Magnetic (Hyper)Thermia or Photothermia? Progressive Comparison of Iron Oxide and Gold Nanoparticles Heating in Water, in Cells, and In Vivo

Ana Espinosa,* Jelena Kolosnjaj-Tabi, Ali Abou-Hassan, Anouchka Plan Sangnier, Alberto Curcio, Amanda K. A. Silva, Riccardo Di Corato, Sophie Neveu, Teresa Pellegrino, Luis M. Liz-Marzán, and Claire Wilhelm*

Magnetic hyperthermia (MHT) and photothermal therapy (PTT) are emergent state-of-the-art modalities for thermal treatment of cancer. While their mechanisms of action have distinct physical bases, both approaches rely on nanoparticle-mediated remote onset of thermotherapy. Yet, are the two heating techniques interchangeable? Here, the heating obtained either with MHT or with PTT is compared. The heating is assessed in distinct environments and involves a set of nanomaterials differing in shape (spheres, cubes, stars, shells, and rods) as well as in composition (magnhemite, magnetite, cobalt ferrite, and gold). The nanoparticle's heating efficacy in an aqueous medium is first evaluated. Subsequently, the heating efficiency within the cellular environment, where intracellular processing markedly decreases MHT, is compared. Conversely, endosomal sequestration could have a positive effect on PTT. Finally, iron oxide nanocubes and gold nanostars are compared in MHT and PTT in vivo within the heterogeneous intratumoral environment. Overall, two distinct therapeutic approaches, related to high dosage allowing MHT and low dosage associated with PTT, are identified. It is also demonstrated that PTT mediated by magnetic nanoparticles has an efficacy that is comparable to that of plasmonic nanoparticles, but only at significant nanoparticle dosages. At low concentrations, only plasmonic nanoparticles can deliver a therapeutic heating.

1. Introduction

The prospects of nano-physical treatments in cancer therapies rely on the nanoparticles potential to exert a physical effect on target tissues (e.g., emit heat or deploy a mechanical force) after being excited by a remote source of energy (e.g., light,[1] magnetism,[2] radiofrequency, or electric field[3]). Nanoparticles allying the effects of specific inorganic components (made of a magnetic and/or a metallic part) with the action of molecular constituents (i.e., chemotherapy drugs, specific receptor ligands, antibodies, polymer coatings), could thus exert a highly localized action.[4] The latter could be triggered by a physical stimulus with precise temporal and spatial control, which could minimize potential adverse reactions occurring in healthy tissue.

In nanoparticle-mediated thermal therapies, heat dissipation phenomena are produced by the fluctuations of magnetic moment in magnetic nanoparticles (magnetic hyperthermia, MHT)[5] or...
charge-density oscillations in metallic nanoparticles\cite{6} (plasmonic photothermia, also called photothermal therapy, PTT).\cite{7} In MHT, magnetic nanoparticles, considered as individual magnetic dipoles, orient their magnetic moments after exposure to an alternating magnetic field (AMF). The magnetic energy is dissipated through the relaxation of the nanoparticles moment to equilibrium, either through the rotation of the nanoparticle as a whole (Brownian relaxation) or through the rotation of the magnetic moment within the nanoparticle core (Néel relaxation). The heat-generating potential of the magnetic nanoparticles depends on nanoparticles structure, size, and magnetic anisotropy.\cite{8} In PTT, plasmonic nanoparticles absorb energy due to the interactions of light with conduction electrons on the metallic nanoparticle surface and part of this energy is released as heat.\cite{9} When the illumination wavelength is in resonance with the surface plasmon frequency (localized surface plasmon resonance, or LSPR), absorption results in optimal heat dissipation. The LSPR frequency is defined by the nanoparticles size, shape, and local environment.

The ability of magnetic and metallic (plasmonic) nanoparticles to be remotely activated via physical stimuli (alternating magnetic field or light, respectively) and to generate heat, could potentially be used as a tool to damage malignant cells. MHT\cite{10,11} and PTT\cite{12,13} thus recently made a breakthrough either as standalone therapies or as adjuvant treatments of cancer. Similar to conventional hyperthermia treatments (such as those employing thermal chambers, ultrasound, microwave, or radiofrequency), the aim of MHT and PTT is to increase tissue temperatures to the range of 40–45 °C. Yet, unlike in conventional treatments, where the heat is focalized to larger zones of the body, the ambition of MHT and PTT is to locally target cancer cells and selectively heat and kill them without harming the surrounding healthy tissue.

Multiple factors make thermal treatments particularly appealing in oncology:

(i) Effect of hyperthermia on the cellular level: Within the cell, nuclear proteins show the greatest sensitivity to heat, which affects inhibition of DNA repair and replication as well as RNA and protein synthesis.\cite{14} Hyperthermia also results in the disaggregation of microtubules and microfilaments. Cells in the S- and M-phase exhibit the highest heat sensitivity. The exposure to heat results in chromosomal damage, and inefficient mitosis, respectively.

(ii) The tumors and the healthy tissue behave differently in response to heat-induced damage: At tissue temperatures of up to 45 °C, exposure times of 30–60 min and heating rates up to 0.7 °C min\(^{-1}\), the vasculature of healthy tissues readily increases the blood flow (or perfusion), which dissipates heat and prevents excessive heating of tissues.\cite{15} Tumor vasculature, on the other hand, is hyperpermeable, tortuous, disorganized, and overflows with blind ends and abnormal bulges,\cite{16} making tumors much less efficient in heat dissipation. Thus, at the same thermal dose, the healthy tissue surrounding the tumor can remain at a lower temperature than the tumor itself (e.g., the temperature of the tumor might rise up to 50 °C while the periphery stays at 45 °C, thus hyperthermia does not harm adjacent tissues/organs,\cite{17} while blood supply in tumors collapses during or after heating). The exposure to a higher temperature within the malignant mass induces the inhibition of blood perfusion within the tumor and increases the rigidity of red blood cells, which results in vasodilatation, blood stasis, endothelial swelling, disintegration of endothelial lining, and plasma leakage or hemorrhage, with red blood cell and platelet aggregation leading to coagulative necrosis. The altered blood flow also affects oxygen and nutrient delivery within the tumor and prevents the removal of lactic acid, leading to severe tissue acidosis within the tumors.

(iii) Effects on the extracellular matrix: Apart from acting on tumor cells and vasculature, nanoparticle-induced hyperthermia affects the extracellular matrix.\cite{18} Its essential constituents, the collagen fibers, slacken during hyperthermia, which allows a better spreading/penetration of chemotherapeutics.\cite{19}

(iv) Imperceptible hyperthermia: In contrast to the overt hyperthermia of tumors, recent research shows that heating nanoparticles may also lead to cell death without a perceptible macroscopic temperature increase.\cite{20} The subcellular temperature increase, focally localized within lysosomes, might induce cell death through lysosomal death pathways, and thus are likely to be applicable to apoptosis-resistant cancer cells.\cite{21}

Due to all these therapeutic benefits of thermal tumor treatments, and particularly, due to the potent local nanthromic action, magnetic nanoparticles-mediated hyperthermia and nanoparticles-mediated photothermal therapy have been extensively studied in preclinical settings, and have recently also translated into clinical evaluations (clinical trials NCT02033447 (prostate), DRKS00005476 (glioblastoma) for MHT; NCT00848042 (head and neck tumors), NCT01679470 (primary and metastatic lung tumors) for PTT).

Until very recently, the two thermal nanotherapies, MHT and PTT, have been studied and developed separately, resulting in two independent fields of thermal treatments. They have only recently begun to intersect due to the recent discovery and use of the photothermal properties of iron oxide nanostructures\cite{22} or to the use of magneto-photothermal hybrids,\cite{23} which combine both heating features in one object. The approaches of magneto-photothermia have brought improved therapeutic results to thermal nanotherapies combining the advantages of each modality while overcoming their intrinsic disadvantages. For instance, considering magneto-photothermal hybrids can perform MHT in deep-seated tumors overcoming limited light penetration that might restrict PTT, while PTT enables a high heating yield per mg of nanoparticle, reducing total nanoparticle administered dose.

Nevertheless, a toolbox is still needed to select the best nanohenter, leading to the most adequate heat delivery, for each thermal treatment. To address this issue, we performed a comprehensive comparison between MHT and PTT, conducted in environments of increasing biological complexity: in the aqueous environment, inside cells, and finally, within tumors. In addition, we thoroughly assessed the effect of the dose. The comparison was made in relation to the composition of the material (gold, iron oxide, or cobalt ferrite), its shape (spheres, cubes, stars, rods, and shells), and heating modality (PTT or MHT).
2. Selection of State-of-the-Art Magnetic and Plasmonic Nanoparticles: The Comparison of Their Heating Capacities within the Same Experimental Settings

The state-of-the-art nanoparticles intended for thermal therapies include magnetic (mainly iron oxide) or plasmonic (principally gold) nanoparticles, either of which exhibit a heating efficacy that correlates with their nanoscale design.

The features that characterize the heating performance of magnetic nanoparticles include size, shape, anisotropy, magnetic exchange coupling, and saturation magnetization. The heating performance of plasmonic nanoparticles also depends on the size, but the essential parameter governing their suitability for thermal therapies in vivo, is particle shape. The particle shape enables to shift the particles absorption in the near-infrared (NIR) wavelengths (generally between 650 and 1000 nm),[19] where the optical absorption of tissues[19b] is minimal and the treatment therefore attains the maximum penetration depth. Treatment depths of 4–6 mm have been reported for intratumorally injected gold nanoshells,[19a] but the treatment range might differ in other tissues, which exhibit a different pigmentation and different perfusion.

In order to compare MHT and PTT, a panel of eight state-of-the-art nanoparticles were selected and produced (Figure 1). Magnetic particles (Figure 1A) include rock-like 9–11 nm maghemite nanoparticles (IONPs, \( \gamma Fe_2O_3 \)) and cobalt ferrite nanoparticles (CFNPs, CoFe_2O_4), both obtained by coprecipitation; 20 nm magnetite nanocubes (IONCs, Fe_3O_4) made by thermal decomposition; and 25 nm maghemite nanoflowers (IONFs, \( \gamma Fe_2O_3 \)) prepared by means of the polyol process.

All of these selected magnetic nanoparticles display high saturation magnetization values, in the 55–75 emu g\(^{-1}\) range (Figure 1C, and Table S1, Supporting Information). Figure 1D also summarizes nanoparticles’ absorbance, emphasizing

Figure 1. Magnetic and metallic nanomaterials for thermal therapy. A) Transmission electron microscopy (TEM) images of magnetic nanomaterials: iron oxide nanoparticles (IONP, 9 nm), cobalt ferrite nanoparticles (CFNP, 11 nm), iron oxide nanocubes (IONC, 20 nm), and iron oxide nanoflowers (IONF, 25 nm). Scale bar = 50 nm. B) TEM images of metallic nanomaterials: gold nanostars (AuNST, 25 and 85 nm), gold nanorods (AuNR, 10 × 40 nm), and gold nanoshells (AuNS, 150 nm). Scale bar = 100 nm. C) Saturation magnetization at 300 K for all magnetic nanomaterials. Normalized UV–vis–NIR spectra of D) magnetic nanomaterials and E) gold nanomaterials.
privileged near-infrared light absorption of IONCs and CFNPs. The optical absorbance as a function of iron content is shown in Figure S1 (Supporting Information) ([Fe] = 0.75–25 × 10⁻³ M for all magnetic materials).

For plasmonic nanoparticles (Figure 1B), we selected two representative samples of gold nanostars (with overall sizes of 25 and 85 nm), which display redshifted LSPR bands in the NIR, centered at about 650 and 800, respectively (Figure 1E), when increasing particle size. In addition to nanostars, nanorods and nanoshells were also selected, both of them exhibiting plasmon maxima at about 800 nm (Figure 1E). The variation of the absorbance spectra related to gold concentration can be seen in Figure S2 (Supporting Information). Table S2 (Supporting Information) also provides the total molar extinction coefficient ε (expressed in m⁻¹ cm⁻¹), either as moles of Fe or Au, or moles of nanoparticles) of magnetic and plasmonic nanoparticles calculated from the Beer–Lambert law at 680 and 808 nm. Gold nanoparticles have large extinction coefficients, in the range of 10⁸ – 10¹⁰ m⁻¹ cm⁻¹. Similar results were obtained in the literature for gold nanostars,[20] nanoshells,[19a] and nanorods.[21] These values are 4–5 orders of magnitude higher than those of magnetic nanoparticles (ε ≈ 10⁵–10⁶) in the NIR range. Among the magnetic nanoparticles, IONCs possess the highest molar extinction coefficient of 5 × 10⁷ m⁻¹ cm⁻¹ at 808 nm.

Figure 2 summarizes the heating characteristics of all selected nanoparticles groups, measured under equal experimental conditions (identical volumes, tube types, and same MHT and PTT conditions, alternating magnetic field, and laser exposure), in water. The measurements were obtained at [Fe] = 25 × 10⁻³ M (1.4 gFe L⁻¹) for all iron oxide nanoparticles, and at [Au] = 0.75 × 10⁻³ M (0.15 gAu L⁻¹) for gold nanoparticles, which are both representative concentrations used when testing the thermal potential of nanomaterials in aqueous dispersions. For gold nanoparticles, the heating was measured under excitation conditions that correspond to the wavelength of plasmonic resonance for the given nanoparticles group (808 nm for gold nanorods, nanoshells, and 85 nm nanostars, and 680 nm for the 25 nm nanostars). Figure 2A,B shows typical infrared images of the heating for all nanoparticles, and Figure 2C,D shows representative heating curves. The average temperature elevations after 1 min heating are shown for all conditions (Figure 2E,F).

Here, we must emphasize the applied experimental settings. MHT was performed at either 18 mT and 470 kHz (which is closer to the clinical limit, with H × f = 6.7 × 10⁶ A m⁻¹ s⁻¹)[25] or 24 mT and 900 kHz, which increases the thermal capacity of the particles, but is clinically less relevant. However, the safety limit for MHT is still a matter of debate, and may be shifted in the near future. On the other hand, PTT was performed in the near-infrared first biological transparency window, at 680 or 808 nm, and for two laser power densities (0.3 or 1 W cm⁻²), considered in the range of tolerable laser powers.[23b] Importantly, available preclinical PTT studies generally use laser powers densities of 1 W cm⁻² or higher, up to 3–4 W cm⁻².[23b] Such high laser power can induce heating with core temperature over 60 °C, and result in ablative tumor lesions.[23b] In addition, lasers can be used in other medical applications at much higher power,[24] where tissues are destroyed after direct irradiations. Such an example is laser surgery, where laser powers in the range of 10–100 W are used. Herein, we focused our study to assess the effects of nanoparticles-induced heating at low laser power densities, where the effects of laser-only-induced tissue damage are limited.

Under our experimental conditions: (i) magnetic nanoparticles yield similar heat generation, regardless of the type of activation (magnetic or photonic); and (ii) plasmonic nanoparticles exhibit a comparable photoactivated heat generation, but at 30 times lower molar concentration. The internal comparison between magnetic nanoparticles mediated MHT and PTT (Figure 2E) indicates that PTT, at the laser power density of 1 W cm⁻², leads to higher temperatures for IONCs and CFNPs, and to comparable temperatures at 0.3 W cm⁻². Conversely, IONFs, which display a high magnetic heating response, exhibit a small photothermal efficiency. This is due to the low absorption of these nanoparticles in the NIR region (see Figure S1, Supporting Information), which is a representative feature of maghemite-based nanoparticles (γFe₂O₃, as IONPs). In contrast, maghemite (Fe₃O₄) or cobalt ferrite (CoFe₂O₄) crystals exhibit higher absorbance in the NIR, due to electronic transitions (δ-d transition, charge transfer transition) between Fe²⁺ and Fe³⁺ ions in magnetite and magnoeto-optical transitions of Co²⁺ ions located in tetrahedral and octahedral coordination in the cobalt ferrite.[25]

While the temperature increase may depend on the experimental conditions (though it remains comparable among materials within the same environments), the concentration-renormalized slope of the temperature curve provides an absolute value representing the material’s heating capacity (also known as the specific absorption rate, SAR) (Figure 2G,H). The SAR, defined as the power absorbed per mass of material, is expressed in watt per gram (W g⁻¹). In the present study, we determined the SAR per gram of iron pertaining to magnetic nanoparticles, or the SAR per gram of gold, contained in plasmonic nanoparticles. Considering magnetic nanoparticles, they reach a maximum of 400 W gFe⁻¹ (for IONC and CFNP) in both MHT and PTT modalities, when the lowest exposure parameters (18 mT and 470 kHz or 0.3 W cm⁻²) are applied. For MHT, when the magnetic field and frequency are increased (up to clinically irrelevant ranges), the SAR approached values of 1 kW gFe⁻¹. This value is among the highest value for nanoparticles used in MHT,[25] with current limit close to 3–4 kW gFe⁻¹.[26]

In contrast, herein, in the case of PTT, magnetic nanoparticles (IONC and CFNP) can overcome 1 kW gFe⁻¹ at laser power densities of 1 W cm⁻². For plasmonic nanoparticles, the SARs (Figure 2H) reach impressive values of 3 or 10 kW gAu⁻¹, at low laser power densities of 0.3 or 1 W cm⁻², respectively.

The following conclusions were drawn from this first-tier testing in aqueous medium, we ascertained the following. First, plasmonic nanoparticles mediated PTT has a higher thermal yield than magnetic nanoparticles mediated MHT, as plasmonic nanoparticles resulted about 20 times more efficient, when the thermal efficacy was expressed in watts per gram of material (iron or gold). This is consistent with their much higher total molar extinction coefficient reflecting a higher absorption part (even though scattering effects cannot be neglected for large gold nanoparticles[60]). And second, irrespective of the fact that magnetic nanoparticles were used thus far mostly for MHT, we here confirm and substantiate more recent approaches
that suggest their use as agents intended for PTT,\cite{17a,26,27} by showing that PTT values obtained at 1 W cm\(^{-2}\) can be higher than the MHT-mediated ones.

From now on, in order to distinguish the heat generation after either laser exposure of plasmonic nanoparticles, or laser exposure of magnetic nanoparticles, we will refer to “plasmonic PTT” or “magnetic PTT.”

3. The Significance of Concentration: A Frequently Disregarded Parameter

The measurements obtained in the aforementioned first-tier testing were made at a specific concentration, as it is often the case in most reported studies. Nevertheless, how does the heating vary as a function of particle concentration? Is the SAR...
an independent and absolute parameter, which is completely unrelated to the concentration? This is the case for MHT, with exceptions either at high concentrations, where magnetic interactions can impact the final heating efficiency, or under uncontrolled aggregation. In PTT, on the other hand, we could expect that concentration does have an impact when the light absorption increases with concentration according to the Beer–Lambert law.

Indeed, as described in detail elsewhere, during the initial phase of heating (where heat dissipation can be neglected), the light-to-heat energy transfer can be written as \( I_0 \cdot S(1-10^{-A}) \cdot \eta = m_{\text{sample}} \cdot C \cdot dT/dt \), where \( I_0 \) (W) is the incident laser power, \( S \) (cm\(^2\)) is the illuminated area, \( A \) is the absorbance of the sample at the irradiation wavelength, \( \eta \) is the photothermal conversion efficiency from irradiation laser energy to thermal energy, \( m_{\text{sample}} \) is the sample mass (here the mass of water), \( C \) denotes the specific heat capacity (\( C_{\text{water}} = 4 \times 10^3 \) J g\(^{-1}\) K\(^{-1}\)), and \( dT/dt \) denotes the initial slope of the temperature increase. All samples tested here follow the Beer–Lambert law \( A = \varepsilon \cdot c \cdot L \), with \( \varepsilon \) (M\(^{-1}\) cm\(^{-1}\)) the molar extinction coefficient (provided in Table S2, Supporting Information), \( c \) the concentration of absorbing nanoparticles, and \( L \) the path length. Consequently, at high concentration, \( 10^{-A} \) becomes negligible, and the initial heating \( dT/dt \) remains almost constant with increasing concentrations. Note also that the SAR is expressed as \( m_{\text{sample}} \cdot C / m_{\text{Fe or Au}} \cdot dT/dt \) (see the Experimental Section), and can thus be theoretically written as

\[
\text{SAR}_{\text{theor}} = \frac{1}{m_{\text{Fe or Au}}} \left[ I_0 \cdot S \cdot (1-10^{-A}) \cdot \eta \right]
\]

Figure 3 compares the heating efficiency of magnetic and gold nanoparticles, with increasing mass concentrations of 0.05, 0.5, and 5 g L\(^{-1}\). Concentrations are expressed in terms of grams of iron for magnetic nanoparticles, or as grams of gold for plasmonic nanoparticles. This comparison was conducted on 20 nm iron oxide nanocubes and 25 nm gold nanostars (similar nanoparticle size). As we can see on the thermal images (Figure 3A), the nanostar-mediated plasmonic PTT is the only one to be efficient at low concentrations (0.05 g\( \text{Au or Fe} \) L\(^{-1}\)). In contrast, at high concentrations (5 g\( \text{Au or Fe} \) L\(^{-1}\)), the difference between MHT and magnetic PTT is less pronounced and the difference between magnetic PTT and plasmonic PTT is negligible. The averaged calculated SAR (Figure 3C) confirms that for MHT, the SAR is concentration independent, and attains 400 W g\( \text{Fe} \) at 18 mT and 470 kHz. For magnetic and plasmonic PTT, at low concentrations (0.05 g\( \text{Au or Fe} \) L\(^{-1}\)), the SAR is impressively high (3 kW g\( \text{Fe} \) and 25 kW g\( \text{Au} \), at 1 W cm\(^{-2}\)), for magnetic and gold nanoparticles, respectively. By contrast, at 5 g\( \text{Fe} \) L\(^{-1}\), the SAR obtained for both magnetic and plasmonic PTT at 1 W cm\(^{-2}\) is comparable with the one obtained in MHT (in the 500 W g\(^{-1}\) range). If we follow the same reasoning in relation to the high extinction coefficient of gold nanoparticles (Table S1, Supporting Information), plasmonic PTT is more impaired at higher particle concentrations than magnetic PTT. As a result, at low (0.05 g L\(^{-1}\)) doses, plasmonic SAR is eightfold higher than magnetic SAR; at medium (0.5 g L\(^{-1}\)) doses, the difference is only threefold; and at high doses, plasmonic and magnetic SARs are of the same order. The theoretical SAR\(_{\text{theor}}\) are also presented in Figure 3C, superimposed to the experimental values, and are in very good agreement. Finally, the calculated photothermal conversion efficiency of gold nanostars \( \eta = (38 \pm 3)\% \) is logarithmically higher than the one obtained for iron oxide nanocubes \( \eta = (29 \pm 2)\% \).
The intermediate conclusion related to the role of nanoparticles concentration is that at low nanoparticle doses, we can only rely on PTT (magnetic or plasmonic, and only plasmonic at very low doses). In contrast, at high concentrations, MHT takes over its advantage, among all because MHT is not limited in terms of treatment depth. In addition, at high nanoparticles concentrations, magnetic PTT is as advantageous as plasmonic PTT, placing iron oxide nanoparticles at the forefront of thermal therapies, which require local (e.g., intratumoral) injections. Conversely, at low concentrations, for instance, the ones obtained after systemic administration, plasmonic PTT appears as the only approach that might be therapeutically viable.

4. The Effect of Intracellular Processing

As nanoparticles are intended for potential therapeutic applications, it is essential to evaluate the heating behavior in the (cancerous) cells and the intracellular environment.

At first, regardless of their type, the nanoparticles entervia endocytosis and concentrate within endosomes (Figures 4A and 5A, Figures S3 and S4, Supporting Information). This intracellular processing (nanoparticles agglomeration in endosomes) impacts the physical properties of nanoparticles, thus magnetic nanoparticles in cells exhibit lower initial susceptibility and hysteresis loop opening for most nanoparticles (Figure S5, Supporting Information). Cells containing iron oxides or gold were dispersed to reach an equal overall concentration of $25 \times 10^{-3}$ m of iron for magnetic nanoparticles (Figure 4) or $0.75 \times 10^{-3}$ m of gold for plasmonic nanoparticles (Figure 5). Note that these cellular samples concentrations were identical to those previously assessed in aqueous suspensions. Figure 4B illustrates the effect of cellular internalization on the heating production through MHT. Irrespective of the nanoparticle type, heating was found to be drastically reduced. The most striking effects were observed in the case of IONFs, with an 85% heating loss, or for CFNPs, with a 90% heating loss. Such an effect was previously reported, and generally attributed to the inhibition of Brownian relaxation and/or magnetic dipolar interactions due to intraendosomal aggregation.[29,31] The heating decrease of nanoparticles upon confinement of nanoparticles in the endosomes is even more pronounced for nanoparticles for which the anisotropy or the interparticle exchange interactions favor Brownian relaxation.[31b] Nevertheless, a marked (40%) decrease also applies to rock-like IONPs, for which the heating mainly involves Néel relaxation, suggesting that strong interparticle interactions can also be harmful to Néel-relaxing nanoparticles. Besides, even if the decrease of heating in the cellular environment is reduced for mentioned nanoparticles, they initially have a lower SAR. As a consequence, for nanoparticles localized within cells, the SAR never exceeds 60 W g$^{-1}$ (for IONC), and falls below 20 W g$^{-1}$ for IONP, CFNP, and IONF.

The fact that, to date, no nanoparticles were yet described entering the cells (this strategy was used by MagForce AG, The Nanomedicine Company),[32] whereas a second alternative comprises functionalization of magnetic nanoparticles with membrane ligands, which specifically target membrane receptors of a given cell type, with an induced cytotoxic response which does not involve global heating on the cellular level.[16b,33]

Conversely, when we analyze the photothermal heating performance of magnetic nanoparticles (Figure 4C), the situation is different, and even reversed. For all tested nanoparticle groups, heating is maintained, and is even greater once the cells have internalized (confined) the particles. In the particular case of IONCs, we can now juxtapose the PTT-derived intracellular SAR of 1200 W g$^{-1}$Fe$^{-1}$ (obtained with a laser density of 1 W cm$^{-2}$) with the modest 60 W g$^{-1}$Fe$^{-1}$, obtained with MHT (470 kHz, 18 mT). We thus obtain a counterintuitive result: IONCs, considered among the best MHT agents,[8c] have now manifested as 20 times more efficient mediators of PTT.

Regarding more conventional (plasmonic) PTT agents (Figure 5), the effects of cell internalization (Figure 5A) are mostly beneficial, due to plasmonic coupling in endosomes.[7a,17a,34] When 25 nm gold nanostars (AuNST-1) or 85 nm gold nanostars (AuNST-2) are excited at wavelengths resonant with their plasmon band (680 or 808 nm, respectively), heating within cells is comparable to that observed in aqueous dispersions (Figure 5B). However, while small nanostars (AuNST-1), excited with the wavelength of 808 nm, do not heat considerably in suspension, because the excitation wavelength does not correspond to their plasmon band, cell-internalized AuNST-1 do respond to the excitation at 808 nm light. And they do it as efficiently as their 85 nm counterparts, which actually have the plasmon band centered at 800 nm. This result is a direct consequence of plasmon redshift and band broadening, due to plasmonic coupling between neighboring nanostars. The consequences of cell internalization can thus be favorable for plasmonic nanoparticles (e.g., 300% heating increase for 25 nm AuNSTs at the excitation wavelength of 808 nm). This phenomenon is compelling for in vivo applications, because smaller (25 nm) nanostars might have a more favorable biodistribution than larger (85 nm) nanostars, and the laser wavelength to be applied (about 800 nm), would be absorbed by bodily components to a smaller degree.

When a direct comparison is made between the heating of magnetic and plasmonic nanoparticles after cellular processing, we can state that plasmonic nanostars are much more efficient heating agents in PTT (7500 W gAu$^{-1}$, 1 W cm$^{-2}$) than iron oxide nanocubes for MHT (60 W gFe$^{-1}$, 470 kHz, 18 mT). Nevertheless, when magnetic nanocubes are used in PTT, their output (1200 W gFe$^{-1}$, 1 W cm$^{-2}$) is getting closer to that of gold nanostars.

Taken together, these measurements indicate that MHT is impaired after particles internalization by cells, and that even the most efficient magnetic nanoheaters, such as iron oxide nanocubes or nanoflowers, lose their heating power (e.g., up to 95%, as shown for nanoflowers). Conversely, in plasmonic PTT, the heating efficacy can be increased, as evidenced for 25 nm gold nanostars. And, in between the two extremes, in magnetic PTT, the internalization did not negatively affect the heating, but it rather slightly increased the heating within the intracellular environment.
Finally, what can we possibly expect to happen within the tumor, which is the final target of thermal anticancer treatments? As we mentioned above, moderate hyperthermia can sensitize the tumors to chemotherapeutics as well as radiation. In addition, as shown in animal models, it can slow down tumor growth\[15b,19a,35\] and stimulate the immune system,\[16\] but does not lead to complete tumor ablation (or total tumor regression). Tumor ablation is generally observed only when higher temperatures (43 °C and above) are obtained within the tumor core.\[17a,37\]

In comparison between MHT and PTT in vivo, we focused on the 20 nm magnetic iron oxide nanocubes and 25 nm plasmonic gold nanostars. The intratumoral heat increase was
measured 1 h after the intratumoral injection at increasing concentrations (100 µL at 0.05, 0.5, 5 g L\(^{-1}\), which correspond to the following administered doses of about 0.25, 2.5, and 25 mg kg\(^{-1}\) body weight) of either iron oxide or gold nanoparticles.

Remarkable and reassuring, on the day of injection, intratumoral heating responses (Figure 6) were comparable to those observed in aqueous suspensions for the same nanoparticles types (Figure 3). This allowed us to obtain the following results: (i) in the MHT setting, a relevant temperature increase occurs only at the highest particle concentration, and the intratumoral temperature increase is proportional to the injected dose: it falls below 1 °C for the 0.5 g L\(^{-1}\) dose and it equals 7.5 °C for the 5 g L\(^{-1}\) dose; (ii) PTT is efficient at low particles concentration, and increasing particle concentrations are not particularly beneficial, especially for plasmonic PTT. Typically, if we increase the concentration by 100 times (from 0.05 to 5 g L\(^{-1}\)) we only get a threefold temperature increase for nanostars, and fourfold increase for nanocubes. Nevertheless, at concentrations of 0.05 g L\(^{-1}\), nanostars are more efficient, with a temperature increase of 10 °C (at 1 W cm\(^{-2}\) exposure), which is in the therapeutic range. For nanocubes, at such a low dose of 0.05 g L\(^{-1}\), the temperature increase is only 5 °C (at 1 W cm\(^{-2}\)) in magnetic PTT. At the higher dose of 5 g L\(^{-1}\), both nanostars and nanocubes provide therapeutic heating of 10 and 8 °C, respectively, at laser power as low as 0.3 W cm\(^{-2}\).

To achieve significant MHT, the dose of nanocubes had to be increased to 12.5 g L\(^{-1}\), which is comparable to amounts injected for MHT applied in preclinical xenograft tumor studies. The temperature thus increased by 18 °C. With the same nanocubes at the same dose, magnetic PTT provided a 9 °C increase at 0.3 W cm\(^{-2}\) and it outperformed the MHT temperature with an increase up to 22 °C at 1 W cm\(^{-2}\).

It is important to emphasize that most PTT studies in preclinical settings use laser power densities of 1 W cm\(^{-2}\) and above, even if they generate a higher thermal response within the exposed tissue,\(^{[38]}\) which is not specific to heating nanoparticles. In our setting, the nonspecific heating (obtained in the
absence of nanoparticles), attributed to the laser power density of 1 W cm\(^{-2}\) was of 4.5 °C at 680 nm and 3 °C at 808 nm. In contrast, a power of 0.3 W cm\(^{-2}\) results in almost no nonspecific heating (below 2 °C at 680 nm, below 1 °C at 808 nm). These data show that power densities as low as 0.3 W cm\(^{-2}\) are totally noninvasive, with no thermal response related to the laser itself.

In order to correlate the ratio between the benefits of thermal therapy and the risks of tissue damage, we have calculated a previously reported\[39\] thermal dose parameter, the CEM\(_{43}\) (cumulative equivalent minutes at 43 °C), in order to normalize thermal data from applied hyperthermia treatments. The CEM\(_{43}\) correlates the heating with the thermal damage, as a function of treatment time.\[39\] Table S3 (Supporting Information) provides the CEM\(_{43}\) parameters calculated for IONCs and AuNSTs at the conditions displayed in Figure 6 (\([\text{Fe}]=0, 0.05, 0.5, \text{and } 5 \text{ g L}^{-1}\), at 0.3 and 1 W cm\(^{-2}\)). In this case, we have considered 5 min of treatment for both power densities. Significant acute and chronic skin damage (CEM\(_{43}\) values over 40 min) would only occur using nanoparticles at high concentration (\([\text{Au}]\) at 0.5 and 5 g L\(^{-1}\), and \([\text{Fe}]\) at 5 g L\(^{-1}\)) and the maximal laser power density. In all other cases, thermal damage threshold would not be achieved.

Finally, we addressed the effect of cell internalization in vivo, and compared the heating effects at the day of injection (day 0) and 3 days after injection (day 3). The comparison was made, in this case, at concentrations of 12.5 g L\(^{-1}\) (62.5 mg kg\(^{-1}\)) for nanocubes and 0.15 g L\(^{-1}\) (0.75 mg kg\(^{-1}\)) for the 25 and 85 nm nanostars (Figure 7). These conditions were chosen to obtain a temperature increase of 10–20 °C. Nanocubes submitted to MHT provided a temperature increase of 18 °C at day 0. After laser exposure at 808 nm (0.3, and 1 W cm\(^{-2}\), non-specific heating of 3 °C), we obtained a temperature increase of 22 °C for nanocubes and 8 or 14 °C for 25 and 85 nm nanostars, respectively. At day 3, the heating efficiency of MHT drops below 10 °C increase, while magnetic PTT is slightly more efficient than at day 0 (25 °C increase). For plasmonic PTT, the heating efficiencies of 25 nm AuNST-1 and 85 nm AuNST-2 after laser excitation at 680 or 808 nm, respectively, decrease at day 3. This decrease most likely correlates with nanostars permeation throughout the tumoral interstitial space and gradual clearance from the tumor. The spreading of the particles throughout the tumor matrix has been reported in previous studies and correlates with particle size,\[40\] with gold nanoparticles below 100 nm exhibiting a more pronounced spreading throughout
Figure 7. Evolution of nanomaterials heating within tumors as function of time (in vivo). Infrared thermal images and temperature elevations at day 0 and day 3 for A,C) the 20 nm IONC at [Fe] = 12.5 g L\(^{-1}\) \((250 \times 10^{-3} \mu\text{g})\) after MHT and PTT treatments and for B,D) the 25 nm AuNST at [Au] = 0.15 g L\(^{-1}\) \((0.75 \times 10^{-3} \mu\text{g})\) subjected to PTT. E,F). TEM micrographs of IONC and AuNST in tumors at day 0 and day 3. The red asterisk in (E) (bottom, left side) indicates the collagen fibers.
the tumor. At low concentration ranges, concentration variations impact the heating to a greater extent and the permeation of nanoparticles (and the concomitant local decrease in nanostars concentration) results in a decrease of the heating. As the administered dose of nanostars was relatively low, the heating of the tumor at day 3 diminished on the account of the fraction of nanostars that was cleared from the tumor. On the other hand, nanocubes were administered in a tenfold higher dose, where concentration variations have a lower effect on the heating outcome, thus even if a fraction of nanocubes was cleared from the tumor, the amount was too low in proportion to the administered dose, and did not substantially affect the PTT outcome at day 3.

That being said, in addition to particle spreading and gradual clearance from the tumor, the internalization of particles by adjacent cells also comes into play. It first explains the decrease in MHT efficiency from day 0 to day 3. Figure 7E (together with Figures S6 and S7, Supporting Information) provides the intratumoral localization of nanocubes at day 0 (evidencing a mainly extracellular nanoparticle distribution) and at day 3 (showing nanoparticles which are partly internalized within tumor cells). Logically, the significant cell internalization at day 3 translated into a decrease in the heating performance of nanocubes for MHT. Interestingly, as observed in vitro in cells, the magnetic PTT is not affected by cell internalization in vivo, and even favored by the intracellular localization of nanocubes. Concerning plasmonic nanostars, electron microscopy (Figure 7F, and Figures S8 and S9, Supporting Information) shows that nanostars were almost all individual, dispersed in the extracellular matrix at day 0, while they were all internalized within cells at day 3. As a result, and as observed in vitro in cells for small 25 nm nanostars AuNST-1, the heating of the tumors at day 0 and day 3 remained comparable after irradiation at 808 nm, while it decreased at 680 nm. In this case, the remaining nanostars at day 3, internalized by the cells, compensated the total heating outcome by increasing their heating output. 25 nm AuNST-1 then becomes as efficient as 85 nm AuNST-2 at 808 nm.

In summary, in order to attain therapeutically relevant temperature increase in vivo, we require smaller doses of gold nanostars in comparison to iron oxide nanocubes. The issue of a potential toxicity of high doses of magnetic nanomaterials then arises. However, doses of 12.5 g L\(^{-1}\) were previously shown to have no impact on tumor growth,\(^3\)\(^5\)\(^a\)\(^1\) suggesting a low toxicity. Besides, MHT clinical trials are underway (e.g., phase II on glioblastoma multiforme, Magforce) with injected doses per treatment reaching 112 g L\(^{-1}\) of Fe.\(^4\)\(^1\)

Herein, for a temperature increase of 15–20 °C, we need either 12.5 g L\(^{-1}\) (62.5 mg kg\(^{-1}\)) of nanocubes for MHT (at 470 kHz and 18 mT) or 0.5 g L\(^{-1}\) (2.5 mg kg\(^{-1}\)) of nanostars for plasmonic PTT at a laser power density of 1 W cm\(^{-2}\). Nevertheless, if the laser power density has to be decreased to a very low dose of 0.3 W cm\(^{-2}\), only a 10 °C temperature increase can be obtained with gold nanostars or magnetic nanocubes at the high dose of 5 g L\(^{-1}\). Such temperatures are not sufficient for tumor ablation, but might slow down the tumor growth or be used as adjuvant therapy to other nonthermal treatments of cancer. As saturation is already reached at 5 g L\(^{-1}\), increasing particle dose does not lead to an increased heating of the tumor. However, at 5 or 12.5 g L\(^{-1}\) of nanocubes, MHT is feasible and thus a combined magnetic PTT and MHT treatment might be suggested for superficial tumors.

Consequently, if we compare plasmonic or magnetic PTT at 0.3 W cm\(^{-2}\), which can only allow a 10 °C increase, MHT has the advantage of increasing the temperature to a much greater extent (e.g., 20 °C). However, using the 1 W cm\(^{-2}\) condition, high temperature increments (>10 °C) are easily reached with both magnetic nanocubes and gold nanostars, at low (0.5 g L\(^{-1}\)) and very low (0.05 g L\(^{-1}\)) doses, respectively.

This makes PTT particularly attractive for achieving high heating efficiency at moderate doses of nanoparticles. However, the depth penetration shortcoming, which is associated to laser irradiation, still hinders the applicability of this technique. Therefore, in deep-seated tumors, MHT might remain the only option. Nevertheless, the use of optical fibers, such as those used for laser therapeutic endoscopy should encourage further the use of PTT. The Aurolase therapy, in which a laser fiber is inserted into the tumor, already advances of this approach.

### 6. Conclusions

MHT and PTT are two promising emergent treatments against cancer. Until very recently, the development of these two modalities advanced along two parallel paths, with magnetic (iron oxide) nanoparticles leading the field of MHT and plasmonic (gold) nanoparticles “striking the gold” in the field of PTT. Yet, magnetic materials may also generate heat upon exposure to light. In this nanoparticles-based thermal therapy context, it appeared timely to provide a comprehensive comparison of the outcome of magnetic versus plasmonic heating. In a head-to-head basis, we compared different magnetic nanoparticles (iron oxides, cobalt ferrite, spheres, cubes, flowers) with different plasmonic (gold, stars, rods, shells) nanoparticles in aqueous, cellular, and tumoral environment.

For comparison in water, we chose to compare relevant concentrations of in water. SARs of plasmonic nanoparticles (almost 10 000 W g\(\text{Au}^{-1}\), 1 W cm\(^{-2}\)) than for magnetic nanoparticles (max 400 W g\(\text{Fe}^{-1}\)). Nevertheless, this comparison should be taken with caution. While SAR is an absolute indicator, concentration-independent in case of MHT, it decreases with the nanoparticles concentration when PTT is applied. We thus compared the SARs of gold nanostars and gold nanoflares) magnetic nanoparticles for MHT, at 5 g L\(^{-1}\), the SARs are within the same order of magnitude (400 and 800 W g\(^{-1}\) for MHT and plasmonic PTT, respectively). Besides, PTT with magnetic nanoparticles can compete with plasmonic heating, with a SAR of ~3000 and 600 W g\(\text{Fe}^{-1}\) at 0.05 and 5 g L\(^{-1}\) (1 W cm\(^{-2}\), respectively. Another key parameter that should be taken into consideration is the effect of cell internalization. The latter has a negative impact on MHT, while it is beneficial for PTT applications in the near-infrared, and the influence of concentation and internalization (confinement) is remarkable in vivo.

Concerning the excitation source, in order to obtain a therapeutic temperature increase (of about 10 °C) at a physiologically...
inert laser power density (of 0.3 W cm$^{-2}$), plasmonic and magnetic nanoparticles should be used at relatively high doses (0.5 or 5 g L$^{-1}$, equivalent to 2.5 or 25 mg kg$^{-1}$ doses, for plasmonic and magnetic nanoparticles, respectively). The magnetically heated iron oxide nanoparticles can lead to an even higher temperature increase (typically of about 20 °C at the highest iron concentration used in this study, which is 12.5 gFe L$^{-1}$). Such temperatures can, in principle and in preclinical practice, be obtained with gold nanostars at 0.5 gAu L$^{-1}$ or with magnetite nanocubes at 5 gFe L$^{-1}$, and a laser power density of 1 W cm$^{-2}$, but as we mentioned above, this laser power density is less appropriate in clinics. In addition, increasing the concentration of gold nanoparticles at low laser power densities will not lead to a temperature increase above 10 °C, which makes MHT the modality of choice for a temperature increase of 20 °C with no °C of gold nanoparticles at low laser power densities will not lead to an appropriate in clinics. In addition, increasing the concentration of iron nanoparticles at low laser power densities will not lead to a temperature increase above 10 °C, which makes MHT the modality of choice for a temperature increase of 20 °C with no collateral unspecific heating. Yet again, this setting will require a very high dose of iron oxide nanoparticles (12.5 gFe L$^{-1}$). Such a dose will slowly but surely undergo internalization within cells and consequently diminishes the efficiency of MHT in the days following the injection. The effects of cell internalization have a smaller impact when large quantities of magnetic nanoparticles are used for MHT (because the fraction of internalized particles vs the extracellular particles remains small), but the heating still decreases in the days following the particles administration.

Overall these findings enable us to conclude that there are two therapeutic applications for the two modalities: one pertaining to low doses, where PTT is effective, but where temperature increase can be limited to 10 °C or requires a laser power density of 1 W cm$^{-2}$, or, alternatively, a MHT application, where a high dose of magnetic nanoparticles is required.

7. Experimental Section

Synthesis of Magnetic Nanomaterials: Iron Oxide and Cobalt Ferrite Nanoparticles: Iron oxide (magnetite) nanoparticles were synthesized by alkaline coprecipitation followed by a forced oxidation by iron nitrate treatment. Cobalt ferrite nanoparticles were obtained by precipitation of a stoichiometric mixture of Co(II) and iron (III) hydroxide followed by heating at 100 °C for 1 h. After nitric acid treatment, magnetic nanoparticles were capped with citrate ions to ensure their stability in aqueous suspension by electrostatic repulsion (negative surface charges). At the end of the synthesis, both nanoparticles were treated in a highly oxidizing medium with iron(III) nitrate at 80 °C for 45 min to achieve complete Fe$^3+$ ions after degradation of the nanoparticles.

Iron Oxide Nanocubes: To obtain nanocubes of magnetite 20 nm in edge length, first, 1 mmol of iron(III) acetylacetone and 4 mmol of decaionic acid were mixed in 25 mL of dibenzyl ether (according to the protocol detailed by Guardia et al.[46]). The temperature of the solution was increased to 220 °C using a progressive heating of 5 °C min$^{-1}$ and then kept at this temperature for 2.5 h. Finally, the solution was heated to reflux temperature (at a rate of 10 °C min$^{-1}$) and reacted for 1 h. The cubes were rinsed three times and dispersed in 15 mL of chloroform and then mixed with a polyethylene glycol (PEG)–gallol solution (20 mL, 0.05 M in CHCl$_3$) together with triethylamine (2 mL); at the end, the mixture was transferred to water. Mössbauer characterization of this synthesis revealed a pure magnetite structure.[42]

Iron Oxide Nanoflowers: Iron oxide nanoflowers were prepared via the polyol process.[15] A mixture of FeCl$_3$ and 2 mmol of FeCl$_2$ (in a liquid mixture of N-methyl-diacetamide and diethylene glycol) was stirred for 1 h. A sodium hydroxide solution (in polyols) was added to the solution of iron chloride, and the resulting mixture was stirred for additional 3 h. Then, the temperature was increased to 220 °C and the mixture kept reacting for 12 h. The precipitated were separated magnetically and washed with a mixture of ethanol and ethyl acetate. Then, an aqueous solution of iron(III) nitrate was added to the nanoparticles. The resulting mixture was heated to 80 °C for 45 min to complete oxidation of the nanoparticles. After another treatment with 10% nitric acid, the particles were rinsed twice with acetone and diethyl ether and finally dispersed in water. Finally, the nanoparticles were oxidized into magnetite similar to IONPs and CNFs. However, due to the multicore structure, a mixture of magnetite/maghemite could still be present, accounting for the high magnetization (see Table S1, Supporting Information).

Synthesis of Metallic Nanomaterials: Gold Nanostars: Gold nanostars were prepared by following the protocol by Kumar et al.[43] Briefly, an aqueous solution of HAuCl$_4$ (0.041 mL, 100 × 10$^{-3}$ M) was mixed with a solution of polyvinylpyrrolidone (PVP) (15 mL, 10 × 10$^{-3}$ M) in dimethylformamide. The mixture was stirred until complete disappearance of the Au$^{+3}$ charge-transfer-to-solvent (CTTS) band at 325 nm, followed by rapid addition of gold seeds in ethanol under vigorous stirring. To obtain nanostars with 25 and 85 nm of diameter, the time of seeds was found to be 0.292 and 0.159 mL, respectively. The color of the solution changes from colorless to blue within 40 min, indicating the formation of gold nanostars. The samples were centrifuged three times and redissolved in water.

Gold Nanorods: Gold nanorods, with an aspect ratio of ~3.5 (length 41 nm, width 11 nm) were prepared following a well-established seeded growth approach described previously,[2] with minor modification. Briefly, spherical gold seed particles were synthesized by mixing 1 mL of 5 × 10$^{-4}$ M HAuCl$_4$, 1 mL of 0.2 M CTAB, and 0.12 mL of ice cold 0.01 M NaBH$_4$. The suspension was vigorously mixed for 2 min at 1800 rpm on an orbital shaker (Heidolph MultiRerag) and then for 1 h at 1000 rpm. Thereafter, 0.12 mL of the fresh prepared gold seeds was added to a solution containing 50 mL of 0.2 CTAB, 2.5 mL of AgNO$_3$, 4 × 10$^{-3}$ M, 50 mL of HAuCl$_4$ 10$^{-3}$ M, and 0.7 mL of ascorbic acid 0.078 M. The resulting mixture reacted for 30 min at 1200 rpm. The described steps were performed at room temperature. The gold nanorods were separated by centrifugation (10 min at 14 000 rpm) and resuspended in ultrapure water.

Gold Nanoshells: Au silica-nanoshell suspensions were purchased from Nanospectra Biosciences, Inc. (Houston, TX). The nanoshells consist of a 150 nm silica-core diameter, surrounded by an ultrathin 8 nm thick gold shell, conjugated with PEG.

Cell Culture and Internalization Assays: For the in vitro measurements, human prostate cancer cells (PC-3 cells) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and 1% penicillin, and maintained at 37 °C with 5% CO$_2$ until confluence.

The cells were co-incubated for 2 h with the different magnetic colloids, at the following concentrations within the extracellular medium: [Fe] = 2 × 10$^{-3}$ M for iron oxide nanoparticles and for cobalt ferrite nanoparticles, [Fe] = 0.2 × 10$^{-3}$ M for iron oxide nanocubes, and [Fe] = 0.6 × 10$^{-3}$ M for iron oxide nanoflowers. The magnetic particles were dispersed in serum-free RPMI-1640 medium supplemented with 5 × 10$^{-3}$ M sodium citrate. At the end of the incubation, the medium was removed and the cells were rinsed three times with culture medium, and further placed at 37 °C for an additional 2 h chase period.

For the metallic nanoparticles, the cells were incubated at [Au] = 0.02 × 10$^{-3}$ M for 12 h in RPMI medium (for 25 and 85 nm nanostars) and in DMEM medium supplemented with 5% FBS and 1% penicillin.

Particle-loaded cells were detached by means of trypsin-EDTA solution and resuspended in phosphate buffered saline (PBS) in order to obtain 150 μL (~20 million cells) and transferred into a 0.5 mL Eppendorf tube. The cell fraction that was intended for transmission electron microscopy (TEM) analysis was fixed with glutaraldehyde (2%) in 0.1 M sodium cacodylate buffer (7.4 pH) at 4 °C for 60 min, and stained with 1% osmium tetroxide in cacodylate buffer. The cells were subsequently dehydrated with graded solutions of ethanol, impregnated with hexa-methyl-phospho-amide, and embedded in EPON resin supplemented with 3% benzyl-dimethyl-amine.
Elemental Analysis: The concentrations of iron and gold in aqueous dispersions and in cells were measured by elemental analysis using an ICP-AES spectrometer (ICAP 6500, Thermo). The samples were digested in a HNO₃ and HCl solution (10 mL) using appropriate iron and gold standards. The iron load per cell was additionally determined by single-cell magnetophoresis.

Transmission Electron Microscopy: Cells or 1 mm³ tumor tissue pieces were fixed with glutaraldehyde (2%) in 0.1 M sodium cacodylate buffer (7.4 pH) at 4 °C for 60 min or overnight, respectively, and stained with 1% osmium tetroxide and 1.5% potassium cyanoferrate in cacodylate buffer. The cells/tissues were subsequently dehydrated with graded solutions of ethanol, impregnated with hexa-methyl-phosphor-amide, and embedded in Epon resin supplemented with 3% benzyl-dimethyl-amine.

TEM images of aqueous dispersions were obtained with a FEI-Philips TECNAI 12 transmission electron microscope. Thin sections (70 nm) of cells and tumors were observed with a Zeiss EM902 electron microscope operating at 80 keV (MIMA2 - plateau de MET - unité 1196 GPL - Jouy-en-Josas, France).

Thermal Measurements: Thermal measurements of nanomaterials in aqueous dispersion and within cells were performed in Eppendorf tubes (0.5 mL) containing 150 µL of sample. Concentrations were adjusted to [Fe] = 25 × 10⁻³ M (1.2 g L⁻¹) for magnetic nanomaterials and [Au] = 0.75 × 10⁻³ M (0.1 g L⁻¹) for metallic nanomaterials, which are in the range of standard element content to induce an elevation of temperature of 10–20 °C.

An alternating magnetic generator device (DM3, NanoScale Biomagnetics) with a frequency ranging of 470 kHz and 18 mT of amplitude was used to induce MHT. The sample was placed between two magnetic coils.

For PTT, each sample was illuminated from the side with visible and NIR lasers (680 or 808 nm) coupled to an optic fiber (Laser Components Systems, Inc.) was used to monitor the surface temperature of the tumors. During the procedures the animals were anesthetized with a ketamine/xylazine anesthesia. An infrared thermal camera (FLIR SC7000, FLIR Systems, Inc.) was used to monitor the surface temperature of the tumors.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This work was supported by the European Union (Marie Curie Intra-European Project FP7-PEOPLE-2013-740 IEF-62647), by Paris city (Research in Paris), by CNRS and by Paris Diderot University. LML-M acknowledges funding from the Spanish MINECO (MAT2017-86659-R). The authors thank Ana Sánchez-Iglesias for the synthesis of Au nanostars, Christine Pêchoux for TEM preparation and analysis, Karine Desboeufs for ICP measurements, Ana Sánchez-Iglesias for the synthesis of Au nanostars, Christine Pêchoux for TEM preparation and analysis, Karine Desboeufs for ICP measurements, and Ludovic Maingault and Isabelle Le Parco from the animal housing facility at the Jacques Monod Institute for animal experiments. The authors also thank Tom Wyatt for carefully proofreading of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

magnetic hyperthermia, magnetic iron oxide nanoparticles, nanomedicine, photothermal therapy, plasmonic gold nanoparticles

Solid tumors were induced by subcutaneous injection of 1.5 × 10⁶ PC3 human epidermoid carcinoma cells in 100 µL of physiological saline in the left and right flanks. Thermal measurements were performed after two to three weeks, when the tumors reached over 100 mm³. More precisely, for thermal measurements on injection day (day 0), animals with 200 mm³ were selected, and tumors were injected with 50 µL of nanomaterials suspension at different concentrations: [Fe] = 0.05, 0.5, 5, and 12.5 g L⁻¹ and [Au] = 0.05, 0.5, and 5 g L⁻¹, in order to compare the same mass element content. Three tumors were injected at each concentration. In order to evaluate the impact of nanomaterials processing within the tumor, another series of thermal measurements were set up, on independent tumors never treated, at day 3 after injection. For this second condition of heating, nanomaterials were injected within smaller tumors, of 100 mm³ in average, to take into account tumor growth, and perform the final measurement on tumors of equivalent size to the day 0 condition (~200 mm³ tumor volume).

Concerning the intratumoral administration, tumors were held with tweezers and nanoparticles were injected with a syringe in the middle of the tumor. This procedure was used in previous studies and histological data showed that after such administrations tumors preferentially localize within the tumor capsula.[15b]

The animals injected with magnetic materials were subjected to MHT (470 kHz, 18 mT, 5 min) and PTT (808 nm at 0.3 and 1 W cm⁻², 5 min, respectively) and those injected with metallic materials were subjected only to PTT (680 and 808 nm at 0.3 and 1 W cm⁻², 5 and 1 min, respectively. Some collateral tumors were used as controls (noninjected and nontreated). MHT and PTT experiments were conducted using the same instrument and setup as in aqueous dispersion and in vitro studies.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

magnetic hyperthermia, magnetic iron oxide nanoparticles, nanomedicine, photothermal therapy, plasmonic gold nanoparticles

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This work was supported by the European Union (Marie Curie Intra-European Project FP7-PEOPLE-2013-740 IEF-62647), by Paris city (Research in Paris), by CNRS and by Paris Diderot University. LML-M acknowledges funding from the Spanish MINECO (MAT2017-86659-R). The authors thank Ana Sánchez-Iglesias for the synthesis of Au nanostars, Christine Pêchoux for TEM preparation and analysis, Karine Desboeufs for ICP measurements, Mathieu Receveur for conceiving the holder for NIR measurements, and Ludovic Maingault and Isabelle Le Parco from the animal housing facility at the Jacques Monod Institute for animal experiments. The authors also thank Tom Wyatt for carefully proofreading of the manuscript.
[36] a) G. Multhoff, *Int. J. Hyperthermia* **2009**, *25*, 169; b) T. Kobayashi, K. Kakimi, E. Nakayama, K. Jimbow, *Nanomedicine* **2014**, *9*, 1715.

[37] D. P. O’Neal, L. R. Hirsch, N. J. Halas, J. D. Payne, J. L. West, *Cancer Lett.* **2004**, *209*, 171.

[38] M. Motamedi, S. Rastegar, G. LeCarpentier, A. J. Welch, *Appl. Opt.* **1989**, *28*, 2230.

[39] P. S. Yarmolenko, E. J. Moon, C. Landon, A. Manzoor, D. W. Hochman, B. L. Viglianti, M. W. Dewhirst, *Int. J. Hyperthermia* **2011**, *27*, 320.

[40] S. D. Perrault, C. Walkey, T. Jennings, H. C. Fischer, W. C. Chan, *Nano Lett.* **2009**, *9*, 1909.

[41] K. Maier-Hauff, F. Ulrich, D. Nestler, H. Niehoff, P. Wust, B. Thiesen, H. Orawa, V. Budach, A. Jordan, *J. Neuro-Oncol.* **2011**, *103*, 317.

[42] P. Guardia, A. Riedinger, S. Nitti, G. Pugliese, S. Marras, A. Genovese, M. E. Matera, C. Lefevre, L. Manna, T. Pellegrino, *J. Mater. Chem. B* **2014**, *2*, 4426.

[43] P. S. Kumar, I. Pastoriza-Santos, B. Rodriguez-Gonzalez, F. J. G. de Abajo, L. M. Liz-Marzan, *Nanotechnology* **2007**, *19*, 015606.