Biocompatible Magnetic Polymer Carriers for In Vivo Radionuclide Delivery

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A bstract: The magnetic guidance of antiplastic and antibacterial agents as well as x-ray and MRI contrast substances in vivo by means of magnetic particles has been attempted repeatedly during the last 2 decades with more or less success. When using microparticles, the circulation time in the blood, the biodistribution, and to a greater or lesser extent, the specific targeting are determined by the uniformity of size, chemical composition, surface modification, and the electric surface charge. The electrophoretic mobility is an important parameter for the prediction of the usefulness of the prepared particle, modified by chemical and biological molecules. For its success, radionuclide therapy depends on the critical relationship between the amount of radioactive isotopes in the target tissue and in critical normal tissue. Because the implementation of radioimmunotherapy for the treatment of cancer has proven to be considerably more difficult than initially anticipated, we propose the use of magnetic nanospheres for the well directed delivery of radionuclides to a tumor after the intravenous administration of the biodegradable colloidal suspension. Key Words: Biocompatible particles—Magnetic carriers—Biodistribution—Radiotherapy.

Polymer particles possess constantly increasing importance as diagnostic and therapeutic tools in medicine as well as other areas of life sciences (1–5). The discovery of uniform latex particles nearly 50 years ago opened up different and exciting fields and established many new applications. Developed on the basis of small particles in the micro- and nanometer ranges, many in vitro diagnostic tests have become routine. The focus has now been shifted to applications of polymer particles in the controlled and well directed transport of drugs in living systems. The ongoing studies in this area are very promising, and this is partly due to 3 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 extended circulation times in the blood, primarily as a result of their modified and functionalized surfaces, thus influencing the biodistribution and overcoming the reticuloendothelial system (RES) uptake. The targeting of microspheres to areas other than the liver, as well as the modification of their adsorption behavior to blood proteins, is therefore possible (3). Finally, the combination of microspheres with biological active molecules such as proteins, peptides, hormones, lectins, and antibodies and the use of fluorescent and radioactive markers is the basis of a large variety of well established and original concepts for 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 a well 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 (6). Magnetically responsive microspheres are therefore the basis of several new procedures in molecular and cellular biology and have led to new clinical diagnostic and therapeutic concepts.

Over the last 2 decades, the wide size distribution of the available microsphere preparations has been
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cations. Magnetic microspheres require preparations of microparticles with suitable functional groups, by chemical surface changes, or by special coating techniques (2).

**BIOCHEMICAL PROPERTIES OF MAGNETIC NANO- AND MICROPARTICLES**

In general, the various routes employed for the manufacture of magnetic microspheres can be divided into 2 categories, entrapment and impregnation (2). The entrapment route is based on aqueous magnetite particles (5–50 nm) and is particularly suitable for hydrophilic microspheres. This method leads to particles with a magnetic core surrounded by the polymer matrix. In contrast, the impregnation method involves the incorporation of magnetic oxide particles within the pores of preformed microspheres and results in magnetic microspheres with a homogeneous distribution of magnetic oxide particles within the whole particle area.

In contrast to beads for industrial applications of magnetic particles (8), magnetic beads for diagnosis and therapy 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.

The different applications of magnetic microspheres require preparations of microparticles with specific reactive sites for a variety of surface modifications. Magnetic microspheres made from polysaccharides or proteins already contain functional groups such as OH, NH₂, SH, and/or COOH. They can be directly activated or used for the introduction of functional groups.

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 polymer beads have been summarized by Ugelstad et al. (6) and rely heavily on the chemistry of active ester synthesis and the methods of carboxyl-activated microspheres (2). Additionally, synthetic microspheres can be functionalized or activated by the copolymerization of monomers containing suitable functional groups, by chemical surface changes, or by special coating techniques (2).

**PRINCIPLES OF PARTICLE CHARACTERIZATION BY MAGNETOPHORESIS AND ELECTROPHORESIS**

The absolute size, the size distribution, and the degree of aggregation are important physical characteristics of magnetic carriers. To obtain information about the magnetic properties of individual particles and their statistical distribution, half and fully automated microscopic magnetophoresis can be applied (11,12). 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 radiolabelling methods (13). 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 surfaces of particles are important for the prediction of the in vivo effects such as opsonization, altered circulation times, and also biodistribution changes. Examples of surface charge effects have been shown in liposomes as drug carriers (14) and in polymer particles as magnetic resonance (M.R.) contrast agents (15). Impressive results of electrokinetic measurements have been obtained for the polyethylene glycol coating of carboxylic acid modified magnetic dextran or starch nanoparticles. The carboxylic acid groups on the surfaces of dextran or starch nanobeads were therefore esterified with polyethylene glycol in different stoichiometric ratios, varying the ratio of carboxylic acid groups to polyethylene glycol (PEG) from 24:1 to 12:1. Figure 1 shows the electrophoretic mobility of unmodified dextran nanospheres in comparison to the corresponding dextran particles with pentetic acid (DTPA) as poly-carboxylic acid on the surface and PEG coated DTPA dextran nanoparticles as a function of the pHe value. The electrophoretic mobility of the dextran particles increased significantly in the whole pH range of 2–11 when covalently bound DTPA was present. The coating with PEG diminished the influence of the carboxylic acid groups, depending on the density of the PEG chains on the particle surface.
Generally, electrophoretic measurements can be carried out with automated techniques based on single particle measurements by microscopic image tracking and analysis (16), image transduction techniques, or laser Doppler effect techniques. To improve the value of electrokinetic studies, the method of electrophoretic fingerprinting was introduced to analyze different batches of polymer carriers (17). Electrophoretic fingerprints or templates are three-dimensional representations of the mean electrophoretic mobility of a given particle suspension versus the 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 under investigation.

INTERACTIONS OF PARTICLES IN LIVING SYSTEMS

Information about the kinetics and mechanisms of biotransformation of colloidal magnetic suspensions, their aggregation behavior, and the biodistribution upon their introduction into living systems 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 of biological molecules, etc. The natural destination of microspheres is the blood plasma where they circulate, transfer to the interstitial fluid (extravasation) and to lymph fluid (drainage), and then return to the plasma via the lymphatic vessels through chains of lymph nodes. Their clearance from the circulation is mediated by interaction with cells, especially those of the RES system, the 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 adsorption (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 (18) showed by means of different idealized models that the transfer processes of large nonextravasating and small extravasating polymer particles are distinctively different. Although polymers of both classes may have the same constituents, their in vivo localization in liquid compartments and their capability of cooperative interaction with components of the biological systems, e.g., opsonization triggering phagocytosis, interaction with immunocomplexes, and complement activation and therefore their biokinetics, may be essentially different. Additionally, functional groups on the particle surfaces can decrease the circulation times of both small and large particles, primarily by increasing the amount of opsonization and also by enhancing/changing the recognition of receptors present on cell surfaces. Even larger particles with functional groups could therefore 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 times (19). The connection of the surface charge measured indirectly by electrophoresis and the circulation time has been demonstrated for other particles as well, e.g., liposomes with different lipid compositions (20). Some progress was achieved...
in reducing the rapid clearance of small particles by the attachment of poloxamer or polysorbate to non-biodegradable polystyrene and polymethylmethacrylate particles or by the creation of liposomes or other carriers containing glycolipids, albumin, or derivatives of PEG (15). Gref et al. (21) found that the blood circulation times of the particles increase as the molecular weight of covalently linked PEG increases. Five hours after injection, only one-third of the molecular weight of the PEG-coated nanospheres had been captured by the liver in comparison to the uncoated particles. This phenomenon was explained by the increasing thickness of the protective PEG layer, thus preventing opsonization. The minimization of the particle surface interaction with biological systems and the special functional surface modification for targeting are opposites, but they are, nevertheless, the key to the development of useful polymer carriers.

**RADIONUCLIDE DELIVERY**

The success of radionuclide therapy depends on the critical relationship between the amount of radionuclides reaching the target tissue and the amount ending up in the normal tissue most sensitive to that radioisotope. This concept was already enormously popular a decade ago when 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 lock-key situation whereby 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 radiolabelled particles can deposit a dose and produce biological damage over a defined, radioisotope dependent distance. Therefore, the radioactive carrier needs only to be brought near the tumor to 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 have led to only moderate radiolabelled antibody uptake, always less than 2% of tissue, and the low rate of enrichment probably has been largely the result of a large amount of antibodies already having been 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 have acted as binding site barriers for further antibody penetration into the tumor (22). Furthermore, a high percentage of patients developed an immune response towards the antibody, which resulted in its biodistribution by forming immune complexes and did not allow a second antibody application.

Several attempts have been made to overcome these problems, e.g., by the immunoadsorption of circulating antigen, removal of unbound antibody from the plasma by plasmapheresis, enhancement of interstitial antibody-radionuclide transport by vasoactive agents, immunomodulators, preirradiation, and/or heat (23). In addition, the electric charge of the radiolabelled antibodies plays an important role in nonspecific nontargeted organ sequestration and must be adjusted for maximum performance (24). Unfortunately, the benefits of these attempts have been moderate.

A more promising and, at least in the animal experiment, successful application of magnetic radioactive microspheres with a size of 10 to 30 μm was the intracavitary treatment of tumors in animals (25,26). 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. A iso, the use of 4.4 MBq of the radioisotope Yttrium-90 actually led to the total disappearance of 4 of 6 tumors. From these experiments, the conclusion can be drawn that in certain anatomic areas and with adequate magnet setups, the targeting of magnetic radiopharmaceuticals such as radiolabelled magnetic microspheres or liposomes and also magnetic antibody-radionuclide complexes is promising for complete tumor eradication in vivo. Further efforts, 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 the blood by modifying the surface with, for example, PEG (19), and better receptor mediated targeting by conjugating the surface with antibodies or polysaccharides (27).

Further improvements are also needed in the development of strong but small magnets and magnet setups. A new excellent system for the movement of relatively large, about 3 mm long, magnetic seeds has recently been tested by McNeil et al. (28). 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 maki
ing it an ideal delivery system of radioisotopes, for example. For 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 avoid the parenchymal liver uptake by bypassing the fenestration as a result of being larger than 100 nm and also by being small enough to minimize the RES uptake by the liver’s Kupfer 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).

Current and future developments will use magnetic carriers more and more in combination therapies. The first task will always be to deliver the particles to the target area, followed by the action either of releasing a drug (chemotherapy) in a very defined fashion (immediately or over time), of irradiating the area (radiotherapy), or of inductive heating (hyperthermia). Very exciting combinations are possible such as the release of a radio sensitizer in the target area, which then allows the differential killing of cells by external radiation. For the last 20 years, the combination of hyperthermia and radiation has shown very good results in vitro; however, the transition into clinical practice has never worked but could succeed in a combination approach using magnetically targeted hyperthermia (30).

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REFERENCES


