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Exploring multifunctional potential of commercial ferrofluids by magnetic particle hyperthermia

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ABSTRACT

In this work we examine a selection of commercially available magnetic iron oxide nanoparticles as candidates for magnetic particle hyperthermia applications combining their primary modality with additional heat triggered actions. Contrary to lab-made magnetic nanoparticles, commercial ferrofluids may be rapidly pushed through the medical approval processes since their applicability has already been addressed successfully (i.e., formulation, reproducibility, toxicity and quality assurance) in conjunction with the strong companies′ drive in the fast delivery of the new therapy to the patient. Four samples are under study with variable hydrodynamic diameters from two companies (Micromod and Chemicell) consisting of iron-oxide magnetic nanoparticles. The tunable magnetic heating characteristics of the ferrofluids were correlated with particle, field and colloidal solution features. Our work revealed a size-dependent magnetic heating efficiency together with fast thermal response, features that are crucial for adequate thermal efficiency combined with minimum treatment duration and show the potential of such materials as multifunctional theranostic agents.

1. Introduction

Biomedical exploration of novel magnetic nanocarriers with enhanced individual and collective features covering a multifunctional range of applications is still a pursuit for many research groups worldwide [1, 2]. Among various application schemes such as drug-release, MRI contrast agents and biomarkers, magnetic nanoparticles (MNPs) comprise a very promising candidate mainly due to their extreme localization at the nanoscale. Magnetic particle hyperthermia (MPH) is a synergistic technique used in cancer treatment, along with chemotherapy or irradiation [3]. In MPH one can take advantages of heat generated by MNPs when are exposed in an alternating (AC) magnetic field [4]. The regional increase of temperature, when applying inside a small region of the human body, may induce apoptosis or necrosis of the cancer cells, without damaging the normal cells [5].

Recently, a new approach to achieve effective apoptotic results in cancer cells is proposed, by using double-effector nanoparticles that generate reactive oxygen species (ROS) and heat [6]. Moreover, when hyperthermia may be applied in combination with common cancer treatments (e.g. chemotherapy), is proven to enhance the effect of anticancer drug, thus promotes effective therapy schemes with reduced clinical dosage [7]. Beyond the strictly therapeutic use of hyperthermia, it may also be exploited in conjunction with other diagnostics techniques, such as MRI, taking advantages of the multifunctionality of the same magnetic carriers [8].

Most of the published works in the field of biomedical applications of MNPs, utilize magnetic nanoparticles synthesized in-lab, aiming to the material-side properties (sample control and property tuning) [9]. Meanwhile, issues concerning the applicability (formulation, reproducibility, toxicity and quality assurance) are usually postponed for the later stages when MNPs will actually be introduced to biomedical practice [10]. Contrary to home-made magnetic nanoparticles, commercially produced materials may be rapidly pushed through the medical approval processes since commercial drive and company resources are focusing in the delivery of the new therapy to the patient as fast as possible.

In this work, we decided to initiate an examination of four commercially available ferrofluids, consisting of iron-oxide nanoparticles, in an effort to see if beyond their actual modality if they possess measurable heating efficiency. Such a study outlines the potential of market products and readily addresses the multifunctional role in modern theranostics by combination of initial role with an additional heat-triggered action. Eventually, such

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systems may be further implemented in therapies, with faster steps, since major tasks (e.g. biocompatibility and sustainability) have already been undertaken before these substances were released in the market.

2. Materials and methods

Commercially available ferrofluids, consist of magnetic iron oxide nanoparticles of two different companies: a) Micromod [11] Nanomag®-D-spio and b) Chemicell [12] FluidMAG-DX-SSS where SSS corresponds to their mean hydrodynamic diameter, 50, 100 and 200 nm. An overview of their features as provided with the companies′ datasheets is collected in Table 1. In order to measure the iron concentration of each commercial ferrofluid the atomic absorption spectrophotometry was used, by a Perkin Elmer AAnalyst 800 instrument by dissolving a quantity of particles′ dispersion in HCl.

Structural and morphological characterization of commercial MNPs were carried out with X-Ray Diffractometer (XRD), using a SIEMENS D500 X-Ray diffractometer, and with Transmission Electron Microscopy (TEM), using a JEOL 1010 operating at 100 kV. For the specimen preparation a drop of diluted solution was deposited on a carbon coated grid. The mean particle size was estimated by applying the log normal function in the size-statistical distribution derived from a series of TEM images depicting more than 300 MNPs.

Hyperthermia measurements were performed by a water-cooled induction coil machine, with varying field amplitude (16–24 kA/m) and constant frequency of 765 kHz. The frequency used seems relatively high according to “limit” of applied AMFs (Alternating Magnetic Fields) in humans which derives from the product of Hf (our case: 152⩾limit: 4.85×10^{8} A/m s). This threshold for patient discomfort arises from subjecting regions of 15 cm to 13.56 MHz and 35.8 A/m and acts up to date as a general extrapolation guideline to the maximum frequency and amplitude that can be applied in MPH. However, such an extrapolation should be cautiously reconsidered especially in nanoscale applications by accounting additional parameters that affect eddy heating in tissues, namely, the effect of diameter: it is evident that smaller tissue regions could tolerate larger field-frequency products[4].

For hyperthermia characterization solution samples were prepared from the initial commercial product. In each case we prepared six concentrations of 0.1, 0.2, 0.5, 1, 2, 3 mg_{Fe}/mL by adding distilled water as solvent. Each experimental circle included a 600 s heating and a 600 s cooling stage. Thermal efficiency of ferrofluids is quantified by estimating the Specific Absorption Rate (SAR), which refers to the amount of energy converted into heat per time and mass of the magnetic material. SAR was calculated by using the following equation [13]:

$$SAR = c\frac{m_f}{m_{Fe}}\frac{\Delta T}{\Delta t} \quad (1)$$

where c the specific heat of the solution, m_f and m_{Fe} the total solution mass and iron content respectively, $\Delta T/\Delta t$ the initial slope of hyperthermia curve (Temperature vs time). Generally, nanoparticles as heating mediators should have the highest possible SAR values resulting to the smallest amount of MNPs to be targeted to the tumor. Since heat mediation may occur also via non-magnetic pathways, in order to exclude thermal exchange with the environment and isolate the magnetic-origin heating contribution, we have incorporated in SAR calculation a set of adiabatic corrections (by subtracting the solvent signal prior to fitting the cooling curve to provide the law of cooling), as described in detail in our previous works [14, 15]. The energy dissipation rate (as shown in Eq. 1) is a sensitive function of particle and solution features together with the amplitude (H) and frequency (f) of the alternating magnetic field.

Due to the lack of standards for the alternating magnetic field amplitude and frequency, each research group characterizes their particles under different field conditions leading to a wide spectrum of SAR values due to the different experimental setups. To address this issue, we follow what Kallumadil et al. [10] introduced, i.e. the concept of intrinsic loss power (ILP), (see Eq. 2) where SAR is normalized against the frequency and magnetic field strength facilitating direct comparison of the heating potential of MNPs studied in this work with systems measured in different laboratories.

$$ILP = \frac{SAR}{H^2 f} \quad (2)$$

Despite the fact, ILP consideration has some validity constraints based on linear response theory, [16] yet is safe for frequencies up to few MHz and for applied field amplitudes lower than the ferrofluid saturation field (i.e. our case). Thus, it provides a quantifiable measure of heating efficacy irrespective of the experimental infrastructure and comparative evaluations between independent research groups may be conducted.

3. Results and discussion

Fig. 1a shows a representative XRD pattern for sample FluidMAG-DX-100 nm, when the iron oxide spinel structure (magnetite and/or maghemite since their distinction may not be performed with XRD/TEM) is confirmed. By applying Scherrer equation the crystallite size was calculated at 8.4 nm. This is the first hint, that commercial particles are practically, forming aggregates composed of nanosized superparamagnetic particles, as already discussed in our previous work on a Chemicell product [17]. Fig. 1b showing a low magnification TEM observation reveals the morphology of commercial MNPs. As one can see, the primary iron oxide MNPs have almost spherical shape with a mean particle size of ~10 nm, forming aggregates of variable size averaging at ~100 nm. Commonly, during TEM specimen preparation, specifically during drying stage, artificial agglomerates may form. The clusters in the samples under discussion pre-exist even in liquid form in accordance with the product datasheets (see Table 1) and coded under the name “multi domain cores” as a result of equilibrating magnetic, gravitational and electrostatic forces. Fig. 1c is the

Table 1
Characteristics of commercial ferrofluids as provided by the companies′ datasheets.

Product name	Mean hydrodynamic particle size (nm)	Core type	Shape	Coating	Magnetization/ Characteristics	Use
Nanomag®-D-spio	50–110	Iron oxide	Cluster-typed	Dextran iron oxide composites	H_c=0.77 kA/m M_s > 69 emu/g	*Detection purposes in MRI, magneto-immuno assays*
FluidMAG-DX	50, 100, 200	Maghemite	multi-domain core	Dextran matrix	Super-paramagnetic	*Cell separation, MRI-diagnostics, Magnetic drug-targeting*

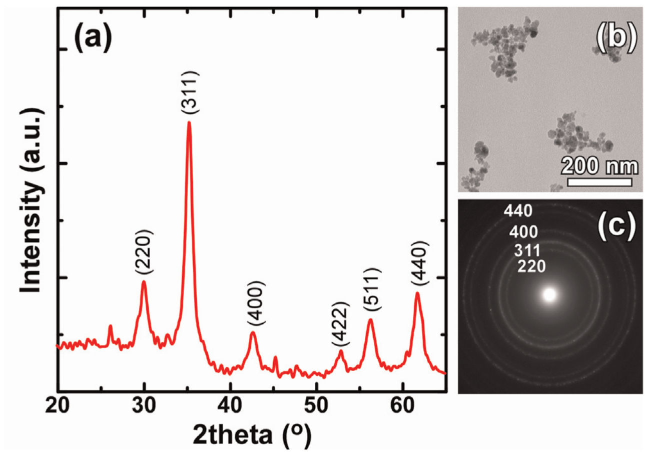

Fig. 1. Structural characterization schemes: XRD pattern (a) and TEM image (b) along with the EDP (c) for sample FluidMAG-DX-100 nm.

corresponding Electon Diffraction Pattern (EDP) of the Fig. 1b confirming the iron-oxide spinel structure of MNPs in good agreement with the XRD pattern.

Hyperthermia measurements were carried out under standard frequency, varying field amplitude and ferrofluid concentration. An indicative set of initial heating/cooling curves is presented in Fig. 2, for sample FluidMAG-DX-100 nm, showing both the heating and cooling stages and reference signal recorded for solution solvent. The potential of this system as hyperthermia heating agent is straightforward, since its heating response, not only reaches the desired hyperthermia region (shaded band denoting the 41–45 °C temperature region that facilitates successful heat penetration of cancer cells) but also surpasses it in quite short time (from 20 to ~200 s, depending on the concentration). SAR values varied from 771 to 1044 W/g for 1 and 0.1 mg_{Fe}/mL respectively and compare relatively well with obtained values in iron-oxide MNPs prepared in-lab [9].

It is well known that thermal response of MNPs solution depends on the properties of MNPs, such as their magnetic features and particle size, as far as on hyperthermia applicable parameters (field amplitude and frequency) [18]. Hence depending on the magnetic profile of MNPs, whether are superparamagnetic or ferro (i)magnetic, the hyperthermia results, as expressed by the temperature rise, may be attributed to the relaxation mechanism (namely Néel or Brown reversal) or hysteresis losses. Brownian relaxation refers to the rotation of nanoparticle inside the medium (water in our case) when exposed to an external applied field and its contribution to the total amount of resulted heat depends on the ability to perform this movement. The measurements protocol followed in present work, is the widely accepted protocol for the hyperthermia measurements, starting with the measurements of the solution as described in detail in our previous publications [14,15,18]. Currently, we are examining selected high-performance commercial samples *in vitro* prior to their *in vivo* evaluation, in an effort to examine the decrease of SAR efficiency when MNPs are immobilized within cells, due to Brownian contribution attenuation [19]. This project is still under study and will be a future manuscript continuing the scope of the current manuscript which is to propose the alternate use of commercially available ferrofluids as hyperthermia agents.

Since one of the major issues in clinical application is to use the minimum dose needed each time without sparing the heating outcome, it is always useful to optimize the thermal performance against solution concentration. Generally, in all samples, the larger the concentration the larger the increase of temperature, achieved in shorter time.

As discussed previously, to provide a more universal approach, ILP values were estimated in all cases and maximum values are depicted in Fig. 3. The sample with optimum thermal response seems to be FluidMAG-DX-100 nm. The reference horizontal line corresponds to the work of Kallumadil et al. [10] for the sample FluidMAG-D with 100 nm hydrodynamic diameter and ILP = 2.01 nHm^2/kg, under 900 kHz and 5.66 kA/m field amplitude. It is well known that coating of MNPs may significantly affect their arrangements, magnetic features and eventually magnetic heating efficiency [9]. The reference sample has starch as a matrix while in our case dextran is incorporated as a biocompatible coating. Dextran, is a typical polysaccharide used for coating of iron oxide MNPs, providing biocompatibility, and is an established coating for clinical applied contrast agents. Furthermore, dextran coating, in the sense of thickness of its surrounding layer, affects magnetic characteristics (saturation magnetization or magnetic interactions) and hence the SAR values of MNPs [20]. ILP values seem to be more sensitive to MNPs size with the 50 nm yielding relatively high values. On the other hand sample of 200 nm seem to possess smaller heating efficiency while the Nanomag®-D-spio sample with a much wider size distribution (denoted also from the company) seem to have the milder heating effect. This may be correlated with the magnetic features i.e. higher magnetization values as MNPs grow in size.

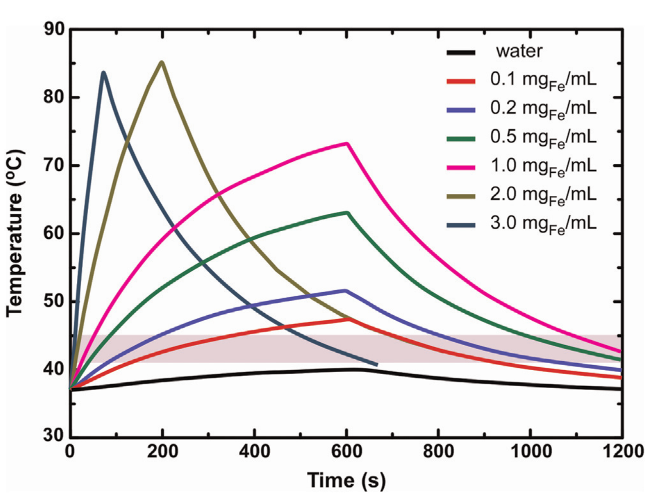

Fig. 2. Time dependent temperature curves for sample FluidMAG-DX-100 nm, for various concentrations (0.1–3 mg_{Fe}/mL), under 24 k A/m field amplitude. Shaded band denotes the desired hyperthermia levels.

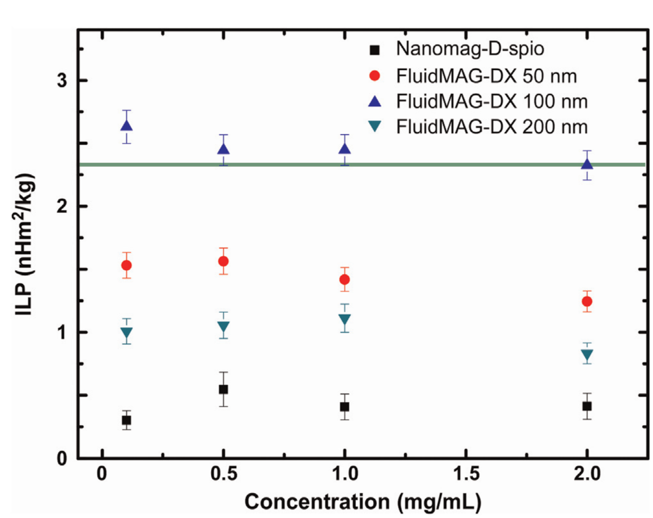

Fig. 3. ILP values vs. concentration, under 20 kA/m field amplitude. Reference line refers to sample FluidMAG-D from Ref. 10.

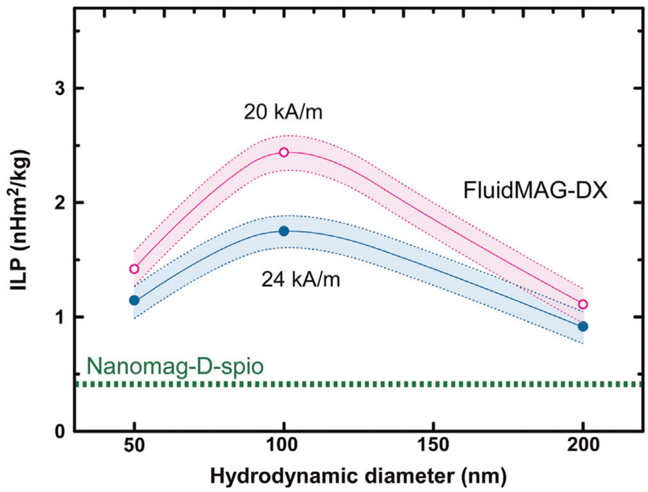

Fig. 4. ILP values (presented as shaded bands including the error bars) for samples FluidMAG-DX-50, 100, 200 nm, measured in concentration 1 mg_{Fe}/mL, under 765 kHz and 20, 24 kA/m. The dotted line corresponds to Nanomag®-D Spio.

On the other hand, the external hyperthermia field should always be in position to manipulate the MNPs coercive field, allowing the transferring of the maximum heating power from the applied filed to the ferrofluid, as recently discussed in a previous manuscript [14]. This seems to be the case for the FluidMAG-DX-100 nm sample. Regarding the concentration effect to thermal response of ferrofluid, from Fig. 3, it seems that for the concentrations examined the ILP values practically remain unaltered. In our case, the heating efficiency is actually driven by the intrinsic MNPs features when solution concentrations are relatively small so as not to allow the dipolar interactions. Meanwhile for much bigger concentrations (> 5 mg/mL) dipolar interactions may lead to MNPs arrangements, thus overrule individual particle contribution and occasionally lead to enhancement of heating efficiency [21].

Since the saturation magnetization, as far as the general magnetic profile affect the heating losses mechanisms, which in turn depends on MNPs core sizes as far as the total hydrodynamic diameter, the ILP variations with the hydrodynamic diameter are presented in Fig. 4 for samples of 1 mg_{Fe}/mL. The regional zone of ILP values is approximated from the two sets of measurements at specific field amplitudes of 20, 24 kA/m for the samples under study where the width of the zone corresponds to the 10% error bar in estimated SAR values. Again analogous reference region for Nanomag®-D-spio is given for comparison. It appears, that the ILP region of Chemicell extends in a region between 1 and 2.5 nHm^2/kg becoming pronounced around the hydrodynamic diameter of 100 nm.

It seems that the arrangement of MNPs in uniform size aggregates appear to provide an additional degree of freedom to fine tune the ILP values as exhibited by the FluidMAG-DX series. In all sample cases the hydrodynamic diameters correspond actually not to individual MNPs but to aggregates, where the dipolar interactions in conjunction with the chemical environment govern the size and its uniformity of these aggregates leading eventually to tunable magnetic heating features as directly seen in Fig. 4. These findings coincide with our recent work for another commercial product from Chemicell [17]. Although, further characterization schemes in more samples from different companies, should be performed in order to manipulate aggregate formation in a beneficiary way, the observed tunable heating efficiency certifies that commercial ferrofluids may also play a role as promising candidates in multifunctional modern theranostics.

4. Conclusions

This work deals with the potential exploitation of a series of commercial iron-oxide ferrofluids, as magnetic hyperthermia agents. Our results show an enhanced heating efficiency that can be tuned by field amplitude and ferrofluid parameters. Thus, if properly tuned, commercial ferrofluids may be also play a role as hyperthermia mediators. More specifically, SAR seems to be aggregate-size dependent and the ferrofluid with the optimum SAR value was the FluidMAG-DX-100 nm, while the concentrations under study did not drastically affect the heating efficiency. Beyond the relative high thermal efficiency of the above possible nano-agents, MNPs should also fulfill other criteria such as biocompatibility and colloidal stability, usually driven mainly by the coating material. The choice of the coating material determines the final hydrodynamic diameter along with the magnetic interaction, and also significantly seems to be a key parameter for the optimum thermal response. In the quest of modern theranostics materials, a novel pathway is opened by exploring the wide range of accessible features in commercial ferrofluids.

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