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Nanosized Magnetic Powder on the Ground of Oxides in Medicine and Biology

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Abstract

A method is presented for producing nanosized oxide ferromagnetics from salt systems with the use of the mechanochemical activation and their fundamental properties are investigated. Results concerning the use of the oxide ferrimagnetics synthesized for the magnetofection of DNA, DNA/RNA sorption, microbial cells, proteins, and contrast agents for MRT diagnostics are reported. It has been found that nanoparticles are non-toxic; their distribution in internal organs and the decrease dynamics, the possibility of using them for medication transport have been demonstrated.

Key words: oxide nanoferrimagnetics, mechanochemical synthesis, adsorption onto nanoparticles, magnetofection

SYNTHESIS AND MAIN PROPERTIES OF NANOSIZED POWDERS OF SIMPLE CUBIC FERRITES

Nanosized powders of simple oxide cubic ferromagnetics (Fe_3O_4, $CoFe_2O_4$, $MnFe_2O_4$ *etc.*), zinc and tin oxides (SnO_2, ZnO) are of great interest for medicine and biology. They can be used to solve problems related to the separation and purification of biological substances, pharmacokinetic studies, targeted medication and gene delivery, contrast enhancement in magnetic resonance imaging, and so on.

Nanosized powders of simple oxide cubic ferrimagnetic – Fe_3O_4, $CoFe_2O_4$, $MnFe_2O_4$ and others, as well as composite $SnO_2 + Fe_3O_4$ have been synthesized by the method of the mechanochemical synthesis from salt systems at the Department of Structural Macrokinetics of the Tomsk Scientific Centre, SB RAS. The size thereof ranges from 3 to 15 nm, powders form loosely-coupled aggregates, and specific surface area is 120–190 m^2/g. The powders are crystalline, they are characterized by a high level of internal elastic microstrains, and they are "active". In case of reducing the size of a structural element from 10^5 to 2–15 nm the oxide ferrimagnetic acquires properties of spin glass with a high blocking temperature. Therewith the saturation magnetization and magnetic anisotropy constant are significantly reduced. The specific magnetization 20–26 $G \cdot cm^3/g$, effective anisotropy field is 520–2500 Oe.

Significant changes in the fundamental magnetic properties of ferrite nanopowders a compared with bulk samples are caused by a significant contribution of the surface anisotropy and magnetoelastic component. The most comprehensive description of the synthesis conditions and fundamental properties thereof are reported by the authors of [1–4].

STUDY THE TOXICITY OF NANOSIZED POWDERS FERRITES

Owing to the promising use of nanosized oxide powders synthesized in biology and medicine they were investigated, primarily, with respect to cytotoxicity. The first works of this kind were carried out *in vitro* at the Shumakov Federal Research Centre of Transplantology and Artificial Organs (Moscow), and demonstrated that the nanosized powders of Fe_3O_4 and $CoFe_2O_4$ are biocompatible and non-cytotoxic.

The toxicity of Fe_3O_4 nanopowder (10 mg/kg) when administered intravenously to male mice was studied at the Institute of Clinical Immunology of the SB RAMS (Novosibirsk) [5]. No visible signs of toxicity were found. The condition of hair-covering, mucous membranes, as well as food and water consumption did not exhibit any changes. The Fe_3O_4 distribution in mice was studied *in vivo*. After a single intravenous injection of the suspension to male mice in RPMI-1640 medium at a dose of 10 mg of Fe_3O_4/g of animal mass, a chemical analysis of iron in the liver, spleen, kidney, lung, brain and urine was performed in 1, 4, 6th days after the administration. The chemical analysis of iron content in the organs studied and biological fluids under investigation a day after the administration demonstrated that Fe_3O_4 is mainly accumulated in the liver (the iron content being more than 10 times higher comparing to the control group), in the spleen and in the lung (the content of iron is more than six times higher, relatively to the control group). Within the following days (at the 4th and 6th day) the level of iron in these organs gradually decreased. It has been found that the Fe_3O_4 coated with polyethylene glycol (PEG) is accu-

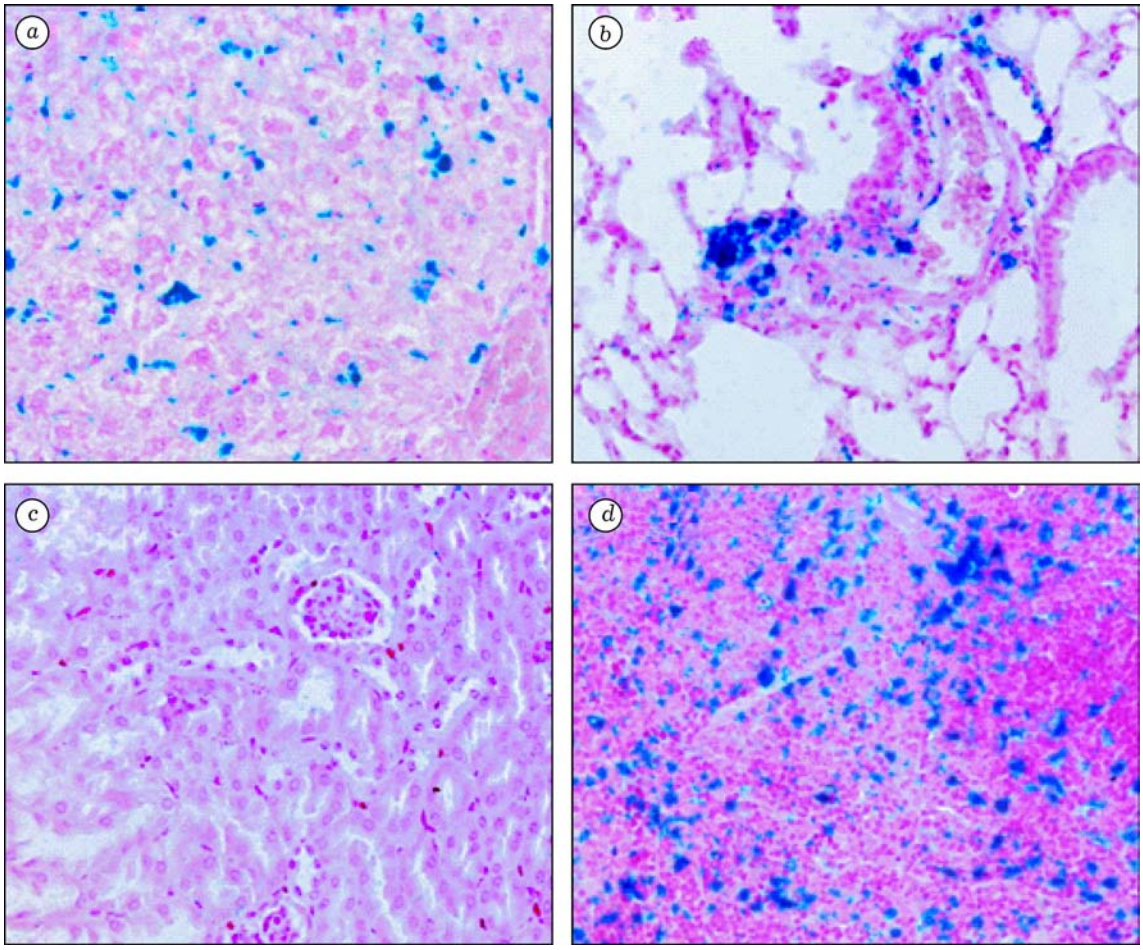

Fig. 1. Histological sections of mouse viscera with administered Fe_3O_4 nanoparticles: *a* – liver, *b* – lung, *c* – kidney, *d* – spleen. Magn. 400.

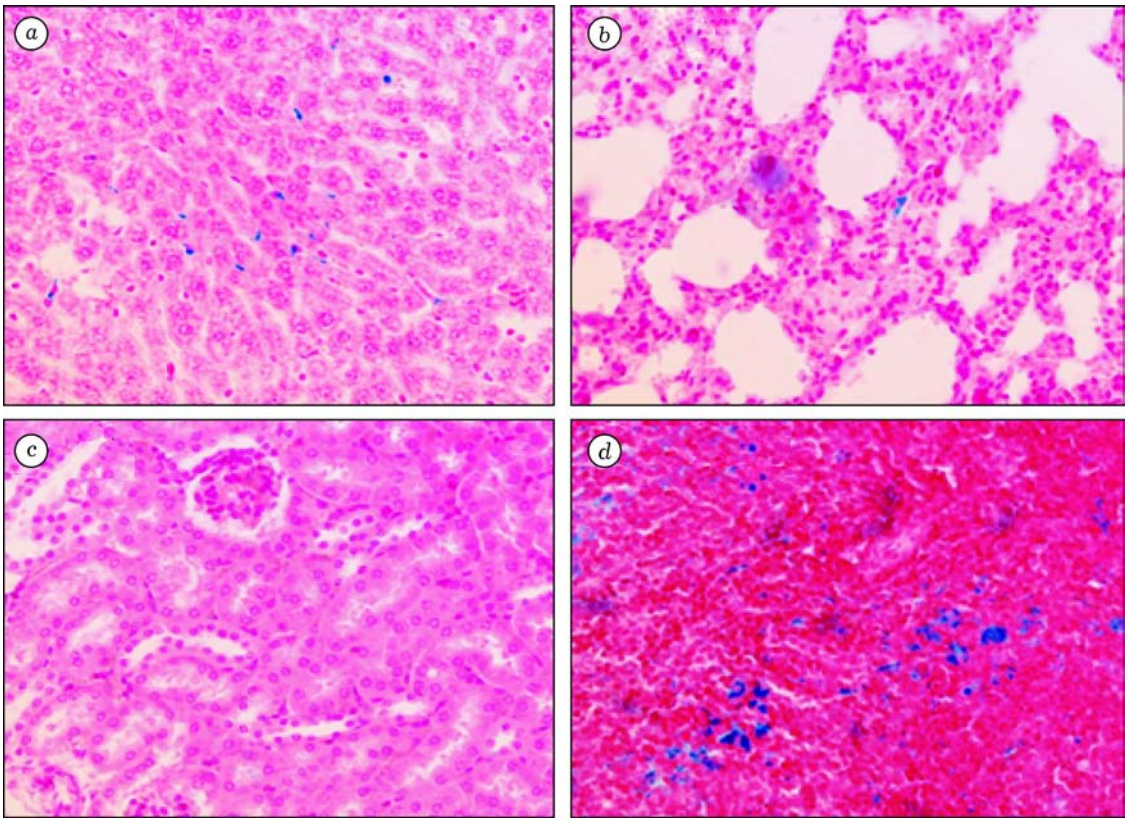

Fig. 2. Photomicrographs of histological sections of mouse viscera with administered Fe_3O_4 nanoparticles and with a magnet sewn in the thigh: *a* – liver, *b* – lung, *c* – kidney, *d* – spleen. Magn. 400.

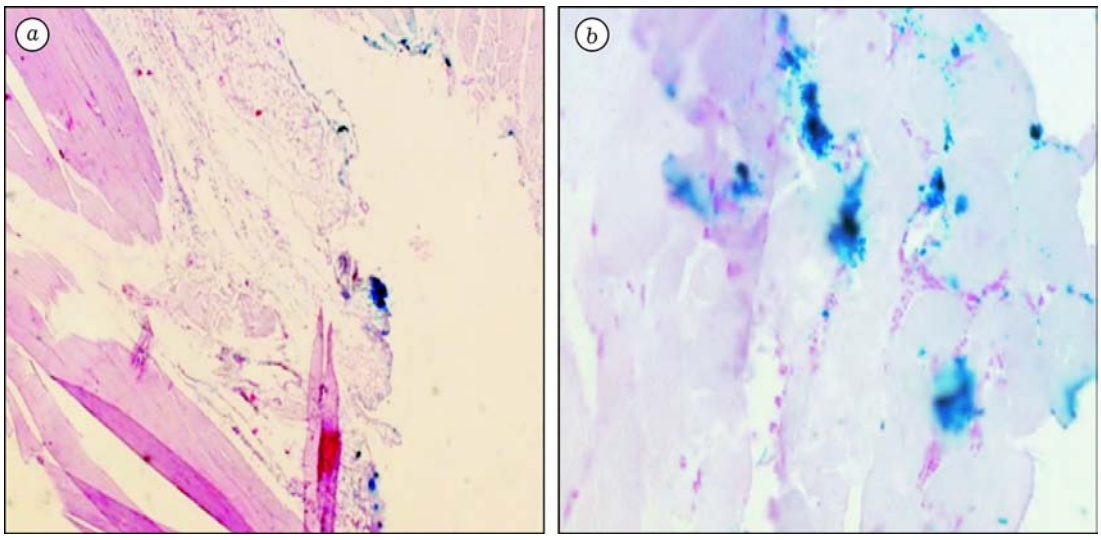

Fig. 3. Photomicrographs of histological sections of mouse thigh muscle with administered Fe_3O_4 nanoparticles, and with a magnet sewn in the thigh. Perls' staining reaction. Magn. 400.

mulated in these organs to a lesser extent as compared to pure Fe_3O_4. The content of iron did not change in the kidney, brain and in the urine of mice under investigation in the case of Fe_3O_4 administered intravenously. Data of to the chemical analysis confirm completely the results of the histological examination of viscera in mice intravenously injected with the suspension of Fe_3O_4. These studies demonstrate that Fe_3O_4 is accumulated mainly within the liver and spleen (Fig. 1).

Reducing the amount of nanoparticles in the viscera can be performed with the help of a magnet sewn in a mouse thigh (Figs. 2 and 3).

SORPTION PROPERTIES OF NANOSIZED FERRITE

Together with the Institute of Petroleum Chemistry of the SB RAS (Tomsk), we investigated the sorption of microorganisms under static and dynamic conditions. The results of several tests are presented in Tables 1 and 2.

It can be seen that the sorption of microorganisms with the use of nanoparticles under static conditions is much higher as compared to silica gel, whereas the use of nanopowders for the sorption under dynamic conditions allows obtaining almost sterile solutions.

We performed experiments concerning of microbial cells and petroleum in the course of sorption under dynamic conditions. It can be seen (Table 3) that both the nanosorbents under investigation exhibit a high adhesion. Introducing the nanosorbents in an oil-contaminated cultural medium provides a simultaneous adsorption of hydrocarbons and an adhesion of the bacterial cells of oil-assimilating microorganisms. Such a combination of the hydrocarbon substrate and the agents of its utilization in the one volume promotes the accumulation of the biomass and the stimulation of the oxygenase activity of microorganisms [6].

USING A NANOSIZED POWDER $SnO_2 + Fe_3O_4$ FOR THE SORPTION OF DNA/RNA

The studies were carried out in collaboration with the Siberian State Medical University (SibSMU, Tomsk). It is established that in the course of the polymerase chain reaction (PCR diagnostics), with the use of $SnO_2 + Fe_3O_4$ nanopowder as a sorbent, the sorption efficiency amounts to 98 %, the efficiency of desorption being equal to 100 %. Therewith, there are no inhibitory properties nanopowder observed with respect to the enzymatic reaction. Increasing the

TABLE 1

Sorption capacity of $SnO_2 + Fe_3O_4$ and $CoFe_2O_4$ sorbents under static conditions with respect to the microorganisms belonging to genus *Micrococcus* (the sorbent mass being equal to 1 g)

Sorbents	Number of microorganisms, million cells			Sorption level,
	in the original suspension	after sorption	sorbed cells	
$SnO_2 + Fe_3O_4$	102	0.012	101.988	99.9882
$CoFe_2O_4$	102	0.018	101.982	99.9824
Silica gel	102	0.360	101.640	99.6470

TABLE 2

Sorption of mobile cells belonging to genus *Bacillus* onto the surface of nanosorbents under dynamic conditions (the mass of the sorbent in a column being equal to 3 g)

Sorbents	Number of microorganisms, million cells		Sorption of microorganisms, million cells/g	Sorption level, %
	in the original suspension	after filtration		
$SnO_2 + Fe_3O_4$	890	0.0050	296.665	99.9994
$CoFe_2O_4$	890	0.0045	296.665	99.9995
Silica gel	890	0.0300	296.656	99.9662

TABLE 3

Adhesion of microbial cells and of polluting petroleum in the course of sorption under dynamic conditions with the use of nanosized ferrite powders

Bacteria, genus	Initial microbial suspension		Microbial suspension after filtration through a column with nanosorbent			
			$SnO_2 + Fe_3O_4$		$CoFe_2O_4$	
	Number of cells, million	Contamination by petroleum, %	Adhesion of microbial cells, %	Contamination by petroleum, %	Adhesion of microbial cells, %	Contamination by petroleum, %
Pseudomonas	102	2.0	99.999	0	100	0
Micrococcus	85	2.0	100	0	100	0

PCR efficiency and the yield of amplificate is observed, which could be connected with a stabilizing effect of nanopowders on the enzymes synthesizing nucleic acids [7]. The results of the studies are protected by a joint patent [8].

EFFECT OF FERRITE NANOPARTICLES ON THE ACTIVITY OF FERMENTS

We investigated an effect of the nanosized oxide powders of cubic ferromagnetics exerted on the stability and activity of the enzymes those are widely used in clinical diagnosis (PCR) and molecular biological studies (together with the SibSMU). It has been found that the enzymatic activity is retained at a room temperature for a long time due to the immobilization on the nanoparticles of cobalt ferrite spinel (Fig. 4).

So, after holding for 40 days the residual activity of the enzyme not sorbed by the nanoparticles does not exceed 3 % of the initial value, whereas the enzyme immobilized on nanoparticles retains at least 40 % of the initial activity during 250 days. The absence of a strong chemical bond between a nanoparticle and enzyme and the magnetic properties inherent in the nanoparticles allow, if necessary, removing the nanoparticles from the reaction mixture or from the enzymatic preparation using a method of magnetic separation [9].

USE OF FERRITE NANOPARTICLES FOR MAGNETOFECTION

Together with the Institute of Chemical Biology and Fundamental Medicine (ICBFM) of the SB RAS (Novosibirsk) we investigated the

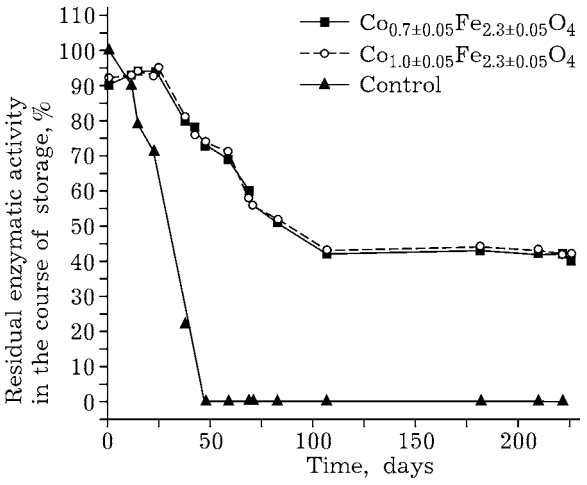

Fig. 4. Effect of cobalt ferrite nanoparticles on enzymatic activity.

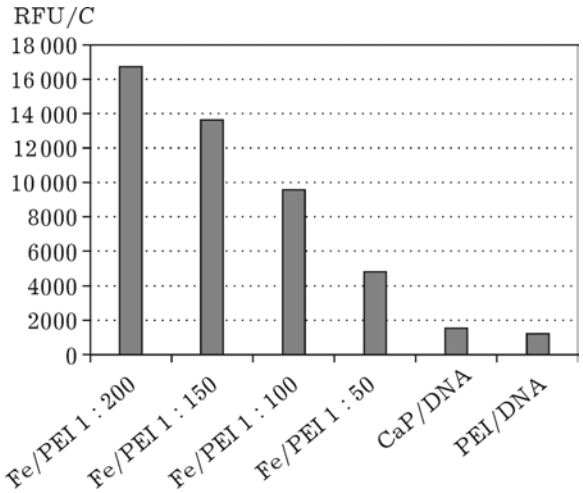

Fig. 5. Magnetofection efficiency depending on Fe_3O_4/PEI particle concentration as compared with CaP/DNA and PEI/DNA complexes.

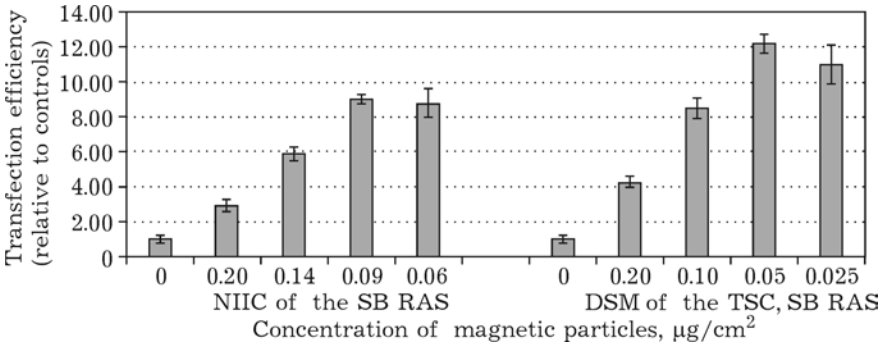

Fig. 6. Comparison of the magnetofection efficiency for two types of nanosized magnetite particles obtained *via* salt solution coprecipitation (the NIIC of the SB RAS) and *via* mechanochemical synthesis (the DSM of the TSC, SB RAS).

efficiency of using the nanoparticles of magnetite Fe_3O_4, and cobalt ferrite spinel $CoFe_2O_4$ as the carriers of DNA constructs into the cell culture *via* magnetofection (MF), *i. e.* the transfection under the action of magnetic field.

It has been found out that with using the identical doses of DNA, the result of magnetofection by magnetite particles is 3–12 times greater than the results of the transfection by calcium phosphate complex CaP/DNA and polyethyleneimine complex with DNA (PEI/DNA) (Fig. 5). It is known that, depending on the method of producing, the nanoparticles differ from each other in some characteristics, in particular, in the sorption and magnetic properties. In this regard, we studied experimentally the efficiency of using magnetite nanoparticles for magnetofection, which nanoparticles were obtained by means of mechanochemical method, and the nanoparticles, synthesized *via* chemical co-precipitation (Fig. 6).

The following tendency was revealed for both types of magnetite: the efficiency of magnetofection increases inversely proportional to the concentration of magnetic particles. After passing the point of optimum concentration, this trend is reversed. However, the maximum efficiency of magnetofection for magnetite particles obtained *via* coprecipitation, corresponds to the concentration value amounting to about 0.09 μg/cm^2, whereas for the particles prepared by mechanosynthesis this value is equal to 0.05 μg/cm^2. Therewith, at the point of the optimal concentration the mentioned value is 25 % higher in comparison with the particles of the first type [10].

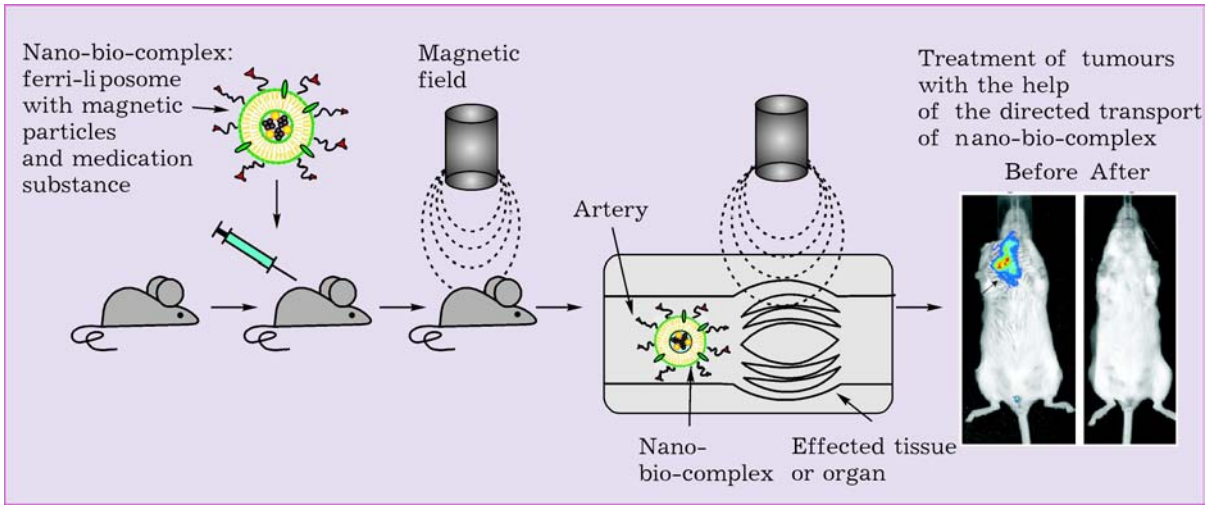

Fig. 7. Treatment of the mouse mammary tumour using directional transport.

USING A NANOSIZED MAGNETITE POWDER FOR THE TRANSPORT OF MEDICATIONS

Together with the Jozef Stefan Institute (Slovenia) we have developed a magnetic nanobiocomplex for a targeted transport of medications to the tumour. As containers for delivery the medications, there were liposomes chosen (Fig. 7). The liposomal nanobiocomplex contains magnetic nanoparticles Fe_3O_4 and a medication in the interior. With the help of a magnet, the ferri-liposomes can be delivered to an organ or tissue, wherein the neoplasm was detected; the ferri-liposomes penetrate into the tumour cells to dissolve and release the pharmaceutical preparation. Thus, there are no toxic effects on the organism as a whole.

Most comprehensively, the results are presented in [11].

CONTRAST AGENTS BASED ON NANOSIZED FERRITE POWDERS

Magnetic resonance imaging tomography (MRT) represents a modern method for the diagnostic imaging of various tissues, wherewith one can detect tumours, inflammation focuses, vascular pathology, and others. In the course of MRI, relaxation time values T1 and T2 are determined. The vast majority of contrast agents have properties inherent in the preparations those exhibits either T1 or T2 relaxation. It is urgent and important to develop contrast agents those could intensively affect both T1 and T2 values, influencing upon both types of relaxation simultaneously.

Together with the Jozef Stefan Institute we have developed a method for MRT contrast enhancement based on the nanosized particles

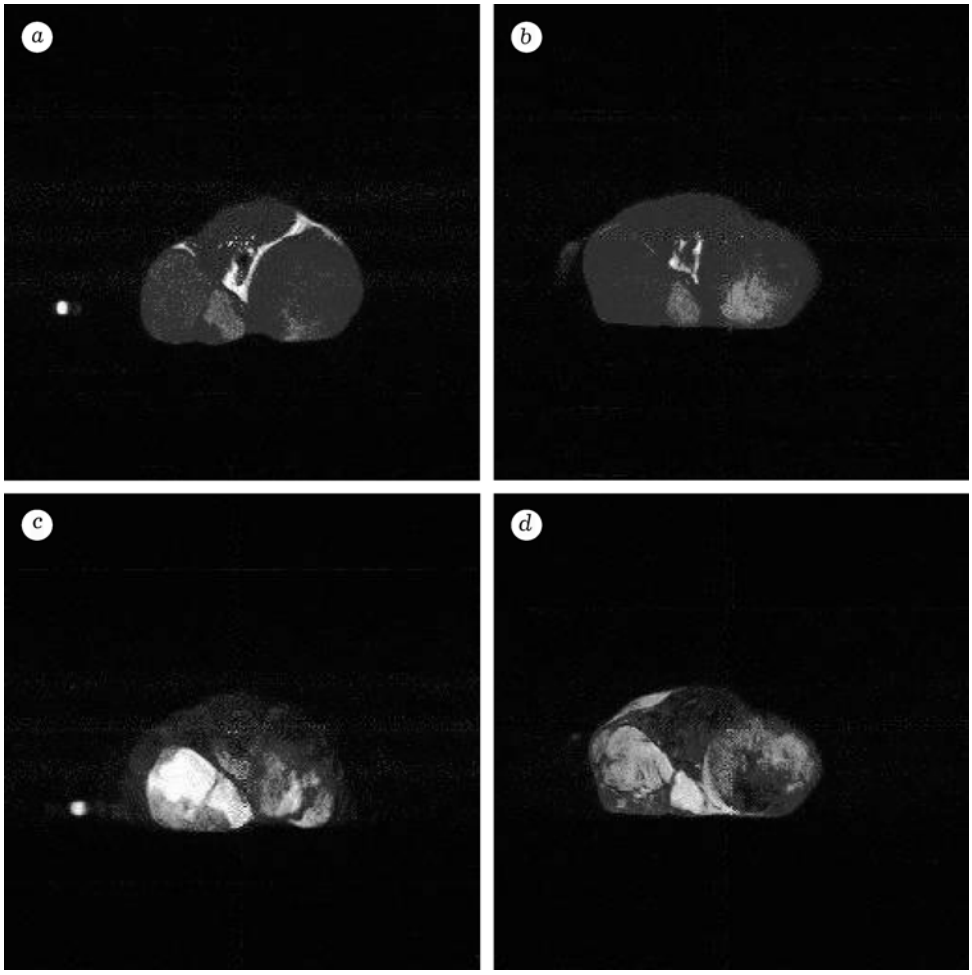

Fig. 8. Magnetic resonance imaging (*in vivo*) of targeted magnetoliposomes in a transplanted mammary tumour in mice: *a*, *b* – T1-MR scanning images; *c*, *d* – T2 MR scanning images.

of magnetite and cobalt ferrite spinel. The studies have demonstrated that the synthesized nanopowders of cobalt ferrite spinel and magnetite can simultaneously improve the relaxation time values T1 and T2 (Fig. 8). The results of the studies are protected by patent [12].

CONCLUSION

Nanosized oxide ferrimagnetics synthesized from salt systems using the method of mechanochemistry are non-toxic; they exhibit good sorption properties both with respect to DNA/RNA and with respect to a variety of microorganisms, and cause increasing the shelf life of enzymes. They can be used for DNA magnetofection into cells, as well as for delivering the medications and contrast agents in MRI. These positive properties of nanosized ferromagnetics open up the prospects for using thereof in biology and medicine.

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