



Magnetic nanoparticles for application in cancer therapy

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ARTICLE INFO

Available online 2 March 2012

Keywords:

Magnetic particle
Iron oxide
Core-shell particle
Magnetic hyperthermia

ABSTRACT

Magnetic particles play nowadays an important role in different technological areas with potential applications in fields such as electronics, energy and biomedicine. In this report we will focus on the hyperthermia properties of magnetite nanoparticles and the effect of several chemical/physical parameters on their heating properties. We will discuss about the need of searching new smaller magnetic systems in order to fulfill the required physical properties which allow treating tumoral tissues more efficiently by means of magnetically induced heat. Preliminary results will be shown about the effect of a biocompatible shell of core-shell magnetite NPs on the heating properties by application of a RF magnetic field.

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1. Introduction

Nowadays multifunctional particles play an important role in different technological areas with potential applications in fields such as electronics [1], energy [2] and biomedicine [3]. Particularly, biocompatible magnetic nanoparticles are widely used in many biomedical applications, such as magnetic resonance imaging, drug delivery, cell and tissue targeting or hyperthermia [4–6]. The magnetic energy absorption of nanoparticle-containing tissues induces a localized heating that allows a targeted cell death at a critical temperature range above 42–45 °C. This temperature increase can be used to selectively kill cancer cells [7,8].

Properly coated iron oxide-based nanoparticles have been extensively studied in hyperthermia experiments because they are thought to be biocompatible and nontoxic, making them suitable for in vivo applications [9,10]. The final properties of the nanoparticles are governed by a big amount of chemical and physical parameters. This makes necessary an intense research in order to optimize their design and provide them with the required physical properties needed to maximize the heat generation. In this way, previous reports have shown that the ability and efficiency of the nanoparticles to induce magnetic heating depends on several factors related to the size, shape, solvent and magnetic properties, as well as the intrinsic characteristics of the applied magnetic field (magnetic field intensity and frequency) [8,11–14]. One important indicator of the efficiency of the nanoparticles under an external magnetic field is the heating power rate, because

an increase of the heating rate would imply lower doses of magnetic nanoparticles administered to the patient and lower time of stay in the body patient. In this sense, the availability of magnetic iron oxide nanoparticles (MNPs) properly designed constitutes for one of the most important subjects of research.

However, one of the main problems from the biomedical point of view is how the nanoparticles can be driven to the damaged tissue. The nanoparticle functionalization plays a crucial role in order to achieve this aim. Although an external magnetic field can be applied to guide the MNPs to specific tissues and organs, inorganic/organic molecule shells can be attached to the nanoparticle surface and trap them by specific bonding to targeted tissues or organs, as well as allow a targetable delivery in a specific area. In fact, aminosilane-coated iron oxide nanoparticles have been recently approved for the treatment of solid brain tumors by means of hyperthermia therapy [15]. In this novel approach, the aminosilane coating causes the nanoparticles aggregate and stay in the damaged tissues, allowing repeated treatments to the patient. Moreover, the non-magnetic functionalized coating plays simultaneously several essential roles: preserve the magnetic properties of the magnetic core, protect the bare nanoparticles and thereby, increasing their biocompatibility and non-toxicity properties and activate the surface for further functionalization steps. Although the silica shell has been one of the most studied coating agents due to its biocompatibility, non-toxicity and ability to be easily functionalized, other polymers/molecules have been used to biofunctionalize the surface of magnetic nanoparticles. [5,10,16]. Aside from exclusive applications to hyperthermia, Rozhkova et al. [17] and Kim et al. [18] have also designed Au-coated Fe–Ni magnetic-vortex microdiscs capable of inducing magnetomechanically cancer-cell destruction

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after their functionalization with proper biomolecules. However, so far, either the effect of non-magnetic shell on the hyperthermia properties of the magnetic cores or the heat diffusion through the functionalizing layer is still unknown. In this report we analyze the need of searching new smaller magnetic systems which allow treating more efficiently tumoral tissues by means of hyperthermia therapy. We also show preliminary results about the effect of changing the coating agent of core-shell nanoparticles on the induced magnetic heating achieved by high structural/magnetic quality core-shell magnetite nanoparticles of average particle size below 10 nm, which have been coated with different inorganic-organic shells.

2. Experimental

Magnetite MNPs of ≈ 10 nm were synthesized by chemical coprecipitation of an aqueous solution containing Fe^{2+} ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 99%) and Fe^{3+} ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 97%) salts in the molar ratio $\text{Fe}^{2+}/\text{Fe}^{3+} = 0.67$ with ammonium hydroxide (NH_4OH , 28%). OAc- and PAA-coated magnetite nanoparticles were obtained by adding different amounts of oleic acid and poly-acrylic acid immediately to the solution after magnetite precipitation. A silica coating on the surface of Fe_3O_4 nanoparticles previously coated with PAA, $\text{Fe}_3\text{O}_4@\text{PAA}@\text{SiO}_2$ was attained basically through the modified Stöber method [15].

Magnetic properties were measured in a vibrating sample magnetometer (VSM) under a maximum applied magnetic field of 1 T at room temperature. Heating properties of the samples were measured by means of a home-made magnetic radio-frequency (RF) power generator operating at a fixed frequency of $f = 308$ kHz and an induced magnetic field of $B = 15$ mT. A cylindrical Teflon sample holder was placed in the midpoint of an ethylenglycol cooled hollow coil (maximum of RF magnetic field), inside a thermally isolated cylindrical Dewar glass under high vacuum conditions (10^{-6} mbar). Measurements were carried out by placing samples in the sample holder and recording the temperature increase versus time with a fiber-optic thermometer during ~ 2 min of applied magnetic field. Morphology and crystal structure of PAA- and silica-coated magnetite nanoparticles were characterized by scanning transmission electron microscopy (STEM) and transmission electron microscopy (TEM) techniques using Hitachi S-5500 (30 kV) and PHILIPS CM-12 (100 kV) microscopes, respectively.

3. Basis of hyperthermia

For multidomain particles (ferro- or ferri-magnetic), heating is achieved mainly by hysteresis losses. Each one of these magnetic domains has a definite magnetization direction, in such a way that under a magnetic field the domains with a magnetization direction along the applied magnetic field axis grow. This process is irreversible and energy losses take place as the amplitude of the AC applied magnetic field increases and decreases continuously.

For particle sizes below the superparamagnetic (SPM) size limit no heating due to hysteresis losses occur (single domain particles). In this scenario, the external magnetic field forces the magnetic moment to reorient along the applied magnetic field axis. Therefore, the heating power is arisen from the energy dissipated in the reversible process of relaxation of the magnetic moments to their equilibrium orientation once the magnetic field is removed. This mechanism is characterized by a specific relaxation time and it is known as Néel relaxation process. In addition to this, the rotational motion of the particles within the solvent constitutes another source of heating and it is known as Brownian

relaxation. As a consequence, temperature increases due to the energy liberated in these mechanisms.

Therefore, the use of biocompatible SPM nanoparticles is highly desirable for biomedical applications, since the absence of coercive forces and remanent fields prevents aggregation phenomena between particles [4] and so increases the residing time inside the human body.

Both Néel and Brownian fluctuations are characterized by different magnetic relaxation times, which determine the heating power of the nanoparticles depending on the frequency of the AMF applied. The total relaxation time can be written as

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

where τ_B and τ_N are the Brownian and Néel relaxation times respectively, which are defined as

$$\tau_B = \frac{3\eta V_H}{k_B T} \quad (2)$$

and

$$\tau_N = \frac{\exp(kV/k_B T)}{\sqrt{(kV/k_B T)}} \quad (3)$$

where η is the viscosity of the solvent in which the particles are dispersed, k_B is the Boltzmann constant, T is the absolute temperature (K), V_H is the hydrodynamic volume of the particle (including the non-magnetic shell if it exists) and V is the magnetic volume. Both mechanisms depend on particle size whereas only the Brownian contribution depends on the viscosity, η , of the carrier solvent. Although several physical parameters have been systematically investigated [13,19] (size, concentration, and solvent viscosity), so far the effect of the coating layer on the heating properties remains unknown.

Our approach in this research includes the synthesis of different biocompatible core-shell magnetite NPs based ferrofluids and it has been focused on the organic/inorganic coating dependence of the heating properties.

4. Results and discussion

Even particle sizes at the nanoscale can be really a problem for the hyperthermia treatment of specific tumoral tissues. The reduction of the size is associated to several problems related to the higher surface/volume ratio. One of the most important in this kind of applications is the reduction of the saturation magnetization as the particle size decreases [20]. The preservation of the bulk magnetic properties is highly desired in order to drive the nanoparticles to the targeted tissues and achieve the required induced heating by application of the minimum external magnetic field as possible.

In this way, new methods of synthesis have been developed to obtain magnetic nanoparticles of lower size retaining their magnetic properties [21]. However, for many materials it continues to be a limiting parameter. In the last years a large interest has been devoted on the magnetic properties that appear in small nanoparticles and clusters of diamagnetic metals, although the origin of such magnetic behavior is not well understood [22,23]. In particular, the appearance of anomalous giant paramagnetism in organic-coated Au nanoparticles below 10 nm was attributed to the ability of capping molecules (i.e. -SH thiol groups) to act as donors or receptors of surface electrons [19]. This anomalous behavior has been tentatively explained on basis to an unfilled Fermi surface band through the giant values of the orbital moment that characterizes its Fermi level [24]. The effect of capping has been clearly evidenced by the fact that uncoated Au nanoparticles of similar sizes do not show permanent localized magnetic moments. Moreover, the

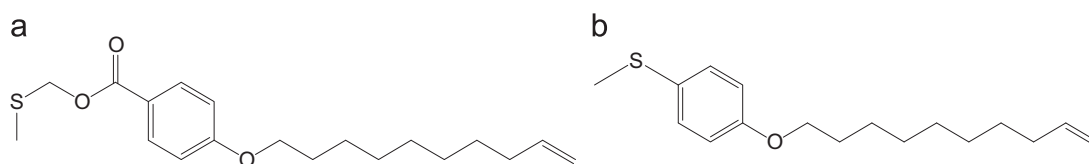


Fig. 1. Thiol-ligands used in Au NPs' synthesis. Ligands (a) and (b) differ on the chemical environment around the S atom.

appearance of magnetism is accompanied by the loss of the plasmonic band, as a consequence of the loss of the metallicity at the surface. However, our results show that other factors should be playing an active role in developing the observed magnetic behavior. We have synthesized Au nanoparticles with a particle size below 6 nm which have been capped with two different thiol-ligands. As shown in Fig. 1 the main difference between them is the different chemical environments around the S atom, which is the atom directly responsible for the ligand–particle bonding (S–Au bond).

In both cases, the resulting S–Au particles showed the absence of the plasmonic band, however, only the Au particles capped with the ligand (a) exhibited a localized permanent magnetic moment. Factors like particle size or self-assembly have been alternatively discarded by comparison of our results with those from the literature, in an attempt to explain the controversial results. Therefore, there should be other parameters playing an essential role in the magnetic localization at particle surface. Since the permanent magnetic moment has been only observed with ligand (a), which has a larger electron-withdrawing of the neighboring CH_2COO group than the benzene ring in ligand (b), we suggest that the responsible factor of this magnetic behavior could be directly related to the strength of the S–Au bond. This is supposed to have a direct effect in the charge transfer between the ligand and the particle surface. In fact, the effect of changing the organic capping on the magnetic properties of very small Au nanoparticles has been previously evidenced, and a particle size around 2.5 nm was found to be the optimal size of DT–Au nanoparticles in order to exhibit the highest saturation magnetization [25]. More recently, also a ferromagnetic-to-diamagnetic transition has been observed as the particle size decreases from the nanometric to cluster size in the SG–Au system [26].

This issue is of crucial interest in many biomedical applications in which the use of very small particles is highly desired in order to act as heat nano-sources in tumoral regions of limited size access. The commonest blood barrier found in most of the tissues is the continuous capillaries type. The separation between the endothelial cells along the basement membrane is the limiting factor of which the efficiency of the hyperthermia therapy will depend. The separation between endothelial cells in this kind of tissues is about 6 nm. If the MNPs are too much big to cross the endothelial barrier through the continuous capillaries, then the local heating will affect only to the external tumoral cells, while the inner ones only will experience a soft thermal effect, not enough to produce death (see Fig. 2a). In the opposite, if the MNPs are small enough to cross the blood barrier, they could penetrate and distribute inside the tumor, provoking a heat diffusion that would kill a bigger amount of neighboring tumoral cells (Fig. 2b). For this reason, the design of smaller MNPs with the required physical properties, capable of crossing the blood barriers and distribute more homogeneously in the targeted tumor, is highly recommended. This fact will contribute to a more efficient treatment of the damaged regions.

The non-magnetic coating not only can affect the magnetic properties of the resulting nanoparticles, but also the heating properties exhibited under a RF applied magnetic field.

We have recently evaluated the influence of colloidal parameters like concentration or solvent viscosity on the specific power absorption of PAA-coated magnetite nanoparticles (10 nm) [16].

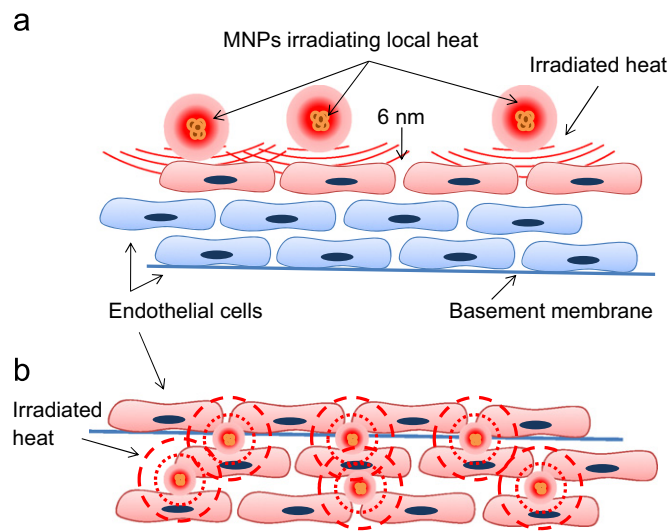


Fig. 2. Continuous capillaries found in most of the tissues: (a) Case in which the MNPs are bigger than the separation between endothelial cells. The magnetically induced heating created by the MNPs only affects the most external cells (in red), while the inner ones (in blue) do not experience important temperature increases. (b) Case in which the MNPs or clusters are smaller than the separation between the endothelial cells, crossing the blood barrier and distributing homogeneously into the tumor. The efficiency of the heating treatment is highly increased due to higher amount of cells which experienced a temperature increase above 42 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

We have observed different behaviors for dispersions containing uncoated- and PAA-coated nanoparticles. This indicates that the effect of non-magnetic coating is more important than expected and can be used to tune the heating properties of nanoparticle systems. Fig. 3 shows HRTEM and TEM micrographs for PAA and silica coated magnetite nanoparticles of 10 nm, respectively. The PAA-coated nanoparticles showed superparamagnetic (SPM)-like behavior with a magnetization saturation of about 60 emu/g, which increases to a range of ~75–80 emu/g once the amount of PAA is subtracted (which represents ~25% of the total mass), very close to the bulk magnetization (90–100 emu/g) (not shown data).

Fig. 4 shows different heating behaviors obtained in water dispersions containing core-shell magnetite nanoparticles of around 10 nm by changing their coating agent (Oleic acid (OA); poly-acrylic acid (PAA); silica (SiO_2)). It is important to remark that the magnetic particle concentration and the frequency of the alternating magnetic field were kept unchangeable.

The results evidence a reduction of the temperature raise for the coated nanoparticles in comparison to the uncoated ones. In the case of the particles coated with an inorganic silica shell the heat diffusion is completely quenched. This could be associated to the very low thermal conductivity shown by silica.

Since biocompatible silica-coated magnetite nanoparticles are widely investigated for potential applications in biomedicine, this result should be taken seriously into account in the case of using them as heat generating sources.

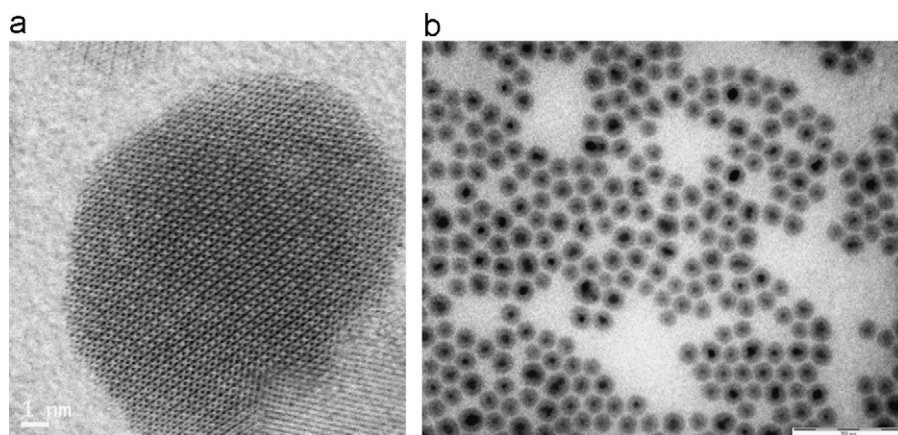


Fig. 3. (a) HRTEM and (b) TEM micrographs of PAA- and silica-coated magnetite nanoparticles of ~ 10 nm.

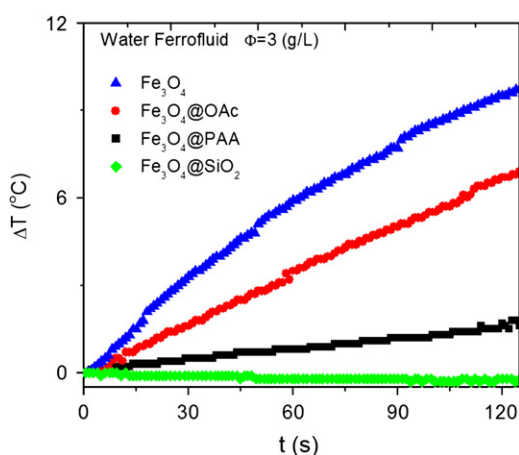


Fig. 4. Temperature increase with time for different coated magnetite nanoparticles. The magnetic particle concentration was fixed at 3 g/l.

5. Conclusions

Efficient design of multifunctional magnetic nanoparticles is a challenge since many aspects have to be controlled. In this work we have put on evidence the determining role of the coating materials used as shells for nanoparticles, which are intended to be used as biocompatible actuators. They can provide stability to the particles, make them biocompatible and even change their physical properties.

The magnetic character of very small Au nanoparticles (below 3–5 nm) can be easily controlled by using specific thiol-ligands, allowing in this way the functionality of “magnetic” gold for biomedical applications.

Organic and inorganic coatings play also an active role on the magnetic hyperthermia performance of small magnetite nanoparticles (around 10 nm) being an important parameter to take into account in the design process of biocompatible applications.

Acknowledgments

This work was partly supported by the European Community's under the FP7-Cooperation Programme through the MAGISTER project “Magnetic Scaffolds for in vivo Tissue Engineering” Large

Collaborative Project FP7—21468. <http://www.magister-project.eu/> and by the Science and Innovation Ministry, Spain (Project NanoBioMed CONSOLIDER-INGENIO 2010).

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