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Alternative low frequency magnetic field theranostics: recent advances, safety and hazards

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Abstract. The paper presents a brief review and comparative analysis of low frequency (nonheating) and radio-frequency electromagnetic nanomedicine technologies. The former are shown to have a considerable advantage over the latter ones: a higher flexibility and penetrating ability, easier to dose and control, easier to localize, as well as safer and less costly. This makes their employment promising for building a new technological platform for low frequency magnetic theranostics with a wider range of options, i.e. possessing a wider multimodality than traditional radio-frequency methods.

1. Introduction

Natural and technogenic electromagnetic fields are important factors of life on Earth (Figure 1). Their influence on many processes in living matter is very prominent [1-10]. The Earth magnetic field (MF) with the flux density of $B_E \approx 50 \mu T$ as well as its low frequency variations with a $10^{-4} - 10^{-2} B_E$ amplitude, mainly induced by the increased Solar activity seems to be not only safe but also essential or, at least, useful for the biosphere function and evolution. It is known that in a number of cases after complete shielding or cancellation of external MFs depression of some functions of living organisms [11, 12] including humans [13] was observed. Investigations of adverse impacts of the Earth MF cancellation on humans [14], carried out for decades, suggest bringing a special term 'The Magnetic Field Deficiency Syndrome' as applied to the 'developed countries' population who spend much time in magnetically shielded office and apartment buildings. Thus, for example, Russiansanitary regulations generally allow for no greater than twofold decrease of the Earth's natural MF inside manmade constructions [15].

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10⁶

10⁵

 10^{7}

 10^{8}

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Figure 1. The map of natural and artificial magnetic fields (MF).

 10^{2}

10

 10^{3}

f, Hz

 10^{4}

The recent century numerous artificial MF sources were added to the natural ones, among them: power lines, telecommunication facilities, electric transport, electric furnaces and other electric industrial equipment, medical and household electro/radio devices. The aforementioned and similar man-made sources of permanent and alternating MF produce fields comparable or even stronger than natural ones. Technogenic electromagnetic fields may be considered as certain factors of the environmental pollution. Some experts in hygiene and ecology even consider them the major pollution agents, especially in urban areas where the artificial electromagnetic background is much more intense than that in the rural areas and the ratio between the exposition period and general life time approaches 100%.

The data provided by a number of independent research groups prove that technogenic MFs may have a pronounced influence on health of industrial personnel and general public [1, 2, 8-10]. In this context, statutory documents issued by various state and international authorities limit the maximum allowed induction of alternating magnetic field (AMF) *B* for various groups of population depending on the field frequency *f* and exposition time, i.e. time spent under the influence of the field. Thus, according to the sanitary regulations of the International Commission on Non-ionizing Radiation Protection (ICNIRP)[16] *B*·*f* product at work places is limited to a constant 25 mT·Hz within the range from tens to hundreds hertz and to 2000 mT·Hz within the range of hundreds kilohertz, whereas for general public this constants are 5 mT·Hz and 920 mT·Hz respectively.

Fields with a flux density exceeding the allowed maximum values by about two orders of magnitude are considered hazardous. These guidelines vary significantly in different countries and international organizations, but everywhere an analogous dependence B(f) is observed. In other words, it is generally accepted that an increase in f leads to the rise in negative effect of AMF on human organism and on biosphere. To some extent it can be explained by the fact that high values of $B \cdot f$ lead to the increased ability of AMF to heat any substances including biological tissues. As a result, nonspecific thermal effects can be added to the specific "magnetic" ones. Basically electromagnetic field dissipates in any initially unexcited medium which results in heating the medium. However, in

easily generated experimental fields with the induction ≤ 1 T, any prominent heating of biological objects is registered only in AMF with frequency values higher than certain characteristic f_T . Since dissipation of AMF energy occurs during every cycle of the field oscillations, then, with other factors being equal, the amount of heat produced in tissues under the influence of radiofrequency MF (RF MF) at a rough approximation is proportional to f. Depending on the organism's size, type of tissue, thermal exchange conditions and other factors, the frequency of 10^4 - 10^5 Hz can be taken as f_T value for homoeothermic animals. Accordingly, AMF with $f < f_T$ is commonly referred to as 'non-heating' field, while AMF with $f > f_T$ is referred to as 'heating' field. Non-specific thermal influence mechanisms being added to the specific 'magnetic' ones trigger complementary risks such as possible increase in biochemical and catalytic reactions rates, biological liquids thinning, secondary structure alteration and denaturation of proteins, phase transitions and permeability increase in membranes, cell apoptosis and necrosis and etc. That is why for safety reasons, it is demanded that the higher AMF frequency is, the lower field induction should be.

Natural or artificial magnetic structures present in the organism can lower the threshold of the registered AMF effect considerably. Study and recording of such effects becomes more and more important and timely since employment of synthetic magnetic nanoparticles (MNPs) stimulated by AMF keeps growing in biomedical applications.

Recent decades witnessed a sharp increase in use of functionalized MNPs (f-MNPs) both in biomedical studies and clinical applications for various methods of biostructure visualization, diagnostics and therapy [17-19]. In particular, they allow increasing the image contrast in magnetic resonance imaging (MRI), whereas in the targeted drug delivery strategy, they support targeted transport, controlled drug release, malignant cells ablation by means of RF magnetic hyperthermia (Figure 2) and other therapeutic treatments [20-24].

All aforementioned techniques utilize f-MNPs as the mediators enhancing localization and magnitude of RF AMF effect on object structure targeted elements. Beside the discussed threats and dosage and control difficulties of overheat, aforementioned technique has another drawback. Heat transfer through tissues results in heat spreading over a long distance from heat generation point source once MNPs are considered in all biomedical applications. During typical exposition times $t_e \approx 10^2 - 10^3$ s, the thermal wave passes through $R_T = (\chi t_e)^{1/2} \approx 3 - 10$ mm from MNP [25,26], where $\chi \approx 10^{-7}$ m²s is a typical value of living tissue thermal diffusivity constant. Since χ varies within one order of magnitude for all tissues, no RF magnetic hyperthermia technique enhancement could localize thermal field stronger than within 1 mm region. It completely denies its capability to be local or selective on the cellular and even more on molecular scale.

Matching the parameters of AMF used in clinical practice with the limits set by sanitary regulations shows that the former are considerably higher. This stimulates the search for and development of safer electromagnetic methods for diagnostics and therapy.

Considering these circumstances and other limitations of the traditional methods for diagnostics and therapy in RF MF, several new approaches have been recently suggested. It is proposed to use lower frequency fields to stimulate MNPs (Figure 2), since the maximum allowable fields here are much lower than those for RF MF. Various applications of LF MF with f-MNPs can lay the physical foundation for the new generation of means for safer magnetic theranostics with enhanced capabilities and modality. IOP Conf. Series: Materials Science and Engineering **98** (2015) 012016 doi:10.1088/1757-899X/98/1/012016



Figure 2. Innovative nanotechnology of theranostic assisted by f-MNPs and low frequency nonheating magnetic field (LF MF) vs traditional biomedical technologies

The paper presents a brief review on the principles, advantages and problems of these approaches for biomedical applications both *in vitro* and *in vivo*.

2. Multimodal visualization and diagnostic techniques

For the past several decades magnetic resonance imaging (MRI) remains one of the most widely used approaches to acquiring and visualizing information on the inner tissues in clinical practice. The main idea of the approach is a studying the spatial peculiarities of magnetic moment relaxation of hydrogen nuclei in a strong uniform MF on thoroughly designed RF pulse forcing spin system transition into an exited state. An image contrast originates from the difference in environment of hydrogen nuclei as their concentration is high and more or less uniform throughout the tissues. Technically, the object under study is placed into a strong and extremely uniform steady MF with a relative inhomogenity around 10⁻⁶ root mean square or even better to achieve a particularly high spatial resolution leading both to energy levels splitting depending on the nucleus spin projection on the field direction and to spin rotation with a well defined frequency known as Larmor precession. Additional RFMF short pulse on this frequency induces a significant rearrangement of the nuclei spin system followed by a gradual relaxation of the system to the equilibrium state. Specifics of this relaxation process provide

the mechanism of contrasting tissues and its regions distinguished by state or functionality such as tumors localization.

MRI approach is extremely versatile because of high complexity of relaxation processes within the nuclei spin system that provides a wide variety in selecting the excitation pulse parameters, type and processing algorithms of the recorded response depending on a particular object under study and the study aim. In some cases, the image contrast is enhanced by agents, which strongly affect relaxation processes in the nuclei spin system. Such agents could be both individual paramagnetic ions or their compounds and superparamagnetic f-MNPs. The most widely used ion is gadolinium, but it is quite toxic for living organisms even being enveloped in chelate complexes, so alternatives are sought for in many ways. In particular, other paramagnetic ions, oxide nanoparticles, nanoparticles based on zeolites or polymers and functionalized by various atoms and ligands have been proposed and tested [27].

To combine capabilities of MRI and other approaches to malignant tumor visualization and treatment, one seeks to design MNPs that could stand as an MRI contrasting agent and therapeutic or another diagnostic procedure agent simultaneously. In particular, the authors of [28] propose a specific design of a multilayered nanocrystal structure made of lanthanides on the carbon nanotube matrix that can be used both as an MRI contrasting agent and a fluorescent label carrier to visualize tumor during its surgical treatment. In [29], MNPs combining MRI contrasting agent properties with a photodynamically controlled drug release carrier are designed. Those MNPs are based on graphene nanosheets functionalized by i) iron nanoparticles to provide superparamagnetic properties, ii) photosensitive fluorescent molecules that release atomic oxygen or free radicals upon light quantum absorption to affect the targeted cell and iii) hydrophilic ligands to facilitate MNPs dispersion in water solutions.

The other group of internal organ visualization techniques is based on thermoacoustic waves generation, i.e. fast pulsed localized heat generation causing acoustic waves generation. It is proposed to use f-MNPs in the regime similar to hyperthermia as a heat generation medium [30]. To achieve theoretical resolution about 1 mm one should use pulse rate about 1 MHz, MF frequency about 10 MHz and field intensity about 10 mT [31].

In the recent decade, an alternative method of MNP based visualization called magnetic particle imaging (MPI) has been developed [32]. It requires neither highly uniform MF nor satisfaction of the resonance condition and uses the magnetic moment of MNP itself as a main target of MF manipulations. MNP detection occurs due to high non-linearity of Langevin magnetization curve of superparamagnetic particles, so MNP ensemble being excited on arbitrary frequency, usually about several kHz, emits an RF signal on multiple frequencies. The parameters of this signal are directly related to media viscosity, and registration of MNP tethering to some large objects is also possible. On the other hand, it is possible to acquire information on MNPs themselves or their aggregation if they are located in the well known media for MNP characterization.

Such response signal is emitted only if external MF traverses zero point or at least approaches close enough to it, so the spatial resolution can be provided by applying an additional high gradient steady MF. Due to the ratio of MNP magnetic moment value μ to the one of proton being in the range of millions, MPI sensitivity could be better than MRI one. The MPI operation speed is much higher than for MRI too, since f-MNPs magnetic moment relaxation is provided by intermolecular viscous forces resulting in a relaxation time being in the range of microseconds, whereas the proton magnetic moment relaxation in MRI case utilizes magnetic mechanisms resulting in relaxation time about one second without a contrasting agent and a few tenth of a second with it. Another MPI advantage when MNPs are used as an agent for targeted drug delivery or other therapy techniques is the usage of those therapeutic MNPs for imaging, so that one can definitely obtain actual information on therapeutic MNPs localization and some data on environment properties of those MNPs. Thus, MPI is able to exceed other visualization techniques in some medical applications in the near future as summarized in the following table. IOP Conf. Series: Materials Science and Engineering 98 (2015) 012016 doi:10.1088/1757-899X/98/1/012016

Table. Comparison of different medical imaging methods [33].				
	Methods			
	X-Ray computer	Magnetic	Positron	Magnetic
Perfomance	tomography	resonance	emission	particle imaging
	(CT)	imaging (MRI)	tomography	(MPI)
			(PET)	
Spatial resolution, mm	0.5	1	4	< 1 ^a
Sensitivity	low	low	high	high
Measurement time, s	1	10-1000	100	<0.1 ^b
Ionizing radiation	yes	no	yes	no

^a Expectations based on calculations by Weizenecker et al. [34].

^b As measured by Weizenecker et al. [35].

3. Targeted drug delivery and cancer treatment by using f-MNPs in non-heating LF MF

In the set of papers on the targeted drug delivery and remote control of the therapeutic agent (TA) carriers AMF utilization for mechanical activation of f-MNPs was suggested [36-43]. In [44,45], the results of activity control of enzyme macromolecules through f-MNPs activated by uniform AMF with the frequency from a few tens to several hundred hertz were reported. Any heating was naturally absent at this sufficiently low AMF frequency (as monitored by sensors continuously). Instead of this, macromolecule deformation forced by f-MNPs brought into oscillating rotational motion by MF was the dominating mechanism.

In [46], the quantitative magneto-mechanical models of such single molecule actuation were described (Figure 3). According to the reported computational data, AMF with the frequency up to 1000 Hz and flux density up to 0.5 T easily obtainable in the laboratory environment can result in various kinds of deformations in the macromolecules being part of f-MNP aggregates. These deformations are sufficient to change the topology of macromolecules and, therefore, to control their biochemical properties. There are four types of such deformations: tension, compression, shear, and torsion. Not only macromolecules linking MNPs in aggregate, but also those in the polymeric coating of each f-MNP ever appearing between the particles undergo them. In [47], the estimation of the effect of f-MNPs in the external uniform LF MF on vesicle and live cell bilayer membrane structure was given. It was shown that in case of the vesicle MNP influence could result in their disruption or permeability increase. The effect may be applicable in targeted delivery of vesicle-encapsulated TA. With respect to cell membrane, f-MNPs can exert the force sufficient to cause specific responses from cell receptors as well as to disturb routine function of ionic channels and to change membrane structure, leading to metabolism disruption. This opens great opportunities for controlled and selective killing of malignant cells in cancer therapy. General regularities of such biostructure stimulation were also discovered, and physical approach to optimization of AMF application mode, including field parameters, size and shape of f-MNPs, was substantiated. The authors also made estimations that show several times increase in the rate of TA macromolecules washing-out from polymeric coating under the external low-frequency uniform AMF influence. The observed increase can also be useful in targeted TA delivery applications. The effect is conditioned by the force of hydrodynamic resistance of the medium to the particle's rotational oscillations. In spite of f-MNP oscillations, this force is always directed from the center of the particle.

It is necessary to emphasize that all above mentioned technologies based on magneto-mechanical actuation have strict frequency limit determined by f-MNP hydrodynamic radius and viscosity of surrounding media. Depending on the media type (water, physiological liquids, intracellular media etc.) and f-MNP geometry, this limit lies within the range from several hertz to several kilohertz, but anyway, it is several orders of magnitude less than one of hyperthermia. Therefore, to implement the nanomechanical conception of control on biological objects, the frequency providing for the minimal AMF power dissipation must be chosen, while in the hyperthermia applications, on the contrary, dissipation enhancement is of primary importance.

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Drug release from vesicle

Cancer cell apoptosis

Figure 3. The main routes of f-MNPs utilization in the low frequency magnetic field (LF MF) for targeted therapeutic agent (TA) delivery. Here μ is the magnetic moment of f-MNP, *L* is the torque applied to f-MNP, MM is the therapeutic agent macromolecule, and F_{MM} is magneto-mechanical force causing macromolecule deformation.

4. Conclusion

An innovative area of magnetic-bio-medical science employing f-MNPs activated by uniform nonheating LF MF is very promising for practical applications. This platform allows developing extremely versatile biomedical technologies to fulfill various functions: i) f-MNPs characterization, ii) diagnostics and visualization of the object's inner structure and f-MNPs distribution within, iii) all the major processes of targeted delivery and on-demand remote control of TA. These technologies can be used both separately and in combination with RF magnetic hyperthermia with magnetonanomechanical and thermal effects induced in any proportion to each other. Moreover, magnetonanomechanical actuation of transmembrane structures of malignant cells (receptors, ionic channels) can selectively trigger their apoptosis with further ablation without using any toxic drugs (non-drug therapy).

MPI in LF MF has a higher speed of operation which allows recording the processes dynamics with the speed higher than 30 frames/sec, a more rapid response and better space resolution than typical 1 mm resolution of MRI and the necessary equipment is significantly less expensive. The magnetic-nanomechanical approach in targeted drug delivery by means of LF MF also has a number of advantages in comparison with technically resembling RF hyperthermia: it can provide selectivity and localization at the nanometer-, instead of millimeter scale, its effective implementation involves safer LF MFs which need less powerful energy sources and have higher tissue penetrating abilities. Sanitary regulations allow using LF MF of much higher values than those of RF MF (approximately in inverse proportion to its frequency). The LF MF exposure dosage can be easily controlled helping to avoid overdosage and hazardous side effects. Finally, combination of methods for f-MNPs characterization, visualization, diagnostics and therapy by means of non-heating LF MF allows developing more versatile and less costly means for theranostics carried out within the same device.

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