Monatshefte für Chemie 133, 737–759 (2002)

Monatshefte für Chemie
Chemical Monthly

Invited Review

Magnetic Nanoparticles and Biosciences

Ivo Šafařík[1,2,*] and **Mirka Šafaříková**[1]

[1] Laboratory of Biochemistry and Microbiology, Institute of Landscape Ecology, CZ-37005 České Budějovice, Czech Republic
[2] Department of General Biology, University of South Bohemia, CZ-37005 České Budějovice, Czech Republic

Summary. Magnetic nanoparticles represent an interesting material both present in various living organisms and usable for a variety of bioapplications. This review paper will summarize the information about biogenic magnetic nanoparticles, the ways to synthesize biocompatible magnetic nanoparticles and complexes containing them, and the applications of magnetic nanoparticles in various areas of biosciences and biotechnologies.

Keywords. Magnetic nanoparticles; Biosciences; Magnetic properties; Nanostructures.

Introduction

Nanotechnology involves the study, control, and manipulation of materials at the nanoscale, typically having dimensions up to 100 nm. This is a truly multidisciplinary area of research and development. The large interest in nanostructures results from their numerous potential applications in various areas such as materials and biomedical sciences, electronics, optics, magnetism, energy storage, and electrochemistry [1].

Magnetic nanoparticles having connections to biological systems and bioapplications usually exist or can be prepared in the form of either single domain or superparamagnetic magnetite (Fe_3O_4), greigite (Fe_3S_4), maghemite (γ-Fe_2O_3), various types of ferrites ($MeO{\cdot}Fe_2O_3$, where $Me = $ Ni, Co, Mg, Zn, Mn, ...), iron, nickel *etc*. Synthetic magnetic nanomaterials are most often available in the form of magnetic fluids (ferrofluids) which have already found many interesting applications (sealing, damping, heat transfer, levitation of magnetic and nonmagnetic objects, production of computers, loudspeakers, measuring devices *etc*.) [2]. Potential applications of biocompatible magnetic fluids in various areas of biosciences and biotechnologies are also very promising as will be shown in this paper.

* Corresponding author. E-mail: safarik@uek.cas.cz

The first magnetic nanoparticles necessary to create a stable magnetic fluid were prepared in the early 1960s [3]. On the contrary, various organisms living on Earth have been able to synthesize them for a long time. These biogenic magnetic nanoparticles have in many respects even better properties than nanoparticles prepared in the laboratory.

The purpose of this review article is to show the close connection between inorganic magnetic nanoparticles and living systems.

Biogenic Magnetic Nanoparticles

In 1962, *Lowenstam* first discovered biochemically precipitated magnetite as a capping material in the radula (tongue plate) teeth of chitons (marine mollusks of the class Polyplacophora) [4]. *Lowenstam* was able to demonstrate the biological origin of this material through a variety of radioisotope tracing studies and by detailed examination of the tooth ultrastructure. Prior to this discovery, magnetite was thought to form only in igneous or metamorphic rocks under high temperatures and pressures. In the chitons, the magnetite serves to harden the tooth caps, enabling the chitons to extract and eat endolithic algae from within the outer few millimetres of rock substrates. Metabolic iron is at first transported to the posterior end of the radula sac and is then deposited as the mineral ferrihydrite within a preformed proteinaceous mesh, forming one or two distinct rows of reddish teeth. This ferrihydrite is converted rapidly to magnetite *via* an unknown process [5].

In 1975, *Blakemore* discovered magnetotactic bacteria [6], which now represent the most intensively studied biomagnetic system. Magnetotactic bacteria form a heterogeneous group of *Gram*-negative prokaryotes with morphological and habitat diversity which have an ability to synthesize fine (50–100 nm) intracellular membrane-bound ferromagnetic crystalline particles (Fig. 1) consisting of magnetite (Fe_3O_4) or greigite (Fe_3S_4) which are covered with an intracellular phospholipid membrane vacuole, forming structures called 'magnetosomes' [7]. Various morphological types of magnetotactic bacteria such as cocci, short or long rods, vibrios, spirilla (Fig. 2), and multicellular forms have been isolated from sediments in diverse aquatic environments, *e.g.*, marine, river, lake, pond, beach, rice paddies, drains, wet soil, deep see, and estuary [8]. Chains of magnetosomes act as simple compass needles which passively torque the bacterial cells into alignment with the earth's magnetic field and allow them to seek the microaerophilic zone at the mud/water interface of most natural aqueous environments. These bacteria swim to the magnetic north in the northern hemisphere, to the magnetic south in the southern hemisphere, and both ways on the geomagnetic equator [5]. Excellent up-to-date reviews covering all aspects of the topic exist [7–9].

Extracellular production of nanometer magnetite particles by various types of bacteria has also been described [10]. Magnetite-bearing magnetosomes have been found in eukaryotic magnetotactic algae, each cell containing several thousand crystals [11]. Biogenic nanometer magnetite particles have been found in marine and lake sediments [12].

The behaviour of various other organisms is also influenced by changes of the magnetic field. It has been shown that some animals, including ants [13],

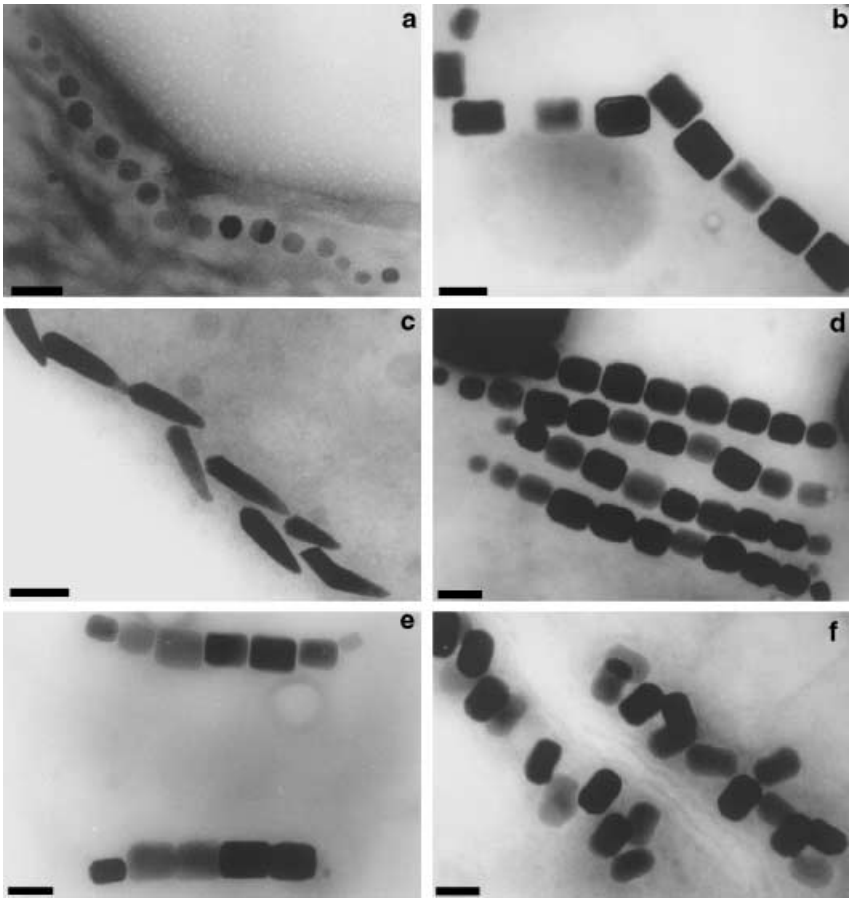

Fig. 1. Electron micrographs of crystal morphologies and intracellular organization of magnetosomes found in various magnetotactic bacteria. Shapes of magnetic crystals include cubo-octahedral (a), elongated hexagonal prismatic (b, d, e, f), and bullet-shaped morphologies (c). The particles are arranged in one (a, b, c), two (e), or multiple chains (d) or irregularly (f). The bar is equivalent to 100 nm. Reproduced with permission of Dr. *D. Schüler*, Germany, from Ref. [7]

honeybees [14], homing pigeons [15], salmon [16], and others use geomagnetic field information for orientation, homing, and foraging. Several hypotheses have appeared trying to explain the mechanisms of magnetoreception [17]. It seems apparent that biomineralized magnetite nanoparticles can interact with the geomagnetic field, monitoring information on its intensity and direction and being thus the main part of a highly evolved, finely tuned sensory system [18]. Identifying the presence of magnetite particles in different organisms whose behaviour is influenced by the geomagnetic field is a first step towards demonstrating that biogenic magnetite is involved in geomagnetic field detection [19]. Table 1 shows some typical examples of living organisms containing magnetic nanoparticles within their cells and organs.

Rainbow trout (*Oncorhynchus mykiss*) has been used for extensive study of the magnetoreception mechanism. The key behavioural, physiological, and anatomical components of a magnetite-based magnetic sense have been demonstrated.

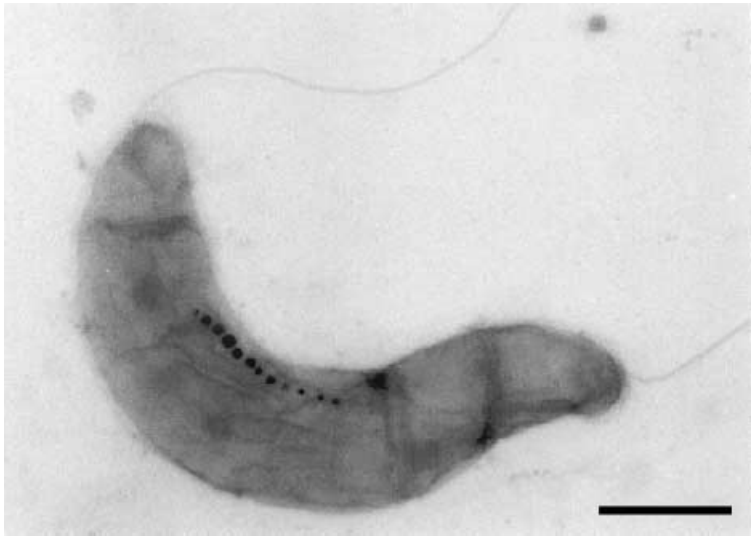

Fig. 2. Electron micrograph of a *Magnetospirillum gryphiswaldense* cell exhibiting the characteristic morphology of magnetic spirilla. The helical cells are bipolarly flagellated and contain up to 60 intracellular magnetite particles in magnetosomes which are arranged in a chain. The bar is equivalent to 0.5 μm. Reproduced with permission of Dr. *D. Schüler*, Germany, from Ref. [7]

Receptor cells containing single-domain magnetite nanocrystals are located within a discrete sublayer of the olfactory lamellae. These crystals were mapped to individual receptors using confocal and atomic force microscopy. It was confirmed that several magnetic crystals are arranged in a chain of about 1 μm within the receptor, and that the receptor is a multi-lobed single cell. These results are consistent with a magnetite-based detection mechanism, as 1 μm-chains of single-domain magnetite crystals are highly suitable for the behavioural and physiological responses to magnetic intensity [20, 21].

The information given so far is connected with the physiological occurrence of magnetic nanoparticles in given tissues and organs. However, it has been shown that many neurodegenerative diseases are connected with the disruption of normal iron homeostasis in the brain. Nanoscale magnetic biominerals (primarily magnetite and maghemite) may be associated with senile plaques and tau filaments found in brain tissue affected by these diseases. These findings have important implications for our understanding of the role of iron in neurodegenerative diseases as well as profound implications for their causes. In addition, the presence of biogenic magnetite in affected tissue should also provide improved mechanisms for early detection through the modification of MRI pulse sequences [22].

Recently, an interesting observation has been presented by NASA researchers. During the detailed study of Martian meteorites, magnetite nanoparticles were found which were similar to those present in magnetotactic bacteria (Fig. 3). *Thomas-Keprta et al*. [23] postulated six criteria that characterize biologically produced magnetite crystals. The simultaneous presence of all six characteristics — *i.e*., a definite size range and width/length ratio, chemical purity, crystallographic perfection, arrangement of crystals in linear chains, unusual crystal morphology, and

Table 1. Examples of organisms synthesizing magnetic nanoparticles (MNP)

Type of organism	General name	Latin name	Localisation of MNP	Type of MNP	References
Micro-organisms	Magnetotactic bacteria	*Magnetospirillum sp.*	magnetosomes	Fe_3O_4	[8]
			magnetosomes	Fe_3S_4	[149]
	Algae		cells	Fe_3O_4	[11]
Protozoa			cells	Fe_3O_4	[150]
Insect	Honeybee	*Apis mellifera*	abdomen	Fe_3O_4	[14]
	Migratory ant	*Pachycondyla marginata*	abdomen	Fe_3O_4	[13]
	Termites	*Nasutitermes exitiosus*	thorax, abdomen	Fe_3O_4	[151]
		Amitermes meridionalis	thorax, abdomen	Fe_3O_4	[151]
Fish	Atlantic salmon	*Salmo salar*	lateral line	Fe_3O_4	[152]
	Sockeye salmon	*Oncorhynchus nerka*	skull	Fe_3O_4	[153]
	Rainbow trout	*Oncorhynchus mykiss*	olfactory lamellae	Fe_3O_4	[21]
	Chum salmon	*Oncorhynchus keta*	head	Fe_3O_4	[16]
Amphibians	Eastern red-spotted newt	*Notophthalmus viridescens*	whole body	Fe_3O_4	[154]
Birds	Bobolink	*Dolichonyx oryzivorus*	upper beak	Fe_3O_4	[155, 156]
	Homing pigeon	*Columba livia*	upper-beak skin	Fe_3O_4	[15, 157]
Mammals	Common Pacific dolphin	*Delphinus delphis*	dura mater	Fe_3O_4	[158]
	Human	*Homo sapiens*	brain, heart	Fe_3O_4	[159–163]

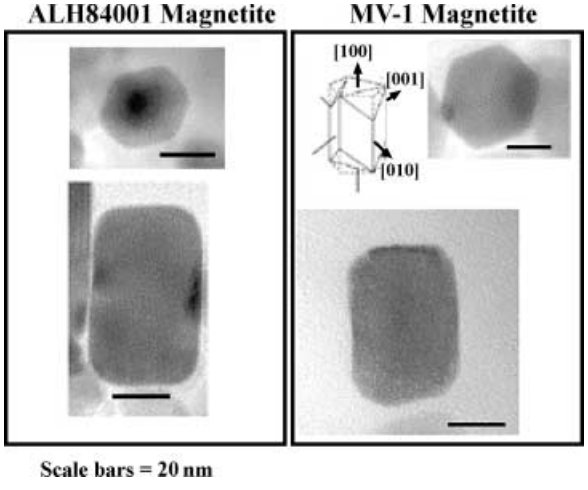

Fig. 3. Typical examples of magnetic nanoparticles found in meteorite ALH84001 (originating from Mars) and in magnetotactic bacterium MV-1. Reproduced with permission of Dr. *K. L. Thomas-Keprta*, USA

elongation of crystals in the [111] crystallographic direction — should constitute evidence of biological origin. During the research, magnetic nanoparticles present in meteorite ALH84001 fulfilled all six criteria. These findings led to the hypothesis that they could be in fact microfossils of former Martian magnetotactic bacteria [23, 24]. Subsequently another hypothesis has appeared suggesting life on Earth could originally have arrived here by way of meteorites from Mars, where conditions early in the history of the solar system are thought to have been more favourable for the creation of life from nonliving ingredients. It was calculated that under the optimal conditions the temperature within the meteorite did not exceed 40°C; the transport time from Mars to Earth could take only a few years, and thus meteorites could transfer life between planets [25].

Biocompatible Magnetic Nanoparticles and Complexes Containing Them

Various types of magnetic fluids (ferrofluids) are often used as the starting material to prepare the target nanomaterials. Ferrofluids are colloidal solutions of magnetic iron oxide (or ferrite) nanoparticles (around 10 nm in diameter) in either a polar or non-polar liquid. These particles are said to be 'superparamagnetic', meaning that they are attracted by a magnetic field but retain no residual magnetism after the field is removed. The first ferrofluids were prepared by grinding micrometer-sized magnetic particles in a ball mill for several weeks and subsequent transfer of nanometer particles into kerosene. The particles were stabilized with oleic acid to prevent clumping. Nowadays, however, the chemical synthesis of ferrofluids preferably employs coprecipitation of ferric and ferrous salts with alkaline solution and subsequent treatment under hydrothermal conditions. To form ferrites, other divalent ions are used instead of ferrous ions [26].

Biocompatible ferrofluids normally use water as a carrier medium, but paraffin or vegetable oils based ferrofluids may be acceptable in some case. In the water phase, the magnetic nanoparticles can be stabilized (in order to prevent their unwanted agglomeration) by ionic interactions [27, 28], a bilayer of an appropriate surfactant (*e.g.* fatty acids) [29, 30], aspartic and glutamic acid [31], *meso*-2,3-dimercaptosuccinic acid [32], citrate [33], peptides [34], *etc*. Alternatively, the coprecipitation of ferrous/ferric ions is performed in the presence of an appropriate biopolymer, such as dextran [35, 36] or polyvinyl alcohol [36].

Many modified procedures have been described for ferrofluid preparation. Synthesis of magnetic nanoparticles using the restricted environments offered by surfactant systems such as water-in-oil microemulsions (reverse micelles) provides an excellent control over particle size, inter-particle spacing, and particle shape. The controlled environment of the reverse micelle also allows sequential synthesis which can produce a core-shell type structure [37, 38]. Alternatively, metallic iron nanoparticles were synthesized in reverse micelles of cetyltrimethylammonium bromide (*CTAB*) using hydrazine as a reducing agent. After addition of an aqueous gold solution, a metallic gold coating on the outer surface of the iron particles was formed. The gold shells on the iron particles provide functionality with thiol-functionalized substrates [39].

Magnetoliposomes are magnetic derivatives of liposomes and can be prepared by entrapment of ferrofluids within the core of liposomes [40, 41]. Affinity magnetoliposomes can be produced by covalent attachment of ligands to the surface of the vesicles or by incorporation of target lipids in the matrix of structural phospholipids [42]. Alternatively, magnetoliposomes are prepared using the phospholipid vesicles as nanoreactors for the *in situ* precipitation of magnetic nanoparticles [43]. Vesicles of another type are constituted by didodecyldimethylammonium bromide, contain an ionic magnetic fluid, and have a diameter of about 1 μm [44].

Micrometric particles (*e.g.* materials used for column chromatography) have been post-magnetized by circulation of ferrofluid through the column chromatography carrier [45]. Dynabeads (magnetic polystyrene particles of a diameter of 2.8 or 4.5 μm produced by Dynal, Norway) are prepared in magnetic form after introduction of $-NO_2$ or $-ONO_2$ groups to the matrix and subsequent reaction with Fe^{2+} salts. During the reaction, iron hydroxides precipitate inside the pores, and after heating they are transformed into nanoparticles of γ-Fe_2O_3 [46]. Ferrofluids can be added to reaction mixtures used to create various magnetic polymeric microparticles [47, 48]. Ferrite plating enables magnetization of various biocompatible materials by directed precipitation of magnetic iron oxides [49, 50]. Another type of magnetic polymeric particles has been prepared by electrostatic adsorption of negatively charged magnetic nanoparticles on positively charged polymer particles and subsequent encapsulation of the prepared complex [51, 52].

Magnetic nanocomposites have been prepared using cross-linked synthetic polymers and different polysaccharides like alginate or cellulose as gel matrices [53–55]. Another procedure is based on the nucleation and growth control of crystalline iron oxide particles in organic matrix through the reaction control of a

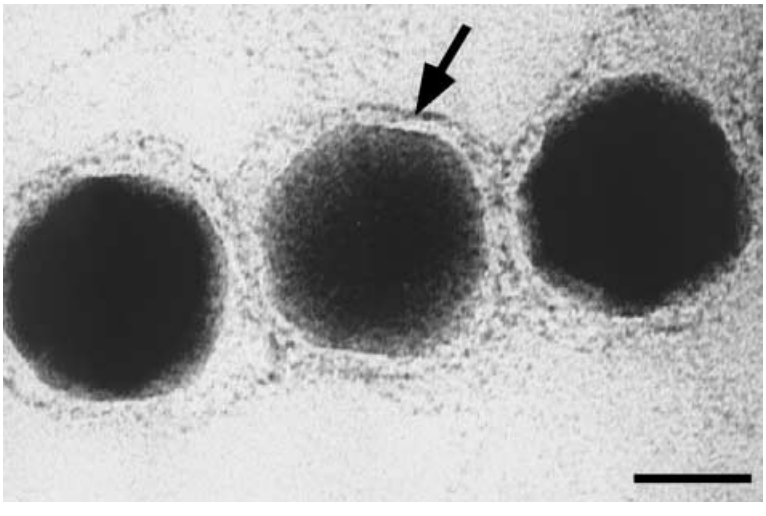

Fig. 4. Magnetosome particles isolated from *M. gryphiswaldense*. The magnetite crystals are typically 42 nm in diameter and are surrounded by the magnetosome membrane (arrow). The bar is equivalent to 25 nm. Reproduced with permission of Dr. *D. Schüler*, Germany, from Ref. [7]

Table 2. Examples of commercially available biocompatible magnetic nanoparticles

Product name	Composition	Particle size (nm)	Application	Manufacturer or supplier
Combidex	Magnetic iron oxides – dextran	17–20	Magnetic resonance contrast agent	Advanced Magnetics, USA
Endorem[a]/ Feridex[b]	Magnetic iron oxides – dextran	100–250	Magnetic resonance contrast agent	Advanced Magnetics, USA
MicroBeads	Magnetic iron oxides – dextran	50	Separation and labelling of cells and molecules	Miltenyi Biotec, Germany
Nanomag	Magnetic iron oxides – dextran	100	Magnetic labelling	Micromod Partikeltechnologie, Germany
Resovist	Magnetic iron oxides – dextran	57	Magnetic resonance contrast agent	Schering AG, Germany

[a] Commercial product name in Europe; [b] commercial product name in the United States

metallorganic precursor with a combination of the hydrolysis and polymerization below 100°C. The reaction conditions influence the size and crystallinity of magnetic nanoparticles in the organic matrix [56].

Bacterial magnetite nanoparticles obtained from magnetotactic bacteria after disruption of the cell wall and subsequent magnetic separation have been used for a variety of bioapplications. Due to the presence of the lipid layer (Fig. 4) the particles are biocompatible, their suspensions are very stable and the particles can be easily modified [8, 57].

Magnetic derivatives of the iron storage protein ferritin (magnetoferritin) have also been prepared. A magnetic mineral has been synthesized within the nanodimensional cavity of horse spleen ferritin using controlled reconstitution conditions [58].

Several types of biocompatible magnetic nanoparticles are commercially available, especially those used as magnetic resonance contrast agents or magnetic labels. A non-complete selection of these products can be found in Table 2.

Application of Magnetic Nanoparticles in Biosciences

Many different types of magnetic micro- and nanoparticles and molecular magnetic labels have been used for a great number of applications in various areas of biosciences and biotechnologies [59–62]. The majority of review papers considers magnetic micro- and nanoparticles to be equally important. Of course, in many areas magnetic microparticles with diameters above 1 μm are used (*e.g.* for immunomagnetic separation of pathogenic microorganisms in food and clinical microbiology), whereas for other applications magnetic nanoparticles are necessary. The following chapters will focus on the bioapplications of magnetic nanoparticles and the most important complex material containing them. Applications of dynabeads (containing magnetic nanoparticles within the bead structure)

will not be considered in this review; there are several sources where information about these particles can be found [63, 64].

Immobilization and modification of biologically active compounds

Immobilization of enzymes, antibodies, oligonucleotides, and other biologically active compounds is a very important technique used in various areas of biosciences and biotechnology. Biologically active compounds immobilized on magnetic carriers can be removed from the system by using an external magnetic field or can be targeted to the desired place. The immobilized compounds can be used to express their activities in a desired process (*e.g.* immobilized enzymes) or can be used as affinity ligands enabling to capture or modify the target molecules or cells.

Magnetic nanoparticles obtained from magnetotactic bacteria have been used for the immobilization of a variety of enzymes, such as glucose oxidase and uricase [65], antibodies [66–68], oligonucleotides [69, 70], *etc.* Colloidal aqueous suspensions of superparamagnetic nanoparticles (9 nm in diameter) composed of maghemite and forming an ionic ferrofluid have been covalently coupled with lectins, enzymes, or antibodies using specific thiol chemistry [71, 72]. Magnetic nanoparticles activated with 3-aminopropyltriethoxysilane have been used for the immobilization of various enzymes, antibodies, and protein A after glutaraldehyde treatment [73, 74]. Dextran-based biocompatible magnetic nanoparticles (*ca.* 50 nm in diameter) produced commercially by Miltenyi Biotec, Germany, are available with many covalently immobilized molecules (*e.g.* annexin V, antibiotin antibody, antifibroblast antibody, anti-FITC antibody, anti-HLA-DR antibody, antihuman epithelial antigen antibody, antihuman melanoma-associated chondroitin sulfate proteoglycan antibodies, antimouse DX5 antibody, antiphycoerythrin antibody, antibodies against various CD markers, goat antimouse, antirabbit, and antirat IgGs, various types of rat antimouse IgG and IgM antibodies, protein A, protein G, streptavidin, and some others; see http://www.miltenyibiotec.com).

Enzymes can be made soluble and active in organic solvents by chemical modification with the amphipathic macromolecule polyethylene glycol (*PEG*). The *PEG*-enzyme conjugates can be also conjugated to magnetic nanoparticles. Alternatively, the magnetite-*PEG* conjugate is prepared first and then conjugated to the target enzyme. The magnetically modified enzymes stably disperse in both organic solvents and aqueous solutions. Magnetically modified lipase catalyzes ester synthesis in organic solvents and can be easily recovered by magnetic force without loss of enzyme activity [75, 76]. Other enzymes such as *L*-asparaginase [76] and urokinase [77] have also been modified in this way.

Antibodies can be modified in a similar manner. Magnetite-labelled antibodies are expected to be applicable clinically as a therapeutic agents for the induction of hyperthermia [78].

Magnetoliposomes containing magnetic nanoparticles entrapped within the cavity have been used for the immobilization of membrane-bound enzymes [79] or antibodies [80] as well as for the entrapment of various drugs [81]. The catalytic activity of isolated lipid-depleted membrane-bound enzymes, such as cytochrom

c-oxidase, has been substantially enhanced after their incorporation in magnetoliposomes.

Production of a protein (enzyme, antibody, protein A) – magnetite complex by genetically engineered magnetotactic bacteria *Magnetospirillum sp.* AMB-1 has been proposed recently [82]. The *magA* gene, encoding an integral iron translocating protein situated in the membrane of *Magnetospirillum sp.* magnetic nanoparticles, has been fused with genes encoding the desired protein. The desired protein-*magA* fusion gene has been cloned into *Magnetospirillum sp.*, and after cultivation, magnetic nanoparticles bearing the desired protein on the particle membrane surface have been isolated. The *magA* protein may thus be used as an anchor for the site-specific expression of foreign proteins on bacterial magnetite particle membranes by gene fusion, thus obviating the need for immobilization of the proteins using chemical reagents [83]. Bacterial magnetic nanoparticles containing protein A [84], luciferase [85], and acetate kinase [85] have already been constructed.

Isolation of biologically active compounds

The isolation and separation of specific molecules belongs to the major problems in biosciences. Affinity ligand techniques represent currently the most powerful tool available to the downstream processing both with respect to selectivity and recovery. Batch magnetic isolations may be faster than standard liquid chromatography procedures, and the target molecules can be separated from untreated samples containing impurities. Although magnetic microparticles are usually employed for this purpose, especially when working with larger volumes of solutions and suspensions, magnetic nanoparticles have been used to a greater extent recently.

Isolation of eukaryotic poly(A)$^+$ m*RNA* can be performed using oligo(d*T*) immobilized on synthetic magnetic nanoparticles [86]. Alternatively, oligonucleotides immobilized on bacterial magnetic particles can be used for the same purpose [70]. Protein A immobilized on magnetic nanoparticles has been used to purify monoclonal antibodies [87]. Alcohol dehydrogenase and lactate dehydrogenase have been isolated using ferrofluid-modified 5′-AMP-sepharose 4B as an affinity adsorbent, whereas ferrofluid-modified 2′,5′-ADP-sepharose 4B was used to isolate glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. IgG and anti-human serum albumin antibodies have been isolated using ferrofluid-modified protein A sepharose and human serum albumin sepharose, respectively [45, 88].

Biocompatible two-phase systems, composed for example from aqueous dextran and polyethylene glycol phases, have been used for the isolation of biologically active compounds, subcellular organelles, and cells for many years. To speed up the phase separation the addition of ferrofluids to the system is useful. In a magnetic field such additives will induce a faster phase separation. Dextran-stabilized ferrofluid added to an aqueous two-phase system containing polyethylene glycol and dextran totally partitioned to the dextran phase. After mixing of the two-phase system, it was possible to reduce the separation time by a factor of 35 by applying a magnetic field to the system [89]. Other types of surface modified ferrofluids have also been successfully used [90].

Determination and detection of biologically active compounds and xenobiotics

An enormous amount of analytical procedures for the detection of biologically active compounds is available; among them, antibody-based techniques are exceptionally important. Magnetic modifications of standard immunoassays can be successfully used for the determination of various biologically active compounds and xenobiotics. In these techniques, specific antibodies or antigens are covalently immobilized on fine magnetic particles. Magnetically based assays are usually faster and more reproducible than the standard microtitration plate based assays and have proven to be simple, rapid, and sensitive. The detection systems can be based on the use of enzymes, radioisotopes, fluorescent substances, or chemiluminescence. The possibility of automation of the analytical procedures is especially important.

Magnetic nanoparticles with immobilized antimouse IgG antibody or protein A have been applied to enzyme-linked immunosorbent assay (ELISA) of mouse IgG. The assay time could be shorten substantially in comparison with the conventional method [91]. Using bacterial magnetic nanoparticles as a carrier, a highly sensitive mouse IgG assay was developed having a good relationship between the luminescence intensity and mouse IgG concentration in the range of $1–10^5$ fg/cm^3 [66]. Antibody-conjugated bacterial magnetic particles have been prepared to determine a model food allergen lysozyme using a high-performance and rapid chemiluminescence immunoassay and a fully automated system [92]. A fully automated sandwich immunoassay for the determination of human insulin using an antibody – protein A – bacterial magnetic particle complexes and an alkaline phosphatase-conjugated secondary antibody has been described recently [83].

Bacterial magnetic nanoparticles have been used for the identification of cyanobacterial *DNA*. Genus-specific oligonucleotide probes for the detection of target strains were designed from the variable region of the cyanobacterial 16S r*DNA*. These oligonucleotide probes were immobilized on magnetic particles *via* streptavidin-biotin conjugation and employed for magnetic capture (hybridization) against digoxigenin-labelled cyanobacterial 16S r*DNA*. Bacterial magnetic particles were magnetically concentrated, spotted in a microarray device, and the fluorescent detection was performed. The cyanobacterial genera were successfully discriminated [69]. A similar approach has been used for the discrimination between Atlantic and Pacific subspecies of the northern bluefin tuna (*Thunnus thynnus*) [93].

Alternative immunoassays or assays employing other binding molecules (*e.g.* lectins) can employ magnetic nanoparticles as ferromagnetic labels instead of enzymes, radionuclides, *etc.* After binding the labelled antibody or lectin to the target analyte, the magnetically labelled complex can be captured by larger particles with immobilized specific antibodies against another analyte epitope, and after sedimentation the amount of magnetically labelled analyte in the sedimented fraction is measured with an appropriate transducer (*e.g.* a magnetic permeability meter). The possible advantage of this approach includes very low interference from the sample matrix, as the transducer is only sensitive to ferromagnetic substances which rarely are present in the sample [94, 95].

Very sensitive superconducting quantum interference device (SQUID) magnetometers have been tested to measure antigen-antibody interactions. In this system, antibodies are labelled with magnetic nanoparticles, and the antibody-antigen reaction is measured by detecting the magnetic field from the magnetic nanoparticles present in the complex. At present, 4×10^6 magnetic markers (diameter = 50 nm), corresponding to 520 pg of magnetic material, can be detected [96–98].

Modification, detection, isolation, and study of cells and cell organelles

The immunomagnetic separation of cells has become an important tool used especially in cell biology and medicine [59]. A substantial amount of experiments has been performed using magnetic nanoparticles bearing antibodies specific to the cell surface epitopes.

In general, two different modes of separation can be used. In the direct method, an appropriate affinity ligand coupled to magnetic nanoparticles is applied directly to the sample. During the incubation magnetic affinity particles are bound to the target cells, and thus stable magnetic complexes are formed. In the indirect method, a free affinity ligand (in most cases an appropriate antibody, often biotinylated) is first added to the cell suspension. If possible, the excess of unbound affinity ligand (antibody) is removed after incubation, and the labelled cells are then captured by magnetic nanoparticles bearing an affinity ligand against the primary label (*e.g.* secondary antibodies or streptavidin). In both methods the resulting magnetic complex is separated using an appropriate magnetic separator [59].

Both positive isolation (when target cellular subsets are magnetically labelled and subsequently separated) and negative isolation (when targets are purified by removing all other contaminating cells) can be performed. Cells of various types can be isolated using equipment and dextran-based magnetic particles from *e.g.* Miltenyi Biotec, Germany, or StemCell Technologies, USA.

Magnetic nanoparticles used to label the cells have no negative effect on the viability of the attached cells, and the isolated cells remain phenotypically unaltered. The extremely small size of the magnetic nanoparticles (*ca.* 50 nm) avoids mechanical stress for the cells and allows short incubation and fast processing times. The particles form a stable colloidal suspension and do not sediment or aggregate in magnetic fields. Their size and composition (iron oxide and polysaccharide) make the particles biodegradable, and typically they do not activate cells or influence cell functions and viability. Cells retain their physiological function. Nanoparticles detachment is not required, so positively selected cells (*i.e.* magnetically labelled ones) can be used immediately after separation for analysis and subsequent experiments. Magnetically labelled cells can be simultaneously stained by fluorochrome conjugated antibodies, facilitating quality control and analysis of the separation. The bound nanoparticles do not affect the light scattering of labelled cells. The purity of the sorted fractions can be determined directly after magnetic separation by flow cytometry. The separation procedure is also compatible with fluorescence microscopy, PCR, or FISH.

Very pure cell populations with excellent recovery and viability can be isolated; typical purities reach 95–99.9%, and $> 90\%$ recovery can be achieved depending

Fig. 5. The automated system for clinical isolation of human cell subsets CliniMACS. Reproduced with permission from materials provided by Miltenyi Biotec, Germany

on the cell frequency and the level of marker expression. Larger amounts of cells can be isolated using automated systems developed by Miltenyi Biotec, Germany; CliniMACS (Fig. 5) allows cells to be separated in a closed, sterile system, whereas AutoMACS enables high throughput sample separation, *e.g.* for further flow cytometric analysis or sorting.

A great variety of cell types has been isolated up to now. Especially important is the process of detection and removal of circulating tumour cells using an immunomagnetic procedure [99]. Another important process is the selective separation of CD34 + cells (stem cells) which opens new possibilities for stem cell transplantation and genetic manipulation of the hematopoietic system [100]. Nanoparticles obtained from magnetotactic bacteria with immobilized antibodies have been used for the detection and removal of *Escherichia coli* [67].

Annexin V is a Ca^{2+}-dependent phospholipid binding protein with high affinity for phosphatidylserine (*PS*) which is redistributed from the inner to the outer plasma membrane leaflet in early apoptosis. If immobilized to magnetic nanoparticles it can be used for the purification of apoptotic and non-apoptotic cells [32].

Not only whole cells, but also various cell organelles can be selectively separated using magnetic biocompatible nanoparticles. The lysosome fraction was isolated from the amoeba *Dictyostelium discoideum* after feeding with dextran-based nanoparticles and subsequent homogenization [101, 102]. A similar procedure was used to isolate lysosomes from human epidermal keratinocytes [103]. Plasma membranes from Chinese hamster ovary cells have been isolated after binding of wheat germ agglutinin immobilized on magnetic nanoparticles to the cell surface, followed by cell disintegration and magnetic separation [104].

A complex of magnetic cationic liposomes and a plasmid was used for transfection of selected animal cells. After incubation, the cells were subjected to magnetic separation, and transformed cells were selected. No other marker is required, and the separation can be performed just after the transformation [105].

Bacterial magnetite particles of 50 to 100 nm diameter were used as *DNA* carriers for the ballistic transformation of the marine cyanobacterium *Synechococcus*. Particles were bombarded into the cyanobacterial cells using a particle gun. Successful transformation and gene expression were confirmed by Southern hybridization and CAT assays, respectively. Magnetic particles were also observed in the cyanobacterial cells by transmission electron microscopy. These results suggested that magnetic nanoparticles can be used as carriers for introducing *DNA* into bacterial cells [106]. Subnanoparticulate magnetic labels such as erbium ions or magnetoferritin have also been used to modify the cell surface [59].

Applications of magnetotactic bacteria

Magnetic particles produced by magnetotactic bacteria have found various practical applications, and cultivation of magnetotactic bacteria can thus be an important process for the production of fine-grade, homogeneous, and biocompatible magnetite under mild conditions at normal temperature and pressure. Only a limited number of magnetotactic bacteria have been isolated in pure culture so far. For larger-scale cultivation, aerotolerant strains are preferred. *Magnetospirillum gryphiswaldense* and *Magnetospirillum* AMB-1 are tolerant to atmospheric air if grown from large inocula. The maximum cell yields reported so far are $0.34\,g/dm^3$ (dry weight; this corresponded to 4.5 mg of bacterial magnetic particles) for *Magnetospirillum* AMB-1 grown using a fed-batch culture system in a $4\,dm^3$ fermentor [107] and $0.33\,g/dm^3$ (dry weight) for *M. gryphiswaldense* grown in a $100\,dm^3$ fermenter [108]. Magnetic particles are usually released from the cells after disruption by ultrasonication or French press. Subsequent techniques for the isolation and purification of magnetosome particles from *Magnetospirillum* species are based on magnetic separation [109, 110] or a combination of a sucrose-gradient centrifugation and a magnetic separation technique [111]. These procedures leave the surrounding membrane intact, and magnetosome preparations are apparently free of contaminating material. Owing to the presence of the enveloping membrane, isolated magnetosome particles form stable, well-dispersed suspensions [7].

Both the cells of magnetotactic bacteria and the isolated magnetic particles have found interesting applications. The cells were used for the nondestructive domain analysis of soft magnetic materials [112] or to locate magnetic poles on meteoritic magnetic grains [113]. Experiments with possible applications of magnetotactic bacteria for radionuclide recovery have also been performed [114, 115]. In the near future, cultivation of genetically engineered magnetotactic bacteria producing magnetic nanoparticles with attached specific proteins can be expected.

Drug and radionuclide targeting

Drug and radionuclide targeting, *i.e.* predominant active compound accumulation in the body target zone independent from the method and route of drug

administration, may resolve problems currently associated with systemic drug administration. One of the possible schemes for drug targeting includes magnetic targeting. For this purpose, the drug or radionuclide can be immobilized in biocompatible magnetic nano- or microspheres or in magnetoliposomes. Typically, the intended drug and an appropriate ferrofluid are formulated into a pharmaceutically stable formulation. This is then usually injected through the artery that supplies the target organ or tumor in the presence of an external magnetic field. Prolonged retention of the magnetic drug carrier at the target site alleviates or delays the RES clearance and facilitates extravascular uptake. This process is based on competition between forces exerted on the particles by the macro- and microcirculation, the characteristics of the magnetic particles (size, configuration), and the applied magnet. To effectively retain the magnetic drug carrier, the magnetic forces must be high enough to counteract linear flow rates within the organ or tumour tissue (between 10 and 0.05 cm/s depending on vessel size and branching patterns) [116–118].

Current technologies of magnetic drug targeting allow the localization of up to 70% of the administrated dose in the target tissue, with minimal interaction and toxicity to normal cells. An up to eight-fold increase in drug concentration in the target tissue after administration of only a third of the drug dose has been observed [116].

Special types of ferrofluids stabilized with anhydroglucose polymers have been developed which enabled chemoadsorptive binding of various drugs, cytokines, *DNA* fragments, and other molecules. Animal studies have demonstrated good tolerance of the magnetically targeted ferrofluid: low concentrations of the magnetic fluid delivered high drug doses and allowed for effective tumor therapy. Clinical studies have been performed on patients with various types of tumors using a magnetic fluid with bound epirubicin. The amount of ferrofluid applied to the patients was 0.5% of the estimated blood volume. The ferrofluid was well tolerated, and in some of the patients the therapeutic procedure was partially effective [116, 119].

Magnetic fluids can be used to prepare various types of magnetic biocompatible and biodegradable (bio)polymer particles and magnetoliposomes that can be used to encapsulate various drugs and radionuclides [120, 121]. Thermosensitive magnetoliposomes can release the entrapped drugs after selective heating caused by an electromagnetic field [122, 123].

In animal experiments, the local prevention of thrombosis in arteries of dogs and rabbits has been achieved by the intravenous application of the autologous red blood cells loaded with ferromagnetic colloid suspension and aspirin if a strong SmCo magnet was secured externally to the artery where the thrombus was initiated [124].

Magnetic fluid hyperthermia

During cancer therapy many procedures have been used. Hyperthermia is a promising approach to cancer therapy based on the heating of the target tissue to temperatures between 42 and 46°C, thus generally reducing the viability of cancer cells and increasing their sensitivity to chemotherapy and radiation.

Unlike chemotherapy and radiotherapy, hyperthermia itself has fewer side effects. Magnetic fluid hyperthermia is based on the fact that subdomain magnetic particles produce heat through various kinds of energy losses during application of an external AC magnetic field. If magnetic particles can be accumulated only in the tumor tissue, cancer specific heating is available.

In 1979, *Gordon et al.* [125] have used for the first time a magnetic fluid based on dextran magnetite nanoparticles to treat mammary tumour bearing rats. Since then, various types of biocompatible magnetic fluids [126, 127], cationic magnetoliposomes [128], or affinity magnetoliposomes [129] have been used for hyperthermia treatment, and several review papers are available on this topic [127, 130].

An interesting possibility of cancer treatment is the combination of hyperthermia treatment followed by chemotherapy or gene therapy. In this case, magnetic nano- or microspheres or magnetoliposomes containing a drug are first used to cause hyperthermia using the standard procedure; subsequently, the released drug acts on the injured cancer cells. This combined treatment might be very efficient [131]. Alternatively, magnetoliposomes have been used to cause hyperthermia which also resulted in the expression of interferon-β from the gene under the control of a heat inducible promoter, inserted to the tumors cells using standard liposomes [132].

At present, systems for magnetic fluid hyperthermia therapy are under development, and the phase I of the clinical testing is under preparation [127].

Contrast-increasing materials during magnetic resonance imaging

Currently, magnetic resonance imaging (MRI) belongs to standard medical examination methods. MRI is essentially proton NMR performed on tissue. In MRI, image contrast is a result of the different signal intensity each tissue produces in response to a particular sequence of the applied radiofrequency pulses. This response depends on proton density and magnetic relaxation times so as MRI contrast depends on the chemical composition (especially on the concentration of water or lipid molecules) and molecular structure of the tissue and is usually manipulated by adjusting the instrumental parameters [133]. In early 1980s it was recognized that target-specific superparamagnetic particles can serve as a dramatic source of exogenous contrast and have rapidly become an important and indispensable tool for the non-invasive study of biological processes with MRI. Superparamagnetic magnetite-dextran nanoparticles change the rate at which protons decay from their excited state to the ground state. As a result, regions containing the superparamagnetic contrast agent appear darker in an MRI than regions without the agent. For instance, when superparamagnetic nanoparticles are delivered to the liver, healthy liver cells can uptake the particles whereas diseased cells cannot. Consequently, the healthy regions are darkened, and the diseased regions remain bright [134]. A novel use of these nanoparticles deals with tracking cells *in vivo*. Rat T-cells were labelled with superparamagnetic Fe_3O_4 nanoparticles. Inflamed rats' testicles attracted the magnetite-attached T-cells and caused a decrease in the MRI signal from the testicles [135]. Alternatively, antibody-conjugated magnetite nanoparticles can serve as a target-directed magnetic

resonance contrast agent [136]. Polyethylene glycol-modified magnetoliposomes with a diameter of 40 nm and containing 1–6 superparamagnetic iron oxide crystals per vesicle have been found to have excellent properties as a bone marrow-seeking MR contrast agent [137].

Miscellaneous and potential medical applications

Recently, the direct antitumour effect of biocompatible cobalt-ferrite-based magnetic fluid on bitch mammary tumor cells has been studied. It was observed that tumor cells intensively endocytosed magnetic nanoparticles and became overloaded by them. This situation led to a massive necrosis of tumour cells [138].

Two methods have been proposed for the treatment of the acquired immunodeficiency syndrome (AIDS) by introducing magnetoliposomes with coupled human immunodeficiency virus (HIV) receptor proteins into the blood stream. In the first procedure, HIV bound to magnetoliposomes should be separated from the patient's body using the arterio-venous shunt connected with a high gradient magnetic separation device where the complex should be removed [139]. The second procedure is based on the inductive heating of the HIV-magnetoliposome complexes in an AC magnetic field which should inactivate the AIDS virus [140].

Silicone magnetic fluid has been suggested for use in eye surgery to help in the course of retinal detachment repair. The procedure should employ a magnetized encircling scleral buckle holding in place a magnetic fluid providing 360° encircling internal tamponade [141, 142].

A possible procedure leading to the damage of the target cells has been described. Magnetic nanoparticles complexed with an appropriate antibody will bind to the cell membrane of the target cell. After application of an external rotating magnetic field of rapidly changing polarity, the ferrofluid particles will be drawn into a circular path, and an axial spin will be induced as each particle aligns itself with the magnetic force lines. A portion of these magnetic fluid particles will be drawn into the target cell membrane and into the cytoplasm causing brief perforations of the cell membrane of the target cells. If enough mechanical damage is done to the plasma membrane or to the intracellular structures, cell lysis may result, but in any case the brief disruptions of the target cell membrane can be used to selectively introduce membrane impermeant cytotoxic or antiretroviral substances into the target cell while relatively sparing normal cells [143].

A brain tumor position sensing method using a magnetoimpedance micromagnetic sensor in combination with magnetic fluids has been proposed. Sensing of magnetic fields generated from magnetic nanoparticles accumulated in the tumor tissue could be an effective way to accurately detect the tumor position during the surgical operation [144].

An artificial sphincter muscle using magnetic fluid has been developed in Japan for use in an artificial anus. The device has been used successfully in dogs, and application in humans is foreseen [26]. Ferrofluid has also been used as a seal for an axial flow pump during the development of an implantable artificial heart [145].

Applications in other biosciences

The influence of biocompatible magnetic fluids on the physiological functions of various plants have been tested. The presence of magnetic nanoparticles in growth media caused different kinds of modifications in plant growth, organogenesis, life cycle, and cell structure. For example, root and leaf induction was accelerated in 2–6 days in the presence of ferrofluid. Positive effect of ferrofluids have also been observed in *in vitro* plant regenerates which were grown in hypogravity conditions [146, 147].

Magnetic field sensitive polymer gels containing magnetic nanoparticles exhibit a quick controllable change of shape caused by the change of magnetic field. This property can be used to mimic muscular contractions. The peculiar magnetoelastic properties of these gels may be used to create a wide range of motion and to control a smooth and gentle shape change and movement similar to what is observed in muscles [148].

Future Trends

As can be seen, magnetic nanoparticles represent an extremely interesting group of inorganic nanomaterials, having close connections to living systems and their components. Their importance has even exceeded the planet Earth, and magnetic nanoparticles might be among the first proofs of the presence of extraterrestrial life.

Further studies of magnetic nanoparticles biomineralization processes will be interesting not only from the point of view of basic research, but also with respect to large-scale synthesis of magnetic biocompatible nanoparticles. Biotechnology production of either unmodified or genetically engineered bacterial magnetosomes may be of great interest and become one of the standard processes for magnetic nanoparticle preparation.

Separation processes employing magnetic modification of originally diamagnetic components followed by magnetic separation will be used more frequently. These techniques will become standard procedures in biology and clinical laboratories.

Magnetic assays, especially those employing magnetic nanoparticles as specific labels, will certainly find more applications in the near future. The progress in this area will be supported by the further development of computer hardware technology which will enable the detection of minute amounts of magnetic labels.

Probably the most important applications of magnetic nanoparticles in the area of biosciences can be expected in medicine and related disciplines. Magnetic drugs, antibiotics, radionuclides, genes, *etc*. targeting, magnetic fluid hyperthermia, detection of cancer cells, isolation of stem cells, possible influence on biological functions by specific types of ferrofluids, improvement of diagnostic procedures (such as MRI), development of clinical biochemistry assays based on the application of magnetic nanoparticles – these all and most probably several other procedures will be further developed to employ the unique properties of magnetic nanoparticles. Not only the individual procedures, but also their combinations (such as magnetic drug targeting combined with hyperthermia) can lead to very interesting results.

The combination of nanotechnologies and biosciences will be one of the leading areas of research and development in the 21st century; magnetic nanoparticles will certainly play an extremely important role.

Acknowledgements

This work was supported by the Ministry of Education of the Czech Republic (grant project No. OC 523.80) and the NATO Science Programme (Collaborative Linkage Grant No. LST.CLG.977500).

References

[1] Huczko A (2000) Appl Phys A – Mater Sci Process **70**: 365
[2] Raj K, Moskowitz B, Casciari R (1995) J Magn Magn Mater **149**: 174
[3] Rosensweig RE (1982) Sci Amer **247**: 136
[4] Lowenstam HA (1962) Bull Geol Soc Am **73**: 435
[5] Kirschvink JL, Hagadorn JW (2000) In: Bauerlein E (ed) The Biomineralisation of Nano- and Micro-Structures. Wiley-VCH, Weinheim, p 139
[6] Blakemore R (1975) Science **190**: 377
[7] Schüler D, Frankel RB (1999) Appl Microbiol Biotechnol **52**: 464
[8] Matsunaga T, Sakaguchi T (2000) J Biosci Bioeng **90**: 1
[9] Schüler D (2000) In: Bartlett DH (ed) Molecular Marine Microbiology. Horizon Scientific Press, Wymondham, p 157
[10] Zhang CL, Vali H, Romanek CS, Phelps TJ, Liu SV (1998) Am Mineral **83**: 1409
[11] Torres de Araujo FF, Pires MA, Frankel RB, Bicudo CEM (1986) Biophys J **50**: 375
[12] Peck JA, King JW (1996) Earth Planet Sc Lett **140**: 159
[13] Wajnberg E, Acosta-Avalos D, El-Jaick LJ, Abracado L, Coelho JLA, Bakuzis AF, Morais PC, Esquivel DMS (2000) Biophys J **78**: 1018
[14] El-Jaick LJ, Acosta-Avalos D, De Souza DM, Wajnberg E, Linhares MP (2001) Eur Biophys J Biophys Lett **29**: 579
[15] Winklhofer M, Holtkamp-Rotzler E, Hanzlik M, Fleissner G, Petersen N (2001) Eur J Mineral **13**: 659
[16] Ogura M, Kato M, Arai N, Sasada T, Sakaki Y (1992) Can J Zool **70**: 874
[17] Lohmann KJ, Johnsen S (2000) Trends Neurosci **23**: 153
[18] Kirschvink JL, Walker MM, Diebel CE (2001) Curr Opin Neurobiol **11**: 462
[19] Acosta-Avalos D, Wajnberg E, Oliveira PS, Leal I, Farina M, Esquivel DMS (1999) J Exp Biol **202**: 2687
[20] Walker MM, Diebel CE, Haugh CV, Pankhurst PM, Montgomery JC, Green CR (1997) Nature **390**: 371
[21] Diebel CE, Proksch R, Green CR, Neilson P, Walker MM (2000) Nature **406**: 299
[22] Dobson J (2001) FEBS Lett **496**: 1
[23] Thomas-Keprta KL, Bazylinski DA, Kirschvink JL, Clemett SJ, McKay DS, Wentworth SJ, Vali H, Gibson EK, Romanek CS (2000) Geochim Cosmochim Acta **64**: 4049
[24] Friedmann EI, Wierzchos J, Ascaso C, Winklhofer M (2001) Proc Natl Acad Sci USA **98**: 2176
[25] Weiss BP, Kirschvink JL, Baudenbacher FJ, Vali H, Peters NT, Macdonald, FA, Wikswo JP (2000) Science **290**: 791
[26] Berkovski B, Bashtovoy V (eds) (1996) Magnetic Fluids and Applications Handbook. Begell House, New York
[27] Massart R (1981) IEEE Trans Magn **17**: 1247
[28] Berger P, Adelman NB, Beckman KJ, Campbell DJ, Ellis AB, Lisensky GC (1999) J Chem Educ **76**: 943

[29] Shen LF, Laibinis PE, Hatton TA (1999) J Magn Magn Mater **194**: 37
[30] Shen LF, Laibinis PE, Hatton TA (1999) Langmuir **15**: 447
[31] Sousa MH, Rubim JC, Sobrinho PG, Tourinho FA (2001) J Magn Magn Mater **225**: 67
[32] Halbreich A, Roger J, Pons JN, Geldwerth D, DaSilva MF, Roudier M, Bacri JC (1998) Biochimie **80**: 379
[33] Domingo JC, Mercadal M, Petriz J, De Madariaga MA (2001) J Microencapsul **18**: 41
[34] Tiefenauer LX, Kuhne G, Andres RY (1993) Bioconjugate Chem **4**: 347
[35] Molday RS, Mackenzie D (1982) J Immunol Methods **52**: 353
[36] Pardoe H, Chua-anusorn W, Pierre TGS, Dobson J (2001) J Magn Magn Mater **225**: 41
[37] O'Connor CJ, Seip CT, Carpenter EE, Li SC, John VT (1999) Nanostruct Mater **12**: 65
[38] O'Connor CJ, Kolesnichenko V, Carpenter E, Sangregorio C, Zhou WL, Kumbhar A, Sims J, Agnoli F (2001) Synthetic Met **122**: 547
[39] Seip CT, O'Connor CJ (1999) Nanostruct Mater **12**: 183
[40] De Cuyper M, Joniau M (1988) Eur Biophys J **15**: 311
[41] De Cuyper M, Joniau M (1993) J Magn Magn Mater **122**: 340
[42] Rocha FM, de Pinho SC, Zollner RL, Santana MHA (2001) J Magn Magn Mater **225**: 101
[43] Sangregorio C, Wiemann JK, O'Connor CJ, Rosenzweig Z (1999) J Appl Phys **85**: 5699
[44] Menager C, Cabuil V (1994) Colloid Polym Sci **272**: 1295
[45] Mosbach K, Andersson L (1977) Nature **270**: 259
[46] Prestvik WS, Berge A, Mork PC, Stenstad PM, Ugelstad J (1997) In: Häfeli U, Schütt W, Teller J, Zborowski M (eds) Scientific and Clinical Applications of Magnetic Carriers. Plenum, New York London, p 11
[47] Yanase N, Noguchi H, Asakura H, Suzuta T (1993) J Appl Polym Sci **50**: 765
[48] Ding XB, Sun ZH, Wan GX, Jiang YY (1998) React Funct Polym **38**: 11
[49] Abe M (2000) Electrochim Acta **45**: 3337
[50] Margel S, Gura S, Bamnolker H, Nitzan B, Tennenbaum T, BarToov B, Hinz M, Seliger H (1997) In: Häfeli U, Schütt W, Teller J, Zborowski M (eds) Scientific and Clinical Applications of Magnetic Carriers. Plenum, New York London, p 37
[51] Sauzedde F, Elaissari A, Pichot C (1999) Colloid Polym Sci **277**: 846
[52] Sauzedde F, Elaissari A, Pichot C (1999) Colloid Polym Sci **277**: 1041
[53] Ziolo RF, Giannelis EP, Weinstein BA, Ohoro MP, Ganguly BN, Mehrotra V, Russell MW, Huffman DR (1992) Science **257**: 219
[54] Llanes F, Ryan DH, Marchessault RH (2000) Int J Biol Macromol **27**: 35
[55] Marchessault RH, Ricard S, Rioux P (1992) Carbohyd Res **224**: 133
[56] Hirano S, Yogo T, Sakamoto W, Yamada S, Nakamura T, Yamamoto T, Ukai H (2001) J Eur Ceram Soc **21**: 1479
[57] Matsunaga T, Takeyama H (1998) Supramol Sci **5**: 391
[58] Meldrum FC, Heywood BR, Mann S (1992) Science **257**: 522
[59] Šafařík I, Šafaříková M (1999) J Chromatogr B **722**: 33
[60] Šafařík I, Šafaříková M, Forsythe SJ (1995) J Appl Bacteriol **78**: 575
[61] Šafaříková M, Šafařík I (2001) Magn Electr Sep **10**: 223
[62] Šafařík I, Šafaříková M (1997) In: Häfeli U, Schütt W, Teller J, Zborowski M (eds) Scientific and Clinical Applications of Magnetic Carriers. Plenum, New York London, p 323
[63] Cell Separation and Protein Purification, Information booklet, Dynal, Oslo, Norway, 1996
[64] Biomagnetic Techniques in Molecular Biology, Information booklet, Dynal, Oslo, Norway, 1998
[65] Matsunaga T, Kamiya S (1987) Appl Microbiol Biotechnol **26**: 328
[66] Matsunaga T, Kawasaki M, Yu X, Tsujimura N, Nakamura N (1996) Anal Chem **68**: 3551
[67] Nakamura N, Burgess JG, Yagiuda K, Kudo S, Sakaguchi T, Matsunaga T (1993) Anal Chem **65**: 2036
[68] Nakamura N, Matsunaga T (1993) Anal Chim Acta **281**: 585

[69] Matsunaga T, Nakayama H, Okochi M, Takeyama H (2001) Biotechnol Bioeng **73**: 400
[70] Sode K, Kudo S, Sakaguchi T, Nakamura N, Matsunaga T (1993) Biotechnol Tech **7**: 688
[71] Sestier C, DaSilva MF, Sabolovic D, Roger J, Pons JN (1998) Electrophoresis **19**: 1220
[72] Halbreich A, Roger J, Pons JN, da Silva MF, Hasmonay E, Roudier M, Boynard M, Sestier C, Amri A, Geldwerth D, Fertil B, Bacri JC, Sabolovic D (1997) In: Häfeli U, Schütt W, Teller J, Zborowski M (eds) Scientific and Clinical Applications of Magnetic Carriers. Plenum, New York London, p 399
[73] Shinkai M, Honda H, Kobayashi T (1991) Biocatalysis **5**: 61
[74] Shi YJ, Shen RN, Lu L, Broxmeyer HE (1994) Blood Cells **20**: 517
[75] Tamaura Y, Takahashi K, Kodera Y, Saito Y, Inada Y (1986) Biotechnol Lett **8**: 877
[76] Yoshimoto T, Mihama T, Takahashi K, Saito Y, Tamaura Y, Inada Y (1987) Biochem Biophys Res Commun **145**: 908
[77] Yoshimoto T, Ohwada K, Takahashi K, Matsushima A, Saito Y, Inada Y (1988) Biochem Biophys Res Commun **152**: 739
[78] Suzuki M, Shinkai M, Kamihira M, Kobayashi T (1995) Biotechnol Appl Biochem **21**: 335
[79] De Cuyper M, De Meulenaer B, Van der Meeren P, Vanderdeelen J (1995) Biocatal Biotransform **13**: 77
[80] Shinkai M, Suzuki M, Iijima S, Kobayashi T (1995) Biotechnol Appl Biochem **21**: 125
[81] Babincová M (1995) Pharmazie **50**: 702
[82] Matsunaga T, Kamiya S, Tsujimura N (1997) In: Häfeli U, Schütt W, Teller J, Zborowski M (eds) Scientific and Clinical Applications of Magnetic Carriers. Plenum, New York London, p 287
[83] Tanaka T, Matsunaga T (2000) Anal Chem **72**: 3518
[84] Matsunaga T, Sato R, Kamiya S, Tanaka T, Takeyama H (1999) J Magn Magn Mater **194**: 126
[85] Matsunaga T, Togo H, Kikuchi T, Tanaka T (2000) Biotechnol Bioeng **70**: 704
[86] Sakamoto A, Abe M, Masaki T (1999) FEBS Lett **447**: 124
[87] Shinkai M, Kamihira M, Honda H, Kobayashi T (1992) Kagaku Kogaku Ronbunshu **18**: 256
[88] Griffin T, Mosbach K, Mosbach R (1981) Appl Biochem Biotechnol **6**: 283
[89] Wikstrom P, Flygare S, Grondalen A, Larsson PO (1987) Anal Biochem **167**: 331
[90] Suzuki M, Kamihira M, Shiraishi T, Takeuchi H, Kobayashi T (1995) J Ferment Bioeng **80**: 78
[91] Shinkai M, Wang J, Kamihira M, Iwata M, Honda H, Kobayashi T (1992) J Ferment Bioeng **73**: 166
[92] Sato R, Takeyama H, Tanaka T, Matsunaga T (2001) Appl Biochem Biotechnol **91–93**: 109
[93] Takeyama H, Tsuzuki H, Chow S, Nakayama H, Matsunaga T (2000) Marine Biotechnol **2**: 309
[94] Kriz CB, Radevik K, Kriz D (1996) Anal Chem **68**: 1966
[95] Kriz K, Gehrke J, Kriz D (1998) Biosens Bioelectron **13**: 817
[96] Enpuku K, Minotani T (2001) IEICE Trans Electron **E84C**: 43
[97] Enpuku K, Minotani T, Gima T, Kuroki Y, Itoh Y, Yamashita M, Katakura Y, Kuhara S (1999) Jpn J Appl Phys **38**: L1102
[98] Enpuku K, Hotta M, Nakahodo A (2001) Physica **C 357–360**: 1462
[99] Bilkenroth U, Taubert H, Riemann D, Rebmann U, Heynemann H, Meye A (2001) Int J Cancer **92**: 577
[100] Kato K, Radbruch A (1993) Cytometry **14**: 384
[101] Rodriguez-Paris J, Nolta KV, Steck TL (1993) J Biol Chem **268**: 9110
[102] Temesvari L, Rodriguez-Paris J, Bush J, Steck TL, Cardelli J (1994) J Biol Chem **269**: 25719
[103] Glombitza GJ, Becker E, Kaiser HW, Sandhoff K (1997) J Biol Chem **272**: 5199
[104] Warnock DE, Roberts C, Lutz MS, Blackburn WA, Young WWJ, Baenziger JU (1993) J Biol Chem **268**: 10145
[105] Nagatani N, Shinkai M, Honda H, Kobayashi T (1998) Biotechnol Tech **12**: 525

[106] Takeyama H, Yamazawa A, Nakamura C, Matsunaga T (1995) Biotechnol Tech **9**: 355
[107] Matsunaga T, Tsujimura N, Kamiya S (1996) Biotechnol Tech **10**: 495
[108] Schüler D, Baeuerlein E (1997) J Phys IV **7**: 647
[109] Gorby YA, Beveridge TJ, Blakemore RP (1988) J Bacteriol **170**: 834
[110] Okuda Y, Denda K, Fukumori Y (1996) Gene **171**: 99
[111] Schüler D, Baeuerlein E (1997) In: Trautwein A (ed) Bioinorganic Chemistry: Transition Metals in Biology and Coordination Chemistry. Wiley-VCH, Weinheim, p 24
[112] Harasko G, Pfutzner H, Futschik K (1995) IEEE Trans Magn **31**: 938
[113] Funaki M, Sakai H, Matsunaga T, Hirose S (1992) Phys Earth Planet Inter **70**: 253
[114] Bahaj AS, James PAB, Moeschler FD (1998) J Magn Magn Mater **177**: 1453
[115] Bahaj AS, Croudace IW, James PAB, Moeschler FD, Warwick PE (1998) J Magn Magn Mater **184**: 241
[116] Lubbe AS, Bergemann C (1997) In: Häfeli U, Schütt W, Teller J, Zborowski M (eds) Scientific and Clinical Applications of Magnetic Carriers. Plenum, New York London, p 457
[117] Widder KJ, Senyei AE (1983) Pharmacol Therapeut **20**: 377
[118] Torchilin VP (2000) Eur J Pharm Sci **11**: S81
[119] Lubbe AS, Bergemann C, Riess H, Schriever F, Reichardt P, Possinger K, Matthias M, Dorken B, Herrmann F, Gurtler R, Hohenberger P, Haas N, Sohr R, Sander B, Lemke AJ, Ohlendorf D, Huhnt W, Huhn D (1996) Cancer Res **56**: 4686
[120] Pulfer SK, Gallo JM (1997) In: Häfeli U, Schütt W, Teller J, Zborowski M (eds) Scientific and Clinical Applications of Magnetic Carriers. Plenum, New York London, p 445
[121] Kuznetsov AA, Filippov VI, Alyautdin RN, Torshina NL, Kuznetsov OA (2001) J Magn Magn Mater **225**: 95
[122] Viroonchatapan E, Sato H, Ueno M, Adachi I, Tazawa K, Horikoshi I (1997) J Control Release **46**: 263
[123] Babincová M, Babinec P (1997) Cell Mol Biol Lett **2**: 3
[124] Orekhova NM, Akchurin RS, Belyaev AA, Smirnov MD, Ragimov SE, Orekhov AN (1990) Thromb Res **57**: 611
[125] Gordon RT, Hines JR, Gordon D (1979) Med Hypotheses **5**: 83
[126] Brusentsov NA, Gogosov VV, Brusentsova TN, Sergeev AV, Jurchenko NY, Kuznetsov AA, Kuznetsov OA, Shumakov LI (2001) J Magn Magn Mater **225**: 113
[127] Jordan A, Scholz R, Maier-Hauff K, Johannsen M, Wust P, Nadobny J, Schirra H, Schmidt H, Deger S, Loening S, Lanksch W, Felix R (2001) J Magn Magn Mater **225**: 118
[128] Yanase M, Shinkai M, Honda H, Wakabayashi T, Yoshida J, Kobayashi T (1998) Jpn J Cancer Res **89**: 463
[129] Le B, Shinkai M, Kitade T, Honda H, Yoshida J, Wakabayashi T, Kobayashi T (2001) J Chem Eng Jpn **34**: 66
[130] Jordan A, Scholz R, Wust P, Fahling H, Felix R (1999) J Magn Magn Mater **201**: 413
[131] Shinkai M, Suzuki M, Yokoi N, Yanase M, Shimizu W, Honda H, Kobayashi T (1998) Jpn J Hyperthermic Oncol **14**: 15
[132] Bouhon IA, Shinkai M, Honda H, Mizuno M, Wakabayashi T, Yoshida J, Kobayashi T (1999) Cancer Lett **139**: 153
[133] Bulte JWM, Brooks RA (1997) In: Häfeli U, Schütt W, Teller J, Zborowski M (eds) Scientific and Clinical Applications of Magnetic Carriers. Plenum, New York London, p 527
[134] Martin CR, Mitchell DT (1998) Anal Chem **70**: A322
[135] Yeh TC, Zhang WG, Ildstad ST, Ho C (1995) Magn Reson Med **33**: 200
[136] Suzuki M, Honda H, Kobayashi T, Wakabayashi T, Yoshida J, Takahashi M (1996) Brain Tumor Pathol **13**: 127
[137] Bulte JWM, de Cuyper M, Despres D, Frank JA (1999) J Magn Magn Mater **194**: 204
[138] Sincai M, Ganga D, Bica D, Vekas L (2001) J Magn Magn Mater **225**: 235
[139] Babincová M, Machová E (1998) Z Naturforsch **C 53**: 935

[140] Müller-Schulte D, Füssl F, Lueken H, De Cuyper M (1997) In: Häfeli U, Schütt W, Teller J, Zborowski M (eds) Scientific and Clinical Applications of Magnetic Carriers. Plenum, New York London, p 517
[141] Dailey JP, Phillips JP, Li C, Riffle JS (1999) J Magn Magn Mater **194**: 140
[142] Voltairas PA, Fotiadis DI, Massalas CV (2001) J Magn Magn Mater **225**: 248
[143] Peasley KW (1996) Med Hypotheses **46**: 5
[144] Uchiyama T, Mohri K, Shinkai M, Ohshima A, Honda H, Kobayashi T, Wakabayashi T, Yoshida J (1997) IEEE Trans Magn **33**: 4266
[145] Mitamura Y, Fujiyoshi M, Yoshida T, Yozu R, Okamoto E, Tanaka T, Kawada S (1996) Artif Organs **20**: 497
[146] Butnaru G, Terteac D, Potencz I (1999) J Magn Magn Mater **201**: 435
[147] Butnaru G (2000) In: Proc Conf "Propagation of Ornamental Plants", Sofia, p 35
[148] Zrinyi M (2000) Colloid Polym Sci **278**: 98
[149] Heywood BR, Bazylinski DA, Garrattreed A, Mann S, Frankel RB (1990) Naturwissenschaften **77**: 536
[150] Bazylinski DA, Schlezinger DR, Howes BH, Frankel RB, Epstein SS (2000) Chem Geol **169**: 319
[151] Maher BA (1998) Proc R Soc Lond Ser B – Biol Sci **265**: 733
[152] Moore A, Freake SM, Thomas IM (1990) Philos T Roy Soc B **329**: 11
[153] Walker MM, Quinn TP, Kirschvink JL, Groot C (1988) J Exp Biol **140**: 51
[154] Brassart J, Kirschvink JL, Phillips JB, Borland SC (1999) J Exp Biol **202**: 3155
[155] Beason RC, Dussourd N, Deutschlander ME (1995) J Exp Biol **198**: 141
[156] Beason RC, Semm P (1996) J Exp Biol **199**: 1241
[157] Hanzlik M, Heunemann C, Holtkamp-Rotzler E, Winklhofer M, Petersen N, Fleissner G (2000) Biometals **13**: 325
[158] Zoeger J, Dunn JR, Fuller M (1981) Science **213**: 892
[159] Kirschvink JL, Kobayashi-Kirschvink A, Woodford BJ (1992) Proc Natl Acad Sci USA **89**: 7683
[160] Schultheiss-Grassi PP, Dobson J (1999) Biometals **12**: 67
[161] Schultheiss-Grassi PP, Wessiken R, Dobson J (1999) Biochim Biophys Acta **1426**: 212
[162] Dobson J, Grassi P (1996) Brain Res Bull **39**: 255
[163] Grassi-Schultheiss PP, Heller F, Dobson J (1997) Biometals **10**: 351

Received October 4, 2001. Accepted November 19, 2001