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Targeted Transport of Drugs by Iron Oxide Nanoparticles

N. L. Shimanovskii

Pirogov National Research University, ul. Ostrovityanova 1, Moscow, 117997 Russia
e-mail: shiman@rsmu.ru

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Abstract—Iron oxide nanoparticles are paramagnetic, and, therefore, they can be used as magnetic resonance (MR) contrast agents in medicine. To this end, a supermagnetic iron oxide SPIO and a superparamagnetic iron oxide USPIO were synthesized as ultramicroparticles, as well as iron oxide MIOH as single crystals.

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INTRODUCTION

The SPIO particles are magnetized when exposed to external magnetic field and exhibit no magnetic properties when the field is removed. Superparamagnetism usually arises in a 10–20-nm particle. Such a nanoparticle contains only one magnetic domene, and it can be considered as a single superspin which possesses a high magnetic susceptibility. Therefore, SPIO nanoparticles give a stronger and faster magnetic response to magnetic fields than bulk magnets with a weak residual magnetization [1, 2].

Iron oxide nanoparticles can present interest for medicine not only as MR contrast agents. The can be used as vehicles to deliver drugs, because these nanoparticles can be accumulated in a target part of the body under external magnetic field. When the field is removed, the nanoparticles are no longer accumulated in one place and rapidly distributed over all organs, after which they metabolize and removed from the body.

The present review analyzes the possible uses of iron oxide nanoparticles as carriers for chemo-therapeutic agents or radioactive isotopes for cancer therapy, as well as for delivery of genes into cells and delivery of peptides and antibodies to their sites of action.

Physicochemical Properties of Organotropic Iron Oxide Nanoparticles

The morphology of iron oxide nanoparticles is determined by the conditions of their formation and the nature of surfactants used in the synthesis. Relatively large surfactants, such as oleylamine acid or adamantane, much affect, for steric reasons, the shape of the synthesized iron oxide crystals [3]. It was found that rod-shaped or nonspherical nanoparticles longer circulated in blood than spherical particles [4]. Spherical nanoparticles can be faster excreted with urine, whereas rod-shaped particles are more efficiently captured by macrophages [5]. Furthermore, the shape of particles may determine their toxicity. As shown, spherical and warm-shaped iron oxide nanoparticles exhibit a higher cell toxicity than rod-shaped nanoparticles or nanocrystal clusters [6].

The size of nanoparticles is an essential factor of their half-life in the blood stream [7]. For example, particles less than 10 nm in size are largely eliminated via kidney, whereas particles larger than 200 nm are accumulated in the spleen or captured by phagocytes. In both cases, the plasma level of nanoparticles rapidly decreases. Nanoparticles with a size ranging from 10 to 100 nm longer circulate in the blood stream and slower absorbed by the reticuloendothelial system. They can also penetrate very small capillaries [8]. The magnetic properties of iron oxide nanoparticles, too, are size-dependent, and this, in its turn, determines their contrasting power in MR therapy, as well as their ability for magnetic hyperthermia and magnetic cell separation [7]. The ability of nanoparticles to penetrate tumor tissue and to be retained in it is also size-dependent. Iron oxide nanoparticles smaller than 2 nm are unsuitable for medical applications because of their ability to penetrate cell membranes, which may entail damage of intracellular organelles. Thus, it is quite important to control the size of iron oxide nanoparticles during their synthesis.

The size of nanoparticles is measured by means of transmission electron microscopy (TEM), dynamic

light scattering, and X-ray diffractometry. The synthesized nanoparticles are most commonly inhomogeneous and vary in size. Therefore, not infrequently one should resort to fractionation techniques: centrifugation and exclusion chromatography [9–11].

The surface charge of nanoparticles determines stability of their colloid solution. Nanoparticles with high positive or negative ζ-potentials form stable solutions and can be stored for a long time. The charge of nanoparticles is also responsible for their capacity to be internalized by target cells [12].

Positively charged iron oxide nanoparticles are stronger accumulated in human breast cancer cells than negatively charged ones. At the same time, the absorption level of nanoparticles depends on the type of cells [13]. Particles with a hydrophobic surface are readily adsorbed on protein surface (opsonization) and absorbed by circulating macrophages [14], thus being rapidly cleared from blood plasma. Particles coated by hydrophilic polymers, such as poly(ethylene glycol) (PEG), become invisible (stealth particles) for reticuloendothelial cells or circulateng macrophages, which enhances their therapeutic efficiency due to prolonged blood half-life [15]. The molecular surface modification of magnetic nanoparticles by various compounds having functional groups, impart new properties to such particles and extends range of their potential biomedical and industrial applications.

Of importance is also the oxidation degree of iron ions on the surface of nanoparticles. Thus, trivalent iron ions favor formation of spherical nanoparticles, whereas bivalent metal ions favor nanorod formation [16].

In the course of receptor-mediated endocytosis nanoparticles conjugate with various ligands, including monoclonal antibodies [17]. Receptors of such proteins as transferrin, lacroferrin, albumin, insulin, and growth factors are expressed on the surface of mammal cells, and this fact explains why just those proteins are used as target molecules for targeted transport of nanoparticles [18, 19]. In particular, iron oxide nanoparticles conjugates with lactoferrin, transferrin, and ceruloplasmin were synthesized [20–22]. The lactoferrin, ceruloplasmin, and insulin conjugates smaller than 20 nm are highly magnetized. Nanoparticles of different sized differently interact with cells (see fugure). Unconjugated iron oxide nanoparticles are internalized by fibroblasts, which entails damage of the cell memebrane and disorganization of the cell cytoskeleton. At the same time, nanoparticles with conjugated proteins attach to cells but undergo no apparent endocytosis.

The bioavailability of iron oxide nanoparticles with a dextran coating is presumed to be associated with the fact that dextran is similar to the glycocalix (cell coat) [24].

Having a high surface area-to-volume ratio, iron oxide nanoparticles show tendency for agglomeration and sorption of blood proteins. As mentioned above, noncoated nanoparticles have a hydrophobic surface, and, therefore, they are rapidly absorbed by the reticuloendothelial system, mostly Kupffer liver cells.

The coating of nanoparticles by amphiphilic polymers, such as PEG, minimizes their ability to absorb proteins and prolongs the circulation time [25]. Inductively coupled plasma emission spectroscopy was used to show that PEGylated iron oxide nanoparticles are less absorbed by mouse macrophages than unmodified nanoparticles. However, modification facilitates nanoparticle absorption by breast cancer cells (BT20). The possible absorption mechanism is associated with the fact that PEG, due to its solubility in both polar and nonpolar solvents, is readily soluble in cell membranes [26].

The optimal size of iron oxide nanoparticles for intravenous injection and prolonged circulation in the blood stream is between 10 and 100 nm [27].

The physical properties and crystal chemical parameters of iron oxide nanoparticles depend on the method of their synthesis [28]. Table 1 summarizes the methods of synthesis of iron oxide nanoparticles with specification of their advantages and disadvantages.

Iron Oxide Nanoparticles as Drug Carriers

Iron oxide nanoparticles can be used as drug carriers only if they do not affect the functional characteristics of the drugs and ensure drug release in a target site of the body. Of particular importance for bioavailability of nanoparticles is their coating. Selected coating materials are presented in Table 2.

Loading iron oxide nanoparticles can be performed either by conjugation of drug molecules with the nanoparticle surface or by their incorporation into the coating material.

The conjugation of ligands with the surface of polymer-coated nanoparticles can involve covalent binding via the surface amino or hydroxy functional

groups of the nanoparticle coating. Furthermore, the covalent binding can also involve such groups as iodolactyl or maleimide, as well as bifunctional compounds like pyridyl disulfide. Such an approach to binding with nanoparticles is expedient in the case of peptide and protein drug substances which are sensitive to oxidative degradation [14].

Other drugs can bind to the surface of iron oxide nanoparticles due to reversible interactions, like electrostatic or hydrophobic. Nanoparticles coated with a cationic polymer poly(ethyleme imine) enter electrostatic interactions with a negatively charged DNA molecule, which implies their use for transfection (introduction of nucleic acids into cells) [75]. Analogously, nanoparticles coated with dextran having negatively charged groups can bind peptides via electrostatic interactions [89]. The hydrophobic parts of peptides can bind lipophilic ligands. These ligands can be released on degradation of such complexes. Bioconjugation can be provided by a high affinity between certain molecules, for example, between the protein streptavidin and the vitamin biotin. Unlike electrostatic and hydrophobic interactions, binding due to a high affinity between partner molecules giver rise to the most stable noncovalent complexes, whose existence is scarcely dependent on the medium (pH and ionic strength).

For drug delivery systems on the basis of iron oxide nanoparticles to work in an optimal way, binding between their surface and a drug to be delivered should not be either too weak (in this case, only a little drug can be absorbed and transported) or too strong (a covalent bond may prevent drug release in a target site of the body). Catalysts, like copper, used to form a nanoparticle surface–drug bond, may cause toxic effects if not removed. In certain cases, the orientation of ligands on their binding with the surface of magnetic nanoparticles may prove difficult to control. For example, Veiseh et al. [14] observed inactivation of ligands on binding of iron oxide nanoparticles containing surface carboxy groups with ligands having several amino groups.

The second methods of loading drug molecules in iron oxide nanoparticles consists, as mentioned above, in the incorporation of ligands into particles during synthesis of the latter, which allows one to go around problems associated with a low efficiency of binding and a low stability of complexes. Highly efficient loading of particles with ligands and a high stability of loaded iron oxide nanoparticles with preserved magnetic properties can be provided by means of magnetoliposomes [90].

Magnetoliposomes are nanosized spherical formations comprising magnetic nanoparticles with a shell of phospholipid bilayer membranes. Magnetolipyosomes can encapsulate both hydrophilic and hydrophobic ligands. Magnetoliposomes as drug carriers offer a number of advantages. First, the surface of liposomes

Table 1. Methods of fabrication of iron nanoparticles

Method	Reaction temperature, °C	Solvent	Particle size range, nm	Shape
Coprecipitation	20–90	Water	15–200	Spherical or rhombic
Microemulsification	20–50	Organic	4–12	Spherical or cubic
Hydrothermal synthesis	220–220	Water/ethanol	520	Spherical
Sol-gel synthesis	200–400	Organic	20–200	Spherical
Electrochemical deposition	70–100	Organic	3–8	Spherical
Sonochemical synthesis	25	Water	10–30	Spherical or rod-like
With the use of polyols	120–280	Organic	5–40	Spherical
Thermolysis	100–320	Organic	3–20	Spherical
Spray pyrolysis	400–700	Organic	5–60	Spherical, aggregation into coarser particles
Laser-induced pyrolysis	1100	Organic	5–30	Spherical with minor aggregation
Biomimetic synthesis (bacteria- or fungus-mediated)	–	–	50–100	Spherical, cluster, cubic octahedral

Table 1. (Contd.)

Advantages of method	Disadvantages of methods	Possibility to fabricate variable-size particles	References
The simplest efficient method. Control of particle size by varying pH and ionic strength. The method can be modified for synthesis in the presence of dextran and other materials which impart bioavailability in *in vivo*. Almost all commercially available iron oxide preparations for MR imaging are produced by this method.	Large particles size distribution range. Aggregation and crystallization may reduce particle.	Yes	[29–32]
Adequate, universal, and simple method. Surfactant stabilization allows particle size control. Uniform particle size distribution.	Adequate nanoparticle crystallization is hard to provide because of the low temperature. Complicated purification from surfactants. The yield of nanoparticles is lower than in the co-precipitation procedure. High solvent consumption.	No	[29, 31, 33, 34]
The solubility and reactivity of metal salts and complexes increases with temperature and pressure. Control of the growth and agglomeration dynamics of metal nanoparticles. Synthesis of ferrites.	Rigid reaction conditions. Undesirable oxidation of metal nanoparticle surface. Long reaction time.	Yes	[31–33, 35]
Suitable for preparation of powders of magnetic materials. Synthesis of particles of the desired shape and size, which is useful for synthesis of hybrid nanoparticles	Monodisperse nanoparticles are hard to obtain. Toxic reagents, complicated synthesis. Wide particle size distribution.	No	[32, 36]
Particle size control by current density. Uniform particle size distribution	High cost of synthesis. Complicated purification from surfactants.	No	[29, 37]
Synthesis of monodisperse nanoparticles of various shapes. The nanoparticles are useful for bioengineering.	Complicated control of particle size. Organometallic precursors may enhance *in vivo* toxicity.	No	[31, 38, 39]
Nanoagglomeration of iron oxide nanoparticles of definite shape and size. High-boiling polyols can be used as solvents and reducers.	Increased particle size may result in nonuniform shape and size. Wide particle size distribution.	Yes	[40–43]
Control of particle size (± 5%) and crystallization process. Easy synthesis of monodisperse nanoparticles. The use of polar solvents (pyrrolidin-2-one), allows, along with thermolysis, various ligands to be involved to impart biocompatibility to nanoparticle surface	Rigid synthesis conditions. The surfactants (specifically, oleic acid) used in the process hinder subsequent surface modification of nanoparticles. Organometallic reagents enhancing *in vivo* toxicity are replaced by metal salts. Heating to very high temperatures leads to formation of iron oxide on nanoparticle surface	Yes	[31–33, 35, 43– 45]
Simple, fast, and continuous synthesis of fine particles with predictable size, shape, and composition. Stable superparamagnetic a-Fe and FeCo nanoparticles incorporated in silica or alumina particles	Careful control of synthesis conditions. High cost of synthesis.	Yes	[29, 31, 46]
Continuous synthesis of small particles. Narrow particle size distribution. Scarce aggregation. High efficiency, alternative to co-precipitation for synthesis of MR contrast agents	High production cost. Careful control of synthesis conditions	Yes	[29, 31]
Wide range of applications of the synthesized nanoparticles. Biocompatibility and stability of particles due to the phospholipid bilayer. Possible modification of particles. Possible use of magnetic particles for immobilization of enzymes, antibodies, and oligonuclotides	Lack of shape and size control. Low yield of magnetic nanoparticles	No	[13, 29]

Table 2. Coating materials for iron oxide nanoparticles, ligands for their surface modification, and possible uses

Coating material	Nature of tissue accumulation	Target molecule	Target tissue	Particle size
Polylactic acid	Active	Herceptin (HER2 antibodies)	Human breast cancer	50–200 nm
		Anti-CD20 monoclonal antibodies (rituximabum)	CD-20 antigen (non-Hodgkin lymphoma)	
	Passive	–	–	
Poly(ethylene glycol)	Active	N-truncated A10 RNA aptamer	PSA	10–50 nm
		Human anti-VCAM-1 antibodies	VCAM-1 as marker of early atherosclerosis	
		Chlorotoxin	MMP-2	
		Folium acid	Folium acid receptor	
		Methotrexate	Folium acid receptor	
	Passive	–	–	
Dextran	Active	Monoclonal antibodies A7	Human colorectal cancer	10–200 nm
		Herceptin	Human breast cancer	
	Passive	–	–	
Chitosan	Active	ANP antibodies	ANP receptors	20–100 nm
		CEA antibodies	CEA	
	Magnetic targeting	–	–	
Silicon dioxide	Active	Annexin V	Phosphatidyl serine	10–300 nm
	Passive	–	–	
Silane	Active	Methotrexate	Folium acid receptor	10–200 nm
		Arg-Gly-Asp (RGD) Peptide	Integrin a V b 3	
	Passive	–	–	
Glycerol monooleate	Active	Herceptin	Her2/neu receptors (breast cancer)	100–200 nm
Albumin	Active	Anti-EGFR antibodies	EGFR	100–200 nm
	Passive	–	–	
Liposomes	Active	Antibodies to transferrin receptors (single-stranded fragments)	Transferrin receptor	20–200 nm
Gold	Magnetic targeting	–	–	30–100 nm

including magnetic particles can be modified by target molecules, which ensures their delivery to a target site of the body. Second, magnetoliposomes with a high content of nanoparticles can, possessing magnetic susceptibility, can be retained for a long time in the required sites of the body, for example, in tumors. Third, the encapsulation in liposomes endows iron oxide nanoparticles with a better bioavailability. Fourth, the liposomal barrier protects the encapsulated drugs from the environment and metabolic degradation [91].

Nanoparticles iron oxide can be loaded into liposomes in two ways: by incubation of prepared liposomes and nanoparticles under external exposure [92, 93] or by directly embedding nanoparticles into the hydrophilic core of liposomes, which ensures a high degree of uniformity of nanoparticles with a size of about 15 nm [67]. However, the phospholipid shell of iron oxide nanoparticles may adversely affect their magnetic and physicochemical properties. On exposure to a strong magnetic field, drugs release from

Table 2. (Contd.)

Properties of modified particles	Application	Advantages of coated particles	References
Improved biocompatibility, lack of hemolytic activity, uniformity	Radiotherapy, drug imaging and delivery	Biodegradability Biodegradability, possibility of conjugation with other polymers (PEG) with desired variation of magnetic properties	[47, 48–50]
Improved dispersiveness and circulation time, ensures drug internalization, reduced toxicity, reduced enzyme inactivation	Imaging and drug targeting to tumors (brain cancer, breast cancer)	Efficient surface coating, easy drug internalization, reduced drug cleavage	[8, 13, 51–53]
Enhanced biocompatibility, prolonged circulation time, reduced aggregation	Drug imaging and delivery. Conjugation with urokinase for targeted thrombolysis	High affinity of iron oxide surface due to coating	[13, 54, 55]
Enhanced biocompatibility, prolonged circulation time, reduced aggregation	Drug imaging and delivery. Conjugation with urokinase for targeted thrombolysis	High affinity of iron oxide surface due to coating	[13, 54, 55]
Enhanced biocompatibility. Easy functionalization due to amino and hydroxy functional groups	Nonviral drug delivery, drug therapy, hyperthermia, tissue engineering, targeted photodynamic therapy	Abundant raw materials, low cost and accessibility of the method	[13, 56, 57]
Provides immobilization of enzymes, platform for radical polymerization with other molecules	Controlled drug delivery, imaging, separation of biomolecules, imaging of apoptosis	Large surface area, no organic , solvent are involved, which reduces toxicity	[13, 58]
Increased magnetization, reduced degradation rate of coated particles	Imaging of breast cancer and drug delivery, assessment of tumor blood vessels. Magnetic separation and purification of DNA	Accelerates penetration into biological matrix, can much improve protein immobilization	[52, 59–63]
Enhanced biocompatibility, particles can be functionalized by other compounds	Stable release of encapsulated drugs, ability to deliver highly hydrophobic antitumor drugs	Does not affect the magnetic properties of Fe_2O_3, high capture efficiency, can reduce drug cleavage	[64, 65]
Enhanced biocompatibility, reduced toxicity, drug stabilization	Drug delivery and cell separation, imaging of esophageal squamous cell cancer	Does not affect cell proliferation	[66]
Prolonged circulation time, enhanced specificity of nanoparticles and their uptake by tumor cells, coating prevents enzymatic degradation of encapsulated drugs	Use in gene medicine, drug delivery, hyperthermia, and imaging	Simple and easy surface modification. Increased drug load does not decrease the superparamagnetic properties of nanoparticles	[14, 67, 68]
Increased colloid stability, reduced surface toxicity, strong binding to the surface of the self-organizing enzyme monolayer	Magnet-mediated targeted enzyme therapy, nitroreductase delivery	Strong magnetic response, uniform coating. Protection of iron oxide core from oxidation, excellent optic properties	[69–71]
Lack of particle aggregation, reduced toxicity, coating provides chemical functionalization	Drug delivery, especially to brain, contrasting and imaging *in vivo*	Aminopolyvinyl alcohol is highly efficiently absorbed by cells	[72–74]
Colloid stability at high salt concentrations, high density of positive charge	Delivery of nonviral genes, magnetofection	Efficient and rapid delivery of genetic material, exibits proton sponge effect	[75–-77]

Table 2. (Contd.)

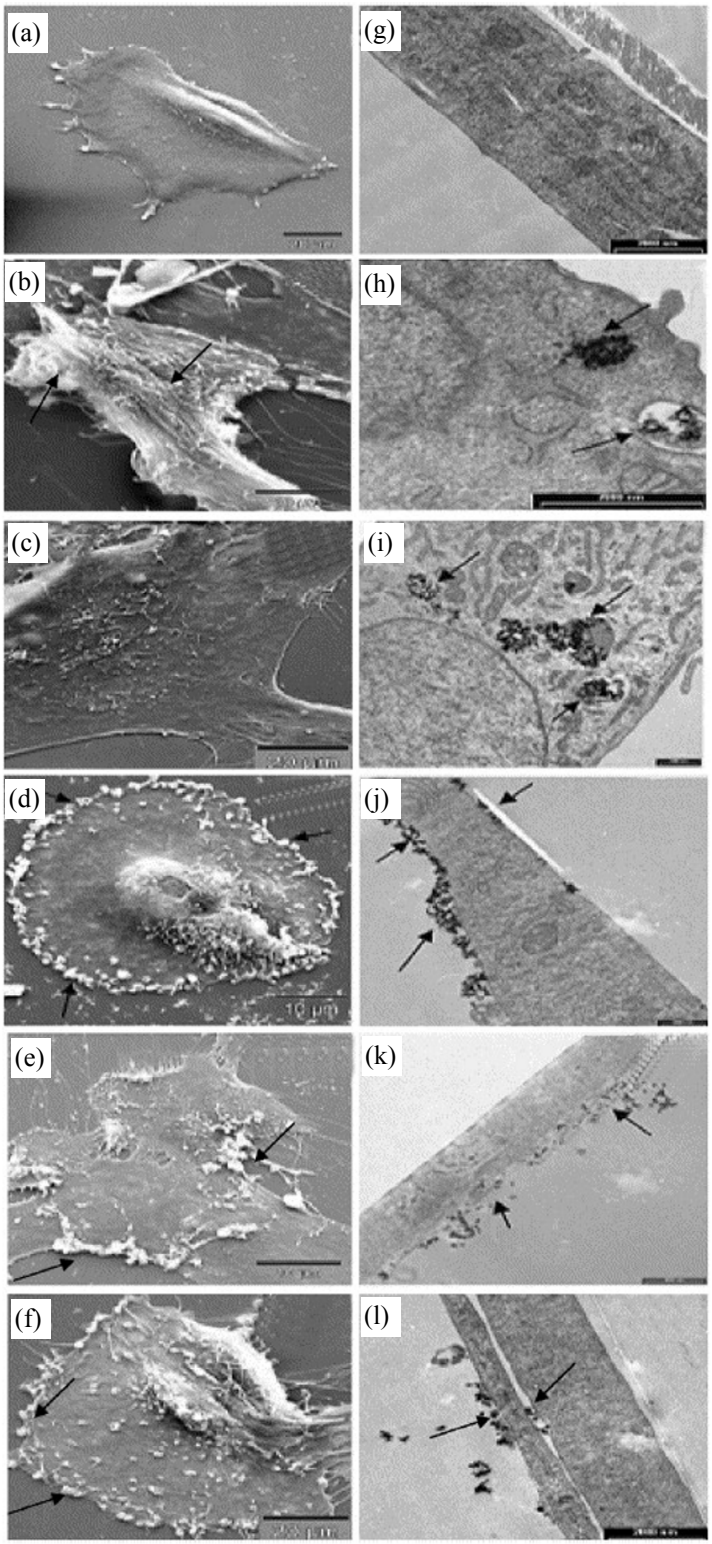
Properties of modified particles	Application	Advantages of coated particles	References
Biocompatibility, coating favors longer circulation, protects from fast absorption by the reticuloendothelial system	Drug targeting, cell separation, intravascular imaging and contrasting	Easy accessibility, biocompatibility, lack of immunogenicity	[78]
Hydrophilicity and biocompatibility, easy functionalization with drugs due to a great number of amino groups	DNA isolation, drug targeting	Natural polymer, efficient drug loading	[79, 80]
Enhanced colloid stability, prolonged circulation	Drug delivery, imaging, and contrasting	Uniform coating, efficient drug loading	[81]
Coating facilitates isolation of genetic material and amplification	Purification of mRNA/DNA. Isolation of specific gene sequences. Immunomagnetic analysis	Simple and easy in implementation, can be automated	[47, 82]
Colloid stability	Stable drug release, arterial chemoembolization	Maintaining drug concentration in a required body site for a long time, particles preserve sufficient magnetic susceptibility	[47, 83]
Reduced cytotoxicity, enhanced cellular uptake, reduced aggregation	Imaging of vessels, perfusion of lymph nodes, receptors, and specific targets	Easy synthesis, highly efficient uptake	[12]
Coating provides uniform distribution and reduced aggregation, improved biocompatibility with synovial tissues	Intravascular treatment of inflammatory diseases (arthritis, osteoarthritis)	Adequate magnetic retention under magnetic field	[84–86]
Improved biocompatibility, reduced aggregation	Imaging and contrasting, radiotherapy	Natural polymer, can be modified by other polymers, such as PEG	[87, 88]

magnetoliposomes because of the equalization of nanoparticle concentration in the bilayer and disruption of the membranes exclusively in a target tissue.

One more class of nanocomposites with iron oxide nanoparticles include magnetodendrimers which are suitable for visualization and tracking of cell migration by MR therapy [94–96]. Carboxylated polyamidoamine dendrimers can be used for coating of iron oxide nanoparticles and stabilization of their suspensions [97]. Lamanna et al. [98] developed a method for preparation of dendronized iron oxide nanoparticles with a hydrodynamic diameter smaller than 100 nm. The presence in the dendrimer of carboxylate or ammonium groups makes possible their labeling by a fluorescent dye and visualization by MR therapy and fluorescence microscopy [98].

Application of Iron Oxide Nanoparticles in Targeted Cancer Therapies

Chemotherapeutic agents are known to cause numerous side effects because of nonselective action. The function of iron oxide nanoparticles is to deliver antitumor agents selectively to cells and spare healthy cells. Therewith, a possibility arises to reduce the doses of chemotherapeutic drugs, because they are delivered directly to target cells. For better pharmacological properties a number of anticancer drugs (paclitaxel, methotrexate, mitoxantrone, doxorubicin)



Micrographs of human fibroblasts: (a, g) control and after incubation with (b, h) uncoated iron oxide nanoparticles (UIONs), (c, i) pegylated UIONs, (d, j) UIONs conjugated with lactoferrine, (e, k) ceruloplasmin, and (f, l) insulin. Uncoated iron oxide nanoparticles form vacuoles in cells and damage cell membranes; pegylated UIONs are internalized in great amounts and cause no toxic effects; protein-conjugated UIONs undergo no endocytosis and attach to cell membranes (arrows) [23].

were included in the composition of magnetic [99, 100]. Iron oxide nanoparticles coated by such polymers as amino polyvinyl alcohol and pullulan (a polysaccharide polymer) demonstrate a stronger capacity to interact with human cancer cells and exhibit a weaker cytotoxic effect on healthy cells [12, 72]. Enhanced retention in cancer cells was also observed in the case of PEG-modified cross-linked starch coated iron oxide nanoparticles [87]. In view of the fact that surface modification of iron oxide nanoparticles can much reduce their superparamagnetic properties, Yang et al. [68] proposed to encapsulate drug-loaded nanoparticles in cationic liposomes. Such nanocomposites were functionalized by single-stranded fragments of antibodies to transferrin receptors which are expressed by tumor cells. Nanoimmunoliposomes can efficiently deliver iron oxide nanoparticles to tumor cells on systemic administration.

Quite promising results in certain therapies were obtained with paramagnetic iron oxide nanoparticles and magnetoliposomes which served as a kind of magnetically controlled containers for targeting and immobilization of biologically active agents. Such delivery systems are of particular importance for diagnosis and treatment of oncological diseases. As a rule, tumor cells contain surface antigens which differ from antigens of normal cells of the same type. Owing to this difference, nanoparticles functionalized by antibodies to such tumor antigens bind exclusively to tumor cells. Antitumor drugs or radionuclides can be loaded in magnetic targeted carriers. Such magnetic complexes as magnetic fluids are injected into an artery supplying blood to an injured organ or tumor, or, if possible, are injected directly into tumor. Further on the target site is exposed to external magnetic field with strictly defined parameters. As a result, due to their affinity to surface tumor antogens, iron oxide nanoparticles are localized on the surface of tumor cells.

Iron oxide nanoparticles can be used to reduce multiple drug resistance of tumor cells which characteristically produce hyperexpression of ATP-dependent carriers tending to pump out a great fraction of hydrophobic chemotherapeutic agents from cancer cells. In one of the experiments, doxorubicin was covalently conjugated to polyethyleneimine by a pH-sensitive hydrazone bond with the terminal amino groups of the PEG coating of nanoparticles. Such doxorubicin-loaded nanoparticles were accumulated in cells resistant to chemotherapeutics due to the fact that doxorubicin was not pumped out from tumor cells with multiple drug resistance [101].

Wang et al. [102] developed multifunctional iron oxide nanoparticles suitable for hyperthermia, MR therapy, and drug targeting. These nanoparticles were synthesized by the precipitation of iron salts in the presence of aqueous ammonia, and the resulting nanoparticles were coated with β-cyclodextrin and a pluronic polymer. Thus stable nanoparticles were obtained, which were readily captured by cells, where they release the encapsulated anticancer agent curcumin. The nanoparticles showed contrasting activity in MR therapy and efficiency in cancer therapy. In view of their high dispersity, magnetic nanoparticles are heated when exposed to alternating magnetic field, and, therefore, they proved to be good candidates for local hyperthermia of tumor cells. Moreover, such nanoparticles exhibit good haemocompatibility, which makes them an excellent tool for drug targeting [103].

Yang et al. [104] obtained multifunctional warm-shaped vesicles loaded simultaneously with iron oxide nanoparticles and doxorubicin, as candidates for targeted cancer therapy and MR imaging. These stable warm-shaped vesicles were synthesized by the double emulsion technique from three heterofunctional polymers. The outer surface of these polymer vesicles is formed by the hydrophilic PEG chains conjugated with methoxyfolic acid which is responsible for targeted delivery to tumor tissue. In inner surface of vesicles is formed by short hydrophilic PEG fragments bearing acrylate groups, cross-linked via free radical polymerization for enhanced in vivo stability. In these vesicles, iron oxide nanoparticles reside in the inner aqueous medium at a concentration of 48.0 wt %, whereas doxorubicin, being a hydrophobic substance, resides in the hydrophobic membrane of the warm-shaped vesicles at a concentration of 9%. Such multifunctional warm-shaped vesicles exhibit expressed cytotoxicity with respect to the HeLa cell line, as they are retained by cells due to folic acid receptor–mediated endocytosis. Therewith, in view of the high concentration of nanoparticles inside vesicles and clusterization, these systems exhibit a better relaxation ability than Feridex (commercially available MR contrast agents) [104]. Such theragnostic (therapy + diagnosis) nanocomposites hold great promise for application as pharmaceuticals for personalized medicine.

Another approach makes use of radionuclides, in particular, β-emitters, leaded in iron oxide nano-

particles, as anticancer agents. These agents function by damaging DNA by free radicals, thereby causing apoptosis of target cells [105]. The problem here is to develop an efficient radionuclide–nanoparticle complex which would accumulate in a high concentration in malignant cells, not affecting healthy cells in the body. The therapies using such complexes may prove much more efficient that the existing external-beam radiotherapies which equally affect both healthy and cancer cells.

Cancer cells can also be killed by unloaded nanoparticles, by realizing the possibility of their local heating under electromagnetic field. Cells show signs of apoptosis, when their internal temperature reaches 41–47°C. Complete necrosis is observed at temperatures close to 50°C.

Targeted magnetic hypethermia involves accumulation and concentration of magnetic nanoparticles in intracellular vesicles (endosomes). Under the action of high-frequency alternating magnetic field, these particles warm up, thus inducing local cell death [106]. The main problem is to ensure selective delivery of nanoparticles to tumor cells and optimize their ability to absorb electromagnetic radiation for therapeutically sufficient magnetic hyperthermia. There is some information on the possibility to enhance the heating capacity of iron oxide nanoparticles by an order of magnitude by increasing their size to 12–14 nm [107]. Gonzalez-Fernandez et al. [108] showed that Fe_2O_3 nanoparticles with a size of about 30 nm exhibit the highest heating capacity. Coating attenuated the heating effect, and, consequently, it should be minimized.

Magnetoliposomes can be used both for local hyperthermia and for concurrent targeted delivery of chemotherapeutic agents to tumor cells. The targeting of the cytostatic methotrexate to skeletal muscles, Zhua et al. [109] fabricated thermally sensitive magnetoliposomes which released up to 80% of the encapsulated drug within 30 min as the temperature was raised from 37 to 41°C. The accumulation of methotrexate in tissues of the locomotor system was much enhanced by exposure to external static magnetic field, which led to heating to 41°C. These thermally sensitive magnetoliposomes characteristically show, along with high organ affinity due to magnetic properties, ability to rapidly release encapsulated drugs as a response to hyperthermia [109].

Zhang et al. [110] developed paclitaxel-loaded freeze-dried negatively charged magnetic liposomes for targeted parentheral (consecutive) therapy of breast cancer. Pharmacokinetic studies showed that these magnetic liposomes selectively accumulate in tumor tissue under external magnetic fields applied at the tumor site. The antitumor activity of the liposomal paclitaxel is higher compared to a usual paclitaxel formulation containing Cremophor EL and ethanol. The half-life of the liposomal paclitaxel is up to 19.37 h (that of the usual paclitaxel formulation is 4.11 h). Consequently, paclitaxel incorporated in magnetic liposomes makes possible efficient targeted chemotherapy with less side effects [110].

Arthritis Therapy

At present arthritis most commonly treated by corticosteroids which are injected into joints. However, repeated injections pose a risk of joint infections and may lead to development of crystals inside the joint, thereby complicating the course of arthritis. Conjugation of corticosteroids with iron oxide nanoparticles might provide an approach to this problem.

Under external magnetic field magnetic conjugates can be retained for a long time in a required body site, being slowly absorbed by macrophages and the reticuloendothelial system of lymph nodes [84]. Such behavior of magnetic conjugates was demonstrated in a sheep experiment with intra-articular injection of iron oxide nanoparticles fluorescently functionalized by amino polyvinyl alcohol [111]. Retention of particles in the joint was provided by applying static magnetic field (NdFeB). The resulting data gave evidence for the bioavailability of these nanoparticles which resided within the synovial membrane for 5 days.

The possible use in the targeted therapy of arthritis of synoviocyte-biocompatible poly(lactic-co-glycolic) acid microparticles for encapsulation of iron oxide nanoparticles and the anti-inflammatory drug dexamethasone acetate was described [84, 85].

Tests on golden hamsters showed a positive dynamics after the treatment of implanted osteosarcoma by cationic magnetoliposomes loaded with the anticancer drug doxorubicin. It was found that the drug concentration was 4 times higher than in the case of traditional administration route; tumor regression and appreciable metastasis reduction were noted.

Gene Therapy

Gene therapy is most commonly performed by traditional protocols involving delivery of a necessary gene by means of viral vectors. However, this approach fails to solve all safety problems. An alternative strategy is basd on the use of cationic gene delivery systems which form complexes due to electrostatic interaction with the negatively charged DNA [76]. Iron oxide nanoparticles coated with cationic polymer proved to be an efficient tool for gene transfection. It was shown than polyethyleneimine-coated iron oxide nanoparticles react with DNA to form cation–anion complexes able to cross the endosomal barrier and deliver a required gene to cell core. Application of static or alternating electromagnetic fields allows more efficient transfection by reducing free diffusion of these particles. The complexes of polyethyleneimine with iron oxide nanoparticles are less toxic than polyethyleneimine–DNA complexes [75].

Namgung et al. [77] described the synthesis of thermally cross-linked SPIO nanoparticles–polyethyleneimine–DNA complexes proved to be highly efficient in gene transfection to vessel endothelial cells. These complexes were used to success to inhibit expression of the type 1 plasminogen activator inhibitor involved in pathogenesis of various vascular disorders, including vascular inflammation and atherosclerosis. Hwang et al. [112] could load heparin-coated iron oxide nanoparticles in an adenoviral vector. Such systems were found to serve as efficient and rapid vehicles for gene delivery to target cells, which opens up new possibilities for application of iron oxide nanoparticles in gene therapy [112].

Stem Cell Therapy

To be used in therapy, stem cells should be delivered to an injured tissue site, where they start to grow and differentiate, thereby restoring the functions of injured tissues. However, this therapy envisions extensive diagnostic and surgical interventions. Iron oxide nanoparticles makes possible solution of many problems associated with tracking migration of stem cells and their targeting. Stem cells labeled by iron oxide nanoparticles can be tracked by MR therapy [113, 114]. Labeling stem cells by nanoparticles can be accomplished in several ways. First, by incubation of cells with nanoparticles which passively penetrate into cells by phagocytosis. However, However, the phagocytic activity is not always able to ensure a sufficient nanoparticle accumulation level [115]. Second, by modification of nanoparticles with transfection reagents, such as SuperFect, poly-L-lysine, and protamine or lipofectamine, which strongly enhances nanoparticle accumulation in stem cells [116–118]. Third, by using magnetic field that simultaneously renders the cell membrane more permeable for nanoparticles [119]. Furthermore, iron oxide nanoparticles can be conjugated with cell surface.

Horak et al. [120] revealed accumulation in rat bone marrow stromal cells of mannose-modified iron oxide nanoparticles by optical and electron microscopy. It was found that poly-L-lysine with a high molecular weight (38100 Da) is an efficient surface modifier for nanoparticles for their optimal internalization in rat bone marrow stromal cells. Therewith, nanoparticles localize in cell endosomes and lysosomes. Such modified iron oxide nanoparticles can serve as s promising tool for noninvasively tracking migration of transplanted cells in the living body [121].

Iron oxide nanoparticles can be used for stem cell targeting. Yang et al. [122] modified iron oxide nanoparticles by ligands of two types: The first type had an affinity for stem cells and the second type, for myocardial infarction. With such bifunctional nanoparticles and external magnetic field, stem cells could be target to the myocardial infarction site [122].

Lewin et al. [123] coated 5-nm iron oxide nanoparticles with aminated dextran to increase their size to 45 nm and modified them with fluorescein isothiocyanate and a complex of diethylenetriaminepentaacetic acid with In-111. The resulting particles having three labels (magnetic, luminescent, and radioisotope) efficiently internalized into hematopoietic and neural progenitor cells, not affecting the viability and differentiation of hematopoietic stem cells [123].

Iron Oxide Nanoparticles as Magnetic Resonance Contrast Agents

Iron oxide nanoparticles, due to their paramagnetic and superparamagnetic properties, proved to be quite useful contrast agents in magnetic resonance tomography (MRT), one of the key methods of nanoinvasive diagnostics of a broad range of internal diseases. Along with perfection of MRT instruments and software, new organotropic and safe MR contrast agents are being developed. By affecting tissue and

organ proton parameters, these agents enhance image contrast, thereby increasing the diagnostic information content. The first MR contrast agents introduced in clinical practice were gadolinium-containing substances. Their disadvantage was that they could induce development of nephrogenic systemic fibrosis in patients with renal failure and inflammatory diseases [124].

Conjugates of SPIO nanoparticles with specific antibodies can serve as organotropic contrast agents. In particular, the possibility to identify myocardial infarction by means of antimyosine antibodies conjugated with monocrystalline iron oxide nanoparticles (MION) was reported.

Iron oxide ($Fe_2^{+3}O_3$) nanoparticles have a crystalline structure, and they strongly accelerate tissue proton relaxation. The diameter of nanoparticles is smaller than that of erythrocytes, and this allows nanoparticles to penetrate the capillary network. The charge and particle size of the developed formulations of iron oxide nanoparticles ensure their selective capture by cells of the reticuloendothelial system. Once the iron oxide nanoparticles have degraded, iron atoms are incorporated into erythrocyte hemoglobin. The quantity of iron in the iron oxide nanoparticles entering the body during diagnostics is much smaller than the total iron depot.

Iron oxide nanoparticle formulations are classed with tissue-specific contrast agents, they allow contrasting of the liver, spleen, and bone marrow, improvement of imaging of diffuse and focal spleen lesions, as well as differentiation of metastasized from normal lymph nodes. The SPIO particles make it possible to gain a valuable information about malignant liver tumors, as well as hemangiomas, cystic lesions, and nodular hyperplasias. The iron-containing agent endorem (ferumoxide) allows visualization of liver metastases in 95% of cases.

The use of iron oxide nanoparticles in the diagnosis of focal liver lesions in 900 patients was reported. The sensitivity and specificity of the MR therapy in combination with iron oxide nanoparticles are 93.8% and 91.5%, respectively. Such combination allows good imaging of bone marrow tumors and differentiation of tumors from hyperplasias [125, 126].

Iron oxide nanoparticles smaller than 20 nm are suitable for MR angiography. For example, the AMI-277 (sinerem) formulation improves imaging of the renal artery and right coronary artery. Furthermore, the AMI-277 formulation allowed imaging of the aorta, lower hollow vein, and portal vein in 16 patients within 45 min.

The NC100150 (klariskan) formulation can be used to perform coronary MR angiography and obtain images of central, segmental, and subsegmental arteries. The SPIO nanoparticles makes possible imaging of early brain lesions, identification of myocardial ischemia sites, and assessment of renal functions and hemodynamics.

Dextran-coated iron oxide nanoparticles (ferumoxtran) show good contrast characteristics even at a low magnetic field intensity. Ferumoxtran makes it possible to obtain better brain images than gadolinium contrast agents. Thus, with ferumoxtran, additional, compared to a gadolinium agent, cancer foci could be revealed in in five of seven patients.

Analysis of the information about iron-containing contrast substances allows us to conclude that these formulations hold a great promise for clinical use as organotropic agents, and we can expect extension of their proposed clinical uses and development of new efficient and safe contrast diagnostic agents [126].

Nanoparticles Iron Oxide for Hyperthermia

At present active work is being done on the development of the technology of production of iron oxide nanoparticles for local magnetic hyperthermia of cancer. To this end, thermosensitive magnetic nanoparticles which are safe for the organism and well retained in target organs by magnetic field. Such nanoparticles transform the energy of alternating magnetic field into heat and cause heating of the tissue they are residing in. Drug release into the tumor site is provided by the ability of iron oxide nanoparticles to heat up under alternating magnetic field of a definite strength and direction. Thermosensitive magnetic nanoparticles having a magnetic phase transition point (Curie point) are used. In practice this means that at higher temperatures these particles are no longer sensitive to magnetic field [126].

As known, tumor cells are much more sensitive to hyperthermia than normal cells, and their sensitivity to antitumor drugs increases with increasing temperature. In this case, magnetically sensitive nanocontainers make possible local controlled hyperthermia of the tumor site (magnetically induced heating of particles to 42–45°C and sometimes even to 47°C) and release of

the pharmacological or radioactive therapeutic agent into this site [126]. It is important that such therapy allow one to avoid or substantially reduce severe side effects always associated with cancer therapy.

As shown in animal experiments, targeted delivery of loaded iron oxide nanoparticles ensures an 8-fold local increase of drug concentration in injured tissue at a dose comprising 1/3 of a traditional therapy dose. Even though the use of iron oxide nanoparticles for cancer hyperthermia has already been known for no less than 10 years, the main results in this field have been obtained in animal experiments. For iron oxide nanoparticles or magnetoliposomes to be introduced in clinical practice, thorough research into health effects and safety of magnetic particles is necessary, which is a challenging task. The size, shape, and composition of nanoparticles should not prevent blood circulation, they should not agglomerate with each other and formed blood elements, should undergo a fairly rapid endocytosis, destroy in lysosomes, and clear from the body (it is considered that nanoparticles should not stay in the body longer than 10 days). There is some information that an attenuated protocol can be based on liposomal or gold-coated iron oxide nanoparticle formulations. The gold coating facilitates the subsequent functionalization of iron nanoparticles that should be sufficiently small (10–300 nm) and improves their biocompatibility for cells and tissues. There still have been scarce concrete data on the use of iron oxide nanoparticles in cancer therapy, even though experiments on animal *in vivo* and on cell cultures gave optimistic results.

CONCLUSIONS

At present a great body of information is available on the design and successful use of magnetically controlled carriers in biology and medicine. The progress in this field, which is at the interface between physics, chemistry, biology, and medicine, should be driven by further development of technics, especially magnetic sensor systems capable of detecting microquantities of magnetic labels, as well as by extension of the list of targeted receptor-specific molecules fir functionalization of iron oxide nanoparticles.

Iron oxide nanoparticles have opened up new possibilities for drug targeting, including enhanced selectivity of action and better tolerability of chemotherapy, overcoming membrane barriers, magnetic field–assisted retention of nanocomposites in a target body site, surmount of multidrug resistance, and simultaneous diagnostics and therapy (theragnostics).

Combining magnetic nanotechnologies and biomedical sciences will undeniably become of the leading progressive fields of research and practical use of iron oxide nanoparticles in XXI century.

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