"Biomedical applications of magnetic nanoparticles II: Electromagnetic radiation"

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Magnetic nanoparticles are used in "in-vivo" diagnosis and therapy on the bases of their interaction with electromagnetic radiation. Here we will concentrate in the tomography using magnetic resonance imaging (MRI). In this case the magnetic moments of the magnetic nanoparticles used as contrast agent, produces a local change of the proton resonance which enhance the MRI contrast. Other aspect which we will cover is the magnetic fluid hyperthermia (MFH), which uses magnetic nanoparticles as heat generators to induce localized cell death. The physical basis of these techniques relies on the interaction with external electromagnetic fields.

The first therapeutic applications of magnetic devices to humans can be chased back to the 16th century, when Austrian physician Franz Anton Mesmer (1734-1815) developed his theories about magnetic fluids. He sustained the influence of invisible 'universal fluids' on the human body (after the Newtonian ideas of 'aether' associated to gravitational forces and tidal cycles), and proposed his theory of 'animal magnetism' gaining notoriety across Europe. Since then Mesmerism (a therapeutics based mainly on hypnotism) has triggered a sustained flood of both research and 'supernatural' quackery.

Pushed by advances in the synthesis of biocompatible magnetic nanoparticles (MNPs) in a reproducible way, the concept of targeting magnetic nanospheres inside microscopic living organisms regained interest and finally became a reality. The size of MNPs is comparable to the DNA or subcellular structures, then, they can be used for cell separation strategies using magnets as external driving forces. Similarly, recent advancements on binding chemistry of biological units onto MNPs surface and the engineering of particle's surface/shape have opened new exciting possibilities for drug delivery with high selective vectors. Nonetheless, *in vivo* applications entangle subtle problems related to the response of a living organism to alien objects (i.e., NPs-drug assemblies). For example, even if a perfectly selective drug delivery system could be designed (e.g., by using some monoclonal antibody-loaded particles), any real experiment has to overcome the problem of immunological reactions triggered by the invading NPs within the host, mainly from the reticuloendothelial system (RES).

At present, most applications of MNPs are based on the following physical principles:

a) The application of controlled magnetic field gradients (i.e., a magnetic force) around the desired target location for remotely positioning MNPs in organs or tissues (targeting, magnetic implants, magnetic separation applied to the sequencing of DNA, etc).
b) The utilization of the magnetic moment of the MNPs as a local perturbation of the proton nuclear resonance (contrast media for Magnetic Resonance Imaging, MRI).
c) The magnetic losses of nanometric particles in colloids for heating purposes (magnetic hyperthermia)

Electromagnetic radiation

Electromagnetic (EM) radiation is a fundamental tool in cancer therapy, extensively used for both diagnostics and therapy. Physical interactions between EM waves and living matter can be very different depending on the portion of the electromagnetic spectrum considered. A variety of clinical tools have been established in physical medicine based on direct emission and detection of EM waves such as x-ray radiography, computer tomography scanning (CT scan) and gamma-ray radiotherapy from radioactive isotopes. Many other techniques rely on indirect uses of EM radiation such as positron-emission tomography (PET), magnetic resonance imaging (MRI), and microwave hyperthermia (MWH).

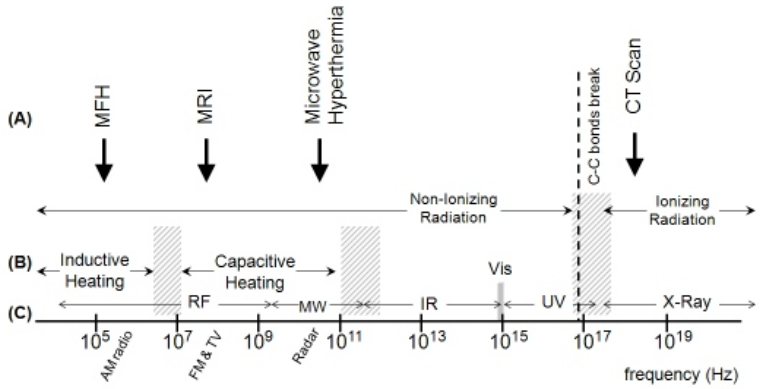

Figure 1. A. Frequency ranges for some of the most used diagnostic/therapy equipments (MFH = Magnetic Fluid Hyperthermia, MRI = Magnetic Resonance Imaging). B. The respective main physical mechanisms at each frequency range. Also shown in (C) is the common nomenclature for the electromagnetic waves at each region: RF = radiofrequency; MW = microwaves; IR = infrared; Vis = visible; UV = ultraviolet and X-Ray.

Figure 1 schematizes the different ranges of the EM spectrum used by different techniques, and also puts comparatively some physical and biological phenomena occurring within each region. The importance of this "EM landscape" is connected to absorption of energy by biological units, since the shorter the wavelength, the higher the energy content. Organic materials composed of long-chained molecules with C-C (or C=C) backbones and other carbon bonds like C-H, C-N, can absorb EM radiation at some specific frequencies that are, consequently, biologically dangerous. As an example, covalent bonds can be broken at approximately 10^{12} Hz ($\lambda \approx 300$ nm, in the UV range). Larger units have more complex (secondary, tertiary) structures, and may be bound to other units by entanglement alone, secondary forces or chemical bonds. Due to this variety of binding forces, living matter displays several 'frequency windows' where interaction with EM radiation can destroy biological units and/or metabolic functions. The frequency ranges employed by the techniques of Figure 1 are usually grouped in two coarse classes: those based on non-ionizing radiation (basically radiofrequency and microwaves), and those using ionizing radiation (high-energy X-rays and gamma-rays). The limit between these areas is defined by the energy threshold to break C-C, C-H and C-N covalent bonds, which would imply the breaking of fundamental organic molecules as DNA, RNA, proteins, etc…

Hyperthermia

For a piece of metal subjected to low- and medium-frequency alternating fields (> 10^2 – 10^3 Hz, for example the case of nuclei of electrical motors) the main mechanisms of power losses are magnetic hysteresis and parasitic currents (eddy currents). On the other hand, in ceramic materials the dissipation of power is mainly originated in processes of nucleation, growth and extinction of magnetic domains. For single-domain particles in physiological conditions the situation differs radically, because a) the magnetic saturation is reached by coherent rotation of the total magnetic moment of each particle; and b) the hysteresis cycles are theoretically reversible and thus they do not entail magnetic losses. In addition to coherent rotation to be considered for single-domain particles, physiological conditions allow mechanical rotation of the particles as a response to the external magnetic field, at least for low frequencies. It follows that for colloidal dispersions the analysis of the heat transference processes must include the effects of both the Brownian motion and fluid. For biomedical applications based on the increase of temperature as magnetic fluid hyperthermia (MFH) therapy, it is clear that the mechanisms of power losses in colloids must be identified before new, more efficient therapeutic materials can be designed to *maximize* the generation of heat.

The heating capacity of a magnetic material or electromagnetic device is quantified through the specific absorption power rate (SAR), defined as the amount of energy converted into heat per time and mass. In terms of the usual experiments and parameters for magnetic colloids, the loss power per gram of Fe_3O_4 is obtained from the heating curves within the initial ΔT temperature rising interval through the definition

$$SAR = C_S \frac{\Delta T}{\Delta t} = \frac{Q}{\rho_t} \ , \qquad \textbf{(I)}$$

where C_S is the sample heat capacity, defined as a mass-weighted mean value for a given concentration of magnetic material, calculated as

$$C_S = \frac{m_{Fe}\, c_{Fe} + m_l\, c_l}{m_{Fe} + m_l} \qquad \textbf{(II)}$$

with c_{Fe}, m_{Fe} and c_l, m_l being the specific heat capacities and masses of magnetic material and liquid carrier, respectively. The last member shows the relationship between the functional definition of SAR and eq. IX.

Within oncology therapeutics, hyperthermia is a general term for the rise of temperature above the physiologic level (in the 40°C -45°C range) within a targeted tumor without damaging the surrounding healthy tissue. The rationale of this therapy is based on solid evidence from preclinical data that the antitumor cytotoxicity of radiation can be enhanced by previous temperature increase of cells or tumor tissues. It is accepted that at the cellular level hyperthermia provokes morphological and physiological changes, such as the loss of integrins from the cell surface, which is thought to be a perturbing effect on metabolic pathways preceding cell death. The actual mechanisms active during hyperthermia treatments seem to be similar to those of radiation regarding cell cycle sensitivity and hypoxia. The most extended method for reaching temperatures above the systemic values (i.e., 37·5°C) is based on the application of microwaves, although therapies involving laser or ionizing radiation have also been successfully applied to heat

up malignant tissues. All these strategies are capable of easily rise the intracellular temperature to the degree needed for thermo-ablation, but also they all have undesired collateral effects such as ionization of genetic material (radiation) or lack of selectiveness (microwaves) that affect the surrounding healthy tissues.

Magnetic hyperthermia can be defined as the rise of temperature that can be accomplished remotely by means of external alternating magnetic field acting on MNPs at the targeted location.

It is important to note that the EM radiation used by MFH belongs to a frequency region where the heating effects on living tissues are negligible. Therefore, differently from other hyperthermia methods, MFH needs a heating agent (i.e., the magnetic nanoparticles) placed at the targeted cells in order to produce the temperature increase. This difference if the main reason of the potential advantages of MFH over alternative strategies, since MNPs can be in principle attached exclusively to (or even introduced into) tumoral cells to heat them with minimum influence on the surrounding healthy tissues. Therefore the success of this approach depends critically of the ability to attach a given particle on those cells that are to be killed (i.e., the 'targeting problem').

The underlying physical mechanisms of MFH are related to the energy dissipation when a ferromagnetic material is placed on an external alternating magnetic field. In physiological conditions there are different effects to be considered for power losses: a) magnetic losses through domain wall displacements (in multi-domain particles), Néel relaxation (in single domain particles); and b) energy loss from mechanical rotation of the particles, acting against viscous forces of the liquid medium (Brownian losses).

Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is the most successful among the imaging techniques currently available. It is a non-invasive, non-destructive modality that can reconstruct both 2D and 3D images of an internal living structure, without limitation in volume or depth of the analyzed target. Since the 1980s, the implementation of dedicated hardware for MRI scanners has reduced the image acquisition time from the many-hour down to the second-scale, widening the applications to include fast dynamic processes. Additionally, signal processing and the development of high-sensitivity RF detectors have shifted the spatial resolution limit from the cm scale to about 10 μm, enabling *in vivo* imaging at microscopic resolution. The above advantages have made MRI to become a most valuable technique for cancer diagnosis and therapy.

Any resonant technique is based on the existence of physical entities (e.g., electrons, nuclei, or molecules) that can be promoted from their ground state (taken as the zero-energy, E_0) to higher-energy excited states with E_1, E_2,…E_n. In the case of MRI, the resonant physical entities are the hydrogen nuclei (protons) that exist abundantly in living tissues. Protons placed in a homogeneous magnetic field B_0 can absorb electromagnetic (EM) waves carrying energies E_ν satisfying $E_\nu = \Delta E$, where ΔE is the energy difference $\Delta E = E_1 - E_0$ between two nuclear levels. Note that MRI involves a *magnetic* coupling between the magnetic-component of the EM waves and the *magnetic* moment of the resonant hydrogen nucleus (nuclear spin). Therefore the MRI is a *nuclear* resonance technique that gives information based on (but not restricted to) the *magnetic* properties of the biological samples. The signal from relaxation of the excited protons is captured through currents induced over a specific arrangement of pick-up coils, and finally the whole relaxation process is reconstructed computationally to obtain temporal or spatial

(2D and 3D) images of the desired organ/tissues. The pick-up coils are needed to transmit and/or receive the MR signal, and for optimum signal-to-noise ratio the coils should cover only the working volume to be observed. Many types of pick-up coils have been designed for minimum noise (e.g., caging coils for head and knee local studies), balancing observation volumes and sensitivity.

The energy $E_\nu = h\nu$ (where ν id the frequency and h is the Planck constant) of the EM waves used for resonant excitation will depend on the applied static field, B_0, through the relation

$$\upsilon = \frac{\gamma}{2\pi} B_0 \qquad \textbf{(III)}$$

where the γ = 267.66 MHz/Tesla is Larmor frequency of the proton. Current commercial MRI platforms employ dc fields B_0 between 1 and 3 T, so that required RF frequencies are in the 50-100 MHz range. But companies have started to develop 7, 8 and 9 Tesla systems that imply the use of frequencies within the ~ 0.3 GHz range. At these high RF frequencies dielectric coupling of the EMF with biological material cannot be neglected (see Figure 1), and indeed heating effects have been observed in phantoms using 8 Tesla MRI platforms. The strategy used to visualize and track target cells by MRI is to tag them with a contrast agent, a ferrofluid containing biocompatible MNPs.

SPION-based contrast agents

The purpose of injecting CA's is to *change* the relaxation rates (called T1 and T2) of the surrounding hydrogen atoms of the tagged cells, to the extent that a measurable change in signal intensity (contrast) is observed between particle-charged and normal tissues. The resulting differences in signals from various body tissues enable MRI to differentiate organs and to contrast benign and malignant (particle-loaded) tissues.

Gadolinium(III) was the first magnetic material clinically used as a contrast agent and still remains the foremost material in terms of total volume employed around the world. This fact is probably related to the slow and costly process of preclinical validation of a new material intended for human uses, which delays the marketing of new products. However, different alternatives are being increasingly reported as good candidates for CAs, such as additional lanthanide ions and iron oxide nanoparticles. Commercial contrast agents based on SPIONs are composed of a iron oxide core of 5 to 10 nm diameter (usually magnetite Fe_3O_4 or maghemite γ-Fe_2O_3), coated with a polysaccharide such as dextran for stabilization purposes, which results in a hydrodynamic size of ~150 nm diameter.

From the physical side, the performance of CAs regarding how it influences the relaxation of neighbor protons is proportional to the square of the saturation magnetic moment (M_S) of the particles, thus the design of new CAs requires optimized magnetic materials with large M_S values. All contrast agents based on MNPs make use of the large magnetic moment of iron-oxide subdomain particles, which can be 10^3 times larger than a single paramagnetic atom. The proximity of the magnetic particles to the desired target tissue is also a crucial parameter, since the magnitude of the interaction between a magnetic particle and neighboring protons is proportional to the sixth power of the inverse of the distance raised to (r^{-6}),

The efficiency of any MRI contrast agents as an early-diagnosis tool is intimately related to its capability of giving the strongest signal capable to be detected with the smallest amount of magnetic material. Current CAs are composed of passive MNPs and

thus there are far from the above specification. In general terms, the ideal efficiency would imply

- Selective binding to target cells to provide a local, specific enhancement;
- Improved relaxational properties to decrease the detection threshold to low than 1mmol Fe/kg.
- Prominent signal–to–noise enhancement to allow high resolution levels;
- Long circulating half-life (hours) to expand the imaging time window;
- Acceptable toxicity profile to be biologically safe;
- Ease of production and clinical use to be economically and commercially sustainable;

Note of the authors: This lecture is a summary of the review paper "Magnetic Nanoparticles for Cancer Therapy" Current Nanoscience, in press

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