Magnetic Nanoparticles for Biomedicine

Ivo Šafařík, Kateřina Horská, and Mirka Šafaříková

Abstract Biocompatible materials exhibiting different types of response to external magnetic field have already found many important applications in various areas of biosciences, biotechnology, medicine, environmental technology etc. In most cases they can be described as composite materials, where the magnetic properties are caused by the presence of iron oxides nano- or microparticles. Such materials can be efficiently separated from difficult-to-handle samples and targeted to the desired place, applied as contrast agents for magnetic resonance imaging or used to generate heat during exposure to alternating magnetic field.

Keywords Maghemite • Magnetic iron oxides • Magnetic particles • Magnetic separation • Magnetite

1 Introduction

Biocompatible materials exhibiting response to external magnetic field have found many interesting applications in various areas of biosciences and biotechnology, including different medical disciplines. The broad family of magnetic field-controllable materials includes both nano- and microparticles, high aspect ratio structures (nanotubes, nanowires), thin films, etc. Ferrofluids (magnetic

I. Šafařík (✉)
Department of Nanobiotechnology, Institute of Nanobiology and Structural Biology of GCRC, Academy of Sciences of the Czech Republic, Na Sádkách 7, 370 05 České Budějovice, Czech Republic
and
Regional Centre of Advanced Technologies and Materials, Palacký University, Šlechtitelů 11, 783 71, Olomouc, Czech Republic
e-mail: ivosaf@yahoo.com

K. Horská and M. Šafaříková
Department of Nanobiotechnology, Institute of Nanobiology and Structural Biology of GCRC, Academy of Sciences of the Czech Republic, Na Sádkách 7, 370 05 České Budějovice, Czech Republic

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fluids), magnetorheological fluids, magnetic polymers, magnetic inorganic materials, magnetically modified biological structures, magnetic particles with bound biomolecules etc. can serve as typical examples. In many cases magnetically responsive composite materials consist of small magnetic particles (most often formed by magnetite, maghemite or various ferrites), usually in the nanometer to micrometer range, dispersed in a polymer, biopolymer or inorganic matrix; alternatively magnetic particles can be adsorbed on the outer surface of diamagnetic particles (Safarik and Safarikova 2009b).

Magnetic properties of such materials enable their applications in numerous areas (Arruebo et al. 2007; Safarik and Safarikova 2009a), namely:

- Magnetically responsive nano- and microparticles and other relevant materials can be selectively separated (removed) from the complex samples using an external magnetic field (e.g. using an appropriate magnetic separator, permanent magnet, or electromagnet). This process is very important for bioapplications due to the fact that absolute majority of biological materials have diamagnetic properties which enable efficient selective separation of magnetic materials.
- Magnetic particles can be targeted to the desired place and kept there using an external magnetic field. These properties can be used e.g. for sealing the rotating objects or in the course of magnetic drug targeting.
- Magnetic particles can generate heat when subjected to high frequency alternating magnetic field; this phenomenon is employed especially during magnetic fluid hyperthermia (e.g., for cancer treatment).
- Magnetic iron oxides nanoparticles generate a negative T2 contrast during magnetic resonance imaging thus serving as efficient contrast agents.
- Magnetorheological fluids exhibit great increase of apparent viscosity when subjected to a magnetic field.
- Magnetic nano- and microparticles can be used for magnetic modification of diamagnetic biological materials (e.g. cells or plant-derived materials), organic polymers and inorganic materials, and for magnetic labeling of biologically active compounds (e.g. antibodies, enzymes, aptamers etc.).

In most cases synthetic (laboratory-produced) magnetically responsive nano- and microparticles and related structures have been developed, however, biologically produced magnetic particles (e.g., magnetosomes produced by magnetotactic bacteria) have been successfully used for selected bioapplications (Arakaki et al. 2008). This short review chapter shows typical examples of biocompatible magnetic materials synthesis and typical examples of their biomedical applications.

2 Synthesis of Magnetic Nanoparticles

Many chemical procedures have been used to synthesize magnetic nano- and microparticles applicable for bioapplications, such as classical coprecipitation, reactions in constrained environments (e.g., microemulsions), sol-gel syntheses, sonochemical and microwave reactions, hydrothermal reactions, hydrolysis and thermolysis

of precursors, flow injection syntheses, electrospray syntheses and mechanochemical processes (Laurent et al. 2008; Lin et al. 2006; Zheng et al. 2010).

The simplest and most efficient chemical pathway to obtain magnetic particles is probably the coprecipitation technique. Iron oxides, either in the form of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3), are usually prepared by aging stoichiometric mixture of ferrous and ferric salts in aqueous alkaline medium. The chemical reaction of Fe_3O_4 formation is usually written as follows:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O$$

However, magnetite (Fe_3O_4) is not very stable and is sensitive to oxidation which results in the formation of maghemite (γ-Fe_2O_3).

The main advantage of the coprecipitation process is that a large amount of nanoparticles can be synthesized. However, the control of particle size distribution is limited. The addition of chelating organic anions (carboxylate or α-hydroxy carboxylate ions, such as citric, gluconic, or oleic acids) or polymer surface complexing agents (dextran, carboxydextran, starch, or polyvinyl alcohol) during the formation of magnetite can help to control the size of the nanoparticles. According to the molar ratio between the organic ion and the iron salts, the chelation of these organic ions on the iron oxide surface can either prevent nucleation and then lead to larger particles or inhibit the growth of the crystal nuclei, leading to small nanoparticles (Berger et al. 1999; Laurent et al. 2008).

Classical coprecipitation method generates particles with a broad size distribution. Synthesis of iron oxide nanoparticles with more uniform dimensions can be performed in synthetic and biological nanoreactors, such as water-swollen reversed micellar structures in non-polar solvents, apoferritin protein cages, dendrimers, cyclodextrins, and liposomes (Laurent et al. 2008).

Hydrothermal syntheses of magnetite nanoparticles are performed in aqueous media in reactors or autoclaves where the pressure can be higher than 2,000 psi (ca 13.8 MPa) and the temperature can be above 200°C. In this process, the reaction conditions, such as solvent, temperature, and time, usually have important effects on the products. The particle size of magnetite powders increased with a prolonged reaction time and the higher water content resulted in the precipitation of larger magnetite particles (Laurent et al. 2008).

The sol-gel process is a suitable wet route to the synthesis of nanostructured metal oxides. This process is based on the hydroxylation and condensation of molecular precursors in solution, originating a "sol" of nanometric particles. Further condensation and inorganic polymerization leads to a three-dimensional metal oxide network denominated wet gel. Because these reactions are performed at room temperature, further heat treatments are needed to acquire the final crystalline state. The main parameters that influence the kinetics, growth reactions, hydrolysis, condensation reactions, and consequently, the structure and properties of the gel are solvent, temperature, nature and concentration of the salt precursors employed, pH, and agitation (Laurent et al. 2008).

The polyol process is a versatile chemical approach for the synthesis of nano- and microparticles with well-defined shapes and controlled sizes. Selected polyols (for example, polyethylene glycol) used as solvents exhibit high dielectric constants,

and can dissolve inorganic compounds. In addition, due to their relatively high boiling points, they offer a wide operating-temperature range for preparing inorganic compounds. Polyols also serve as reducing agents as well as stabilizers to control particle growth and prevent interparticle aggregation (Laurent et al. 2008). Non-aggregated magnetite nanoparticles (7 nm in diameter) were synthesized during the reaction of triethylene glycol with $Fe(acac)_3$ at an elevated temperature (Cai and Wan 2007).

Recently a novel synthesis of magnetite nanoparticles based on a flow injection synthesis (FIS) technique has been developed. The technique consists of continuous or segmented mixing of reagents under laminar flow regime in a capillary reactor. The FIS technique has shown some advantages, such as a high reproducibility because of the plug-flow and laminar conditions, a high mixing homogeneity, and an opportunity for a precise external control of the process. The obtained magnetite nanoparticles had a narrow size distribution in the range of 2–7 nm (Laurent et al. 2008; Salazar-Alvarez et al. 2006).

Spray and laser pyrolysis, typical representatives of aerosol technologies, are continuous chemical processes allowing for high rate production of nanoparticles. In spray pyrolysis, a solution of ferric salts and a reducing agent in organic solvent is sprayed into a series of reactors, where the aerosol solute condenses and the solvent evaporates. The resulting dried residue consists of particles whose size depends upon the initial size of the original droplets. Maghemite particles with size ranging from 5 to 60 nm with different shapes have been obtained using different iron precursor salts in alcoholic solution (Laurent et al. 2008).

A wide variety of chemical reactions accelerated by microwave irradiation of reactants have been observed. Recently a simple, quick and cost effective microwave method to prepare relatively uniform magnetite nanoparticles (80 ± 5 nm) directly from Fe^{2+} salts has been developed; the formation of magnetic nanoparticles using microwave method requires only a few seconds or minutes. Also magnetite nanoparticles doped with silver nanoparticles can be prepared using this procedure (Zheng et al. 2010).

Nanosized magnetite powders can also be synthesized via a mechanochemical reaction. Ball milling of ferrous and ferric chlorides with sodium hydroxide led to a mixture of magnetite and sodium chloride. To avoid agglomeration, the excess of NaCl is usually added to the precursor before ball milling. To prepare different size of particles, the as-milled powders were annealed at temperatures ranging from 100°C to 800°C for 1 h in appropriate atmosphere (Lin et al. 2006).

3 Stabilization of Magnetic Particles

To obtain biocompatible magnetically responsive materials it is usually necessary to stabilize the prepared iron oxide nanoparticles by appropriate modification of their surface or by their incorporation into appropriate biocompatible matrix.

The modified magnetic nanoparticles should be stable against aggregation in both a biological medium and a magnetic field.

Several compounds with carboxylic, phosphate and sulfate functional groups are known to bind to the surface of magnetic particles and stabilize them. Citric acid can be successfully used to stabilize water-based magnetic fluids (ferrofluids) by coordinating via one or two of the carboxyl residues; this leaves at least one carboxylic acid group exposed to the solvent, which should be responsible for making the surface negatively charged and hydrophilic. Other ferrofluids can be stabilized by ionic interactions, using e.g., perchloric acid or tetramethylammonium hydroxide (Berger et al. 1999; Laurent et al. 2008).

In most cases biocompatible (bio)polymers are used for magnetic particles stabilization and modification. Ideal natural or synthetic polymeric materials used for particles stabilization should have several important properties; they should be biocompatible and for many applications also biodegradable, non-toxic, non-thrombogenic, non-immunogenic and inexpensive. The "ideal" magnetically responsive (bio)polymer biocompatible composite nanoparticles should have the following typical properties: particles diameter below 100 nm, stability in blood, no activation of neutrophils, no platelet aggregation, avoidance of the reticuloendothelial system, noninflamatory behavior, prolonged circulation time, possible immobilization of appropriate biologically active compounds (e.g., antibodies) and scalable and cost-effective production (Lockman et al. 2002). Dextran, a polysaccharide polymer composed exclusively of α-D-glucopyranosyl units with varying degrees of chain length and branching has often been used as a polymer coating mostly because of its excellent biocompatibility. The formation of magnetite in the presence of dextran 40,000 was reported for the first time in 1980s (Molday and Mackenzie 1982). The same procedure has been used for the preparation of approved magnetic resonance contrast agents Ferumoxtran-10 (Combidex, Sinerem); this material has a small hydrodynamic diameter (15–30 nm), and shows a prolonged blood residence time, which allows this USPIO ("Ultrasmall superparamagnetic iron oxides") to access macrophages located in deep and pathologic tissues (such as lymph nodes, kidney, brain, osteoarticular tissues, etc.). Other common biopolymer coatings are formed e.g. by carboxymethylated dextran, carboxydextran, starch, chitosan, alginate, arabinogalactan or glycosaminoglycan, while polyethylene glycol (PEG) and polyvinyl alcohol (PVA) represent biocompatible synthetic polymers (Laurent et al. 2008).

Magnetic nanoparticles often form a magnetic part of magnetically responsive composite microparticles formed from various synthetic polymers, biopolymers, inorganic materials, microbial cells or plant materials (Safarik and Safarikova 2009b). Superparamagnetic monodisperse microparticles composed of polystyrene matrix with entrapped maghemite nanoparticles (approx. 8 nm in diameter; Fonnum et al. 2005), known as Dynabeads (Invitrogen), have been used in enormous amount of bioapplications, especially in molecular biology, cell biology, microbiology and protein separation.

4 Examples of Biomedical Applications of Magnetic Particles

Magnetically responsive nano- and microparticles have many already established and potential applications in various areas of biosciences, biotechnology and environmental technology. Biomedicine related applications are mainly based on the utilization of selected properties, namely magnetic separation, magnetic targeting, heat production and MRI contrast increase.

4.1 In Vitro Application of Magnetic Particles

Magnetic separation is a very simple, fast, efficient and gentle tool for the rapid isolation of target molecules, cell organelles and cells from complex biological mixtures and crude samples such as blood, bone marrow, tissue homogenates, urine, stools and other biological materials. The molecules and cells isolated by magnetic separation are usually pure, viable and unaltered. The gentle test tube magnetic separation is the technology of choice when there is a need for high yields of pure and biologically active compounds and biological structures in small scale (Šafařík and Šafaříková 1999, 2004).

Magnetic separations have been mainly employed for the immunomagnetic separation (IMS) of eukaryotic target cells (e.g., cancer cells, stem cells or T-lymphocytes). The progress of this procedure has been enabled by the development of monoclonal antibodies and the improved characterization of cells specific antigens. It is now possible to separate many specific cell types from mixed populations in blood, bone marrow and other sources without cell loss or damage. By positive isolation the cells of interest are magnetically labeled by specific particles and subsequently isolated for analysis. For some downstream applications (e.g., mRNA or DNA isolation) the beads can remain attached to the isolated cells. For other applications (e.g., FACS analysis) magnetic microbeads have to be removed from the cell surface. By negative isolation (= depletion), unwanted cells are removed prior to analysis of the remaining population. Primary coated Dynabeads and some other magnetic particles are ready-to-use products for a wide variety of cell surface markers. In addition, secondary coated magnetic particles offer an excellent possibility to make beads with the reactivity of choice using mouse, rat, sheep or rabbit antibodies (Šafařík and Šafaříková 1999).

The magnetic separation procedure can be scaled up if large quantities of living cells are required. Several commercially available devices enable to separate target cells from larger volume of blood or cells suspensions. The CliniMACS System (Miltenyi Biotec, Germany) is based on MACS Technology which employs MACS MicroBeads (nano-sized superparamagnetic particles approximately 50 nm in diameter, composed of an iron dextran matrix coupled to specific antibodies) and a magnetic separation unit. CliniMACS permits the automated separation of cells on

a clinical-scale level in a closed and sterile system. The key components of the CliniMACS Plus Instrument are the integrated microcomputer, the magnetic separation unit, the peristaltic pump and various pinch valves. The Isolex® 300 System (Baxter, USA) is a semi-automated magnetic cell separation system designed to select and isolate CD34 positive cells, ex *vivo,* from mobilized peripheral blood using anti-CD34 monoclonal antibody and superparamagnetic microspheres (Dynabeads). The device consists of an instrument for separating Dynabeads from mobilized peripheral blood mononuclear cell (MNC) suspensions and an associated disposable set for providing the fluid path.

IMS of important pathogenic bacteria (*Salmonella, Legionella, Listeria* and verotoxigenic strains of *Escherichia coli* (e.g., O157, EPEC/VTEC O103, O111 and O145)) and protozoan parasites (*Cryptosporidium, Giardia*) is efficiently used in medical, food and water microbiology. Both micrometer and nanometer scale magnetic particles with immobilized specific antibodies are used as labels. IMS enables to shorten the detection time which is a very important feature from the medical point of view. As an alternative to IMS, magnetic particles with immobilized lectins have been used for similar purpose (Šafařík and Šafaříková 1999).

Magnetic particles with immobilized annexin V have been employed for the simple and efficient separation of apoptotic cells from normal culture. This procedure is based on the fact that 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 apoptotic or dead cells. Once on the cell surface, PS becomes available for binding to annexin V and any of its conjugates.

Magnetic targeting of nucleic acids bound to magnetic particles into the recipient cells is the basis of magnetofection which is a simple and highly efficient transfection method. The magnetic iron oxide nanoparticles are usually coated with specific cationic molecules which can associate with the gene vectors (DNA, siRNA, ODN, virus, etc.). The magnetic particles are then concentrated on the target cells by the influence of an external magnetic field generated by magnets. The cellular uptake of the genetic material is accomplished by endocytosis and pinocytosis, two natural biological processes. Consequently, membrane architecture and structure stay intact, in contrast to other physical transfection methods that damage the cell membrane. The nucleic acids are then released into the cytoplasm by different mechanisms depending upon the formulation used. Coupling magnetic nanoparticles to gene vectors of any kind results in a dramatic increase of the uptake of these vectors and consequently high transfection efficiency. Transfected cells can be separated from the non-transfected ones using an appropriate magnetic separation technique (Plank et al. 2003; Scherer et al. 2002; Safarik and Safarikova 2009a).

Magnetic separation of nucleic acids and proteins enables direct isolation and purification of target biomolecules from difficult-to-handle biological samples, such as cell homogenates or body fluids. Magnetic separation techniques have several advantages in comparison to traditional separation procedures; the laboratory-scale process is very simple, and all steps of the purification can take place in one test tube without expensive liquid chromatography systems (Berensmeier 2006; Šafařík and Šafaříková 2004).

Magnetic particles can be efficiently used for the detection and determination of target analytes in clinical biochemistry. Many devices have been developed such as electrochemical immunoassay systems for the simultaneous measurements of several proteins, flow injection analysis (FIA) employing enzymes immobilized on magnetic particles, magnetic separation immunoassay for digoxin with flow injection fluorescence detection, sequential injection analysis with a chemiluminescence detector for the determination of vitellogenin or immunoassay for sequential injection analysis (SIA) (Aguilar-Arteaga et al. 2010). Efficient preconcentration of target analytes from large volumes of water or biological samples can be achieved using Magnetic solid phase extraction (Šafaříková and Šafařík 1999).

4.2 In Vivo Application of Magnetic Particles

Magnetic drug targeting employs magnetic biocompatible nanoparticles containing a drug which could be injected intravenously, transported to a site of action (e.g., cancerous tumor or arterial blockage) and be retained at the site by application of a magnetic field gradient. This form of drug delivery is advantageous in that a specific site in the body can be targeted by the magnetic field gradient, the doses required for systemic drug delivery are reduced, localized drug levels can be increased significantly with reduced potential toxic side effects at non-targeted tissues, and a prolonged release of high localized drug concentrations at a required site can be obtained (Vatta et al. 2006).

Magnetic fluid hyperthermia, based on the fact that subdomain biocompatible magnetic particles produce heat through various kinds of energy losses during application of external AC magnetic field, is a promising approach to cancer therapy due to the heating of the target tissue to the temperatures between 42°C and 46°C that generally reduces the viability of cancer cells and increases their sensitivity to chemotherapy and radiation. Unlike chemotherapy and radiotherapy, hyperthermia itself has fewer side effects. Different types of magnetic biocompatible nanocomposites such as dextran-stabilized magnetic fluid, other types of biocompatible magnetic fluids, aminosilane-modified nanoparticles, cationic magnetoliposomes or affinity magnetoliposomes have been used for hyperthermia treatment (Goya et al. 2008; Safarik and Safarikova 2009b).

Superparamagnetic particles are used as magnetic resonance imaging (MRI) contrast agent in diagnostics applications. Particulate magnetic contrast agents include ultrasmall particles (USPIO – “Ultrasmall superparamagnetic iron oxides”; diameter between 10 and 40 nm), small particles (SPIO – “Small superparamagnetic iron oxides”; diameter between 60 and 150 nm), and oral (large) particles (diameter between 300 nm and 3.5 μm). Commercially available iron oxides based contrast agents are usually stabilized by dextran, carboxymethyl dextran, carboxydextran or styrene divinylbenzene copolymer (Laurent et al. 2008).

A crucial aspect of successful cell transplantation is tracking and monitoring the grafted cells in the transplant recipient. To screen cells *in vivo*, superparamagnetic

iron-oxide nanoparticles have been used to label the cells, enabling subsequent MRI visualization *in vivo,* due to the selective shortening of T_2-relaxation time, leading to a hypointense (dark) signal. MRI, as a noninvasive method, may then be used not only to evaluate whether the cells have been successfully engrafted, but also monitor the time course of cell migration and their survival in the targeted tissue. This information may further help to optimize the transplantation procedure in terms of the number of required cells, the method or site of cell administration and the therapeutic time window after injury during which transplantation will be most effective (Syková and Jendelová 2005).

Detailed information about *in vivo* applications of magnetic particles has been described in other chapters of this book.

5 Conclusions

Magnetic nano- and microparticles have been intensively studied for many years. Magnetically responsive biocompatible materials represent an extremely important group of stimuli responsive materials with high potential both in research and application area. Various areas of biomedicine have already found a substantial benefit due to the application of magnetic nano- and microparticles, both for *in vitro* and *in vivo* procedures. In fact, many different types of magnetic materials are available, however, only a small part of them can be used for possible biomedical applications. Further progress in this area could be expected if cost effective biocompatible magnetic particles would become available. Safety and biocompatibility studies of magnetically responsive materials, in particular long-term toxicity studies, have to be carried out. The potential of magnetic nanomaterials will probably expand when complex magnetic nanoparticles and drugs containing (nano)systems will be constructed, enabling simultaneously their magnetic navigation, MRI detection and heat production.

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