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Applications of Magnetic Targeting in Diagnosis and Therapy—Possibilities and Limitations: A Mini-Review

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INTRODUCTION

SMALL PARTICLES IN THE MICRO- AND NANOMETER RANGES possess increasing importance as diagnostic and therapeutic tools in medicine, as well as other areas of life sciences (for reviews see refs. 1–5). The discovery of uniform latex particles by polymer chemists of the Dow Chemical Company nearly 50 years ago opened up different exciting fields and established many new applications. Developed on the basis of small particles, many *in vitro* diagnostic tests such as phagocytosis and latex agglutination tests have become routine, and the same is true for the use of microspheres in high-pressure liquid chromatography (HPLC) applications. Further developments are still ongoing, and the focus has now shifted to applications of polymer particles in the controlled and directed transport of drugs in living systems.

The ongoing studies in this area are very promising, and this is partly due to three factors: First, there are now biocompatible polymer particles available that contain exact drug amounts and release them in a controlled fashion. Second, these microspheres have enhanced circulation time in the blood, which is mainly due to their modified and functionalized surface, thus influencing the biodistribution and overcoming the reticuloendothelial system (= RES) uptake. Microsphere targeting to areas other than the liver, as well as modifying their adsorption behavior to blood proteins, is therefore possible.(3) Finally, the combination with biologically active molecules, such as proteins, peptides, hormones, lectins, or antibodies, and the use of fluorescent and radioactive markers is the basis of the large variety of well-established and original concepts of future biomedical applications.

The development of magnetically responsive microspheres brought an additional driving force into play. Magnetic forces can be used to move the particles in directed fashion, to target and to hold them *in vivo* at anatomical sites with restricted access, and also to track and separate cells and protein molecules *in vitro* for the directed particle movement, targeting and holding at the restricted anatomic sites by *in vivo* experiments as well as for cell and protein tracking and separation *in vitro*.(6) Therefore, magnetically responsive microspheres are the basis of several new procedures in molecular and cellular biology and have led to new clinical diagnostic and therapeutic concepts.

For the last two decades, the wide size distribution of the available microsphere preparations was limiting *in vivo* studies with magnetic particles due to rapid RES uptake. Therefore, it was only natural that new developments in the production methods for uniformly sized particles and in the prolongation of blood circulation times brought renewed interest to the old ideas of magnetic drug delivery. This minireview seeks to outline the possibilities and limitations of magnetic targeting in drug, radiation, hyperthermia, and genetic therapy. These concepts are treated more extensively in a book written by the participants of the first international meeting on the scientific and clinical applications of magnetic carriers, which was organized by the Cleveland Clinic and the University of Rostock in September 1996.(7)

CLASSIFICATION AND APPLICATIONS OF MAGNETIC NANO- AND MICROPARTICLES

Many industrial applications of colloidal iron oxide solutions, so-called ferrofluids, have been described and are used in loudspeakers, recording technology, as paint ingredients, and in electrochemical devices.(8) Pharmaceutical ferrofluids differ from these industrial colloidal preparations in that preparations for biomedical use must be water-based, biocompatible, nontoxic, and nonimmunogenic.

The application of magnetically responsive microspheres for the biophysical targeting of antitumor agents and other drugs has already been reviewed by Widder *et al.*(9) and Gupta and Hung,(10) respectively. A comprehensive overview of the application of magnetic particles in biomagnetic separation processes was given by Uhlen *et al.*(11) Tables 1 and 2 provide

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TABLE 1. BIODEGRADABLE MAGNETIC PARTICLES

Matrix of magnetic particles	*Biomedical application*	*References*
Erythrocytes	Drug targeting	12,13
	Cell separation	14
Liposomes	Drug targeting	15,16
Phospholipides	Immobilization of membrane-bound enzymes	17–20
Albumin	Drug targeting	21–23
	Cell separation	24
Starch	Drug targeting	25
	MRI	26–28
	Radiotherapy	29
Poly(lactic acid)	Radiotherapy	30–32
Dextran	Cell separation	33,34
	Enzyme immobilization	35
	MRT	36–39
	Local hyperthermia	40–42
	Drug delivery	43
	Immunoenzyme assay	44
Chitosan	Drug targeting	45,46
Polyalkylcyanoacrylate	Drug targeting	47
Polyethylene imine	Drug targeting	48

a short summary of the different types of magnetic particles, together with examples of specific biomedical applications. Magnetic particles are usually classified in biodegradable (Table 1) and nonbiodegradable (Table 2) particles.

SURFACE MODIFICATION AND FUNCTIONALIZATION

The different applications of magnetic microspheres require preparations of microspheres with specific reactive sites for a variety of surface modifications. Magnetic microspheres made from polysaccharides or proteins already contain functional groups such as OH, NH_2, SH, and/or COOH. They can be directly activated or used for the introduction of functional groups. Typical methods of polysaccharide activation are (i) the oxidation of the polysaccharides by periodate to form corresponding polyaldehydes,[44] (ii) the preparation of an azid of the polysaccharide,[55] (iii) the activation by cyanogen halides or organic cyanates to form the corresponding imidocarbonate derivatives,[55] (iv) the preparation of bromo- or chlorohydroxypropyl derivatives through a reaction with bromo- or chloroepoxypropane,[55] and (v) the activation of hydroxy groups via their tosylates.[56]

In general, particles prepared with polysaccharides or with synthetic polymers can be functionalized or activated after the particles are formed. The most important methods for the activation of synthetic magnetic polymers have been summarized by Ugelstad *et al.*[56] and rely heavily on the chemistry of active ester synthesis and the methods of carboxyl-activated microspheres.[57–59] Additionally, synthetic microspheres can be functionalized or activated by copolymerization of monomers containing suitable functional groups, by chemical surface changes or special coating techniques.[2]

PARTICLE AND SURFACE CHARACTERIZATION

The absolute size, the size distribution, and the degree of aggregation are important physical characteristics of magnetic carriers. First measurements are always done by light and electron

TABLE 2. NON-BIODEGRADABLE MAGNETIC PARTICLES

Matrix of magnetic particles	*Biomedical application*	*References*
Ethylcellulose	Arterial chemoembolization	49
Synthetic polymers (*e.g.*, polystyrene, polymethylmethacrylate)	Magnetic separation of specific bacteria, Viruses and parasites	50
	mRNA purification	51
	Isolation of specific genomic sequences	50
	Purification of the templates from contaminants that inhibit the PCR	50
	Detoxification of the blood	52,53
	Magnet-assays	54

microscopy in combination with image analysis. Such absolute data are necessary for the calibration of the faster and statistically more reliable measurements with light scattering and photon correlation spectroscopy.[60] Atomic force microscopy is also valuable for the characterization of the atomic surface structure, the surface modifications, and the alterations taking place during the adsorption of biological molecules. To get information about the magnetic properties of individual particles and their statistical distribution, half- and fully-automated microscopic magnetophoresis can be used.[61,62]

The magnetic responsiveness of magnetic particles in a liquid stream can be measured with a very simple constant-flow apparatus in which the particle accumulation is detected by optical or radiolabeling methods.[63] The magnetophoretic velocity depends on the Brownian motion, the fluid velocities and the very strong inhomogeneous magnetic fields applied to the system. Depending on the velocity profile in the tube, the viscosity of the medium, the particle size, and the particle magnetization, the percentage of retention of the particles can be calculated.[64] Using physiological flow conditions (0.05–10 cm/s) and a unipolar magnetic arrangement, the retention of small particles down to 100 nm has been confirmed.

Exact measurements of the surface charge of particles are important for the prediction of the *in vivo* effects such as opsonization, altered circulation times and also biodistribution changes. Examples for surface charge effects have been shown in liposomes as drug carriers[20] and in polymer particles as MR contrast agents.[65] The measurements can be realized with automated techniques based on (i) single-particle measurements by microscopic image tracking and analysis,[66] (ii) image transduction techniques, or (iii) laser Doppler effect techniques. To improve the value of electrokinetic studies, the method of electrophoretic fingerprinting to analyze different batches of polymer carriers has been introduced.[67] Electrophoretic fingerprints or templates are three-dimensional representations of the mean electrophoretic mobility of a given particle suspension versus pH and ionic strength of the media. These fingerprints represent a surface, described by isomobility lines over all pertinent electrochemical conditions. They are very sensitive to surface modifications and adsorption processes, and the correlation between these fingerprints and the biodistribution is currently being studied.

CELL SEPARATION AND PURIFICATION

Magnetic separation with magnetic particles is a widespread technique that is already successfully adapted for biological applications. After specific attachment of strongly magnetizable microparticles, biological cells or molecules can be tracked in an inhomogeneous magnetic field. Winoto-Morbach and Tchikov[24] have developed an automated microscopic analysis system for the rapid counting of labeled and unlabeled cells. They demonstrated that the binding of only one magnetic microsphere via at least one (1) receptor lead to magnetophoretic movement of a lymphocyte.

The possibility to separate specific cells bound to magnetic microspheres in an inhomogeneous magnetic field allows for the separation or purification of cell subpopulations (for review see ref. 11). This can be done easily in a tube batch by batch, applying separate loading and elution steps[34] or continuously in a chamber with laminar flow conditions under the influence of an inhomogeneous magnetic field.[68] The magnetic moments, induced by the magnetic field, will force the magnetically labeled biological cells or molecules to deviate toward the direction of the magnetic forces. The focused stream of magnetic particles is then collected into separate vials. This technique is used for many important medical applications in a continuously growing field. Examples are the removal of tumor cells from bone marrow, the separation of subpopulations of lymphoid cells, the isolation of "single" tumor cells or bacteria for diagnosis. Additionally, the purification and fractionation of biological molecules using magnetic separation are already well-established methods.[11,69,70]

BIODISTRIBUTION

Information about the kinetics and mechanisms of biotransformation of colloidal magnetic suspensions, their aggregational behavior, and the biodistribution upon introduction into living system is essential for *in vivo* applications in medicine. Interactions between polymer particles and biological systems depend on the polymer structure, the surface composition, size, electric surface charge, hydrophilicity, adsorption behavior of biological molecules, *etc.* The natural fate of microspheres is to circulate with blood plasma, transfer to the interstitial fluid (extravasation) and to lymph (drainage), and then to return to plasma via lymphatic vessels through chains of lymph nodes. The clearance from circulation is mediated by interaction with cells, especially of the RES system, extracellular matrix, blood proteins, and renal filtration. Particles larger than 5–10 nm cannot penetrate the endothelium by small vascular pores or by transcytotic vesicles after binding to transcytosis-associated receptors. The permeability of these barriers, however, can be increased temporarily by several exogenic factors such as drugs, immune modulators, heat, and radiation.

The *in vivo* kinetics and biodistribution of the particles can be altered by blood protein absorption (opsonization) or by direct cellular recognition. These processes are associated with rapid aggregation or phagocytosis resulting in the distribution of the material mainly in the liver and spleen. As an example, albumin or galactose microspheres used in clinical studies for imaging are cleared from the blood within seconds, preventing their use in many imaging and other applications.

Papisov[38] showed by means of different idealized models that the transfer processes of large non-extravasating and small extravasating polymer particles are distinctively different. Although polymers of both classes may consist of the same constituents, their *in vivo* localization in liquid compartments and capability of cooperative interaction with components of the biological systems, *e.g.,* opsonization triggering the phagocytosis, interaction with immunocomplexes, complement activation, and therefore their biokinetics may be essentially different. Additionally, functional groups on the particle surface can decrease the circulation time of both small and large polymers mainly by increasing the amount of opsonization and also by enhancing or changing the recognition of receptors present on cell surfaces. Therefore, even larger particles with functional groups

could be targeted to areas with pathological processes, which often show increased vascular permeability.

Papisov *et al.* concluded that negatively charged and essentially neutral particle surfaces provide the longest circulation time.[37] The connection of the surface charge measured indirectly by electrophoresis and the circulation time has been demonstrated for other particles too, *e.g.,* liposomes with different lipid compositions.[19] Some progress was achieved in reducing the rapid clearance of small particles by the attachment of poloxamer or polysorbate to nonbiodegradable polystyrene and polymethylmethacrylate particles, or by the creation of liposomes or other carriers containing glycolipids, albumin, or derivatives of polyethylene glycol (PEG).[65] Gref *et al.*[71] found that the blood circulation time of the particles increases as the molecular weight of the covalently linked PEG increases. Five hours after injection, only one-third of the high-molecular-weight PEG-coated nanospheres were captured by the liver in comparison to the uncoated particles. This phenomenon was explained by an increased thickness of the protective PEG layer, thus preventing opsonization. The minimization of the particle surface interaction with biological systems and the special functional surface interaction with biological systems and the special functional surface modification for targeting are opposites, but they nevertheless are key factors for the development of useful polymer carriers.

MAGNETIC RESONANCE CONTRAST ENHANCEMENT

Magnetic resonance imaging (MRI) has demonstrated excellent diagnostic sensitivity for the distinction of normal and pathologic tissue due to the intrinsic differences of their hydrogen atom relaxation times. Various magnetopharmaceuticals, small particles that modify the proton relaxation parameters, have been developed to increase both diagnostic sensitivity and specificity.[72] The selective distribution of a contrast agent in normal and pathological tissue depends on the surface properties of the nanoparticles and results in increased contrast and therefore improved detection of disease. The targeting efficiency for specific anatomical sites can be enhanced by surface modifications using biological active substances such as antibodies or receptor ligands. Such altered nanoparticles will then seek, find, and bind to the target tissue, thus enhancing the MRI contrast and allowing for easy detection. The use of monoclonal antibodies for the receptor-mediated uptake of colloidal contrast agents is such an approach.[73]

DRUG TRANSPORT

One of the major challenges in cancer therapy is the delivery of antineoplastic agents to remote, difficult-to-reach anatomic sites. Thus, specific cell targeting enhances the delivery efficiency and at the same time reduces the toxicity and side effects to normal tissue. Many different methods currently are being tested, such as pH-sensitive and thermosensitive liposomes, which selectively release the cytotoxic agents in the target area due to pH changes occurring there[74] or due to (forced) local hyperthermia,[75] to name a few.

In 1978 a new concept of targeted drug delivery was introduced by Widder *et al.*[9,76] They developed different magnetic pharmaceuticals, such as magnetic erythrocytes, magnetic albumin, and polymer microspheres, for the delivery of cytotoxic drugs. Their intravenous application in the presence of a magnet externally applied to the tumor areas leads to increased tumor uptake of the drug as compared to the same experiment without applied magnetic field and field gradient. The drug is then released locally from the magnetic carrier and exerts its pharmacological effect at the cellular level of the tumor tissue, sparing the normal tissue. This attractive method of drug localization depends on an abundant vascular supply of the targeted tissue and an accessibility to strong magnetic fields. Only in such cases where a srong inhomogeneous magnetic field can be brought close enough to or induced in the blood vessels and capillaries of the tumor can magnetic guiding of particles be successful.

The principle of injecting magnetic carriers into the supplying vein and subsequent retention has been demonstrated successfully in many different *in vivo* systems. The magnetic carriers were injected into the tail vein,[27,28,77–79] right/left ear,[25] kidney,[47,80,81] and lung.[80,81] All of the above authors found a 3- to 10-fold increase of microsphere retention in the target tissue; however, quite a large amount of the particles ended up in the liver. This effect was put to use by one group who explored intrahepatic targeting and hyperthermic treatment of cancerous areas in the liver with an on-line liver perfusion system, injecting thermosensitive magnetoliposomes and applying an external magnetic field.[82]

The highest increase of the therapeutic index of the associated drug, namely up to 10- to 100 fold, was demonstrated in tail vein experiments in rats and has not been shown by any other drug-targeting device.[77–79] From the protracted retention of the carrier after removing the magnetic field for more than 1 day, Widder has concluded that the carrier might be lodged in the vascular endithelium or had possibly traversed the vascular basement membrane into the interstitial tissue.[9] This would be desirable because the microspheres would then serve as extravascular depots releasing the drug at the desired site. Alternatively, partial thrombosis due to conglomeration of the microspheres could also be responsible for the continued retention of carrier.

Other work in medical *in vivo* applications of magnetic carriers includes Orekhova *et al.*'s use of magnet-loaded red cells to concentrate locally and intra-arterially in a constant magnetic field of a miniature magnetic positioned outside of the artery.[13] These carriers were loaded with an anti-thrombotic drug for the prevention of clot formation after surgery.

Further success was achieved by Allen *et al.*,[83] who developed a magnetic drug-releasing system based on polymer particles with a diameter of 1–2 μm, which can be guided to a depth of 10 cm by external magnetic fields of less than 15,000 oe. This distance has not been achieved before in biological systems, and the first clinical studies are now underway. Additionally, Gallo *et al.* achieved an enhanced delivery of chemotherapeutic drugs to brain tumors by using magnetic chitosan microspheres and magnetic guidance by a factor of 3–5.[84]

HYPERTHERMIA

Colloidal magnetic particle suspensions exposed to a strong alternating magnetic field can be used for heat induction.[40–42] The power absorption depends in the case of multidomain ferrite particles on the hysteresis curve and in the case of ferrofluids, which are subdomain particles of 1–100 nm, on mechanical particle rotation. Both mechanisms lead to energy loss into the surrounding area which expresses itself as locally induced hyperthermia only at the spot where magnetic particles have been placed or targeted.[40–42,85–89]

Jordan[41] has systematically investigated the influence of specific particle attributes such as size, shape, composition, manufacturing details, and also external parameters such as the magnetic field strength and frequency. It was found that ferrofluids are the most promising particles for hyperthermia due to their superior specific absorption rate. The ferrofluids were then used in a further study[42] to induce hyperthermia in an intramuscularly implanted mouse tumor. For this purpose, a combined magnetic targeting/infiltration procedure was used. Although not all of the magnetic fluid could be retained in the tumor area, a 2.5-fold increase of the tumor iron with a maximum at 30 min after injection was observed.

Magnetic fluid hyperthermia is not limited to cancer therapy. Noninvasive thrombolysis in fine capillaries is currently being discussed. It would be possible with magnetic fluids after puncture at supporting vessels combined with magnetic guidance. And in reversed order, magnetic carriers can even induce blood coagulation by hyperthermic treatment of the target capillaries.

Further enhancements in magnetic fluid hyperthermia are possible by combining it with other treatment modalities such as radiation and/or chemotherapy.[90] Some of the techniques for this purpose already exist, *e.g.,* drugs incorporated into magnetic microspheres can be dissolved in a programmable fashion by local hyperthermia.[40,91] The particles used for hyperthermia must also be optimized further for physical characteristics, and, very importantly, for targeting characteristics to malignant cells by employing all the techniques already discussed above, *e.g.,* the combination with antibodies.

RADIONUCLIDE DELIVERY

The success of radionuclide therapy depends on the critical relationship between the radionuclide amount reaching the target tissue and the amount ending up in the normal tissue most sensitive to that radioisotope. This concept was enormously popular less than a decade ago, and many groups tried to use antibody–radionuclide complexes to treat cancerous tissue. It was thought that the antibody would exclusively and specifically bind to the tumor, similar to the key-lock situation where only one key is able to open the lock. After binding, the radioisotope would irradiate and kill the tumor cells. The advantage of radiation over, for example, cytotoxic drugs is that radiolabeled particles can deposit a dose and produce biological damage over a defined, radioisotope dependent distance. The radioactive carrier therefore only needs to be brought, by means of a strong external magnetic field, near the tumor and will then from there irradiate the tumor cells.

However, the successful implementation of radioimmunotherapy for the treatment of cancer has proven to be considerably more difficult than initially anticipated. Many different factors lead to only a moderate radiolabeled antibody uptake of always less than 2% per gram of tissue, and the low uptake probably was largely due to a large amount of the antibodies being filtered out in the liver soon after injection. The tumor blood flow was also found to be heterogeneous, the antigen was nonuniformly distributed throughout the tumor cells as well as among regions of tumors, and the uptake and binding of the antibodies to tumor cells actually produced areas with high concentrations of antibodies, which then acted as "binding site" barriers to further antibody penetration into the tumor.[92] Furthermore, a high percentage of patients developed an immune response toward the antibody, which results first in altered biodistribution by forming immune complexes and second did not allow a second antibody application.

Several attempts have been made to overcome these problems, *e.g.,* by immunoadsorption of circulating antigen, removal of unbound antibody from the plasma by plasmapheresis, enhancement of interstitial antibody–radionuclide transport by vasoactive agents, immunomodulators, pre-irradiation, and/or heat.[93–95] In addition, the electric charge of the radiolabeled antibodies plays an important role for the unspecific nontarget organ sequestration and must be adjusted for maximum performance.[96,97] Unfortunately, the benefits of these attempts were moderate.

A more promising and, at least in the animal experiment, successful application of magnetic radioactive microspheres sized between 10 and 30 μm is the intracavitary treatment of tumors in animals.[30–32] The amount of radioactivity actually delivered to the tumor increased from 6% of the injected amount to 73%, just by attaching a round, 9-mm diameter and 1-mm-thick magnet on the skin surface above the tumor. And the use of 120 μCi of the radioisotope Yttrium-90 actually lead to the total disappearance of four out of six tumors.

We conclude from these experiments that, in certain anatomic areas and by using adequate magnet setups, the targeting of magnetic radiopharmaceuticals such as radiolabeled magnetic microspheres or liposomes and also magnetic antibody–radionuclide complexes is promising for the complete tumor eradication *in vivo.* Further enhancements, however, are necessary to turn the magnetic guidance of radionuclides in cavities or in the blood circulation into more than just a curiosity. The necessary improvements might include the preparation of particles with more uniform size, higher maximal magnetization, longer circulation times in blood by modifying the surface with, for example, polyethylene glycols (PEG),[36–38,40,71] and better receptor-mediated targeting by conjugating the surface with antibodies or polysaccharides.[43,84,98]

Further improvements are also needed in the development of strong but small magnets and magnet setups. An excellent system for the movement of relatively large, about 3-mm-long magnetic seeds has recently been tested by McNeil *et al.*[99–101] It looks like a helmet containing several superconducting magnets and can move the magnetic seed within the brain with high accuracy to any location, thus making it an ideal delivery system of, for example, radioisotopes. For the case of magnetic radioisotope delivery after intravenous administration, much smaller microspheres must be employed.

Magnetic nanospheres in a size range of 110–140 nm seem to be ideal because they first avoid the parenchymal liver uptake by bypassing the fenestration due to being larger than 100 nm, but second are also small enough to minimize the RES uptake by the liver's Kupffer cells.[29] Additionally, they can be retained by currently available magnets at biological flow rates, as was recently shown in an *in vitro* circulation model system using a gamma-camera for the detection of the radioactive microspheres.[29]

OUTLOOK

Magnetic drug delivery by particulate carriers is an efficient method to obtain high local drug concentrations at localized disease sites. Moreover, many unwanted side effects from nontargeted drugs in the systemic circulation can be reduced by using magnetic carriers. However, much developmental work is still ahead in the area of magnetic targeting. The effective retention of microspheres is restricted to areas relatively close to the body surface, and even in the case where magnetic producing high-magnetic-field gradients are applied. Compact and powerful magnets solving these problems must and can be built based on the new superconducting magnet technique; however, it is not a simple situation because each target organ probably will require a special setup.

The developments in physical, targeting, and surface characteristics of magnetic microspheres are ongoing, and the ideal magnetic carrier looks different for different applications. However, it can be said that ideal magnetic particles should be highly responsive to an external magnetic field, nontoxic, biocompatible, and show low uptake in the reticuloendothelial system (low "background").

Current and future developments will use magnetic carriers more and more for combination therapies. The first step will always be to deliver the particles to the target area, followed by the actual therapy either by releasing a drug (chemotherapy) in a very defined fashion (immediately or over time), by irradiating the area (radiotherapy), or by inductive heating (hyperthermia). Very exciting combinations are possible such as, for example, the release of a radiosensitizer in the target area which then allows for differential cell kill by external radiation. Similarly, the magnetic carriers could be used to deliver boronated compounds to the area in preparation for boron-neutron-capture therapy. For the last 20 years, the combination of hyperthermia and radiation has shown very good results *in vitro*; however, the translation into the clinic never worked, but could succeed in a combination approach using magnetically targeted hyperthermia.

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