

Heating Tumors to Death using Functionalized Fe_3O_4 Magnetic Nanoparticles

R. S. Ningthoujam, R. K. Vatsa

Chemistry Division

and

Amit Kumar, Badri N. Pandey

Radiation Biology & Health Sciences Division

Abstract

Application of Magnetic Nanoparticles (MN) has emerged as a potential mode of hyperthermia, a modality for cancer therapy, in which temperature of a tumor is increased beyond physiological temperature up to 40-43 °C. We have prepared Fe_3O_4 magnetic nanoparticles (Fe_3O_4 -MN), which were surface-functionalized with polyethylene glycol (Fe_3O_4 -PEG-MN) and oleic acid (Fe_3O_4 -OA-MN) to make them water and lipid soluble, respectively; as well as to control their size. The particle sizes for Fe_3O_4 -MN, Fe_3O_4 -OA-MN and Fe_3O_4 -PEG-MN were found to be 12, 6 and 8 nm, respectively. Under induction heating conditions, these MN preparations could achieve hyperthermic temperature. Interestingly, Fe_3O_4 -OA-MN showed higher cytotoxicity in human breast cancer cells as compared to Fe_3O_4 -MN and Fe_3O_4 -PEG-MN, which was further enhanced when MN treated cells were subjected to induction heating.

Introduction

Fe_3O_4 based Magnetic Nanoparticles (MN) have gained increasing attention in the recent past as prospective candidates for diagnosis and targeted therapy of cancer [1,2] due to their biocompatibility and chemical stability under physiological conditions. These MNs provide dual benefits (i) diagnosis of cancer sites due to their potential as contrasting agent using magnetic resonance imaging, and (ii) therapeutic applications because of their heating ability (40-43 °C; hyperthermia) by induction heating for killing the cancer cells. Hence, the MNs could prove as unique weapons to trace out hidden cancer cells in patients, and once the target is located, kill them effectively (Fig. 1). Moreover, MN-based heating systems could also be employed for controlled release of drug from caged drug delivery systems, designed to release their content at defined temperatures, which could be further exploited to combine advantages of nanoparticles based drug delivery and hyperthermia modality of cancer therapy.

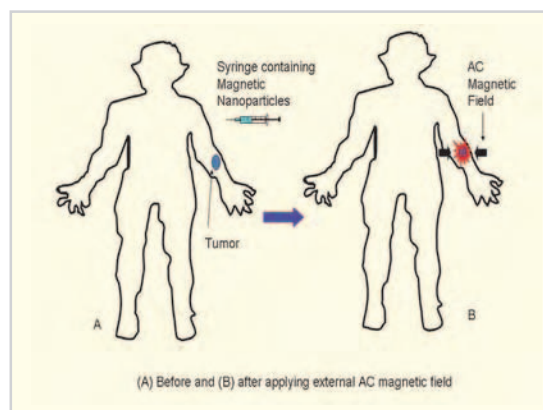


Fig. 1: Schematic outline of application of Magnetic Nanoparticles in Cancer Therapy

Application of hyperthermia to tumor tissue is done in two ways: (i) applying external source of heat (e.g. water bath, microwave, ultrasound, infrared sauna) which is also called 'external or extracellular hyperthermia' and (ii) delivering MN inside the cancer cells followed by application of alternating current (AC) field, which would increase the temperature of tumor mass. The latter approach is known as 'intracellular hyperthermia' (Fig. 2). Cell

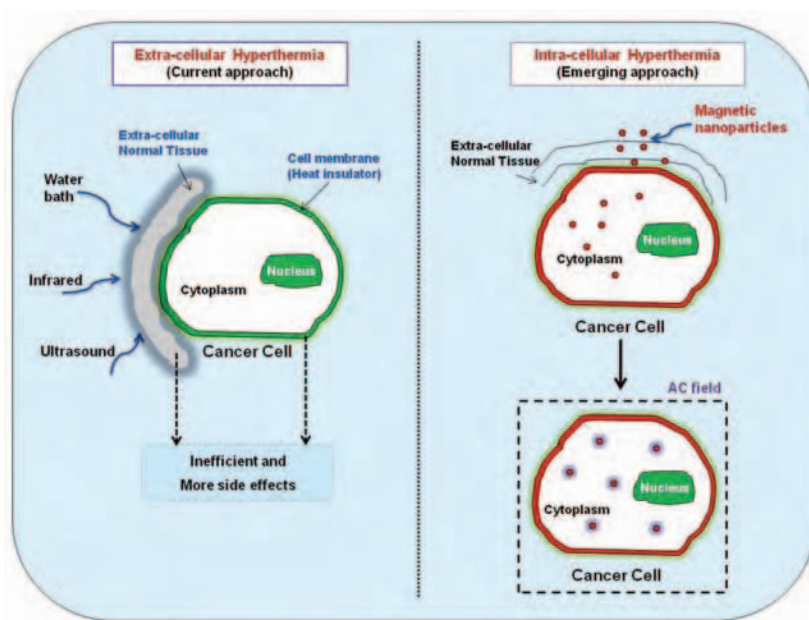


Fig. 2: Extracellular vs intracellular hyperthermia

membranes, with lipid as one of the major constituents, thermally insulate the tumor cells heated from external sources. Consequently, extra heat from external source has to be provided to achieve the therapeutic temperature, which may cause blisters, burns, swelling, blood clots, bleeding under clinical conditions. Therefore, application of hyperthermia using extracellular approach has faced practical problems. On the other hand, intracellular heating using internalized MN at the tumor site provides an efficient approach for hyperthermia application (Fig. 2).

In the present study, we have used Fe_3O_4 based MN, which have Fe^{2+} and Fe^{3+} ions (written as $\text{Fe}^{\text{II}}\text{O}$, $\text{Fe}_2^{\text{III}}\text{O}_3$) and are generally called magnetite. Fe_3O_4 has Curie temperature (T_c) of 585 °C, saturation magnetization (M_s) of 92 emu/g at room temperature and coercivity (H_c) of 323 Oe [1,2]. When size of Fe_3O_4 bulk material is decreased (in the range of nanometers), its intrinsic magnetic property changes. Below a certain dimension, each particle behaves as a magnetic single-domain. Its magnetic anisotropy energy is proportional to the particle volume (KV , K anisotropic constant and V particle volume). For small particles, the surrounding thermal energy (kT) is sufficient to overcome magnetic anisotropy energy (KV) resulting in

spontaneous reversion of magnetization of a particle from one easy direction to other. MN with particle size (~ 5 nm) can have large magnetic moment ($5 \times 10^3 \mu_{\text{BM}}$) and thus 'super' is prefixed to paramagnetic term because overall system shows the paramagnetic nature (i.e. net magnetization ~ 0 emu/g, coercivity ~ 0 Oe) in absence of magnetic field [1-3]. This behavior is called superparamagnetic. Such superparamagnetic particles can be used in diagnosis and targeted therapy of cancer [4,5]. Magnetic nanoparticles suspended in liquid medium, called ferro-fluids, when placed under external AC magnetic field, try to orient the magnetic moment of nanoparticles towards the direction of magnetic field and would result in heat generation involving mechanism(s) like (i) Neel's relaxation; (ii) Brownian rotational loss (iii) hysteresis loss and (iv) eddy current [6].

The major advantages with application of MN smaller than 50 nm are (a) their high effective surface area for easy attachment of ligands, (b) better tissue diffusion and (c) reduced magnetic dipole-dipole interaction. As a result, no magnetization is retained in these particles after removal of magnetic field i.e. their superparamagnetic nature [6]. In-vivo administration of MN requires their controlled size and reduced

agglomeration for targeted delivery to the diseased site. However, MN prepared by chemical route usually agglomerate and are larger in size posing serious practical limitations. Capping the surface of nanoparticles would change their intrinsic physico-chemical properties like surface functionality, charge, reactivity and dimension making them more stable and suitable for *in-vivo* applications. Surfactants like oleic acid (OA) would result in oil dispensible nanoparticles, whereas hydrophilic MN could be prepared by capping agent like polyethylene glycol (PEG). The variation in lipophilicity would affect the cellular interaction of MN, their cellular uptake and hence, the net outcome of their therapeutic efficacy.

In the present work, we employed Fe_3O_4 nanoparticles (Fe_3O_4 -MN) capped either with water soluble PEG (Fe_3O_4 -PEG-MN) or oil soluble OA (Fe_3O_4 -OA-MN). The heating behavior of MN under AC induction and their efficacy to kill human breast cancer cells was investigated.

Experimental Section

Fe_3O_4 MN were prepared by co-precipitation method from Fe^{2+} and Fe^{3+} ions in NH_4OH solution. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ precursors were used for Fe^{2+} and Fe^{3+} ions, respectively. The surface of particles was functionalized with polyethylene glycol (PEG-6000) and oleic acid (OA). These MN were characterized using X-ray diffraction, FT-IR, VSM and Mössbauer techniques [7].

Heating of MN was performed in plastic micro-centrifuge tube (1.5 ml) using induction heater (Easy Heat 8310, Ambrell, UK, frequency 265 kHz) with 6 cm diameter (4 turns) coil as shown in Fig. 3. MN suspended in 1 ml of distilled water were placed at the centre of coil and samples were heated for 10 min at a current of 100-600 A. Magnetic field generated at the centre of the coil was calculated from the relationship: $H = 1.257ni/L$ (in Oe) where,



Fig. 3: Induction heating system

n , i and L denote the number of turns, applied current and the diameter of the turn in centimeters, respectively. The magnetic field could be varied from ~ 80 to 500 Oe by changing the current from 100 to 600 A.

Human breast cancer cell line (MCF7) was obtained from National Centre for Cell Sciences, Pune, India. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS) and antibiotics in humidified atmosphere of 5% CO_2 at 37 °C. The desired number of cells was seeded in complete medium for overnight followed by treatments with different types of MN for morphological studies using confocal microscopy.

Results and Discussion

XRD patterns of Fe_3O_4 -MN, Fe_3O_4 -OA-MN and Fe_3O_4 -PEG-MN showed that these MN were crystalline in cubic structure with lattice parameter $a = 8.39(1) \text{ \AA}$ [7]. Increased broadening of diffraction peaks was observed after capping with OA or PEG due to reduction of particle size. Average crystallite sizes of Fe_3O_4 -MN, Fe_3O_4 -OA-MN and Fe_3O_4 -PEG-MN were found to be 12, 6 and 8 nm, respectively. Chemical bonding between surfaces of particles with capping agent is supported by FT-IR study [7].

Microstructures of these particles were studied by Transmission Electron Microscopy. Fe_3O_4 -MN shows agglomerated particles with individual size of 20 nm (Fig. 4a). The selected area electron diffraction (SAED) of Fe_3O_4 -MN shows the rings, which correspond to the fcc structure (Fig. 4b).

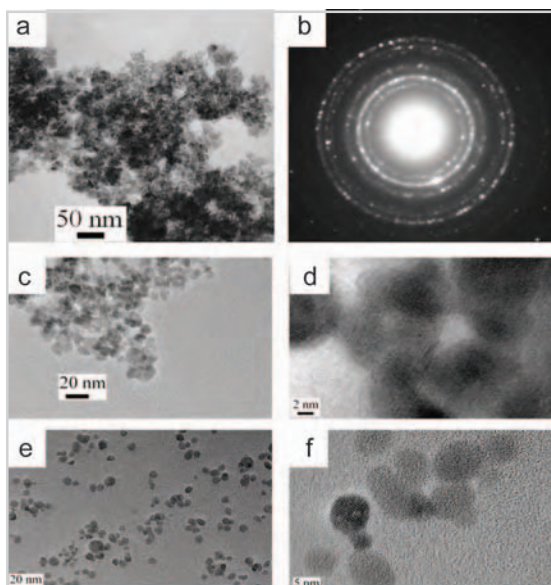


Fig. 4: (a) TEM and (b)SAED images of Fe_3O_4 -MN. (c) TEM and (d) HRTEM images of Fe_3O_4 -PEG-MN. (e) TEM and (f) HRTEM images of Fe_3O_4 -OA-MN.

Agglomeration of particles was reduced after capping with PEG (Fig. 4c, d) and OA (Fig. 4e, f). In order to understand magnetic behavior of these particles, magnetization was recorded up to $\pm 1.0 \times 10^4$ Oe (Fig. 5). Unsaturated magnetization is found for all these samples with very small coercivity (~ 3 Oe) indicating superparamagnetic nature of these particles. Magnetization values at 1.0×10^4 Oe were found to be 59.7, 34.39 and 37.37 emu/g for Fe_3O_4 -MN, Fe_3O_4 -OA-MN and Fe_3O_4 -PEG-MN, respectively. Reduction in magnetization after coating with PEG or OA is related to reduced agglomeration of particles. With reduction of particle size and agglomeration, spin relaxation of domain increases and consequently, magnetization decreases. Mössbauer study supports the increase of superparamagnetic fraction with capping of PEG or OA [7].

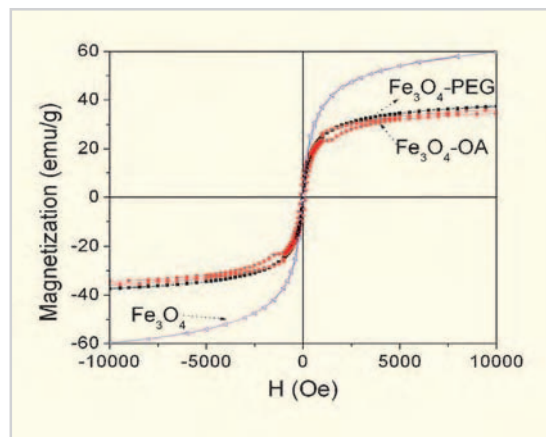


Fig. 5: M vs. H curves for Fe_3O_4 -MN, Fe_3O_4 -OA-MN and Fe_3O_4 -PEG-MN. Here, M is expressed in term of emu per g of Fe_3O_4 content using VSM measurement.

Further, we have studied the heating ability of these MN under AC magnetic field. The MN suspended in distilled water were placed in induction heating coil (see Fig. 3). Fig. 6 shows the rise in temperature with increasing current (100-600 A) applied for 10 min to 10 mg of each sample. To achieve hyperthermic temperature, the required current is 250 A for Fe_3O_4 -MN and 550 A for Fe_3O_4 -OA-MN and Fe_3O_4 -PEG-MN. Also, the results show that the hyperthermia temperature (42°C) could be obtained using 2 mg for Fe_3O_4 -MN and 5 mg for

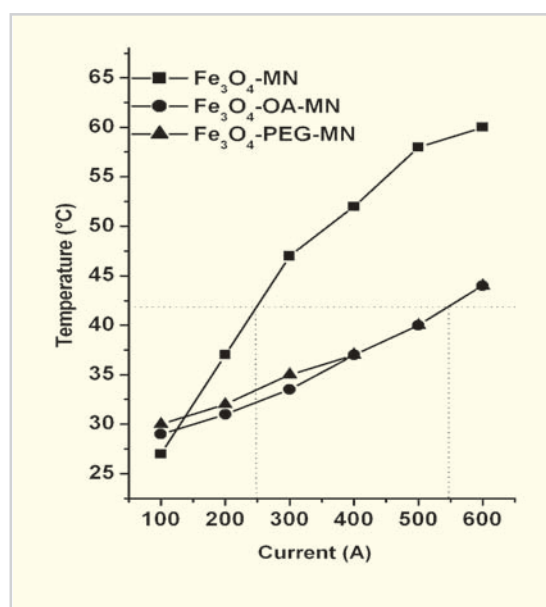


Fig. 6: Heating abilities at increasing current for different MN samples at a particular concentration (10 mg)

Fe_3O_4 -OA-MN or Fe_3O_4 -PEG-MN at 500-600 A. The specific absorption rate (SAR) of these samples were calculated at 400 A (335.2 Oe or 26.6 kA/m). The SAR values for Fe_3O_4 -MN, Fe_3O_4 -PEG-MN and Fe_3O_4 -OA-MN were 38.4, 28.3 and 33.5 W/g, respectively [7].

Morphological alteration was evaluated in MCF7 cells treated with different types of MN with or without induction heating (Fig. 7a-h) to further evaluate their efficacy. Compared to control cells (Fig. 7a,b) and cells treated with Fe_3O_4 -MN and

Fe_3O_4 -PEG-MN (Fig. 7c,e), rounded off and less clustered cells were observed in Fe_3O_4 -OA-MN treated cells (Fig. 7e). Cells treated with Fe_3O_4 -MN under induction heating did not show significant effect on morphology (Fig. 7d), however, effect was more pronounced in Fe_3O_4 -PEG-MN treated cells under same condition (Fig. 7f). It was interesting to observe that the cells showed more rounding off and cell density was remarkably reduced when treated with Fe_3O_4 -OA-MN followed by induction heating (Fig. 7h) suggesting better efficacy of Fe_3O_4 -OA-MN to induce toxicity in cancer cells under induction heating conditions.

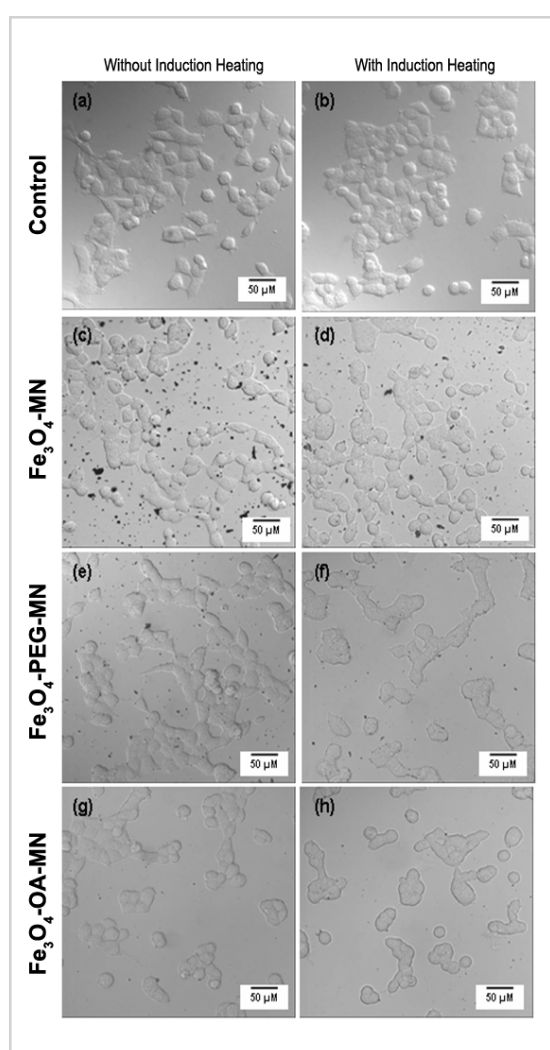


Fig. 7: Effect of different MN treatment on morphological alterations in MCF7 cells with or without induction heating. Untreated control (a-b) and cells treated with Fe_3O_4 -MN (c-d) Fe_3O_4 -PEG-MN (e-f) and Fe_3O_4 -OA-MN (g-h) were observed under bright field mode of confocal microscope.

Conclusions

Reduced agglomeration of Fe_3O_4 magnetic nanoparticles (MN) was found when capped with PEG or OA which results in an increase of the ratio of superparamagnetic to ferromagnetic fraction. Hyperthermic temperature can be achieved at reasonably low concentration (2-5 mg) of MN suggesting their suitability for hyperthermia applications. The alteration in morphology of human breast cancer cells was more effective when MN were capped with OA followed by induction heating. Further experiments are underway to validate these results under *in vivo* animal experiment models.

Acknowledgement

Authors would like to thank Dr. D. Das (Head, Chemistry Division) and Dr. M. Seshadri (Head, Radiation Biology and Health Sciences Division) for their encouragement during this collaborative research work. Authors also acknowledge the help of Shri Manjoo Ali and Smt. R. Vasumathy (confocal microscopy) and Y. Priyabala, Runa Ghosh and Lina Pradhan for conducting some of the experiments for this work.

References

1. B. D. Cullity, "Introduction to Magnetic Materials". London: Addison-Wesley Publishing Company, 1972.

2. G. F. Goya, T. S. Berquo, F. C. Fonseca and M. P. Morales, "Static and dynamic magnetic properties of spherical magnetite nanoparticles". *J. Appl. Phys.* 94 (2003) 3520.
3. R. S. Ningthoujam and N. S. Gajbhiye, "Magnetization studies on e- $\text{Fe}_{2.4}\text{Co}_{0.6}\text{N}$ nanoparticles". *Mater. Res. Bull.* 45 (2010) 499.
4. A. Ito, M. Shinkai, H. Honda and T. Kobayashi, "Medical application of functionalized magnetic nanoparticles". *J. Biosci. Bioeng.* 100 (2005) 1.
5. S. Naqvi, M. Samim, A. K. Dinda, Z. Iqbal, S. Telagoanker, G. J. Ahmed and A. Maitra, "Impact of magnetic nanoparticles in biomedical applications". *Recent Pat. Drug. Deliv. Formul.* 3 (2009) 153.
6. R.S. Ningthoujam, R.K. Vatsa, Amit Kumar and B.N. Pandey. Functionalized Magnetic Nanoparticles: Concepts, Synthesis and Application in Cancer Hyperthermia. In Functionalized Materials. 2012, Editors: S. Banerjee and A. K. Tyagi, *Elsevier Publications* (pages 229-260).
7. R. Ghosh, L. Pradhan, Y. P. Devi, S. S. Meena, R. Tewari, A. Kumar, S. Sharma, N. S. Gajbhiye, R. K. Vatsa, B. N. Pandey and R. S. Ningthoujam, "Induction heating studies of Fe_3O_4 magnetic nanoparticles capped with oleic acid and polyethylene glycol for hyperthermia". *J. Mater. Chem.* 21 (2011) 13388.