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Biomaterials and magnetism

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Abstract. Magnetism plays an important role in different applications of health care. Magnetite (Fe_3O_4) is biocompatible and therefore is one of the most extensively used biomaterials for different applications ranging from cell separation and drug delivery to hyperthermia. Other than this, a large number of magnetic materials in bulk as well as in the form of nano particles have been exploited for a variety of medical applications. In this review, we summarize the salient features of clinical applications, where magnetic biomaterials are used. Magnetic intracellular hyperthermia for cancer therapy is discussed in detail.

Keywords. Magnetism in health care; magnetic biomaterials; magnetic intracellular hyperthermia.

1. Introduction

Magnetism, which is an intrinsic property of every atom, has a profound influence on living organisms. The haemoglobin in our blood is an iron complex and is magnetic in nature. Magnetotactic bacteria are perhaps the first living organisms to orient themselves with the earth's magnetic field[1]. These bacteria are known to contain aligned chains of magnetite particles of various shapes. There is now substantial evidence that all living organisms, including animals and humans, contain magnetic particles and act as magnetic receptors.[2] It is established that the magnetism and magnetic materials have a strong role to play in health care and biological applications[3–8]. Some of the early medical applications of magnetic materials were, for example, the removal of metallic objects from the body of animals and humans.[9] The use of materials in biological environment for implantation or for replacement of a part or a function of the body in a reliable and physiologically acceptable manner was a challenge for the last several decades. Of late the combination of fine particles and magnetism in the field of biology and biomaterials has been found useful in sophisticated bio-medical applications such as cell separation[10–14], drug delivery[15] and magnetic intracellular hyperthermia treatment of cancer[16–18]. Similarly, the development of magnetically responsive micro-spheres can be used *in vitro* to direct the particles so that they remove bound cells and molecules and in-vivo to target and hold the magnetic carriers at specific sites for applications in protein and cell separation. Further the purification of bone

*References in this paper have not been cited in journal format.

marrow cells from tumor cells using immuno-magnetic beads is also an established method in clinical therapy. The development of nano technology along with involvement of magnetism, opened new windows of sophisticated biomedical applications such as diagnostic, therapy etc.[19]

In this article, we review some of the most recent advances in areas, where magnetic materials particularly when of nano size, are used in biological applications. Some of these are:

- Magnetic bioseparation
- Drug delivery
- MRI contrast agent
- Therapy – Hyperthermia treatment of cancer

The section on hyperthermia would include magnetic fluid hyperthermia (MFH) or intracellurar hyperthermia. Though the field of magnetic hyperthermia had been known for several decades, it came into prominence during the last ten years or so. It appears that this mode of treatment perhaps combined with chemotherapy/radiotherapy could prove one of the most suitable for cancer therapy. A more detailed discussion on magnetic hyperthermia for cancer therapy is included in this review.

2. Role of magnetism in biomaterials

Magnetism has a strong role to play in some specific bioapplications. For example, in sorting of cells, interactions between biological cells and magnetic nano particles occur which lead to separation under the action of magnetic field gradient. The characteristics of hard and soft magnetic materials as well as the particle size dependent properties have been exploited for different bio applications. Magnetic properties change dramatically when particle size reduces beyond a critical limit and goes to single domain and sub-domain regions. Below a critical size, it shows superparamagnetic (SP) properties. This is exploited extensively for magnetic bioseparation, MRI contrast agent and drug delivery. For bioapplications (e.g MRI contrast agent, bioseparation etc.), superparamagnetic particles are found superior to ferro/ferri magnetic particles due to absence of remanance. Since a magnetic mateiral exhibits magnetic properties only in the presence of a magnetic field, in bioseparation, it can be removed from suspension by applying a magnetic field. After separation it is easy to redisperse it in a homogeneous mixture in the absence of a magnetic field[20].

In magnetic hyperthermia, the ferro, ferri, as well as superparamagnetic properties of particles are useful. The losses due to magnetization and reorientation of these particles depend upon the type of demagnetization process, which is determined by intrinsic properties such as magnetocrystalline anisotropy and extrinsic properties such as particle size and microstructure. Magnetic hysteresis is a useful attribute of the material. Hysteresis loss represents the energy consumed in cycling a material between positive and negative fields. The area inside the second quadrant of the loop determines the energy consumed in one cycle. The hysteretic power loss of an AC device can be obtained by frequency multiplied by hysteretic loss per cycle. The power loss can be dissipated in the form of heat for hyperthermia applications. When there is a reduction in size this magnetic nano particle behave superparamagnetically. These SP particles would not exhibit hysteresis losses. But Neel relaxation in them is equally useful in generating and dissipating heat. More discussion on these aspects follows in §4.4b.

3. Biocompatibility studies

For any material to be applied for biotechnological usage, it should pass through a strict regimen of various *in vitro* and *in vivo* tests, which qualify the material as "compatible" with its "living" neighbourhood. In other words, when kept in cell culture environment (*in vitro*) or kept in contact with living carriers (tNo.) (*in vivo*), it should not lead to detrimental reactions which change the intrinsic properties (cell growth rate, cell morphology, accumulation of unwanted proteins, overexpression of housekeeping and other genes, denaturation of structural and functional proteins etc.) of the nearby and distant environment over a period of time. These assessments are done with the help of various techniques some of which are listed below.

3.1 *In vitro*

Cell count and cell viability study is done by counting cells over a cell counter (Neubar's Chamber) after diluting (1:1) with trypan blue dye. Dead cells immediately take up the dye while living cells start to take up after some time period within which number of viable cells can be counted.[21] Cell viability can also be assessed by MTT assay in which living cells are differentiated against the dead cells by their capability of metabolizing the chromogen to give blue crystals.[22]

Cell morphology can be assessed by observation under high magnification by light microscope and further by electron microscope. The latter can give the idea of surface adherence of cells on the material if the sample is biocompatible (figure 1).[18]

Polyacrylamide gel electrophoresis (followed by western blotting, if needed) and fast performance liquid chromatography of the supernatant can give an idea of the changes (both in amount and nature) in the secreted protein profiles.[18,21] Agarose gel electrophoresis (followed by southern/northern blotting, if needed) can show the changes at the nucleic acid level.

3.2 *In vivo*

Visual evidence, of any unwanted tNo. reaction, is the initial "test" done usually. This is followed by taking biopsy of nearby and distal tNo. and performing histopathology (followed by immunohistochemistry, if needed) tests, which qualitatively show the extent/nature of tNo. damage.[21,22]

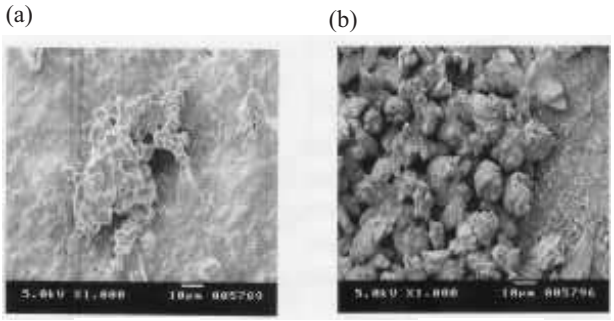

Figure 1. SEM photographs of WBC cells grown on magnetic oxide-based glass surface[18]. **(a)** Not biocompatible, **(b)** biocompatible.

4. Biomedical applications

4.1 *Magnetic bioseparation*

Bioseparation is an important phenomenon for the success of several biological processes. Therefore, prospective bioseparation techniques are increasingly gaining importance. Amongst the different bioseparation techniques, magnetic separation is the most promising.

For a long time magnetic separation has been used for applications other than bioseparations. Typical examples are separation of magnetic colour impurities form kaolin clay, enrichment of low grade iron ore, removal of ferromagnetic impurities form boiler water and so on. The application of these techniques has been restricted and of limited use up to 1970. Later labelling and targeting techniques have become useful for some interesting applications in the areas of bioscience and biotechnology, primarily due to the development of innovative ideas and improved properties of magnetic materials. Presently, the separation technique is regularly used in molecular biology, cell biology, and microbiology. The magnetic separation technique has several advantages with respect to other techniques used for the same purpose.[23]

Magnetic separation of cells and bio molecules is based on the contrast of magnetic susceptibility between separand (magnetic) and medium (containing other nonmagnetic) materials. A few cells or biomolecules have intrinsic magnetic properties. Magnetic bio-separation may be classified into two modes. For the first case, the separand may have sufficient intrinsic magnetic moment (e.g. red blood cells and magnetotactic bacteria) and can be directly separated by applying magnetic fields. Alternately, the cells or biomolecules which are nonmagnetic in nature can be modified by attachment of magnetic responsive entity and thus can be manipulated using an external field.

The separation of cells or compounds may be done by direct and indirect methods. In the direct method, ligands are immobilized on magnetic particles, and incubated with the medium (cells or compounds) for some time. The target cells bind with these ligands and the complex formed can be separated by a magnetic field. On the other hand, in the indirect mode, the target cell initially interacts with the ligand (primary antibody). The secondary antibody is then immobilized on magnetic particles and added to the medium containing the cells. When antibodies with poor affinity or antigen are less accessible, indirect methods might perform better.[24] Magnetic complex is then separated using a magnetic separator. However, after separation the separand may be removed form the immunomagnetic particles for which several techniques are available.[25–26]

The magnetic separation of cells or bio molecules is more effectively done by the superparamagnetic materials, because it exhibits magnetic properties in presence of magnetic field only. In addition, ferromagnetic as well as superparamagnetic particles coated or encapsulated with polymers or liposome can be used for magnetic labelling.[29] For this purpose, magnetite (Fe_3O_4) or haematite (γFe_2O_3) have been extensively used as magnetic carriers.

The isolation of various macro molecules such as enzymes, enzyme inhibitors, DNA, RNA, antibodies and antigens etc. from different sources including nutrient media, fermentation broth, tNo. extracts and body fluids, has been done by using magnetic absorbents. In case of enzyme separation, the appropriate affinity ligands are immobilized on polymer coated magnetic carrier or magnetizable particles.[27–28] Immobilized protein A or protein G on silanized magnetite[29] and fine magnetotactic bacteria[30] can be used for isolation and purification of IgG.[31] Monosized superparamagnetic particles, Dynabeads, have been used in isolation of mRNA, genomic DNA and proteins.[32]

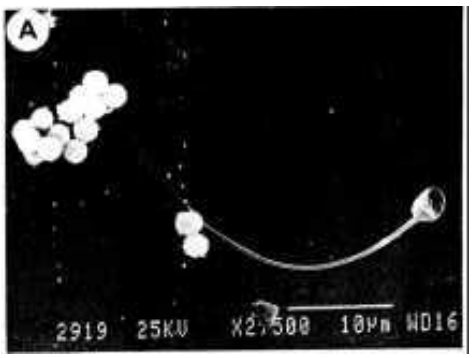

Figure 2. SEM image of binding of sperm cell to magnetic microsphere (from Margel *et al*[39]).

Isolation and separation of cells (prokaryotic, eukaryotic) or antigen have been done by immobilization of specific antibodies against the target (cells or antigen) i.e. by immunomagnetic separation.[33–35] There is abundance of literature available for isolation of different types of cells from blood and the cell sources by this technique.[36–38] In figure 2, we show a typical SEM micrograph demonstrating the binding of immuno-microspheres to the tail of sperm cells (reproduced from ref. 39). Removal of cancer cells from bone marrow is one of the most important applications of magnetic separation. Tumour cell separation from peripheral blood has been performed by immobilization of antibody on silica coated with superparamagnetic iron oxide.[40]

Finely dispersed magnetic absorbent (ferro-carbon particle) has been used for new methods of biological fluid detoxification. A suspension of such absorbent particles is injected into an extracorporeal system. During movement with the bloodstream, it absorbs toxic materials with low, medium and high molecular weights. A high gradient magnetic separator then removes magnetic particles (magnetic particles are then removed by a high gradient magnetic separator) and purified blood is returned to the organism. This is also known as haemosorption and is schematically shown in figure 3. Animal experiments showed the high effectiveness of this technique for the removal of low-molecular weight toxins.[41]

Magnetic bioseparation has been successfully used in the bioscience, biotechnology and biomedical fields, in both the laboratory and on a large scale. Magnetic separation techniques are advantageous compared to other standard techniques. To develop the magnetic separation technique as the best processing method in the near future, an interdisciplinary effort involving a combination of physical, chemical and engineering aspects is essential.

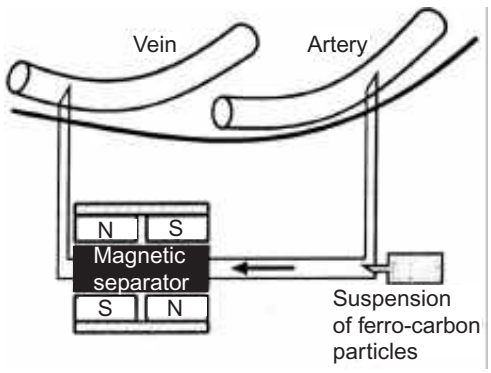

Figure 3. Schematic for magnetic haemosorption (from Michael *et al*[41]).

4.2 *Drug delivery*

4.2a *Magnetic drug targeting:* The activity of most pharmaceuticals or drugs against certain diseases or disease sites suffers from their inability to accumulate selectively in the pathological organ, tNo. or cells. When the drug or pharmaceutical agent is introduced into the body intravenously, it gets distributed throughout the body. To reach the target site, the drug has to cross many biological barriers (organ, cells and its compartments), and hence there is a chance of its getting inactivated. Large quantities or doses have to be administered to get the required therapeutic concentration to a target site. As a result, many negative side effects may be caused by cytotoxic and/or antigenic drugs. Hence, the healthy tNo. gets exposed to higher concentrations of drugs. The situation becomes particularly critical in case of drugs having very low therapeutic indices (e.g. most anticancer drug). However, the above problems may be solved by selectively and quantitatively accumulating the drug to the target site (organ or tNo.). Independent of the site and methods its administration, drug targeting at non-target sites should remain under certain minimum levels to avoid site reactions, whereas it should remain high at the disease site(s).

There are many different approaches to targeted drug delivery, e.g. direct application of drug into the affected zone, use of reactor molecules having high affinity to the affected site and physical targeting.[42] Targeted drug delivery by external physical force (magnetic field) is an innovative new approach, capable of effective drug targeting.

4.2b *Mechanism:* Magnetic drug transport technique is based on the fact that the drug can be either encapsulated in to a magnetic micro-sphere (or nano-sphere) or conjugated on the surface of the micro/nano sphere. When the magnetic carrier is intravenously administered, the accumulation can take place within the area to which the magnetic field is applied and often augmented by magnetic agglomeration.[43] The accumulation of the carrier at the target site allows them to deliver the drug locally. Efficiency of accumulation of magnetic carrier depends on physiological parameters[44] e.g. particle size, surface characteristics, field strength and blood flow rate etc. The magnetic field assists to extravasate the magnetic carrier into the targeted tNo.s. Though little is known about the process of extrusion, magnetic targeted carriers (MTC) are pulled through some kind of pore/ channel opened by the force of the magnet. It has been observed that immobilization of MTC occurs within the tumor area when magnetic field is removed.[45]

Site-directed drug targeting is one way of local or regional antitumor treatment. The efficiency of chemotherapy treatment may be enhanced to a great extent by magnetically assisted delivery of cytotoxic agent to the specific site. There are a large number of magnetic carrier systems which demonstrates increasing drug concentration efficiency at the tumor site.46–47 In case of brain tumours, the therapeutic ineffectiveness of chemotherapy is mainly due to the impervious nature of the blood-brain barrier (BBB), presence of drug resistance and lack of tumour selectivity. Various novel biodegradable magnetic drug carriers are synthesized and their targeting to brain tumour is evaluated *in vitro* and in animal models. New cationic magnetic aminodextran micro spheres (MADM) have been synthesized. Its potentiality for drug targeting to brain tumour is under investigation.[48]

The aim of the specific cell targeting is to enhance the efficiency of drug delivery and at the same time to reduce the toxicity and side effects to normal tNo.s. An immense improvement of existing approaches to diagnosis and treatment of various diseases could be conveyed by targeted delivery of pharmaceuticals. The therapeutic applications of drug targeting are under investigation and some clinical trials are also under way. The numerous results[49–50] show that

the magnetic drug targeting is a promising area in the development of new and cost-effective clinical protocols in the near future. Further "magnetic drug delivery" will undoubtedly rise from its investigative origins, along with nano-biotechnology, to play an important role in improving human health.

4.3 *MRI contrast agents*

Magnetic resonance imaging is considered to be one of the most powerful techniques in diagnostic, clinical medicine and biomedical research. This is an innovative technique that can provide information on the physical and chemical states of tNo.s. The magnetic resonance images are obtained by placing the area of interest within a powerful, highly uniform static magnetic field. Since hydrogen nucleus (single proton) is abundant in the body due to the high water content of the biological tNo.s, the static magnetic field will make most of the protons to align with the field. These protons (nuclear spins) then move out of their alignment by the application of an alternating magnetic filed, which in turn is produced by the radio frequency coil near the specimen (static magnetic field). The resonant frequency of the alternating magnetic field should be in the radio frequency range (15–60 MHz). The nuclei absorb energy from the oscillating magnetic field and undergo transition from the lower energy state to the higher energy state. When the alternating magnetic field is switched off, the nuclei that return to the equilibrium state thereby emitting energy at the same frequency as previously absorbed. Further, this induces a signal in the coil, which is the source of alternating magnetic field. This nuclear magnetization can be transformed to diagnostic images by a series of algorithms. In an MRI image, contrast is due to different signal intensities form each tNo. produced in the presence of RF pulses. This response depends on proton concentration (water content), chemical and molecular structure of the tNo.[51] By varying the number and sequence of the pulsed radio frequency, images based on different tNo. characteristics are possible.[52]

MRI can provide information that differs from other imaging modalities. Its major technological advantage is that it can characterize and discriminate among tNo.s using their physical and biochemical properties. The ability of MRI techniques to get images in multiple planes offers special advantages for radiation or surgical treatment.

Though MRI can provide definite noninvasive diagnoses, the sensitivity or the specificity of such processes can be improved by the addition of contrasting agents. Difference in proton density as well as in the relaxation process of protons in their physiological environment is the source of tNo. contrast. This can be enhanced with the help of contrasting agents. These may be paramagnetic macromolecular compounds, superparamagnetic iron oxide or rare earth metal ion (Gd) complexes.[53] Paramagnetic metal ions reduce the T1 relaxation of water protons and enhance the signal intensity, hence images are brighter. Superparamagnetic iron particles (SPIO) are more effective than monomolecular[54] or macromolecular Gd contrast agents[55] for this purpose.

The most commonly used superparamagnetic material is Fe_3O_4 with different coatings such as dextrans,[56] polymers,[57] and silicone.[58] SPIO causes marked shortening of T2 relaxation and hence reduction of signal intensity (SI) occurs in MR images. So far it has been mainly used as a liver-specific contrast agent for intravenous application. It may be used for detection of metastases in non-enlarged lymph nodes. When contrast agent is interstitially applied, none of it is accumulated in the lesion since metastases do not have an intact phagocytosing system. Thus, a contrast agent induces a signal effect in normal tNo., but not in metastases and therefore contrast is enhanced[59] (figure 4).

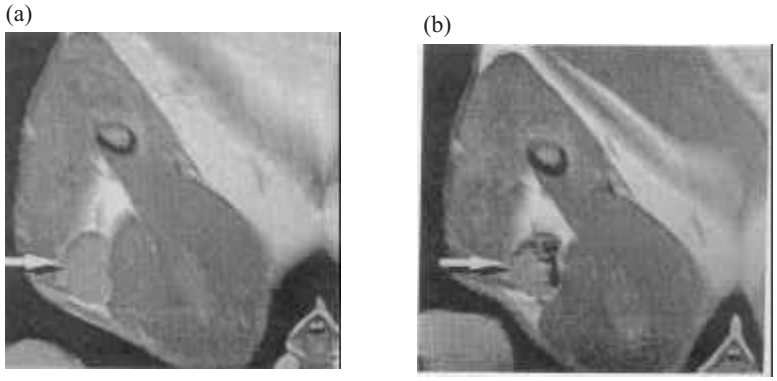

Figure 4. SPIO-enhanced MR lymphograph in a VX2-tumour in a rabbit. The node precontrast image **(a)** shows a homogeneous signal pattern, whereas in the post-contrast image **(b)** large metastasis is visible (from Kresse *et al*[59]).

4.4 *Hyperthermia for treatment of cancer*

Heat treatment of organs or tNo.s, such that the temperature is increased to 42–46°C and the viability of cancerous cells reduces, is known as hyperthermia. It is based on the fact that tumour cells are more sensitive to temperature than normal cells.[60–62] In hyperthermia it is essential to establish a heat delivery system, such that the tumour cells are heated up or inactivated while the surrounding tNo.s (normal) are unaffected. Though different hyperthermia techniques depend upon the heating methods used, each one has certain limitations. Boundary effects limit microwave, ultrasound, and RF hyperthermia. High frequency microwave beams have poor depth penetration and low frequency microwaves are difficult to focus on target areas. Though ultrasound has high penetration and focusing capabilities, applications are limited by strong absorption by bone and high reflection by air filtered cavities (lung etc.). By this technique it is difficult to heat up targets of high perfusion area to the desired temperature due to continuous dissipation of heat. Interstitial technique device is implanted into the tumour, which acts as a heat source by connection with external power sources.[63] In this review, however, we limit our discussion to only magnetic hyperthermia.

4.4a *Magnetic hyperthermia:* Magnetic materials have been extensively used for hyperthermia of biological tNo.s. This is based on the principle that the magnetization process determines the magnetic losses. These losses, depending upon the thermal conductivity and heat capacity of the surrounding medium, can (*in principle*) be dissipated in the form of heat raising the temperature of the surrounding. The losses are of different kinds, which are determined both by the intrinsic and extrinsic properties and the particle sizes. Besides the hysteresis losses, for larger grains, eddy current losses, and relaxation losses for superparamagnetic particles (Neel relaxation) and frictional losses of particles (Brownian movements) have been extensively exploited for hyperthermia. Detailed discussion on these losses and their relevance follows in the next section.

Depending on the approach of investigation (characteristics of magnetic heat sources), the magnetic hyperthermia can be classified as in figure 5.

4.4b *Physics of hyperthermia:* It is important to understand the underlying physics to use hyperthermia as cancer therapy. In an external AC magnetic field, the heating of magnetic oxides with low electrical conductivity is due either to loss processes during the reorientation

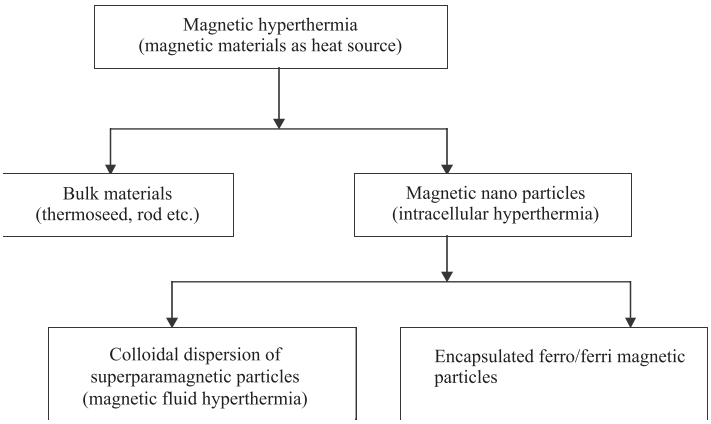

Figure 5. Classification of magnetic hyperthermia.

of the magnetization[64–65] or frictional losses if the particles can rotate in an environment of sufficient low viscosity.[66] Inductive heating of magnetic oxides (i.e. via eddy currents) may be neglected. Magnetic properties of powders may depend significantly on grain size and particles microstructure.

The magnetic reorientation, which is responsible for losses in ferro- or ferrimagnetic particles, depends on the type of demagnetization process. This is determined by intrinsic magnetic properties like magneto crystalline anisotropy and magnetization on one hand and extrinsic properties such as particle size and shape. Size and shape dependence of Hc is well known and it gets maximized when it reaches a critical low size (single domain particle). Similarly, it can be enhanced for accicular particles having large aspect ratios. With decreasing particle size, however, it goes to superparamagnetic region. Due to reduction in particle volume, effective strength of magneto crystalline anisotropy force decreases. This leads magnetization vector to become unstable. If the volume of each particle is V, then the energy barrier ΔE that must be overcome before a particle can reverse its magnetization is KV ergs. On the other hand, fluctuation of thermal energy continuously occurs on a microscopic scale. For very small particles, thermal energy (kT) exceeds the anisotropy forces and spontaneously reverses the magnetization of a particle from one easy direction to the other in the absence of an applied field. Hence, the, magnetization of the assembly will start decreasing. Below the critical size, there is a rapid decrease in remanent magnetization due to relaxation effect, which may be expressed as:

$$M_r = M_i e^{-t/\tau}, \tag{1}$$

where τ is magnetic relaxation time, M_i is remanance of particles not affected by relaxation. This results in vanishing of hysteresis losses near the critical size. The relaxation time for superparamagnetic particles is determined by the ratio of anisotropy energy KV to thermal energy kT and is expressed as

$$\tau = f_o \exp[KV/kT], \tag{2}$$

where f_o (frequency factor) $\sim 10^9\,\mathrm{s}^{-1}$. For superparamagnetic particles specific power loss (due to Neel relaxation) may be expressed as[67]

$$P = (mH\omega\tau)^2/2\tau kTV(1+\omega^2\tau^2), \tag{3}$$

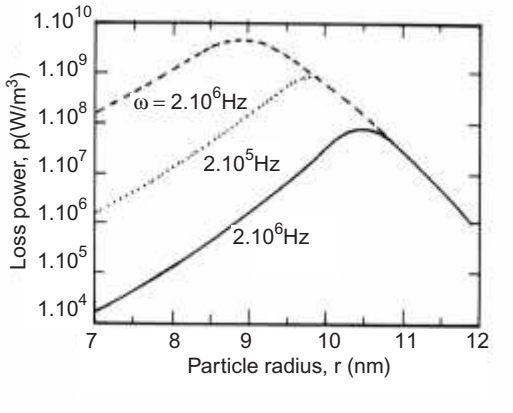

Figure 6. Particle size dependence of power density of losses due to Neel relaxation (from Hergt *et al*[67]).

where m is particle moment and other terms have their usual significance.

The losses according to the above equation increase with the square of the frequency while for $w\tau \gg 1$, the relaxation losses saturate at

$$P = (mH)^2/2kTV\tau. \tag{4}$$

Particle-size dependence of loss power is shown at different frequencies in figure 6. A sharp maxima of loss power at a particular particle size is evident. Figure 7 shows critical particle size region where hysterisis loss vanishes and Neel losses grow as a new loss mechanism.[67]

Magnetic fluid hyperthermia (MFH) utilizes colloidal dispersions of superparamagnetic iron oxide or other magnetic nano particles exhibiting a very high Specific Absorption Rate (SAR) compared to hysteresis losses of larger particles. For magnetic fluid SAR may be defined as kH^2f where k is a materials constant, f is the frequency of operation and H is magnetic field strength. It may be determined by the "rate of temperature rise" methods, using the formula $\mathrm{SAR} = c\mathrm{d}T/\mathrm{d}t$, where c is the specific heat capacity and $\mathrm{d}T/\mathrm{d}t$ is the temperature increase per time[68]. Besides high SAR, these magnetic nano particles should be biocompatible. Magnetite, Fe_3O_4, is known to be biocompatible. However, other magnetic oxides have to be tested for their biocompatibility. Alternatively, they have to be coated with biocompatible materials or entrapped in liposomes before they are injected into a tumour region.

Field homogeneity is of importance, as the SAR of particles may vary. Also, the particle concentration may not be uniform. This may lead to thermal gradients. Furthermore, the power requirement for heat generation in these nanoparticles is much more compared to

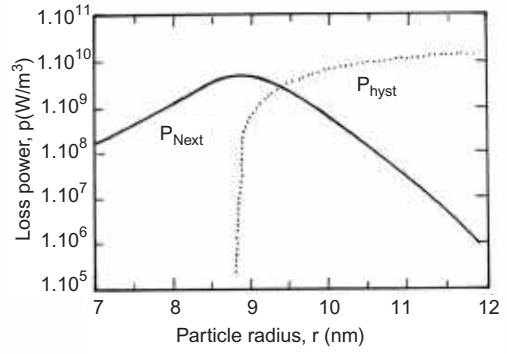

Figure 7. Dependence of magnetic power loss density on particle size for magnetite fine particles. Both Neel losses and hysteresis losses are shown (from Hergt *et al*[67]).

implanted bulk materials. It was found that the nano particles of magnetic oxides absorb much more power than larger magnetic particles exhibiting hysteresis losses. These nano particles also contribute to the power absorption due to Brownian relaxation process besides the Neel relaxation. The core of the particles oscillates within the shell depending upon excitation frequency and core magnetization. Brownian relaxation may also be optimized by viscosity of the fluid and the structure of the shell material.

4.4c *Hyperthermia using bulk magnetic materials:* Magnetic hyperthermia can be induced by two different methods. In the first method, known for several decades, finite size magnetic implants are surgically placed within the tumour site, which absorbs energy from externally applied AC magnetic field and dissipates it in the form of heat to the surrounding tNo.s. These tNo.s can be destroyed, if the temperature rises above 42°C. A large number of bioactive/biocompatible glass and glass ceramics have been exploited for such investigations.[69–73] These are known to form bonds by the formation of an apatite layer on the surface. It is however difficult to get homogenous heat distribution through this method. In such a method it is expected that temperature rise will be observed close to the implanted material and there will be non-uniformity in the temperature distribution in the tumour region. Figure 8 shows a schematic diagram for application of alternating magnetic field to a bone packed with glass ceramics. The temperature of the glass ceramic and the outside of the bone is measured and shown in figure.[74] Three different fluro-optic thermometers have been used for temperature measurements. There is a danger that the temperature may rise more than the requirement and the normal tNo.s may get affected. This can be avoided if the transition temperature (Tc) of these thermo seeds can be tuned between 42–50°C. The tuned curie temperature (Tc) would act as a temperature switch during treatment, and a constant temperature is maintained in the tumour region.[75–77] A major drawback of this procedure (using bulk materials) is that it is an invasive method and required surgical removal after hyperthermia treatment. Therefore, repeated surgery may be required which could be traumatic.

4.4d *Intracellular hyperthermia:* The alternative approach is to use fine particles as heat mediators instead of needles or rods such that hyperthermia becomes noninvasive. When fluids containing submicron-sized magnetic particles (typically 1–100 nm)[78] are injected, these particles are easily incorporated into the cells, since their diameters are in the nanometer range. These magnetic particles selectively heat up tNo.s by coupling AC magnetic field to targeted magnetic nano particles. As a result, the whole tumour can be heated up uniformly.

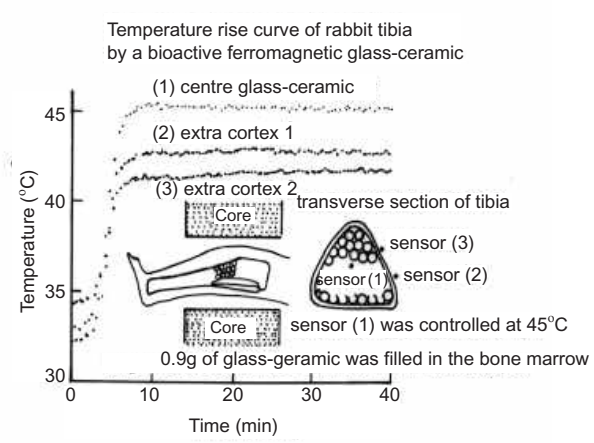

Figure 8. Schematic diagram showing bone heating and corresponding temperature rise (from Ohura *et al*[74]).

This is called intracellular hyperthermia. It has been shown that malignant cells take up nine times more magnetic nano particles than normal cells. Therefore the heat generated in malignant cells is more than in normal cells.[70] Also, as blood supply in the cancerous tNo. is not normal, the heat dissipation is much slower. Hence, the temperature rise in the region of tumour is higher than in the surrounding normal tNo.s. It is therefore expected that this therapy is much more concentrated and localized.

Hyperthermia with small particles started in 1957. Gilchrist *et al*[79] found high concentrations of magnetite particles in lymph nodes near the injection site after it was administered into subserosa of the intestine of dogs. They observed a temperature rise of 4·7°C/min, when lymph node (47 mg ferrite /gm of tNo.) were subjected to AC magnetic field (15–20 kA/m, frequency 1–2 MHz). Best power absorption was reported for particle core size distribution between 20 and 100 nm. Medal *et al*[80] used an AC magnetic field at 120 kHz with a field amplitude of 37 kA/m. The result was not so satisfactory since most of the animals died within 7 minutes of the application of the AC field. Later, in 1965 they published another paper[81] where frequency used was 55 kHz and field strength was increased to 40 kA/m. The report was encouraging as no side effects were observed. Gordon *et al*[82] in 1979 treated mammary tumour-bearing rats, by injecting ferrofluid of dextran magnetite. They used an AC applicator of frequency 450 kHz (38 kA/m field strength). Ferrofluid (100 mg) was slowly injected into the tail vein over 10 minutes. After 48 hours, an AC field was applied for 12 min. They observed a temperature increase of 8°C/min. After one week of therapy, magnetic particles were seen in the liver, spleen, and kidney. In electron microscopic investigation, some intracellular uptake of particles was reported. Prior to 1990, this mode of hyperthermia did not gain importance. This is now popularly known as magnetic fluid hyperthermia (MFH), and is discussed in the following section. Given in chart 1 is the general flow diagram for developing suitable nano magnetic materials for magnetic intracellular hyperthermia.

4.4e *Magnetic fluid hyperthermia (MFH):* Magnetic fluids can be defined as fluids, consisting of ultramicroscopic particles ($\sim$ 100Å) of magnetic oxide.[83] These particles are stabilized by using surfactant to prevent their agglomeration and make stable colloidal suspension in suitable medium (water or hydrocarbon). They behave like true homogeneous fluids and are highly susceptible to magnetic fields.[84] Ferrofluid consisting of superparamagnetic particles of Fe_3O_4 and other magnetic particles, modified/coated with different types of biopolymer or synthetic polymer are used for hyperthermia applications.[83] Since during MFH, the cells may be loaded with magnetic nano particles by virtue of comparable dimension, it is also known as intercellular hyperthermia. Though there had been several *in vitro* and *in vivo* studies, but clinical applications were not thought of. Jordan *et al* (1993) demonstrated that magnetic nano particles (which are superparamagnetic) can be exploited more usefully to absorb power using Brownian and Neel relaxation. In fact, it was observed that these relaxation could generate much more heat compared to conventional ferro/ferri magnetic particles exhibiting hysteresis losses. They have developed ferrofluid consisting of nanoparticls modified by aminosilan which has 10-fold higher uptake by glioblastoma cells than the normal cells.[85] Since then there had been number of reports which brought this therapy close to clinical trials.[85–87] Figure 9 shows sample micrograph showing a fibroblast and prostate carcinoma cell. The malignant cell shows visible pigmentation due to large nano particle uptake.

4.4f *Intracellular hyperthermia using magnetic particles:* The larger magnetic particles show ferri or ferromagnetic properties and comparable heating effects may be achieved with them as with superparamagnetic particles.[98] These particles can be exploited for the intracellular hyperthermia treatment of cancer. The coating of these particles by liposome makes them

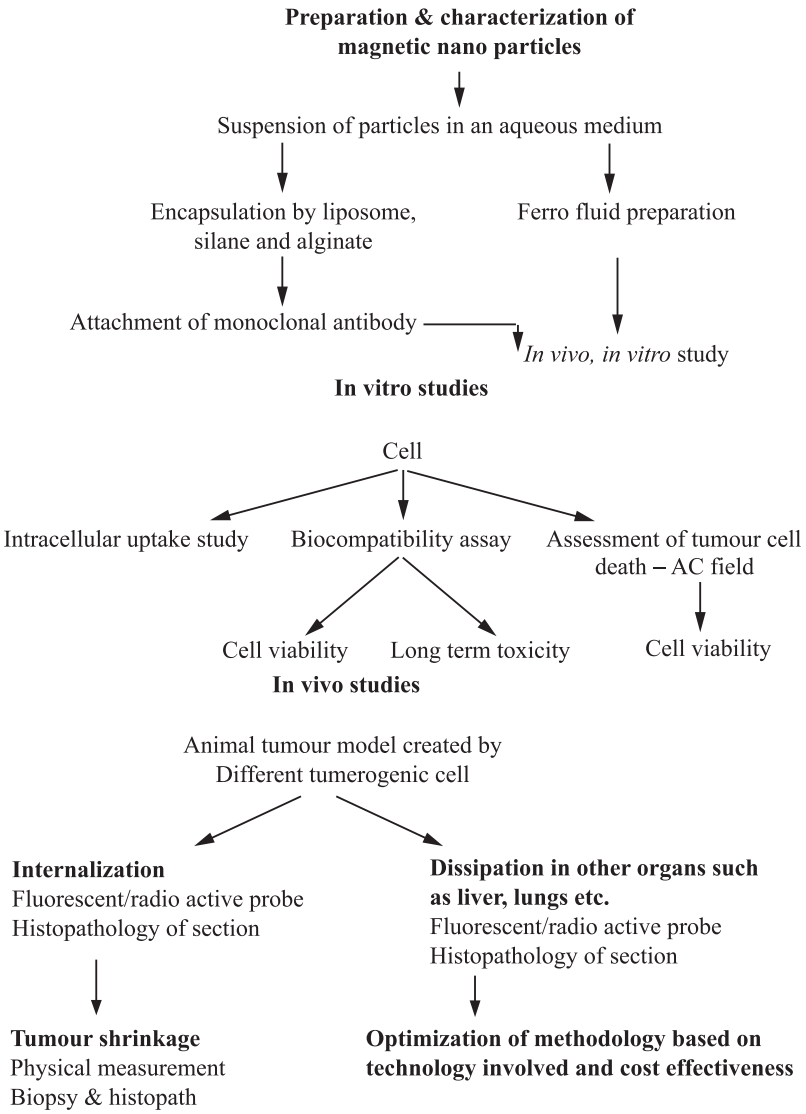

Chart 1. Development of suitable magnetic materials for clinical hyperthermia.

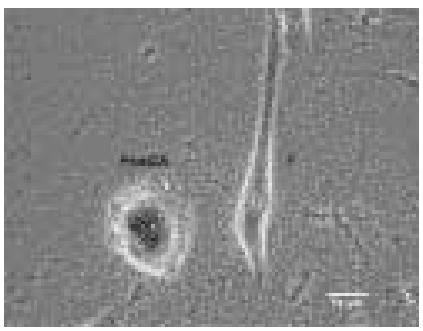

Figure 9. Phase contrast light microscopic picture of a prostate carcinoma cell and a fibroblast cell (from Jordan *et al*[85]).

highly dispersible in aqueous media as well as biocompatible. Magnetoliposomes (MLs) have been investigated both for their magnetic properties and for their applications in hyperthermia simulation.[89–90] The application of magnetic force on the uptake of magnetoliposomes was investigated and enhancement in the uptake has been observed in tumour cells.[91] To enhance the affinity or internalization of magnetic particle to the glioma cell, cationic magnetic liposome (CMLs) have been developed.[92–93] It exhibits ten times higher affinity for glioma cells, due to the positive charge on the surface (cells have negative charge). It has been proved that higher affinity of magnetite to the cells allows complete killing of tumour cells.[94] In vivo studies have been investigated in rats using MCLs. The rat glioma T-9 cells were used to create tumours. After 11 days of transplantation, the MCLs were injected into the tumour. Rats containing MCLs were subjected to hyperthermia treatment by applying AC field with 24 hours intervals. It was observed that the tumour tNo. completely disappeared[95] (figure 10). The drawback of this technique is that MCLs can be absorbed by normal cells also, so administration is limited by direct injection of MCLs in tumour site.[96] This limitation can be overcome by conjugation of antibodies to the magnetic particles. Antibody conjugated magneto liposome[97] and antibody-conjugated polyethylene oxide-magnetic complex were also developed. Insufficient low accumulation of magnetic particles in to the tumour cells leads to the insufficient heating. Shinkai *et al* (2001) further developed unique antibody-conjugated MLs which have high affinity and high heating ability.[97] Its capabilities have been demonstrated by *in vitro* and *in vivo* studies.

To make the hyperthermia as a potential therapy, selective destruction of cancerous cells is important. Though the hyperthermia is based on the fact that the tumour cells are very much sensitive to heat in the range of 42–46°C[60–62], it can assist the therapy to a high degree of selective destruction of cancerous cells. This is possible if targeted delivery of potential magnetic particles to the cancerous cells is made so that it will be much more effective to heat than the normal tNo.s. For this, optimization of magnetic parameters of particles is essential, which includes synthesizing particles having high value of specific absorption rate (SAR).[98–99] This minimizes the required dose of magnetic particles. It is, however, important that the internalization or intracellular delivery of particles is maximized.

Cellular inactivation depends on dose, when heat is applied to the tumour (42–46°C). The critical biological reason for thermal inactivation of cell is not yet known. Hyperthermia may induce many reversible effects on cell and tNo.s.[100] A few minutes after hyperthermia, heat shock protein (hss) is expressed in cell, which results in thermo tolerance of the cells.[101] This leads to an increase of cell survival. Besides hyperthermia can induce alternation in cell cycle and can induce apoptosis.[102–104]

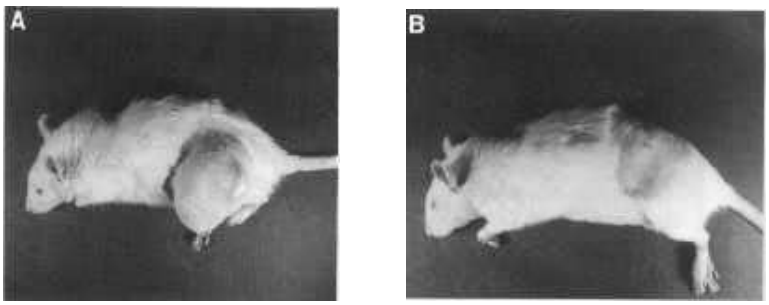

Figure 10. Tumour bearing rats photographed before hyperthermia application **(A)**: after application of AC magnetic field thrice at 24 intervals **(B)** (from Yanase *et al* [95]).

4.4g *Combined therapy:* A combination of chemotherapy or radiation therapy with hyperthermia is found much more effective than hyperthermia itself.[105] Several reasons are given for the enhanced effect. Tumours are poorly vascularised and it can be hard for therapeutic agents to reach their target. Heat increases the perfusion of a tumour and therefore drugs are transported more effectively into the target tNo. In addition, heat makes blood vessels more permeable to drugs. This occurs preferentially in tumours where blood vessels tend to be structurally incomplete. On the other hand, normal blood vessels are surrounded by a basement membrane and other perivascular cells and not significantly affected by heat. It has recently been reported that hyperthermia increases the rate of liposome leakage into tumours by a factor of 2–5 depending on the type of tumour. In normal tNo., however, enhancement of liposome leakage is not reported.

5. Conclusion

Biocompatible magnetic materials find a variety of applications such as in cell separation, drug delivery and hyperthermia. Magnetic forces can be used *in vitro* to direct the particles so that bound cells and molecules can be moved and *in vivo* to target and hold the magnetic carriers at anatomical sites or within cells for applications such as hyperthermia. Sometimes surface modification of these magnetic carriers is necessary for *in vivo* drug delivery or making these biocompatible. Entrapment in liposomes, alginate and some other biopolymers is common for use in control release of drugs or magnetic nano particles for hyperthermia or for local contrast enhancement in MR imaging. For hyperthermia applications, the superparamagnetic as well as ferromagnetic particles have been exploited. The superparamagnetic particle is more suited because its dimension is comparable to the cell dimension. In addition, the Neel loss, which is responsible for temperature rise in superparamagnetic particles is more effective than hysterisis losses in ferro/ferromagnetic particles. However, it is possible to tune the T_c for ferro/ferromagnetic particle, which is an added advantage to regulate the temperature.

The applications of nano materials to biotechnology/biosciences (so-called nanobiotechnology), are gradually increasing, and is a challenging area for future research in health care. From the above brief report on the use of magnetic particles in the bio applications, it is clear that nano materials having suitable magnetic properties find several applications in health care. The use of magnetic materials in bio-fields looks like restricted by the imagination of people who exploit them. So the scope of applications of magnetism (magnetic materials) in the biofield is wide open.

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References*

1. Blakemore R P, Frankel R B 1981 Magnetic navigation in bacteria. *Sci. Am.* 245: 58–65
2. Urs Häfeli 1997 In *Scientific and clinical applications of magnetic carriers* (eds) U Häfeli, W Schütt, J Teller, M Zborowski (New York, London: Plenum) p. 1
3. Gruttner C, Rudershausen S, Teller J 2001 *J. Magn. Magn. Mater.* 225: 1–7

*References in this paper/list are not in journal format

4. Mykhaylyk O, Cherchenko A, Ilkin A, Dudchenko N, Ruditsa V, Novoseletz M, Zozulya, Yu 2001 *J. Magn. Magn. Mater.* 225: 241–247
5. Kim D K, Zhang Y, Kehr J, Klason T, Bjelke B, Muhammed M 2001 *J. Magn. Magn. Mater.* 225: 256–261
6. Jordan A, Scholz R, Wust P, Fahling H, Felix R 1999 *J. Magn. Magn. Mater.* 201: 413–419
7. Koneracka M, Kopcansky P, Antalik M, Timko M, Ramchand C N, Lobo D, Mehta R V, Upadhyay R V 1999 *J. Magn. Magn. Mater.* 201: 427–430
8. Poeckley-Schoeniger C, Koepke J, Gueckel F, Sturm J, Georgi M 1999 *Magn. Reson. Imag.* 17: 383–392
9. Häfeli U O In: *Magnetism in medicine* (eds) W Andrä, H Novak (Berlin: John Wiley and Sons)
10. Chatterjee J, Haik Y, Ching-Jen Chen 2001 *J. Magn. Magn. Mater.* 225: 21–29
11. Popovic Z, Sjostrand J 2001 *Vision Res.* 41: 1313–1319
12. Del Gratta C, Della Penna S, Battista P, Di Donato L, Vitullo P, Romani G L, Di Luzio S 1995 *Phys. Med. Biol.* 40: 671–681
13. Liberti P A, Rao C G, Terstappen L W M M 2001 *J. Magn. Magn. Mater.* 225: 301–307
14. Zborowski M, Malchesky P S, Savon S R, Green R, Hall G S, Lewandowski J J, Nose Y 1987 *Proceedings of the Ninth Annual Conference of the IEEE Engineering in Medicine and Biology Society* (Cat. No.87CH2513-0) 3: 1161–1162
15. Andra W, Nowark H 1998 *Magnetism in medicine (Hand book)* (Berlin: Wiley-VCH)
16. Shinkai M, Yanase M, Suzuki M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T 1999 *J. Magn. Magn. Mater.* 194: 176–184
17. Jordan A, Wust P, Scholz R, Tesche B, Fahling H, Mitrovics T, Vogl T, Cervos-Navarro J, Felix R 1996 *Int. J. Hyperthermia* 12: 705–722
18. Golui S, Datta D, Bahadur D 2000 *Proc. 8th Int. Conf. on ferrites (ICF 8)* Kyoto, Japan, p 105
19. Olsvik-O, Popovic T, Skjerve E, Cudjoe K S, Hornes E, Ugelstad J, Uhlen M 1994 *Clin. Microbiol. Rev.* 7: 43–54
20. Lea T, Vartdal T, Nustad K, Funderud S, Berge A, Ellingsen T, Schmid R, Stenstad P, Ugelstad J 1988 *J. Mol. Rccogn.* 1: 9
21. Kundu P K, Waghode T S, Bahadur D, Datta D 1998 *Med. Biol. Eng. Comput.* 36: 654–658
22. Häfeli U O, Pauer G J 1999 *J. Magn. Magn. Mater.* 194: 76–82
23. Safarik I, Safarikova M 1999 *J. Chromatography* B, 722: 33–53
24. Karumanchi R S M S, Doddamane S N, Sampangi C, Todd P W 2002 *Trends Biotechnol.* 20: *Cell separation and protein purification* 1996 Information booklet. Dynal Oslo
25. Scouten W H, Konecny P 1992 *Anal. Biochem.* 205: 313
26. Ghose M *et al* 1995 *Biotechnol. Tech.* 9: 149–152
27. Burns MA, Graves D J 1985 *Biotechnol. Progr.* 1: 95–103
28. Kobayashi H, Matsunaga T 1991 *J. Colloid Interface Sci.* 141: 505–511
29. Nakamura N, Matsunaga T 1993 *Anal. Chim. Acta* 281: 585–589
30. Widjojoatmodjo M N, Fluit A C, Torensma R, Verhoef J 1993 *J. Immunol. Methods* 165:: 11–19
31. De Cuyper M, De Meulenaer B, van der Meeren P, Vanderdeelen J 1995 *Biocatal. Biotransform.* 13: 77–87
32. *Cell separation and protein purification* 1996 Technical handbook, Dynal, Oslo
33. Olsvik O, Popovic T, Skjerve E *et al* 1994 *Clin. Microbiol. Rev.* 7: 43–54
34. Safarik I, Safarikova M, Forsythe S J 1995 *J. Appl. Bacteriol.* 78: 575–585
35. Naume B, Nonstad U, Steinkjer B, Funderud S, Smeland E, Espevik T 1991 *J. Immunol. Methods* 136: 1
36. Nilsson H, Johansson C, Schynius A 1987 *J. Immunol. Methods* 105: 165
37. Funderud S, Erikstein B, Asheim H C, Nustad K, Stokke T, Blomhoff H K, Holte H, Smeland E B 1990 *Eur. J. Immunol.* 20: 201

38. Margel S, Gura S, Bamnolker H, Nitzan B, Tennenbaum T, Bar-Toov B, Hinz M, Seliger H 1997 In: *Scientific and clinical applications of magnetic carrier*s (eds) U Häfeli, W Schütt, J Teller, M Zborowski (New York, London: Plenum) p. 37
39. Sieben S, Bergemann C, Lübbe A, Brockmann B, Rescheleit D 2001 *J. Magn. Magn. Mater.* 225: 175–179
40. Kutushov M V, Kuznetsov A A, Filippov V I, Kuznetsov O A 1997 In: *Scientific and clinical applications of magnetic carriers* (eds) U Häfeli, W Schütt, J Teller, M Zborowski (New York, London: Plenum) p. 391
41. Vladimir P, Torchilin 2000 *Euro. J. Pharm. Sci.* 11: S81–S91
42. Kuznetsov A A, Filippov V I, Kuznetsov O A *et al* 1999 *J. Magn. Magn. Mater.* 194: 22
43. Lübbe A S, Bergemann C, Brock J, McClure D G 1999 *J. Magn. Magn. Mater.* 194: 149–155
44. Fricker J 2001 *Drug Discovery Today*, 6: 387–389
45. Widder K J, Morris R M, Poore G A, Howards D P, Senyei A E 1983 *Eur. J. Cancer Clin. Oncol.* 19: 135–139
46. Gallo J M, Gupta P K, Hung C T, Perrier D G 1989 *J. Pharm. Sci.* 78: 190–194
47. Pulfer S K, Gallo J M 1997 In: *Scientific and clinical applications of magnetic carriers* (eds) U Häfeli, W Schütt, J Teller, M Zborowski (New York, London: Plenum) p. 445
48. Lübbe A S, Alexiou C, Bergemann C 2001 *J. Surg. Res.* 95: 200–206
49. Alexiou C, Arnold W, Hulin P, Klein R J, Renz H, Parak F G, Bergemann C, Lubbe A S 2001 *J. Magn. Magn. Mater.* 225: 187–193
50. Koening S H 1996 *Acad. Radiol.* 3: 597–606
51. Stark D D 1991 *Radiology* 179: 333–335
52. Su M Y, Mühler A, Lao X, Nalcioglu O 1998 *Magn Reson. Med.* 39: 259–269
53. Weinmann H J, Brasch R C, Press W R *et al* 1984 *Am. J. Roentgenol.* 142: 619–624
54. Schuhmann-Giampieri G, Schmitt-Willich H, Frenzel T *et al* 1991 *Invest. Radiol.* 26: 969–974
55. Weissleder R, Elizondo G, Wittenberg J *et al* 1990 *Radiology* 175: 489–493
56. Bach-Gansmo T, Fahlvik A, Ericsson A *et al* 1994 *Invest. Radiol.* 29: 339–344
57. Marincek B 1996 *J. Belg. Radiol.* 79: 13–17
58. Kresse M, Wagner S, Taupitz M 1997 In: *Scientific and clinical applications of magnetic carriers* (eds) U. Häfeli, W Schütt, J Teller, M Zborowski (New York, London: Plenum) p. 545
59. Cavaliere R, Ciocatto E C, Heidelburger C, Jonson R O, Margottini M, Mondovi B, Moricca B G, Rossi-Fanelli A 1967 *Cancer* 20: 1351–1381
60. Overgaad K, Overgaard J 1972 *Eur. J. Cancer* 8: 65–78
61. Overgaard J 1977 *Cancer* 39: 2637–2645
62. Field S B, Hand J W 1990 *An introduction to the practical aspects of clinical hyperthermia* (London: Taylor and Francis)
63. Kneller E 1966 In: *Encyclopedia of physics, vol. XVIII/2, Ferromagnetism* (ed.) H P J Wijn (New York: Springer-Verlag) pp 438–544
64. Chikazumi S 1964 *Physics of magnetism* (Philadelphia, PA: Lippincott)
65. Shliomis M I 1963 Magnetic fluids. *Sov. Phys. Usp.* 17: 153–184
66. Hergt R, Andrä W, d'Ambly C G, Hilger I, Kaiser W A, Richter U, Schmidt H-G 1998 *IEEE Trans. Magn.* 34: 3745–3754
67. Ordan A, Wust P, Scholz R, Faehling H, Krause J, Felix R, In: *Scientific and clinical applications of magnetic carriers* (eds) U Häfeli, W Schütt, J Teller, M Zborowski (New York, London) p. 569
68. Singh K, Bahadur D 1999 *J. Mater. Sci. (Mater. Med.)* 10: 481–484
69. Jagadish B R, Prabhu N, Bahadur D 1998 *Bull. Mater. Sci.* 21: 133–137
70. Jianhua C, Naru Y 2001 *J. Chin. Ceram. Soc.* 29: 238–244
71. Kawashita M, Takaoka H, Kokuls T, Yao T, Hamada S 2001 *J. Ceram. Soc. Jpn.* 109: 39–44
72. Kokubo T 1999 *Mater. Sci. Forum* 293: 65–82
73. Ohura K, Lkenaga M, Nakamura T, Yamamuro T, Ebisawa Y, Kokuko T, Kotoura Y, Oka M 1990 *Bioceramics (Proc. 3rd Int. Symp. Ceramics in Medicine)* Terre Haute, Indiana,

pp 225–229
74. Giri J, Ray A, Sriharsha T, Dasgupta S, Datta D, Bahadur D 2002 *Proc. 14th MRSI Annual Meeting*, Hyderabad
75. Brezovich I A, Meredith R F 1989 *Radiol. Clin. North Am.* 1: 589–602
76. Cetas T C, Gross E J, Contractor Y 1998 *IEEE Trans. Biomed. Eng.* 45: 68–7
77. Jordan A, Scholz R, Wust P, Fähling H, Felix R 1999 *J. Magn. Magn. Mater.* 201: 413–419
78. Gilchrist R K, Medal R, Shorey W D, Hanselman R C, Parott J C, Taylor C B 1957 *Ann. Surg.* 146: 596–606
79. Medal B S, Shorey W, Gilchrist R K, Barker W, Hanselman R, 1959 *Am. Med. Assoc. Arch. Surg.* 79: 427–431
80. Gilchrist R K, Shorey W D, Hanselman R C, Depeyster F A, Yang J, Medal R 1965 *Ann. Surg.* 161: 890–896
81. Gordon R T, Hines J R, Gordon D 1997 *Med. Hypothesis* 5: 83–102
82. Ramachand C N, Pande P, Kopcansky P, Mehta R V 2001 *Indian J. Pure Appl. Phys.* 39: 683–686
83. Mosbach K, Anderson L 1997 *Nature* 270: 259
84. Jordan A, Scholz R, Maier-Hauff K, Johannsen M *et al* 2001 *J. Magn. Magn. Mater.* 225: 118–126
85. Jordan A, Scholz R, Wust P, Fähling H, Felix R 1999 *J. Magn. Magn. Mater.* 201: 413–419
86. Hilger I, Andrä W, Hergt R, Hiergeist R, Schubert H, Kaiser W A 2001 *Radiology* 218: 570–575
87. Hiergeist R, Andrä W, Buske N, Hergt R, Hilger I, Richter U, Kaiser W 1999 *J. Magn. Magn. Mater.* 201: 420–422
88. Shinkai M, Matsui M, Kobayashi T 1994 *Jpn. J. Hyperthermic Oncol.* 10: 168–177
89. Shinkai M, Suzuki M, Iijima S, Kobayashi T 1994 *Biotech. Appl. Biochem.* 21: 125–137
90. Suzuki M, Shinkai M, Yanase M, Ito A, Honda H, Kobayashi T 1999 *Jpn. J. Hyperthermic Oncol.* 15: 79–87
91. Shinkai M, Yanase M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T 1996 *Jpn. J. Cancer. Res.* 87: 1179–1183
92. Yanase M, Shinkai M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T 1997 *Jpn. J. Cancer Res.* 88: 630–632
93. Yanase M, Shinkai M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T 1998 *Jpn. J. Cancer Res.* 89: 775–782
94. Yanase M, Shinkai M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T 1998 *Jpn. J. Cancer Res.* 89: 463–469
95. Yagi K, Hayashi Y, Ishida N, Ohbayashi M, Ohishi N, Mizuno M, Yoshida J 1994 *Biochem. Mol. Biol. Int.* 32: 167–171
96. Le B, Shinkai M, Kitade T, Honda H, Yoshida J, Wakabayashi T, Kobayashi T 2001 *J. Chem. Eng. Jpn.* 34: 66–72
97. Chou C K 1990 *Int. J. Hyperthermia* 6: 367–370
98. Chan D C F, Kirpotin D B, Bunn P A Jr 1997 In: *Scientific and clinical applications of magnetic carriers* (eds) U Häfeli, W Schütt, J Teller, M Zborowski (New York, London: Plenum) p. 607
99. Streffer C, van Beuningen D 1987 In: *Hyperthermia and the therapy of malignant tumours* (ed.) J Streffer (Berlin: Springer) p 24–70
100. Li G C, Mivechi N F, Weitzel G 1995 *Int. J. Hyperthermia* 11: 459–488
101. Sellins K S, Cohen J J 1991 *Radiat. Res.* 126: 88–95
102. Ohnishi K, Ohnishi T 2001 *Int. J. Hyperthermia* 17: 415–427
103. Ohtsubo T, Igawa H, Saito T, Matsumoto H, Park H J, Song C W, Kano E, Saito H 2001 *Radiat. Res.* 156: 103–109
104. Shinkai M, Suzuki M, Yokoi N, Yanase M, Shimizu W, Honda H, Kobayashi T 1998 *Jpn. J. Hyperthermic Oncol. Cancer. Res.* 14: 15–21