The NPs must meet a number of physical and magnetic characteristics in order to obtain the maximum hyperthermal effect, but also a reduced or even lack of toxicity on healthy cells. This chapter presents just this aspect of biocompatibility/cytotoxicity and nanoformulations of magnetic nanoparticles for their use in superparamagnetic hyperthermia. We will consider the recent nanoformulations that could be used successfully in superparamagnetic hyperthermia, tested in vitro and in vivo, as well as current trends in dual therapy, thermochemotherapy, or thermo-radiotherapy.

Keywords
Magnetic IONPs · Nanoformulations · Toxicity · Superparamagnetic hyperthermia · Cancer therapy

Nomenclature

AMF Alternating magnetic field
B16-F10 Murine melanoma cell line
Bio-MNP Biocompatible magnetic nanoparticle
Bio-SPMNP Biocompatible superparamagnetic nanoparticle
BHK-21 Baby hamster kidney cells
CKD Chronic kidney disease
CT-26 Colorectal cancer cells
DOX Doxorubicin
In alternative therapies against tumors, one of the most promising methods at the present time is superparamagnetic hyperthermia (SPMHT) (Rosensweig 2002; Ito et al. 2003; Matsuoka et al. 2004; Jordan et al. 2006; Johannsen et al. 2007; Hou et al. 2009; Kobayashi 2011; Caizer 2013, 2014; Wang et al. 2017). This method was recently introduced in cancer therapy and is based on increasing the temperature of magnetic nanoparticles (MNPs) dispersed in a tumor to around 42–43 °C, noninvasively, as a result of superparamagnetic relaxation in the alternative magnetic field at a frequency of $10^2$ kHz (Rosensweig 2002). Many studies have been conducted on magnetic hyperthermia (MHT) with MNPs and superparamagnetic nanoparticles (SPMNPs), as well as in vitro and in vivo studies (Ito et al. 2004; Gazeau et al. 2008; Hu et al. 2012; Alphandéry et al. 2013; Caizer et al. 2013; Di Corato et al. 2015; Caizer 2017; Kandasamy et al. 2018a, b; Yan et al. 2018; Zhou et al. 2018; Tian et al. 2019), which show the feasibility of the method in the destruction of tumor cells.

However, studies on MHT with nanoparticles (NPs) having superparamagnetic behavior in the external magnetic field, called SPMHT, have shown that this is more effective than MHT (Pavel et al. 2008; Pavel and Stancu 2009; Caizer et al. 2010). This is due, first of all, to the specific loss power (SLP), which is greater than that of common NPs, which leads to more efficient heating and a higher specific absorption rate (SAR).
Secondly, in the case of SPMNPs, they are smaller in size, usually in a range of approx. 5–20 nm, than those used in common MHT, where NPs are larger (>20 nm), which implies at least two advantages: (i) reduced toxicity and (ii) easier entry of NPs into cells, which makes it the most efficient method, by destroying cells from the inside (intracellular therapy).

In the case of large NPs used in MHT, besides the two aspects mentioned previously, which become disadvantages, in this case, there is another major disadvantage: high magnetic fields must be used to obtain the loss power necessary in hyperthermia. This is because the power dissipated in this case is obtained as a result of the magnetization with hysteresis (hysteresis loop) of the large NPs, the power being proportional to the surface area of the hysteresis loop. High loss power is equivalent to obtaining a large hysteresis loop, and this can only be achieved in high fields. However, the use of high magnetic fields ($H$) raises major difficulties in terms of both obtaining their application at high frequencies ($f$) and their use in human body therapy, where the known admissible dose should not be exceeded, e.g., $Hf < 5 \times 10^9$ Am$^{-1}$Hz (Hergt and Dutz 2007).

After achieving the maximum efficiency of 100% in the destruction of tumors, besides the use of SPMHT in antitumor therapy, with the physical and magnetic aspects of optimizing the method, another very important issue is the nanoformulation of MNPs with various biochemical agents, so as to obtain the most suitable biocompatible NPs for this type of therapy, without toxicity.

A search in the PubMed database on the keywords “iron oxide nanoparticles” led to a considerable number of published articles – over 14,600, with the earliest mentioning iron oxide NPs used in biomedicine (magnetoresponsive indomethacin NPs) dating from 1988 (Malaiya and Vyas 1988), which indicates that this type of NP is ranked among the first NPs studied for biomedical applications. The interest in iron oxide NPs (IONPs) as effective tools in the medical and pharmaceutical domains is still elevated since from the 1 January until 31 July 2019 over 800 studies were published that included the words “iron oxide nanoparticles” (source: PubMed database). The distinct features of IONPs, like superparamagnetism, high colloidal stability, and low toxicity, make these NPs attractive for multiple uses, particularly in the biomedical field: MHT, magnetic resonance imaging (MRI) as contrast mediators, targeted drug delivery systems, gene therapy, magnetic cell splitting, tissue repair, cancer treatment, and so forth (Laffon et al. 2018; Hataminia et al. 2019). It worth mentioning that beyond the many advantages of IONPs, the widespread use of these NPs has raised serious concerns in terms of potential toxicity, causing researchers to devote considerable attention to the appraisal of NPs’ safety profiles (according to the PubMed database, over 1500 published articles investigated IONP toxicity).

Several fundamental physical, chemical, and biological properties should be present for NPs to be considered appropriate for use in clinical practice: (i) charge: the optimal charge of NPs for in vivo applications, including tumor targeting, is near neutral or weakly negative; (ii) zeta potential – establishes a solution’s stability – NPs <100 nm possess a negative net potential, whereas NPs >100 nm present a positive potential; (iii) coating (as polyethylene glycol (PEG), oleate or oleic acid, dextran, chitosan, dimercaptosuccinic acid (DMSA), poly(L-lactic acid), citrate, silica, starch): dictates the circulation half-time of NPs by interfering with the recognition and elimination of intravenously administered NPs via the reticuloendothelial system (RES); (iv) size: IONPs 10–100 nm in size are recommended for intravenous use, while NPs >200 nm and <10 nm are seized by the spleen or discarded by renal clearance; (v) shape: influences the cellular uptake of NPs by macrophages (the spherical shape is internalized faster compared to other shapes); and (vi) functional targeting: assures strong binding to target cells and prolonged contact/retention in target area (Belanova et al. 2018; Durymanov et al. 2015; Arami et al. 2015).

This chapter presents an overview of the latest data concerning the nanoformulations of
magnetic IONPs with a focus on their applications in MHT/SPMHT as an alternative cancer therapy by describing the main mechanisms of action and the therapeutic potential of this technique. Moreover, the fundamental physical and magnetic issues regarding MHT and SPMHT and the in vitro and in vivo fate of magnetic IONPs after their administration in terms of toxicity are also presented and discussed.

22.2 Magnetic Nanoparticles in Alternative Cancer Therapy by Superparamagnetic Hyperthermia

MNPs that are used in MHT or SPMHT are generally ferrimagnetic (FiM), due to both the possibility of their magnetization in high-frequency fields (Valenzuela 1994) and their low toxicity compared to ferromagnetic (FM) NPs. Although the FM NPs have a magnetic moment per particle larger than those of the FiM (Cullity and Graham 2009), due to the parallel orientation and the same orientation of the atomic (or ionic) magnetic moments (Fig. 22.1a) compared to the opposite and unequal orientation of magnetic moments in the case of FiM (Fig. 22.1b), which would be beneficial in MHT, they are not yet used. However, it is not excluded that such NPs as iron (Rosensweig 2002) or their alloys (e.g. iron-platinum (Fe-Pt)) (Habib et al. 2008) may be successfully used in the near future, and even more efficiently, in MHT or SPMHT. Now with modern nanobiotechnology these FM NPs can be made perfectly biocompatible and nontoxic for nanometric sizes, which would be a great advantage in hyperthermia.

22.2.1 Magnetic Behavior in Static Magnetic Field

The magnetization \( \mathbf{M} \), which macroscopically characterizes a material from a magnetic point of view, is given by the vector sum of the atomic (or ionic) magnetic moments \( \mathbf{\mu}_m \) per unit volume \( V \),

\[
\mathbf{M} = \frac{\sum \mathbf{\mu}_m}{V} \quad (22.1)
\]

or

\[
\dot{\mathbf{M}} = \frac{d \mathbf{\mu}_m}{dV} \quad (22.2)
\]

respectively, in the case of a continuous distribution of magnetic moments.

For a magnetic domain with uniaxial symmetry (Fig. 22.2a) having volume \( V \) where the magnetization is uniform and stable, and equal to the spontaneous magnetization (or saturation) \( M_s \) of the material, the magnetic moment of the domain will be

\[
\mu_{md} = \int_{(V)} M_s dV \quad (22.3)
\]

In the case of NPs that are not too large, e.g., <20–25 nm for soft FiM NPs, they are generally of a single domain, so that in the case of spherical approximation (Fig. 22.2b) the magnetic moment of the NP is

\[
m_{NP} = M_s V_{NP} \quad (22.4)
\]

and

Fig. 22.1  Schematic representation of orientation of ionic magnetic moments in NPs with (a) ferromagnetic and (b) ferrimagnetic structure
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where $D$ is the magnetic diameter of the NP.

In an external magnetic field of intensity $H$ (Caizer 2016), a system of single-domain NPs with magnetic moments $m_{\text{NP}}$ (Eq. (22.4)) oriented in all directions at room temperature (Fig. 22.3a) will tend to orient in the direction of the applied magnetic field. The magnetization of the NP system takes place according to a Langevin law, just like the magnetization of a paramagnetic atomic system (Jacobs and Bean 1963):

$$M = n m_{\text{NP}} \left( \coth \frac{\mu_0 m_{\text{NP}} H}{k_B T} - \frac{k_B T}{\mu_0 m_{\text{NP}} H} \right)$$

(22.6)

where $n m_{\text{NP}}$ is the saturation magnetization $M_{\text{sat}}$ of the NP system and

$$L = \left( \coth \frac{\mu_0 m_{\text{NP}} H}{k_B T} - \frac{k_B T}{\mu_0 m_{\text{NP}} H} \right)$$

(22.7)

is a Langevin function with $\xi = \mu_0 m_{\text{NP}} H / k_B T$ argument. In Eq. (22.6) $n$ is NP concentration, $\mu_0$ is the permeability of a vacuum, and $k_B$ is Boltzmann’s constant. Therefore, this behavior in the magnetic field of NPs is called superparamagnetic behavior (Bean and Livingston 1959), being specific to MNPs made up of many atoms (ions) with magnetic moments (e.g., even more than $10^5$ atoms/ions in a magnetic domain), compared to the paramagnetic behavior of individual atoms/ions.

When the magnetic field increases from zero to the maximum value corresponding to the magnetic saturation, the magnetization of the SPMNP system increases according to the Langevin function (Fig. 22.3b), the saturation magnetization being a constant. At the decrease of the magnetic field from the saturation value to zero, the magnetization of the system returns to zero on the same curve (arrows on curve in Fig. 22.3b).
In conclusion, in this case the hysteresis loop is missing, a loop that exists in the case of large NPs, generally >25–30 nm for soft FiM NPs.

For low-magnetization fields, when the condition \( \xi \ll 1 \) is met, developing the Langevin function in series for a small argument, the magnetization of the NP system will be

\[
M = \frac{\mu_0 n m_{NP} H}{3k_B T} \quad (22.8)
\]

According to Eq. (22.8), in this case the variation of the magnetization of the NP system with the applied field is linear, at a constant temperature.

For high magnetic fields, when \( \xi \to \infty \), the Langevin function (Eq. (22.7)) takes the value 1 (\( L \to 1 \)), and the magnetization of the NP system in this case will be

\[
M = n m_{NP} \quad (22.9)
\]

or

\[
M = M_{sat} \quad (22.10)
\]

respectively.

According to Eqs. (22.5) and (22.9), at a fixed concentration, the saturation magnetization of a NP system depends on the size of the NPs (by diameter \( D \)), \( M_s \) being a material characteristic.

### 22.2.2 Magnetic Behavior in External Alternating Magnetic Field.

#### Superparamagnetic Relaxation

In an alternative magnetic field with low frequency (quasi-static), at room temperature, the magnetization and magnetic behavior of the SPMNPs system will greatly depend on the amplitude of the magnetic field, as illustrated in Fig. 22.4, for three practical cases, two limits (a) and (c) and one intermediate (b).

Case (a) corresponds to a situation where the amplitude of the external alternating magnetic field is small (or very small) (\( H < \)), so that the magnetization of the NPs is in the linear area of the Langevin function (Eq. (22.8), Fig. 22.3b). Case (c) is the one corresponding to the very large amplitude of the external alternating magnetic field (\( H >> \)) (corresponding to the magnetic saturation). And case (b) corresponds to the situation when the magnetic field amplitude is moderate (but not very large), so that the magnetization deviates from the linear variation, following a Langevin-type curve.

In SPMHT, cases (a) and (b) are of interest, with case (c) being excluded due to the very large magnetic field, which is practically unusable.

In contrast, in MHT, where large NPs are used, it even case (c) would be of interest since there a hysteresis loop is followed as wide as possible, and this can be done in large or very large magnetic fields to obtain the hyperthermic effect being promoted.

As the frequency of the alternating magnetic field (AMF) increases, the magnetization can no longer immediately follow the variation of the magnetic field, and a delay in the time of the magnetization with respect to the field takes place, a phenomenon known as magnetic relaxation. At higher frequencies (kHz – MHz) the time delay is greater. As a result of this delay, there is dissipation (loss) of energy, which leads to the heating of the NPs.

In the Debye model it is shown that the complex components of magnetic susceptibility (\( \chi = M/H \)) \( \chi = \chi' + \chi'' \) are

\[
\chi' = \frac{\chi_0}{1 + (\omega \tau)^2} \quad (22.11)
\]

and

\[
\chi'' = \chi_0 \frac{\omega \tau}{1 + (\omega \tau)^2} \quad (22.12)
\]

where \( \chi_0 \) is the static magnetic susceptibility, \( \omega = 2\pi f \), where \( f \) is the frequency of the AMF, and \( \tau \) is the relaxation time.

The magnetic relaxation time in the case of NPs dispersed in a fluid generally has two components (Néel 1949; Brown 1963), determined by the rotation of the magnetic moment inside the NP (Néel) and the rotation of the NP in fluid (Brown), under the action of the AMF. However, in the case of SPMHT, when the NPs are fixed in
the tumor (tissue), only the Néel-type relaxation occurs, so that the relaxation time in this case will be

$$\tau = \tau_N = \tau_0 \exp \left( \frac{K V_{NP}}{k_B T} \right)$$

(22.13)

where $K$ is the magnetic anisotropy constant and $\tau_0$ is a time constant that usually has the value $10^{-9}$ s (Back et al. 1998).

In a magnetic field of small amplitude, the static magnetic susceptibility $\chi_0$ can be approximated by the Langevin relation

$$\chi_0 = \frac{3\chi_i}{\xi} \left( \coth \xi - \frac{1}{\xi} \right)$$

(22.14)

where $\chi_i$ is the initial magnetic susceptibility and $\xi$ the Langevin parameter

$$\xi = \frac{\mu_0 m_{NP} H}{k_B T}$$

(22.15)

given by the ratio of the magnetic moment energy of the NP in the magnetic field $H$ and the thermal energy at temperature $T$.

### 22.2.3 Specific Loss Power and Heating Rate

In an AMF with amplitude $H$ and frequency $f$, the loss power is given by the equation (Rosensweig 2002)

$$P = \pi \mu_0 \chi'' f H^2$$

(22.16)

Thus, in the case of NPs with superparamagnetic behavior in an AMF with frequency $f$ and amplitude $H$, the dissipated power can be determined
by considering the component $\chi'$ of the complex magnetic susceptibility of the NP system, given by Eq. (22.12), where $\tau$ and $\chi_0$ are given by Eqs. (22.13) and (22.14). At the same time, in the case of MHT experiments, in order to not have a dependence of the power dissipated by the nature of the material, the SLP will be considered: $P/\rho$, where $\rho$ is the density of material.

In adiabatic conditions it is possible to evaluate the increase of the $\Delta T$ temperature of a system in a time interval $\Delta t$ from the application of an AMF, using the formula

$$\Delta T = \frac{P}{\rho c} \Delta t$$

or heating rate (speed) $T/\tau$, where $c$ is the specific heat. This formula is very important in SPMHT experiments since it allows for the quantitative estimation of the time necessary to reach a temperature of 42–43 °C, which is required in hyperthermia, as a function of other parameters that are in the SLP formula (Eq. (22.16)), respectively, in the magnetic susceptibilities $\chi'$ (Eq. (22.12)) and $\chi_0$ (Eq. (22.14)), and the practical parameters of the external applied magnetic field: $H$ and $f$.

Figure 22.5 shows a concrete case in which the SLP was determined for magnetite NPs ($Fe_3O_4$), which are suitable for SPMHT. Power was calculated computationally in three dimensions depending on the frequency of the magnetic field $f$ and the diameter $D$ of monodisperse NPs for a usual magnetic field $H$ of 10 kA/m (Caizer 2014). The data used in the computed calculation are shown in Table 22.1.

The obtained diagram makes it possible to accurately determine the diameter of the NPs ($D_0$) to be used in SPMHT, corresponding to the maximum dissipated power. Thus, for a diameter of approx. 15 nm is obtained the time temperature dependence in Fig. 22.6 at the same amplitude of the magnetic field and frequency of $f = 150$ kHz.

The result shows, under the specified conditions, that the necessary temperature can be reached in the hyperthermia of tumors of 42–43 °C (hyperthermic temperature) at an interval of 80 s. This time is a very good one without posing a danger of exposure to therapy that will be too long, which can affect healthy tissues.

Figure 22.7 shows an increase of temperature for 30 minutes in MHT by applying AMF with the parameters $H = 21.0$ kAm⁻¹ and $f = 340$ kHz, in the experimental case of hydrophilic graphene-based yolk-shell MNP$s$ functionalized with copolymer Pluronic F-127 (GYSMNP@PF127) conjugated with the drug doxorubicin (DOX) (Rodrigues et al. 2018). For this nanosystem the optimum temperature in hyperthermia is reached for 10–15 minutes.
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22 Magnetic Nanoparticle Nanoformulations. Biocompatibility

MNPs exhibit highly interesting in vivo applications based on their intrinsic magnetic and superparamagnetic properties that enable them to be used in the diagnosis and treatment of malignant pathologies (Williams 2017). Their use requires parenteral administration, intravenous or local, which poses several challenges, mainly in terms of formulation and biocompatibility (Chen et al. 2012). Design maps can be developed to rationally apply hypothermic treatments and choose the most appropriate administration route depending on the intrinsic properties and biodistribution abilities of the NPs (Cervadoro et al. 2013).

A key step in magnetic nanoformulation is the achievement of physical stability of the dispersed NPs. A second aspect is NP biocompatibility, which requires the implementation of effective ways to make NPs unrecognizable by the immune system and expand their biological lifetime.

An important issue is the homogeneous distribution of MNPs at the tumor level, which makes it possible to achieve a uniform and well-controlled temperature pattern without hot spots or unheated tumor regions; a potential solution might involve multipoint injections directly at the tumor level, but overall the practical difficulties are overwhelming (Dutz and Hergt 2014; Kudr et al. 2017).

Systemic administration raises other challenges, such as efficient concentrations at the tumor level and the avoidance of clearance by the immune system. So far, several solutions have been adopted, in particular coating with various organic layers (i.e., synthesis of core-shell nanohybrids) and antibody binding (Ito et al. 2005).

The in situ preparation of functionalized MNPs with short-chain molecules containing carboxylic groups was reported by Kandasamy et al. in 2016 and 2018 (Kandasamy et al. 2016, 2018a, b). MNPs can be subjected to further chemical or biological conjugation without additional surface functionalization (Kandasamy et al. 2016); they showed good biocompatibility.

### Table 22.1 Characteristic observables of NPs and alternating magnetic field parameters (Caizer 2014)

| Sample   | $M_s$ (kA/m) | $K$ (kJ/m³) | $\rho$ (g/cm³) | $\varepsilon$ | $f$ (kHz) | $H$ (kA/m) |
|----------|--------------|-------------|----------------|--------------|-----------|------------|
| Fe$_3$O$_4$ | 477          | 11          | 5.24           | 0.017        | 150       | 10         |

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**Fig. 22.6** Temperature increase in $\Delta t$ time interval for optimal diameter $D_0 = 15$ nm of Fe$_3$O$_4$ SPMNPs in the case of SPMHT with parameters $H = 10$ kHz, $f = 150$ kHz (the result will be published by Caizer (2019)).
and high magnetization and were thoroughly investigated in terms of heating responses and efficacies using a wide range of AMFs and dispersion media (Kandasamy et al. 2018a, b). Spherically shaped superparamagnetic iron oxide nanoparticles (SPIONs) exhibited stronger and faster hyperthermia effects than commercially available ferrofluids and provided higher killing efficiency toward MCF-7 breast tumor cells.

Multifunctionalized MNPs were synthesized by Aires et al. in 2015 by using bovine serum albumin as coating to improve the colloidal stability and magnetic response (Aires et al. 2015). Albumin induces hemocompatibility, delays clearance by the immune system, and deters the adsorption of other proteins; in addition, it allows for the attachment of anticancer drugs, thereby providing the opportunity to combine hyperthermia with drug delivery in the treatment of cancer.

Tagged dextran-coated MNPs containing ferric oxide were synthesized by conjugating the specific anti-human epidermal growth factor receptor (HER2) aptamer to the surface of the NPs (Pala et al. 2014). Using fluorescence microscopy, the authors were able to prove the high specificity of the tagged NPs toward the overexpressing HER2 receptor human adenocarcinoma SK-BR3 cells; also, a significantly lower dose of tagged NPs versus nontagged ones was needed to achieve similar effects of hyperthermia, thereby reducing the occurrence of side effects.

Stable, biocompatible, and easily dispersible MNPs were synthesized by coating magnetite NPs with a temperature- and pH-responsive polymer, poly(2-(dimethylamino)ethyl methacrylate); their heating capacity was investigated, revealing higher SARs than bare MNPs (Reyes-Ortega et al. 2017), thereby enabling the therapeutic use of hyperthermia. Other pH and temperature dual responsive polymer-modified SPIONs were synthesized using glutamic acid and N-isopropylacrylamide as co-monomers; carboxymethyl cellulose-induced SPION water solubility while folic acid served as the tagging agent (Patra et al. 2015). When interacted with an external magnetic field, the modified SPIONs showed very fast and efficient temperature increase, thus exhibiting promising solutions for cancer treatment and diagnosis.

Polyethylenimine (PEI), oleic acid, and Pluronic F-127 were used by Tomitaka et al. in 2012 as coating materials for Fe3O4 NPs in order to prevent NP aggregation and to enable their enhanced permeability and retention effects (Tomitaka et al. 2012a). The three coating materials possess various properties that...
make them suitable for biomedical applications in cancer treatment: (1) PEI is a water-soluble polymer bearing amine groups able to facilitate the binding and transport of nucleic acids; (2) oleic acid facilitates the surface covalent binding of tumor-targeting antibodies; and (3) Pluronic F-127 provides biocompatibility and, consequently, a longer biological lifetime due to its structural similarity with polyethylene glycols. Out of the three coating materials, Pluronic F-127 was selected by the authors as being suitable for the preparation of MNPs that could act as a heat source for hyperthermia; the main reason behind this decision was the fact that Pluronic-coated NPs exhibited heat dissipation in an independent manner from the viscosity of the surrounding environment (Tomitaka et al. 2012b). The NPs thus obtained were further subjected to in vitro studies using HeLa ovarian cancer cell cultures; while no cytotoxicity was recorded, the hyperthermia treatment significantly reduced cell viability by mediating apoptosis through the mitochondrial pathway.

Oleic acid coating was used for the synthesis of biocompatible colloidal suspension of IONPs with magnetic properties, which revealed a lack of toxicity both in vitro and in vivo (Coricovac et al. 2017).

Usually, the most common MNPs are based on ferrite or iron oxide due to their intrinsic magnetic properties; however, in some cases, such as bone malignant pathologies, special NPs that are able to ensure bone substitution as well as hyperthermia must be designed (Li et al. 2018). A biocompatible magnetic biomaterial containing Fe$^{3+}$ and Ni$^{2+}$ (2:1) cosubstituted hydroxyapatite NPs was obtained and evaluated in terms of physicochemical stability, biocompatibility, and hyperthermia potential (Karunamoorthi et al. 2013). The studies revealed significant hyperthermia properties and a low level of toxicity, probably due to the incorporation of cytotoxic groups of the magnetic phase into the biocompatible phases represented by hydroxyapatite and tricalcium phosphate.

Clustered superparamagnetic particles were obtained to prevent the leakage of SPIONs from the capillaries of healthy tissues and to increase their relaxivity and SAR (Hayashi et al. 2013). The clusters were decorated with folic acid and polyethylene glycol in order to promote a high tumor concentration; both clustering and surface decoration were conducted via thiol-ene click reaction. The modified SPION nanoclusters accumulated at a high level at a tumor site, facilitating enhanced MRI contrast; also, the application of an AMF led to a higher tumor temperature compared to the surrounding tissues, in the end significantly reducing tumor volume and meaningfully improving survival time for the experimental mice.

Gadolinium-doped dextran-coated magnetite NPs were investigated as potential theranostic agents due to their ability to enhance MRI contrast as well as to act as hyperthermia mediators (Palihawahadana-Arachchige et al. 2017). Despite the fact that gadolinium may reduce magnetization, thereby hampering hyperthermia efficiency, the authors revealed that by selecting the proper composition of Gd-doped Fe$_3$O$_4$ NPs one may achieve higher SARs; however, future studies are needed to achieve the theranostic use of Gd-doped Fe$_3$O$_4$ NPs as both diagnostic and therapeutic agents.

Magnesium-doped iron oxide SPIONs were designed and synthesized to achieve efficient hyperthermia to completely eliminate tumor tissue (Jang et al. 2017); in addition to being highly biocompatible, magnesium was selected as doping material due to the fact that magnetically induced heating power can be effectively controlled by adjusting the concentration and distribution of Mg$^{2+}$ cations within SPIONs. In vitro and in vivo hyperthermia studies demonstrated SPIONs’ great ability to destroy cancer cells due to their significant heating power within the biologically safe range of AMFs.

Gold-coated SPIONs with suitable physicochemical and biological properties revealed a four- to fivefold increase in released heat compared to bare SPIONs under a low-frequency oscillating magnetic field (Mohammad et al. 2017).
In vitro studies have shown that in the absence of an external magnetic field, gold-coated SPIONs do not act as cytotoxic agents on cancer cells.

The most recent approach in the field of magneto-nanoformulations is the design of hybrid nanosystems (Fig. 22.8), which associate MNPs used either for diagnostic or treatment purposes with anticancer drugs that thus benefit from an improved pharmacological profile; this particular approach has become known as thermo-chemosensitization, that is, the enhancement of chemotherapy efficiency through simultaneous hyperthermia; studies have revealed that MHT induces higher concentrations of anticancer drugs at the tumor level, probably due to the hyperthermia effect on tumor vasculature as well as an increased drug cytotoxicity by mechanisms not yet fully understood (Hervault and Thanh 2014). Overall, one might classify the combined effect of thermochemotherapy as synergistic, in particular when mild hyperthermia is applied; in addition, the potential occurrence of treatment-induced secondary cancers is significantly lower (Hervault and Thanh 2014).

An excellent review concerning the diagnosis, targeting, and treatment of prostate cancer by means of MNPs was published in 2017 by Chowdhury et al.; the review concludes that modified MNPs that combine targeted drug delivery with MHT might represent potential future theranostic solutions for prostate cancer. Most importantly, these theranostic nanoformulations might allow the monitorization of disease progression while the treatment is ongoing based on the “see-and-treat” principle (Chowdhury et al. 2017).

The scientific literature mentions three main nanoformulations for the combined use of MNPs and anticancer drugs: liposomes, micelles, and polymer nanoformulations. Several such nanoformulations and their main characteristics are given in Table 22.2.

22.3.1 Liposomes

Liposomes are spherical nanovesicles that exhibit an aqueous core and a lipophilic outer bilayer and currently represent a very popular option for drug delivery due to several practical reasons: (1) the possibility of entrapping both hydrophilic and hydrophobic drugs in either core or outer layer, (2) improved drug pharmacokinetic and toxicological profiles, and (3) high possibility of fine surface tuning for optimized pharmacological properties (Alavi et al. 2017).

Magnetoliposomes represent an attractive possibility for allowing a combined chemother-
### Table 22.2  Magnetic nanoformulations

| Magnetic nanoparticles | Organic coating component | Experimental records                                                                                                                                                                                                 | Reference                  |
|------------------------|--------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| **Liposomes**          |                          |                                                                                                                                                                                                                     |                             |
| Iron oxide             | Oleic acid, phospholipid bilayer | Excellent targeting ability, thermosensitivity, high responsiveness to alternating magnetic field, and laser processing simultaneously improved tumor cell killing effects; real-time monitoring of cancer progression through fluorescence and MRI | Guo et al. (2018)           |
| Iron oxide             | Phospholipid bilayer     | Cell damage depends on bulk temperature and duration of treatment, but mainly on cell type and thermal energy/cell during magnetic hyperthermia treatment                                                                 | Engelmann et al. (2018)     |
| Iron oxide             | Citric acid, phospholipid bilayer | Targeted delivery, controlled drug release; significant tumor regression                                                                                                                                          | Babincová et al. (2018)    |
| Manganese ferrite nanoparticles (MnFe₂O₄) | Phospholipid bilayer | High encapsulation efficiency superparamagnetic behavior; simultaneous use as nanocarriers and hyperthermia agents | Rodrigues et al. (2017)     |
| Cobalt ferrite nanoparticles | Oleic acid, phospholipid bilayer | Drug release through modification of membrane state; controlled drug release                                                                                                                                   | Nappini et al. (2011)      |
| **Polymer nanoformulations** |                          |                                                                                                                                                                                                                     |                             |
| Iron oxide             | Pluronic F-127           | Short-term, tumor-specific, hyperthermic treatment; long-term sustained drug delivery                                                                                                                              | Wang et al. (2013)          |
| Iron oxide             | Carboxymethylcellulose   | Simultaneous use as nanocarriers and hyperthermia agents                                                                                                                                                    | Carvallho et al. (2019)    |
| Iron oxide             | Poly-N-isopropyl-acrylamide | Controlled synergistic tumor destruction through drug release and hyperthermia                                                                                                                                  | Chang et al. (2014)         |
| Cobalt ferrite nanoparticles | Poly(maleic anhydride-alt-1-octadecene) | High specific absorption rate | Nam et al. (2018)           |
| Cobalt-doped ferrite nanoparticles | Human ferritin protein, poly(ethylene glycol) | Enhanced magnetic anisotropy and hyperthermic efficiency compared to undoped sample                                                                                                   | Fantechi et al. (2014)     |
| **Micelles**           |                          |                                                                                                                                                                                                                     |                             |
| Mn-Zn-ferrite nanoparticles | 2-(2-methoxyethoxy)ethyl methacrylate (MEO₂MA) and oligo(ethylene glycol) methacrylate (OEGMA) | Temporospatial synchronism of thermochemotherapy; synergistic effect, enhanced tumor cell sensitivity, reduced side effects | Li et al. (2018)            |
| Iron oxide             | Poly(ethylene glycol)-poly(lactide) (PEG-PLA) | Higher effectiveness compared to either hyperthermia or chemotherapy alone | Kim et al. (2015)           |
| Iron oxide             | Poly(N-isopropylacrylamide-co-acrylamide)-block-poly(ε-caprolactone) | Responsiveness to magnetic heating at physiologically relevant temperatures; significant MRI contrast enhancement abilities | Kim et al. (2013)           |
| Iron oxide             | Amphipatic chitosan      | Intracellular responsive delivery and thermotherapy; complete regression of primary breast tumor without inducing secondary progression                                                                 | Manigandan et al. (2018)   |
| Iron oxide             | Polystyrenegraft-poly(2-vinylpyridine) copolymer (poly(acrylic acid)-block-poly(2-hydroxyethyl acrylate) double-hydrophilic block copolymer | Significant decrease in cell viability depending on incubation concentration and exposure time to alternating magnetic field, dual-dose effect | Nguyen (2018)               |
apy and MHT and provide biocompatibility and transport facilities for loaded chemotherapeutics without adding systemic toxicity (Gogoi et al. 2014).

Phosphatidylcholine liposomes containing maghemite NPs were obtained using a thin-film hydration method and tested in vitro on healthy and tumor colon cell lines as well as on blood cell lines for biocompatibility (Lorente et al. 2018). That study revealed high cellular uptake with mitochondria accumulation, in particular in cancer cells; also, the application of an external magnetic field induced cell migration, supporting the use of magnetoliposomes for biomedical purposes. The controlled release of the encapsulated drug through MHT is an important aspect when designing magnetoliposomes; the drug encapsulation efficiency seems to depend on the amount of MNPs (Ferreira et al. 2016).

Thermosensitive magnetic nanovesicles were prepared using a biphasic suspension of dextran-coated La_{0.75}Sr_{0.25}MnO_3 (LSMO) and Fe_3O_4 NPs at a ratio of 10:1, where the combination of the two inorganic nanomaterials provides the possibility of self-controlled hyperthermia, while the nanovesicular formulation enables the loading of anticancer drugs, such as paclitaxel (Gogoi et al. 2014). The biphasic nanohybrid formulation induced a synergistic cytotoxic effect during in vitro studies on MCF-7 breast cancer cells. In terms of biocompatibility, in vitro and in vivo studies revealed the absence of adverse effects while the therapeutic efficacy was preserved (Gogoi et al. 2017).

Two newly designed types of magnetoliposomes containing magnesium ferrite NPs were tested as nanocarriers for curcumin, revealing that the drug can be preferentially directed toward the tumor site, where it will be released through membrane cell fusion; the simultaneous application of MHT is possible, allowing a dual cancer therapy (Cardoso et al. 2018).

### 22.3.2 Polymer Nanoformulations

Polymer nanoformulations encompass a huge variety of components and gained their place in the biomedical field mainly due to important design flexibility in terms of polymer choice (biodegradable or nonbiodegradable polymers), functionalization, and synthetic approaches (via direct polymerization or use of preformed polymers) (Banik et al. 2015).

A polymer nanoformulation embedding both MNPs and anticancer drugs was designed by Balasubramanian et al. in 2014. It consists of two anticancer drugs, curcumin and 5-fluorouracil, and MNPs encapsulated in poly(D,L-lactic-co-glycolic acid) and functionalized with two cancer targeting agents (Balasubramanian et al. 2014); synergistic strong and fast citotoxic activity was recorded upon applying MHT on MCF-7 and G-1 cancer cells.

A copolymer of methyl methacrylate/ethylene glycol dimethacrylate/hydroxyl ethyl methacrylate was used as polymer envelope for the simultaneous embedding of magnetite particles and gemcitabine in order to provide a multifunctional platform for the hyperthermia-triggered release of the anticancer drug (Iglesias et al. 2018). The application of an AMF induced MHT at 43 °C for precise time intervals, which substantially improved the drug-release rate, reaching 100% release in less than 4 hours.

### 22.3.3 Micelles

Micelles are nanoformulations synthesized by the spontaneous assembly of surfactant molecules in an aqueous environment, exhibiting a hydrophobic core, which may accommodate lipophilic drugs, and a hydrophilic outer layer provided by the surfactant polar groups (Hervault and Thanh 2014). Thermosensitive micelles can be obtained through the use of a thermo-responsive polymer that can be grafted either on the polar head or the nonpolar tail (Hervault and Thanh 2014).

Block copolymer micelles incorporating hydrophobically modified MNPs were designed by Glover et al. in order to evaluate the hyperthermia efficacy as well as thermally enhanced drug release; the incorporated MNPs were able to preserve their heating properties, reaching hyper-
thermia conditions after 5 minutes and triggering drug release (Glover et al. 2013). Hybrid gold/IONPs were embedded in block copolymer micelles for the purpose of combined hyperthermia and chemotherapy as well as optical imaging (Kim et al. 2009). The inorganic core-shell NPs were self-assembled within the polymer hydrophobic polymer core, which contained block copolymers of poly(N-isopropylacrylamide-co-acrylamide)-block-poly(-caprolactone); these NPs combine the advantages of gold and MNPs while the micelle structure provides water solubility, bioavailability for the loaded components, reduced aggregation, and improved stability in an aqueous biological environment.

22.4 In Vitro and In Vivo Viability/Cytotoxicity of Magnetic Nanoparticle Nanoformulations

22.4.1 Magnetic Hyperthermia

Emerging strategies are required in the oncology domain since conventional therapies (chemotherapy, radiotherapy, and surgery) are invasive techniques with numerous side effects (e.g., myelosuppression, cardiotoxicity, neurotoxicity, peripheral neuropathy, damage to surrounding tissues), and, according to the latest studies, IONPs with superparamagnetic properties represent an alternative with great potential (LeBrun and Zhu 2018; Dulińska-Litewka et al. 2019). Their superparamagnetic potential, together with their increased stability in biological media, biocompatibility, low toxicity, biodegradability, long retention time, capacity to agglomerate under a magnetic field, and accessibility for functionalization, place these NPs among the most studied NPs for biological applications (Vallabani and Singh 2018).

The most currently studied clinical applications of IONPs, particularly in the field of tumorigenesis, are MHT, MRI as contrast agent, and controlled/targeted drug delivery (Dulińska-Litewka et al. 2019), subjects that will be further discussed in what follows.

22.4.1.1 IONP Application as a Contrast Agent in Magnetic Resonance Imaging

MRI is a noninvasive technique that is used to visualize the anatomy of different organs (brain, liver, heart) and to detect the presence of different injuries (like cancer lesions) (Vallabani and Singh 2018; Dulińska-Litewka et al. 2019). A wide palette of contrast agents with magnetic properties has been developed in recent years (e.g., gadolinium, quantum dots with paramagnetic micelles, liposomes), and increased attention has focused on iron oxides with superparamagnetic properties, which proved highly efficacious as contrast agents applied in MRI detection. A magnetic IONP authorized by the U.S. Food and Drug Administration (FDA) as therapy to combat the iron deficit associated with chronic kidney disease (CKD) is ferumoxytol (Feraheme). Besides the therapeutic role of ferumoxytol, this compound also proved to be effective as an imaging tool for labeling stem cells for in vivo monitoring or as a noninvasive method to supervise preclinical and clinical experiments that involve stem cell therapies (Castaneda et al. 2011; Vallabani and Singh 2018). The superparamagnetic behavior and large magnetic moment of IONPs allow them to serve as an agent that produces negative contrast (a type – T2 contrast agent). One of the drawbacks associated with these iron NPs is a dark signal that was detected in T2 images, a signal that is responsible for difficulties in identifying areas of interest. A method to overcome this drawback consists in coating IONPs with citrate, which led to efficient NPs with both positive and negative contrast qualities (Vallabani and Singh 2018).

22.4.1.2 IONPs’ Application as Biosensors

IONPs functionalized as nanozymes represent novel nanotechnology-based tools with applicability to biomedicine. This type of NP preserves
the natural biological properties of enzymes (e.g., superoxide dismutase, oxidases, peroxidases) and in addition comes with multiple benefits, such as augmented stability at different pH and temperature levels, a reduction in synthesis costs, and plural uses as a single platform. The enzyme-like features of nanozymes have expanded the areas of application in the biomedical field: biomarkers for cancer diagnostics (several enzymes are reliable markers for different illnesses, including cancer) and the development of biosensors that can quantify the levels of cholesterol, glucose, urea, and oxygenated water, molecules with key roles in cellular metabolic processes (Gawande et al. 2016; Vallabani and Singh 2018).

22.4.1.3 IONPs’ Application in Photothermal Therapy

The intrinsic properties of IONPs make them suitable tools for both magnetic and photothermal applications. This dual character of IONPs was highlighted by Espinosa et al., who tested the response of iron nanocubes (formulated as an aqueous suspension) to AMF and near-infrared laser irradiation exposure, which consisted of a significant augmentation of heating effects (two-to fivefold) compared to a magnetic field alone. This procedure proved highly efficient both in vitro (triggered cancer cell apoptosis) and in vivo (tumor regression) (Espinosa et al. 2016). Another example of IONPs designed for photothermal cancer therapy was proposed by Chen et al.: crystallized IONPs functionalized with polysiloxane-containing copolymer that possesses an antibiofouling capacity. Intravenous administration of these NPs following laser irradiation resulted in complete tumor regression with no relapse (Chen et al. 2014).

22.4.1.4 IONPs’ Application as Drug Carriers

The multifunctional properties of IONPs were tailored to improve their efficacy as drug carriers for therapies of different illnesses (mainly cancer). A method to augment the effectiveness of breast cancer therapy (chemo- and radiotherapy) was proposed by PirayeshIslamianet al. The method involved testing doxorubicin conjugated with superparamagnetic mesoporous hydroxyapatite nanocomposites and deoxy-D-glucose (PirayeshIslamian et al. 2017; Vallabani and Singh 2018). A recent study conducted by Ye and coauthors proved that Fe3O4 NPs improved the antitumor effect of cryoablation in MCF-7 cells by impairing the capacity of intracellular ice formation during the freezing process (Ye et al. 2017).

22.4.1.5 Magnetic Hyperthermia

The term hyperthermia has a Greek origin and refers to the generation of heat in high concentration, heat that the body fails to dissipate by thermoregulation (Gkanas 2013; LeBrun and Zhu 2018). According to the medical oncology definition, hyperthermia describes a therapeutic approach by which an established region of interest is subjected to an increase in temperature above 40 °C (Perigo et al. 2015) for an extended period of time (Spirou et al. 2018; LeBrun and Zhu 2018). The beneficial effects of rising body temperature in treating illnesses and fighting infection have been known since 1866, when clinicians used different bacteria or other infectious agents to treat syphilis, gonorrhea, epilepsy, and even tumors (face sarcoma and melanoma) by inducing high fever over 40 °C (Bierman 1942; LeBrun and Zhu 2018; Spirou et al. 2018). In recent years, hyperthermia has evolved from an approach involving raising whole-body temperature (using thermal chambers and blankets) (Perigo et al. 2015) to localized hyperthermia that acts by heating a certain region by means of optimized external devices (microwave radiation, implanted electrodes, ultrasounds, and laser irradiation) (Moros et al. 2015); side effects are minimal (LeBrun and Zhu 2018). Moreover, a considerable number of preclinical and clinical studies have been performed (even randomized clinical trials) to verify the outcome of hyperthermia coupled with radiotherapy/chemotherapy for different cancer treatments; this strategy being has no additional adverse effects and offers an improved therapeutic effect (De Haas-Kock et al. 2009; Lutgens et al. 2010; Chang et al. 2018).

Depending on the temperature values applied to achieve hyperthermia within the target cells/
tissues, this process can be classified into diathermia (37–41 °C), a method used to treat/relieve arthritis symptoms, moderate hyperthermia (41–46 °C), which induces protein denaturation and aggregation, DNA crosslinking, which leads to apoptosis as the endpoint, and thermal ablation (46–50 °C), which triggers extensive necrosis, coagulation, and carbonization (Gkanas 2013; Hilger et al. 2005; LeBrun and Zhu 2018). Another term that is used to describe hyperthermia is mild hyperthermia, which refers to temperatures up to 42 °C (Spirou et al. 2018). The methods used to clinically induce local hyperthermia have been improved over time and include radiofrequency, ultrasound, microwave, and laser ablation (a method that is used to treat superficial skin cancers); however, some drawbacks were noted: (i) inefficient as stand-alone therapies, (ii) invasive because it involves inserting one or more probes, (iii) causes thermal injuries to surrounding healthy tissue, and (iv) reduced control and confinement of heat to tumor area (LeBrun and Zhu 2018).

The elevated temperatures that are characteristic of hyperthermia have been associated with several detrimental effects in cells/tissues (several differences have been described between normal and tumor cells due to the acidic microenvironment of tumor cells, but no disparities between intrinsic thermosensitivity were recorded), including cell death via complex molecular pathways (protein and DNA denaturation, impaired mitochondria, interference with intracellular transport, changes in cytoskeleton, alterations of plasma and subcellular organelle membranes, sequential loss of enzyme functions), augmented perfusion within tumors, increased concentration of chemotherapeutic drugs and higher oxygen concentrations leading to a greater response of tumors to radiotherapy, and enhanced immune response of immune cytotoxic cells against tumor cells.

The degree of in vitro cytotoxicity induced by hyperthermia is dependent on temperature and exposure time (Chang et al. 2018; Spirou et al. 2018; LeBrun and Zhu 2018). A relevant aspect to note is the possibility of cells that escape death after hyperthermia to develop thermotolerance due to some major classes of proteins (heat shock proteins) and enzymes that repair the damage and transform these cells into resistant ones (Richter et al. 2010; Chang et al. 2018).

Since hyperthermia is not effective as a single therapy, several studies proposed its use as complementary treatment in combination with radio- and chemotherapy, this combination becoming the subject of several randomized clinical trials that are currently in progress and of others that are finished (Chang et al. 2018). Hyperthermia proved to be a potent radiosensitizer via protein denaturation and inactivation of the proteins responsible for DNA repair, thus suppressing the repair of DNA damage triggered by radiotherapy and augmenting the death of tumor cells (Kampinga and Dikomey 2001; Chang et al. 2018; Spirou et al. 2018). Hyperthermic therapy acts synergistically with different chemotherapeutic agents as cisplatin, cyclophosphamide, and bleomycin, whereas in the case of doxorubicin, 5-fluorouracil, and vincristine, no significant effects were observed. The mechanistic pathways involved in the sensitization of cancer cells to chemotherapeutic agents consist of augmented tumor blood circulation and enhanced vascular permeability, which increases drug concentration at a target site (Song et al. 2005; Chang et al. 2018).

Beyond the multiple advantages and applications of hyperthermia, some inconveniences were asserted, such as a lack of specificity against tumor cells (both healthy and cancer cells are sensitive to heat), inducement of burns in healthy tissues surrounding targeted cancerous tissues, difficulty of reaching an accurate temperature for promoting cytotoxic effects (43 °C for rodent cells and 43.5 °C for human cell lines) at target sites and the occurrence of overheating, unreliable localization, and a narrowed penetration of heat and development of thermotolerance (Moros et al. 2015; Chang et al. 2018; Spirou et al. 2018).

An updated version of hyperthermia, with augmented therapeutic effects and negligible adverse effects, is represented by MHT. MHT is a phenomenon that describes the ability of MNPs to produce heat in the presence of a suitable AMF.
via hysteresis energy loss and Néel or Brown relaxation (Moros et al. 2015). The first reference of this technique for cancer treatment dates from 1957, and between then and now notable progress was recorded in this field, MHT being assessed, at present, in preclinical and clinical trials as adjuvant therapy for different tumors (Moros et al. 2015).

Compared to hyperthermia, MHT brings notable advantages, including offering targeted delivery by tailoring NP surfaces to specific bioactive molecules, providing a controlled temperature increment by means of remote-switchable instruments represented by NPs (Moros et al. 2015), making deeper penetration into tissues by applying an AMF, administering MNPs in different concentrations and for longer durations within the target sites being available for multiple treatment sessions, enhancing heating capacity determined by nanosized magnetic particles, controlling the size and morphology of NPs (by augmenting their biocompatibility and reducing the adsorption of blood proteins – biocorona), and offering multiple drug delivery routes because it is a minimally invasive technique (Perigo et al. 2015; Gkanas 2013; Chang et al. 2016). The disparities in terms of clinical efficiency between hyperthermia and MHT are illustrated in Fig. 22.9.

An ideal magnetic material for MHT is represented by magnetic IONPs (10–100 nm), based on the following considerations: iron is an inorganic element naturally found in organisms that is involved in key cellular processes, has high biocompatibility because it is recycled within organisms via metabolic pathways, superparamagnetic properties, and low toxicity (human body tolerates a dose of 5 mg/kg body mass); another very important aspect is that magnetite and maghemite are activated by AMFs (Perigo et al. 2015; Zahedi-Tabar et al. 2019; Spirou et al. 2018). The magnetic properties of IONPs stem from the existence of iron ions with different valences in their crystal structure; these unpaired ions have oppositely aligned magnetic moments, leading to strong magnetization (Chang et al. 2018).

The application of AMFs stimulates IONPs to produce heat by two mechanisms: hysteresis loss and relaxational losses (Chang et al. 2018; LeBrun and Zhu 2018; Mahmoudi et al. 2018). Hysteresis losses in bulk take place in large IONPs that present multiple magnetic domains in their structure (Chang et al. 2018; LeBrun and Zhu 2018). The heat that results after AMF appli-

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**Fig. 22.9** Comparison between hyperthermia and MHT in terms of therapeutic potential
cation represents a difference in energy owing to the alignment of the magnetic moments of the NPs with the direction of the magnetic field (Kirschning et al. 2012; Chang et al. 2018; LeBrun and Zhu 2018). A decrease in IONP size is directly correlated with the reduction of the number of magnetic domains, with a single magnetic domain remaining at a threshold size (Houlding and Rebrov 2012; Chang et al. 2018).

The heat produced via relaxational losses occurs in superparamagnetic, single-domain NPs with sizes less than 25 nm. Two types of relaxation losses have been described: Néel and Brown relaxations (Chang et al. 2018; LeBrun and Zhu 2018). Néel relaxation can be defined as rapid changes that occur in a particle’s magnetic moment when it is exposed to an AMF, an alignment that is contrary to the particle’s crystalline structure, resulting in heat production. Brownian relaxation consists in frictional heat resulting from the physical rotation of particles within a supporting medium when the particles attempt to realign themselves with a changing magnetic field (Suto et al. 2009; Suriyanto et al. 2017; Ruta et al. 2015; Chang et al. 2018).

MNPs for MHT can be administered intravenously (the NPs are coated with a drug or carrier proteins and targeted for cancer cells) or direct intratumoral injections, a more invasive method with multiple advantages (Chang et al. 2018; LeBrun and Zhu 2018; Dulińska-Litewka et al. 2019; Mahmoudi et al. 2018). Other routes of delivery for the MNPs have also been also described as intraperitoneal (for ovarian, pancreatic, and gastric cancers), intra-arterial (liver cancers), and intracavitary (Chang et al. 2018).

MHT using magnetic IONP efficiency in treating cancer was tested in various models, including in in vitro and in vivo studies. An overview of these models is presented in Table 22.3.

MHT was officially introduced in clinical practice in 2011 for the treatment of glioblastoma in combination with conventional therapies (Dulińska-Litewka et al. 2019). Recent studies proved the use of MHT as a method to treat cancer under different approaches: (i) in combination with brachytherapy for prostate cancer, (ii) to stimulate the immune response in a murine melanoma model, (iii) in combination with radio frequency to treat gastrointestinal diseases (Crohn disease, colitis ulcerosa, and cancer), (iv) in therapy for patients diagnosed with HIV (Dulińska-Litewka et al. 2019).

Besides the numerous advantages and biomedical applications of MHT, this method also has several limitations: it has not been used in a clinical setting for any type of cancer, it makes it difficult to control the deposition of NPs at target sites, NPs are nonuniformly dispersed within tumors/tissues of interest, and it makes it difficult to estimate the proper thermal dose by predicting the temperature-time history (LeBrun and Zhu 2018).

### 22.4.2 Toxicity Assessment of IONPs

According to the ISO standard, the term nanoparticle refers to particles that possess one, two, or three external dimensions in a nanoscale range of 1–100 nm, dimensions that allow them to interact at a cellular (10–100 nm), subcellular (20–250 nm), genetic (10–100 nm), and even protein level (3–50 nm) (Markides et al. 2012).

The use of IONPs for biomedical purposes could be considered a double-edged sword since, on the one hand, IONPs possess multiple advantages (MRI-based clinical applications, targeted carriers due to their susceptibility to manipulation by an external magnetic field, promising therapy for tumor cells via MHT, reduced side effects of conventional drugs), but, on the other hand, their small dimensions facilitate their ability to cross different biological membranes in organisms, leading to unexpected noxious effects (Laffon et al. 2018; Nyström and Fadeel 2012).

In light of several recent studies (Yang et al. 2017; Laffon et al. 2018) that revealed the toxicity of IONPs both in vitro and in vivo, it has become essential to design a strategy for the evaluation of NPs’ toxicological profile by understanding the mechanism of action and the molecular processes involved.

The ability of IONPs to enter cells and accumulate in different cellular organs as lysosomes,
Table 22.3 Applications of MHT using IONPs

| Magnetic iron oxide | In vitro/in vivo studies | Toxicity endpoints induced by magnetic hyperthermia | Reference |
|---------------------|--------------------------|---------------------------------------------------|-----------|
| Cationic liposomes – MCLs | T-9 rat glioma cells | Increased uptake of positively charged MCLs by glioma cells; a significant reduction of cell viability was reached at 43 °C after 40 minutes of magnetic irradiation | Shinkai et al. (1996) |
| Polyethylene glycol-based magnetic hydrogel nanocomposites | M059K glioblastoma cells | Selective cell death of glioblastoma cells by applying thermoablative temperatures (60–63 °C) | Meenach et al. (2010) |
| Iron oxide MNPs – nanomagnetic fluid | U251 human glioma cells | A dose-dependent inhibition of human glioma cell proliferation and presence of chromatin condensation, cytoplasmic vacuoles, and apoptotic bodies | Xu et al. (2017) |
| Monodisperse magnetic iron nanoparticles | B16-F10 – murine melanoma cell line | Mild magnetic hyperthermia did not impair melanoma cell viability but upregulated hsp70 (heat shock protein) gene expression, a protein with key roles in sensibilization of cells to radiotherapy/chemotherapy | Moros et al. (2015) |
| Citric acid-coated zinc-doped magnetite nanoparticles (Zn0.4Fe2.6O4) | Bone-cancer cell line – MG-63 | Significant cell death after mild (42 °C) and extreme (47 °C) magnetic hyperthermia; in addition, even mild hyperthermia triggered differentiation of MG-63 cells to a more mature phenotype with a decreased capacity for self-renewal by upregulating alkaline phosphatase (ALP) expression, an early marker of osteogenesis | Moise et al. (2018) |
| Iron oxide magnetic nanoparticles | DA3, MCF-7, and HeLa | Significant cell death via apoptosis in all cell lines; the most sensitive cell line was MCF-7 (viability % < 3.5%) | Gkanas (2013) |
| Polycarboxylic iron oxide nanoparticles conjugated with doxorubicin | Human breast cancer cell lines – MCF-7 and MDA-MB-231 | Targeted cytotoxicity in breast cancer cells, MCF-7 and MDA-MB-231, by promoting apoptosis and lesser noxious effects on normal human mammary epithelial cells, MCF 10A | Catalano (2018) |
| Fourth-generation dendrimer-coated iron oxide nanoparticles – G4@IONPs | Human breast cancer cell line (MCF-7) and human fibroblast cell line (HDF1) | A significantly reduced viability of MCF-7 cells (36.7% viable cells), whereas HDF1 cells (63.5% viable cells) were less sensitive to magnetic hyperthermia | Salimi et al. (2018) |
| Human-like collagen protein-coated MNPs | Baby hamster kidney BHK-21 cells | No toxic effects on cell viability, suitable for magnetic hyperthermia experiments | Chang et al. (2016) |
| Magnetite nanoparticles | Muscle tissue from cow | Thermoablation (87 °C) induced tissue alterations characterized by light-brown discoloration, pyknotic cell nuclei and degenerated myofibrils | Hilger et al. (2000) |
| Iron oxide MNPs – nanomagnetic fluid | Nude male mice inoculated with U251 human glioma cells | Dose-dependent inhibition of tumor development; tumors with hemorrhage and necrosis | Xu et al. (2017) |
| Magnetic fluid hyperthermia (MFH), combined with external radiation | Dunning model of prostate cancer using Copenhagen rats | Reduced tumor growth after magnetic hyperthermia and radiation | Johanssen et al. (2006) |

(continued)
Table 22.3 (continued)

| Magnetic iron oxide bionanoparticle type | In vitro/in vivo studies | Toxicity endpoints induced by magnetic hyperthermia | Reference |
|----------------------------------------|--------------------------|---------------------------------------------------|-----------|
| Biocompatible superparamagnetic nanoparticles | Rat malignant glioma using RG-2 cells in Fisher rats | A single intratumoral injection with magnetic fluid followed by two thermotherapy treatments determined a prolongation of rat survival; histopathological evaluation indicated large areas of necrosis next to particle deposits, tumor cells with a decreased proliferation rate, and reactive astrogliaosis adjacent to tumor | Jordan et al. (2006) |
| Magnetic fluid hyperthermia | C3H mouse with induced mammary carcinoma | Dextran magnetite magnetic fluid administered intratumorally and exposed to an alternative magnetic field led to decrease in tumor volume and widespread tumor necrosis | Jordan et al. (1997) |
| Magnetic iron oxide nanoparticles (MIONs) | Squamous cell carcinoma | Intravenously administered MIONs under the effect of an AMF heated the tumor cells until ablation temperature, 60 °C, with no toxic effects for healthy surrounding tissue | Huang and Hainfeld (2013) |
| Porphyrin-coated MIONS | Melanoma | Intratumoral injection of MIONS followed by three short 10-minute AMF exposures decreased murine B16-F10 melanoma tumor volume; intravenous injection followed by three consecutive days of AMF exposure also proved efficient at decreasing tumor volume | Balivada et al. (2010) |
| MIONs conjugated to ChL6, an antibody that targets tumor-associated antigen L6 | Breast cancer | Intravenously administered magnetic nanoparticles followed by magnetic hyperthermia induced tumor growth delay in a mouse model of breast cancer using HBT3477 xenografts | DeNardo et al. (2007) |
| Magnetic hydroxyapatite nanoparticles | Colorectal cancer cells (CT-26 cell line) implanted in mice | Tumor growth skrinkage was induced by magnetic hydroxyapatite nanoparticles exposed to AMF | LeBrun and Zhu (2018) |
| Ferrofluid | PC3 tumors in mice | A reduction of tumor volume with areas of necrosis in center of tumor and apoptosis events in periphery | LeBrun and Zhu (2018) |

mitochondria, phagosomes, and vesicles (Catalano 2017) could be responsible for the noxious effects associated with IONP exposure. The main in vitro toxicity endpoints described for IONPs are decreased viability, oxidative stress, DNA damage, mitochondrial alterations, alterations in cell morphology and cell motility, cell membrane disruption, effects that are dependent on multiple factors such as cell type, IONP size, and concentration used, the capping agent, and the exposure time (Laffon et al. 2018; Liu et al. 2014).

The in vitro impact of IONPs has been studied intensively in recent years, but the results obtained were somewhat contradictory, with some studies affirming that IONPs are biocompatible and nontoxic at low concentrations (<100 μg/mL) (Kunzmann et al. 2011) and others reporting noxious effects even at these low doses (Laffon et al. 2018). No cell viability decrease was recorded following IONP exposure in human T lymphocytes, monocytes, primary rat astrocytes and neurons, human macrophages and amniotic fluid cells, murine microglial cells, fibroblasts, and macrophages (Laffon et al. 2018). A significant decrease in normal cell viability depending on concentration and exposure time was recorded in alveolar epithelial A459 cells after FeO3 exposure, in rat astrocytes treated with aminosilane- or starch-coated magnetite, and in human T lymphocytes exposed to IONPs coated with carboxyl or amine groups (Laffon et al. 2018).

In one of our previous studies, it was proved that magnetite and maghemite NPs double-
coated with oleic acid are nontoxic for human immortalized keratinocytes, HaCaT, at concentrations of 25 μg/mL after 24-hour stimulation. Moreover, the NPs had a stimulatory effect on cell migration and proliferation assessed by means of a scratch assay (Coricovac et al. 2017). In a more recent study, our group showed that a higher concentration (50 μg/mL) of magnetite double coated with oleic acid induced a significant decrease in HaCaT cell viability, whereas uncoated NPs had no impact on this parameter. In addition, the decreased viability was accompanied by the presence of a special phenomenon – enucleation characterized by the presence of holes within the cells as the nucleus was drawn out (Fig. 22.10) (Moacă et al. 2019).

Since engineered NPs are being applied more and more widely, it is essential to determine the fate of these NPs after their administration by investigating their safety/hazard potential. In this regard, intense discussions have been held among specialized groups to define a strategy for the evaluation of nanomaterial-induced hazards (Nyström and Fadeel 2012; Landsiedel et al. 2017). A schematic protocol to assess NPs’ toxicological profile is presented in Fig. 22.11.
22.5 Conclusions

Magnetic IONPs are among the first and most studied NPs for clinical use and remain important due to their multiple biomedical applications and to the associated challenges, the topic being always of interest. Though considerable progress has been made in the field of MHT and SPMHT, which uses biocompatible magnetic IONPs, further investigations are still required to overcome the present limitations. Better ways of assessing the toxicity of these NPs since studies that have done so following chronic/long-term exposure are rather scarce.

The key to successful cancer therapy by MHT and, recently, SPMHT, for the complete destruction of tumors, depends on four essential factors: (i) finding the most suitable SPION MNPs for MHT, (ii) establishing the appropriate nanoformulations of MNPs to achieve very good biocompatibility and eliminate toxicity to healthy tissues surrounding tumors, (iii) using high-efficacy SPMHT instead of MHT, and (iv) establishing adequate in vivo protocols for the application of SPMHT in different types of cancer in order to obtain the maximum efficiency in the destruction of tumor cells.

Issue (ii) and, partially, issue (iii) were presented and discussed in this chapter. For issue (iv), more data will be needed in future research in order to be able to outline a steps toward a thorough investigation into the efficacy of the method in cancer therapy and its application to humans in preclinical and clinical settings.

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