Magnetic and Electrical Separation, Vol. 10, pp. 223–252
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THE APPLICATION OF MAGNETIC TECHNIQUES IN BIOSCIENCES

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(Received 12 June 2000; In final form 28 July 2000)

The idea to use magnetic techniques in biosciences is not new, but it has enjoyed a resurgence of interest especially during the last two decades. Magnetic adsorbents, carriers and modifiers can be used for the immobilization, isolation, modification, detection, determination and removal of a variety of biologically active compounds, xenobiotics, cellular components and cells. Magnetic separation and labelling have recently found many useful and interesting applications in various areas of biosciences, especially in molecular and cell biology, microbiology, biochemistry and bioanalytical chemistry. Special attention is being paid to the possible biomedical and clinical applications. Currently, the magnetic selective separation, represents the most often used magnetic technique. It can facilitate or accelerate many separation and purification processes especially in heterogeneous systems. To perform a biomagnetic separation an appropriate magnetic separator and magnetic particles or other magnetic labels are needed. Several types of separators and a wide assortment of magnetic particles of different type and size (usually surface modified) are commercially available. The importance of biomagnetic (separation) techniques has increased in recent years and further development of new important applications is expected in the future. The purpose of this review is to summarise basic elements and prospects of biomagnetic techniques and to highlight possible applications in various bioscience disciplines.

Keywords: Biologically active compounds; Cells; Immunomagnetic assays; Immunomagnetic separation; Magnetic particles; Magnetic separation; Xenobiotics

INTRODUCTION

Magnetic separation techniques have been used for many years in various areas of industry for diverse applications such as minerals

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treatment, beneficiation of coal, removal of weekly magnetic coloured impurities from kaolin clay, removal of ferromagnetic impurities from large volumes of boiler water in both conventional and nuclear power plants, in waste water treatment and in many other applications [1–3]. Since the 1970s new possible applications of the magnetic phenomena for both separation and analysis of various biologically active compounds and cells have been studied, together with the development of other types of manipulations with selected compounds and cells using an external magnetic field. Due to the fact that most compounds and cells present in living organisms are diamagnetic, usually an appropriate modification has to be performed before a biomagnetic technique can be employed.

Magnetic separation (such as isolation and/or removal of specific cells and cell organelles, isolation of nucleic acids, proteins, xenobiotics, heavy metals *etc.*) is the most common approach to exploit magnetic principles in biosciences. However, biomagnetic techniques can be used for various other applications. Magnetic support can be used for immobilization of a variety of biologically active compounds and cells when a magnetic carrier enables simple manipulation with immobilized structures. The principle of magnetic separation can be used for the modification of standard immunoassay analytical methods such as EIA (Enzyme Immuno Assay) used to determine concentrations of analytes in medical diagnostics and environmental analysis. Also other new magneto immunoassay techniques based on magnetic permeability determination have been recently developed. Some of these techniques allow to analyse the target compounds not only in the laboratory, but also at a sampling place and some of them have been automated. Another group of techniques using an external magnetic field has been studied for biomedical and clinical applications, often in connection with tumour therapy.

Magnetic separation has several advantages in comparison with standard separation procedures used in various areas of biosciences. The separation process can often be performed directly in raw samples containing suspended solid material such as body fluids, bone marrow, tissue homogenates, cultivation media, food and clinical samples, waste water, soil suspensions *etc.* Owing to the magnetic properties, target structures captured to specific surface modified magnetic particles or magnetically modified affinity materials can be relatively

easily and selectively removed from the sample in an external magnetic field. The power and efficiency of the magnetic separation procedure is useful for both small and large-scale operations. In biosciences the volume of treated solution or suspension usually ranges from a few microliters to several litres. Magnetic selective separation is a technique that can facilitate or accelerate many separation and purification processes and can be efficiently combined with the majority of other procedures used in biosciences.

Several review papers and booklets describing various aspects of biomagnetic methods and applications are available. The papers are usually oriented on specific topics, such as applications of these techniques in biochemistry [4], in molecular biology [5, 6] in microbiology and cell biology [7–10], on the application of the magnetic field assisted bioreactors [11] *etc.* An important information can be found in books such as Scientific and Clinical Applications of Magnetic Carriers [12], Magnetism in Medicine [13] and in a special issue of the Journal of Magnetism and Magnetic Materials [14]. The purpose of this review is to summarise elements of biomagnetic techniques in general and to show non life sciences experts the importance and possibilities of applications of magnetic techniques in the bioscience disciplines.

PRINCIPLES OF BIOMAGNETIC SEPARATION TECHNIQUES

There are two ways of magnetic manipulation of the target structures when working with biologically active compounds and cells. In the first case, structures to be manipulated exhibit sufficient intrinsic magnetic moment so that magnetic manipulation can be performed without any modification of the target. There are only a few examples of paramagnetic or ferromagnetic biomolecules or cells, such as ferritin, haemoglobin, deoxygenated erythrocytes (in plasma) and magnetotactic bacteria. In all other cases, when working with diamagnetic molecules and supramolecular structures, an appropriate magnetic modification has to be performed. Usually the attachment of magnetic labels of various nature to the target structures or immobilization or adsorption of targets to magnetic carriers or

adsorbents are used. This magnetic modification allows to achieve the required contrast in magnetic susceptibility between the originally diamagnetic structures of interest and other compounds. The linkage of magnetic labels to the target structures is often mediated by affinity ligands or other (more or less specific) interactions (Fig. 1).

Direct and Indirect Techniques

In general, a magnetic affinity separation can be performed in two different modes. In the *direct method* an appropriate affinity ligand coupled to magnetic particles or magnetically modified biopolymer with affinity towards a target structure is applied directly to the sample. During the incubation the magnetic affinity particles or colloids are bound to the target compounds or cells and thus stable magnetic complexes are formed.

In the *indirect method* a free affinity ligand (in most cases an appropriate antibody) is first added to the solution or suspension with the target structure. If possible the excess unbound affinity ligand (antibody) is removed after incubation and the resulting labelled complex is then captured by appropriate affinity magnetic particles. For example if antibodies are used as free affinity ligands, magnetic particles with immobilised secondary antibodies, Protein A or Protein G are used to capture the complex. An other approach often used is based on interactions of free biotinylated affinity ligands and magnetic particles with immobilised streptavidin or avidin. In both methods the resulting complex of magnetic particles with the target structure is washed and recovered using an appropriate magnetic separator.

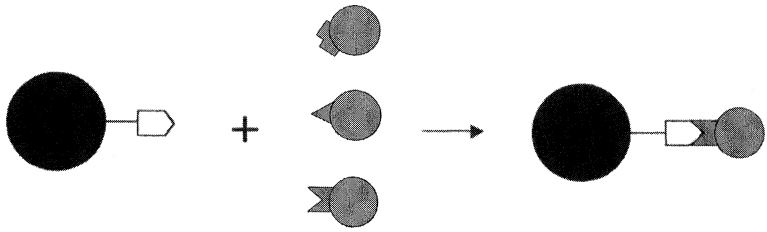

FIGURE 1 A specific interaction of a immobilised affinity ligand with the target structure.

The two methods perform equally well, but in general, the direct technique is faster, more easily controlled and requires less antibodies, while the indirect procedure is more efficient especially if the affinity ligands have poor affinity for the target compounds or cells. A disadvantage of the indirect method is the need for excess antibodies or excess magnetic particles since the removal of free antibodies can be difficult. A general scheme of magnetic separation where antibody is used as an affinity ligand is shown in Figure 2.

Both *positive isolation* (when predefined compounds or cellular subsets are directly isolated) and *negative isolation* (when targets are purified by removing all other contaminating structures) can be performed. If required the target compounds or cells can be eluted or detached from magnetic particles using different procedures starting with highly specific ones (*i.e.*, detachment of cells using specific DETACHaBEAD system, Dynal, Norway) and ending with standard methods used in affinity chromatography or analytical chemistry. Nevertheless, in many applications (especially in molecular biology, bioanalytical chemistry, environmental chemistry and microbiology) isolated targets can be used for further work with magnetic particles still attached, depending on the type of magnetic particles (ferrofluids) and a subsequent technique used. Some types of magnetic particles are compatible with polymerase chain reaction (PCR), ELISA, flow

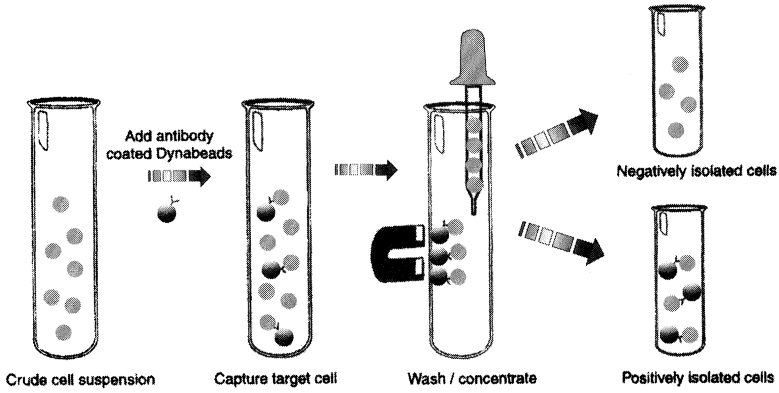

FIGURE 2 A scheme of the magnetic separation of cells when antibody (Y) is used as an affinity ligand and Dynabeads as magnetic particles. Reproduced, with permission, from materials provided by Dynal, Norway [8].

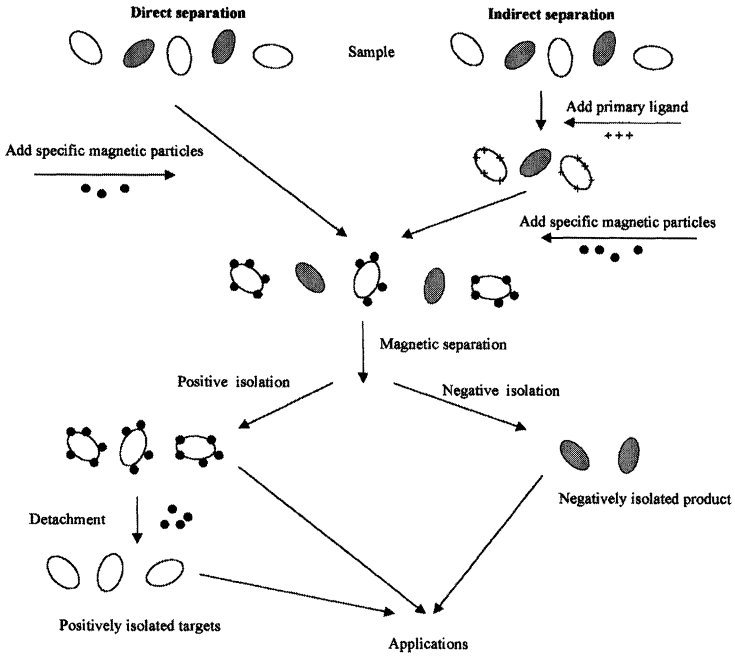

FIGURE 3 A general scheme of biomagnetic separation of target structures.

cytometry, fluorescence microscopy, fluorescence *in situ* hybridisation (FISH), cultivation of microbial cells on nutrient agars *etc.* A general scheme of magnetic separation of molecules and cells is given in Figure 3.

Other Separation Techniques

In most cases *magnetic batch affinity, ion exchange or non-specific adsorption* is used to perform the separation step. This approach represents the simplest procedure available, enabling the whole separation to be performed in one test-tube or flask. If magnetic particles (with diameters > approx. 1 μm) are used, simple magnetic separators can be employed. If magnetic colloids (diameters ranging between tens and hundreds of nanometers) are used as magnetic affinity labels, high-gradient magnetic separators or quadrupole (hexapole) separators have usually to be used to remove magnetic target structures from the system.

Alternatively *magnetically stabilised fluidised beds* (MSFB), which allow continuous separation of target structures, can be used. The use of MSFB is an alternative to conventional column operation, such as packed-bed or fluidised bed chromatography, especially for large-scale purification of biological products. Magnetic stabilisation allows the expansion of a packed bed without the mixing of solid particles. High column efficiency, low pressure drop and elimination of clogging can be attained [15]. Magnetic field assisted bioreactors containing fluidized magnetizable beads are attractive devices for bioprocessing [11].

Biocompatible *two phase systems*, composed for example of dextran and polyethylene glycol, are often used for isolation of biologically active compounds, subcellular organelles and cells. The separation of the phases can be accelerated by the addition of fine magnetic particles or ferrofluids to the system followed by the application of a magnetic field. Magnetically enhanced phase separation usually increases the speed of phase separation by a factor of about 10 in easy systems, but it may increase by several orders of magnitude in difficult systems [16].

EQUIPMENT

Magnetic particles or labels and a simple magnetic separator or another special device are necessary to perform magnetic manipulation of biologically active compounds, xenobiotics, cell organelles and cells.

Magnetic Particles and Magnetic Labels

With the exception of a few naturally occurring paramagnetic/ferromagnetic molecules or cells (*e.g.*, ferritin, deoxygenated erythrocytes, magnetotactic bacteria) biological material to be manipulated is diamagnetic and has to be magnetically labelled or immobilized on magnetic particles in order to be susceptible to magnetic treatment. The magnetic labelling or immobilization can be performed using magnetic particles, magnetic colloids, magnetoliposomes or molecular magnetic labels (such as erbium ions). In most cases magnetic properties of the labels are caused by the presence of small particles

of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3); in some cases also ferrite particles or chromium dioxide particles have been used.

Magnetic Particles (Microspheres)

The diameter of most commercial magnetic particles (microspheres) is typically 1 to 5 micrometres. Particles are often declared to be superparamagnetic to that effect that they exhibit magnetic properties when placed within a magnetic field, but retained no residual magnetism when removed from the magnetic field. Many particles are biocompatible and some of them (based on biopolymers) are biodegradable. Commercially available magnetic particles can be obtained from a variety of companies [7]. In most cases polystyrene is used as a matrix, but carriers based on other polymers such as cellulose, agarose or polyvinylalcohol are available. Magnetic particles typically comprise fine grains of iron oxides uniformly dispersed throughout the interior of a polymer particle, the surface chemistry of which can be modified to provide a range of different linking methods. Alternatively magnetic silica, porous glass or silanized magnetic particles can be used for the same purpose.

Commercial magnetic particles are usually uncoated, activated with a reactive group and coated with affinity ligands. The most often attached affinity ligands are antibodies, oligodeoxythymidine, streptavidin, Protein A and Protein G. Magnetic particles with such immobilised ligands can serve as generic solid phases for immobilisation or separation of specific molecules. In exceptional cases enzyme activity may decrease as a result of usage of magnetic particles with exposed iron oxides. In this case encapsulated microspheres, having an outer layer of pure polymer, can be used. Magnetic particles of different type and size can also be prepared in the laboratory. These particles are usually not spherical and uniform but their advantage is in their significantly lower price. Such types of particles permit to use magnetic separation also in large-scale applications.

Magnetic Colloids (Nanoparticles)

Magnetic colloids (nanoparticles; typical size is approx. 50 to 300 nm) are prepared by a variety of methods which result in the formation of

"flocs" composed of a polymer (typically, dextran, starch or protein) and magnetic iron oxide crystals [17]. Magnetic nanoparticles are often used for the labelling of target structures (usually cells) and as a biodegradable (or biocompatible) carrier for biomedical applications. When working with magnetic nanoparticles, high gradient magnetic separators or quadrupole separators have to be used for their manipulation.

Other Magnetic Labels

Magnetoliposomes are magnetic derivatives of ordinary liposomes (small roughly spherical artificial vesicles consisting of a continuous bilayer or multibilayer of complex lipids enclosing some of the suspending medium) usually prepared by the incorporation of fine magnetic particles into the lipid vesicles [18]. Magnetoliposomes are often used as carriers for immobilisation of membrane-bound enzymes (such as cytochrome c oxidase [19]), for drug targeting [20] or magnetic labelling the target cells [21]. In some cases *magnetic molecular labels* also have been used. Lanthanides, especially erbium in the form of erbium chloride ($ErCl_3$), have been used for labelling a variety of cells, both prokaryotic and eukaryotic [22]. Also ferritin and its magnetic derivative magnetoferritin have been used for the same purpose [23].

Magnetic Separators and Detectors

Recently many commercial magnetic separators have become available, ranging from small laboratory separators based on strong permanent magnets to automated cell sorters able to separate up to 10 million magnetically labelled cells per second. There are two basic types of laboratory magnetic separators, namely batch and flow-through ones.

Batch Portable Magnetic Separators

In most cases the isolation of magnetically labelled structures is performed in a batch mode. *Test tube magnetic separators* equipped with strong rare – earth magnets enable to separate magnetic microparticles from volumes ranging approximately between 5 microlitres and

50 ml. Recently many different types of separators (*e.g.*, see Fig. 4) have become available from different companies. Nanoparticles can be separated with *quadrupole* and *hexapole* magnetic gradient separators (QMGS and HMGS, Immunicon, USA; Fig. 5) from similar volumes. Other types of separators are used for the isolation of targets from wells of standard microtitration plates. Magnetic complexes from larger volumes of suspensions (up to approx. 500 to 1000 ml) can be separated using *flat magnetic separators*.

Flow-through Magnetic Separators

Flow-through magnetic separators are characterised by the flow of the liquid and magnetically labelled structures through the separation system. Laboratory-scale *high gradient magnetic separators* (HGMS) contain small columns, loosely packed with fine magnetic grade stainless steel wool, placed between the poles of strong permanent magnets or electromagnets (Fig. 6). Magnetically labelled structures

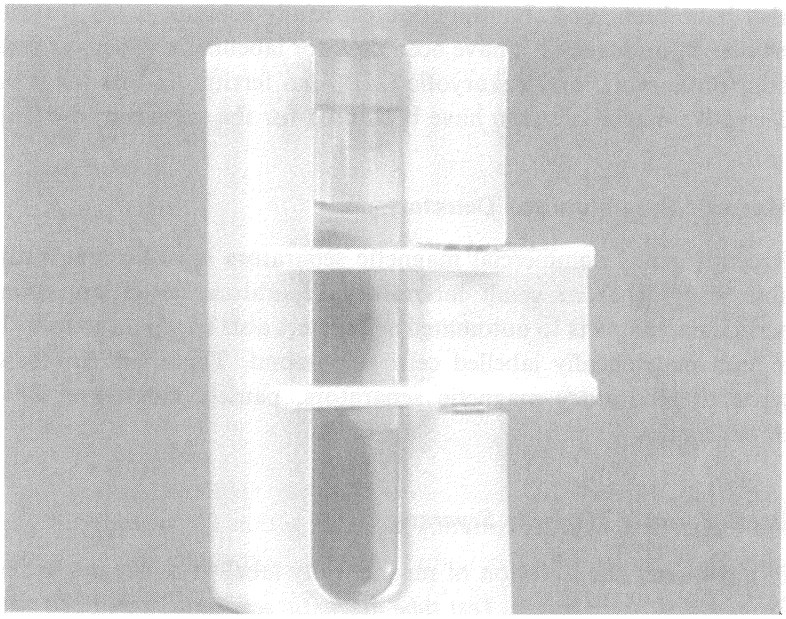

FIGURE 4 A batch portable magnetic separator. Reproduced, with permission, from materials provided by Dynal, Norway.

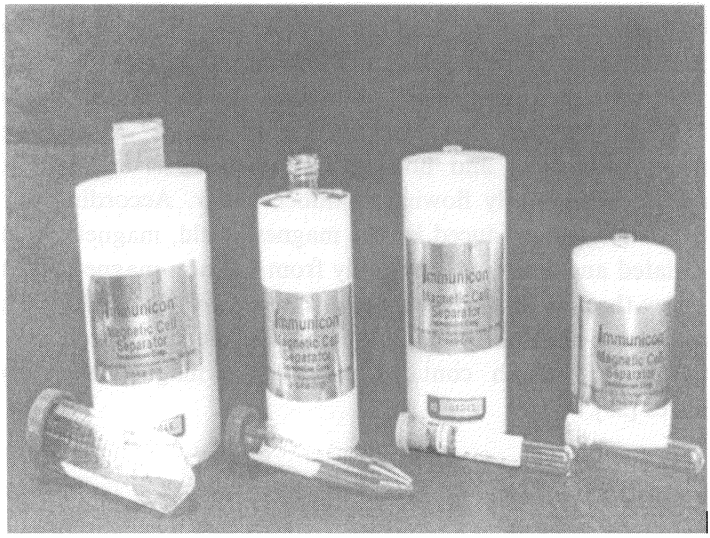

FIGURE 5 Quadrupole and hexapole magnetic gradient separators. Reproduced, with permission, from materials provided by Immunicon, USA.

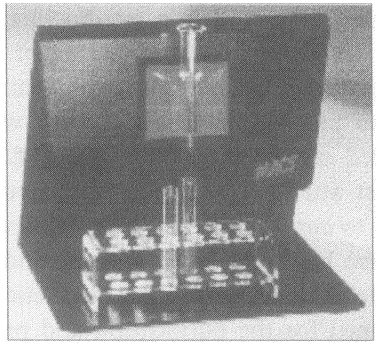

FIGURE 6 A portable high gradient magnetic separator MidiMACS capable of separating up to 10^8 magnetically labelled cells. Reproduced, with permission, from materials provided by Miltenyi Biotec, GmbH, Germany.

are passed through the column, captured within the steel matrix, and after the removal of the column from the magnetic field the captured structures are released. HGMS is used when working with colloidal magnetic particles, especially for the separation of magnetically labelled cells. Recently special devices able to separate $10^{10}-10^{11}$ cells

in one step have become commercially available (Miltenyi Biotec, Germany; StemCell Technologies, Canada). Another type of *continuous magnetic sorters* is based on a similar principle as an electrophoresis counter-flow chamber. In this device a mixture of magnetically labelled and non-labelled particles is injected into a stream of continuously flowing chamber buffer. According to their magnetic moments, induced by the magnetic field, magnetic particles are deviated and collected separately from the non-magnetic particles [24]. Flow-through *quadrupole magnetic separators* focus the magnetic field around a central, cylindrical area. An inlet is splitted into two outlets one of which contains mainly the magnetically labelled structures [25].

Other Special Separation Devices

Further magnetic techniques using special separation devices have also been described but the techniques are not being used so frequently. *Ferrography* is a method of particle separation onto a glass slide based upon the interaction between an external magnetic field and magnetic moments of particles suspended in a free-flowing, open stream. The separated material preserved on a glass slide can be used for further analysis [26]. A more sophisticated instrumentation with carefully controlled flow and magnetic field conditions, which evolved from ferrography, is called *analytical magnetapheresis* [22].

Because the most attractive feature of magnetic particles is their simple adaptability to *automation*, new types of devices based on magnetic separation have been developed recently and others are expected in the future. For example a special Magnetic Particle Processor (Labsystems, Finland) for the purification and processing of nucleic acids or proteins and cells has been introduced into market and some of commercial immunomagnetic assay systems used in medical diagnostics have been automated.

Detectors

The detection of magnetic permeability represents a new approach in the area of immunoassays. (Immunoassays are a group of techniques for the measurement of low concentration of biochemical substances

in biological fluids based on specific antibody-antigen reaction when the concentration is determined using specific, usually enzymatic labels. See also in *Immunomagnetic assays*.) The target biologically active compounds and cells are labelled with superparamagnetic particles and colloids and subsequently their concentration is determined by measuring the local magnetic field. Magnetic Permeability Meter (European Institute of Science, Sweden), Magnetic Assay Reader (Quantum Design, USA) and a prototype of Hand Held Slide Reader (Ericomp, USA) are suited to applications in the field of biochemistry, molecular biology and medical diagnostics.

MAGNETIC SEPARATION

Magnetic Separation of Biologically Active Compounds and Xenobiotics

Magnetic separation (both isolation and removal) of biologically active compounds and xenobiotics have been successfully used in various areas of biosciences, such as molecular biology, biochemistry, analytical chemistry, immunochemistry, enzymology, environmental chemistry *etc.*

The Isolation of Nucleic Acids

Currently the magnetic affinity separation techniques are used especially in molecular biology for the isolation of nucleic acids (both RNA and DNA) and oligonucleotides. Almost all the procedures are based on the same principle, namely hybridisation (*i.e.*, formation of highly specific complexes of nucleic acids according to their complementarity in the base sequences) of immobilised oligonucleotides and target molecules. Specific oligonucleotides immobilised on magnetic particles are bound to specific sites of target nucleic acids in crude cell lysates and then magnetically isolated. Separated nucleic acids can be eluted from the beads and used for further applications (Northern blotting, dot/blot hybridisation, hybridisation probes) or used still attached to magnetic particles (cDNA synthesis, construction of solid-phase cDNA libraries *etc.*). Enzymatic

downstream applications are usually not inhibited by the presence of magnetic particles.

The Isolation of Proteins

In the case of protein separation no simple strategy for magnetic affinity separation exists. Various affinity ligands have been immobilised on magnetic particles, or magnetic particles have been prepared from biopolymers exhibiting affinity for target enzymes or lectins. Immunomagnetic particles, *i.e.*, magnetic particles with immobilised specific antibodies against the target structures, have been used for the isolation of various antigens and can thus be used for the separation of specific proteins. Enzymes are usually isolated using immobilised specific inhibitors, cofactors, dyes or other suitable ligands, or magnetic beads prepared from affinity biopolymers are used. A general procedure, especially from the point of view of recombinant oligohistidine-tagged proteins, is based on the application of metal chelate magnetic adsorbents. Another general procedure employs immobilised Protein A or Protein G for a specific separation of immunoglobulins from ascites, serum and tissue culture supernatants.

The Isolation of Xenobiotics

The isolation of organic and inorganic xenobiotics from environmental and clinical samples using magnetic techniques may find useful applications in the near future. Immobilised copper phthalocyanine dye has an affinity for planar organic compounds, such as polyaromatic hydrocarbons with three and more fused aromatic rings in their molecules, and for triphenylmethane dyes, both groups representing real or potential carcinogens and mutagens. The immunomagnetic separation of some xenobiotics such as pesticides, TNT or PCBs is used as a first step in the course of their immunoassay. Non-specific adsorbents, such as magnetic charcoal have been used for separation of water soluble organic dyes [27].

Magnetic Solid-phase Extraction

A new procedure for the batch preconcentration of xenobiotics and biologically active compounds from larger volumes of solutions or

suspensions, namely magnetic solid-phase extraction (MSPE) has been developed as an alternative technique to standard solid-phase extraction. This procedure based on the adsorption of a target on a relatively small amount of magnetic specific adsorbent permits to handle litre volumes of samples [28]. Using MSPE with subsequent elution, very low concentrations in ppb (μg/l) range of some compounds can be detected.

The Magnetic Isolation of Cells

The isolation and removal of cells and cell organelles can be performed using direct or indirect methods as mentioned above. Another differentiation of magnetic separation techniques is based on the selection of the magnetically labelled cells. In the negative selection cellular subsets are purified by removing all other cell types from the sample and the purification process does not involve any direct contact of magnetic labels with the cells to be isolated. In the positive selection, which is used more often, the target cells are magnetically labelled and then isolated from the cell suspension. Depletion is a method by which one or more unwanted cellular subsets are removed from a cell suspension.

Immunomagnetic Separation

Immunomagnetic separation (IMS) is the most often used magnetic approach for the isolation of prokaryotic and eukaryotic cells. IMS can be performed in all the formats mentioned above; nevertheless the positive direct isolation method is usually used. The indirect technique is recommended when the target cell has a low surface antigen density or a cocktail of monoclonal antibodies is used. Typically 95 to 99% viability and purity of the positively isolated cells can be achieved with a typical yield of 60 to 99%. The depletion efficiency often reaches 99.9% and it leaves the remaining cells untouched. Magnetic particles and labels do not have a negative effect on the viability of the attached cells and the isolated cells are phenotypically unaltered.

Magnetic labels and many types of magnetic microparticles are usually compatible with subsequent analytical techniques such as flow

cytometry, electron and fluorescence microscopy, polymerase chain reaction (PCR), fluorescence *in situ* hybridisation (FISH) or cultivation in appropriate nutrient media. In some cases, however, it is necessary to remove immunomagnetic microparticles from the cells after their isolation. The detachment process can be performed in several ways according to the cell types and the way of their binding to magnetic beads [7].

The time for cell separation is usually less then 60 min depending on the incubation time that usually takes 10 to 30 min. In positive isolation, the purity of cells generally decreases with time, although the yield increases. Non-specific interactions of non-target cells especially with hydrophobic magnetic particles can be expected. These interactions can be partially eliminated using *e.g.*, bovine or human serum albumin, casein and non-ionic tensides such as Tween 20 in washing solutions.

Cell organelles can also be isolated from crude cellular fractions. Dynal (Oslo, Norway) has developed Dynabeads M-500 Subcellular, which are able to isolate rapidly more than 99% of target organelles.

Separation with Other Affinity Ligands

Other affinity ligands attached to magnetic particles can also be used for cell separation [7]. Immobilised antigens can be used for the isolation of antibody expressing or antigen-specific cells, specific lectins can interact with saccharide residues on the cell surfaces and oligosaccharides have been used for rapid isolation of specific lectin-expressing cells. Erbium ions, ferritin and magnetoferritin have been used for the magnetic labelling of both prokaryotic and eukaryotic cells and submicron magnetic particles of γ-Fe_2O_3 adhere to the surface of *Saccharomyces cerevisiae*, making the cells magnetic and amenable to magnetic separation.

There are also a few types of cells (magnetotactic bacteria, paramagnetic form of red blood cells) exhibiting sufficient intrinsic magnetic moment that can be isolated directly without any magnetic modification. Isolation of erythrocytes infected by *Plasmodium* containing paramagnetic hemozoin (component of malarial pigment) has also been published [29].

AN IMPORTANT USE OF IMMUNOMAGNETIC CELL ISOLATION

Cell Biology and Biomedical Research

An immunomagnetic separation is often used in microbiology, cell biology, medical research and parasitology. In cell biology and biomedical research magnetic particles are being increasingly used for the isolation of miscellaneous cell subsets (according to their surface CD markers) directly from tissue homogenates, body fluids and other cell sources. Specific human cell types such as B lymphocytes, endothelial cells, granulocytes, hematopoietic progenitor cells, Langerhans cells, leukocytes, monocytes, natural killer cells, reticulocytes, T lymphocytes and spermatozoa may serve as examples. Isolated cells are usually used for research purposes and diagnostics. Another very important application – immunomagnetic removal of cancer cells from blood and bone marrow of patients suffered from cancer illnesses has been studied in many laboratories.

Microbiology

In microbiology these techniques are especially used for the isolation and detection of pathogenic microorganisms (Fig. 7). Standard food and clinical microbiology procedures usually require four stages and the total analysis time from sampling to obtaining a result can be measured in days. IMS enables the time necessary for detection of some pathogen to be shortened, when the target cells are magnetically

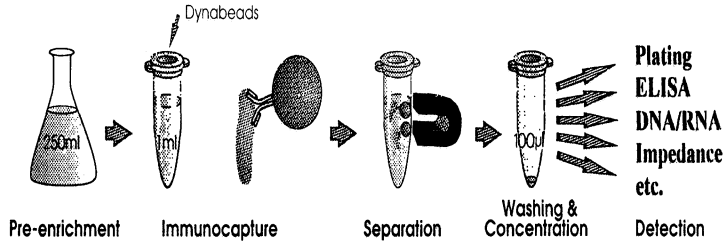

FIGURE 7 The immunomagnetic separation of target microorganisms. Reproduced, with permission, from materials provided by Dynal, Norway [5].

separated directly from the pre-enrichment medium or the sample (Fig. 8). Isolated cells can then be identified by standard microbiological procedures, *e.g.*, inoculation on selective agars or alternatively enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) can be used. The progressive combination of IMS and PCR can shorten the isolation and detection period from days to a few hours. IMS is not only faster but in many cases also gives better results because sub-lethally injured and stressed microbial cells can more reliably be detected. Several tens of various microorganisms have already been separated [7]. For some important microbial pathogens (*e.g.*, *Salmonella*, *Listeria*, *Escherichia coli* O157 – Fig. 9) commercial specific immunomagnetic particles are available and new ones have been under development.

A large number of microorganisms has an affinity to ingest or precipitate ion species onto their surfaces. In the case of magnetic ionic species, magnetic separation methods could be applied to remove the ion-loaded organisms from the surroundings. This process could be used in the mineral processing industry in addition to the treatment of effluents from the nuclear industry, in the recovery of precious metals and other industrial plants [30]. Also magnetotactic bacteria were used

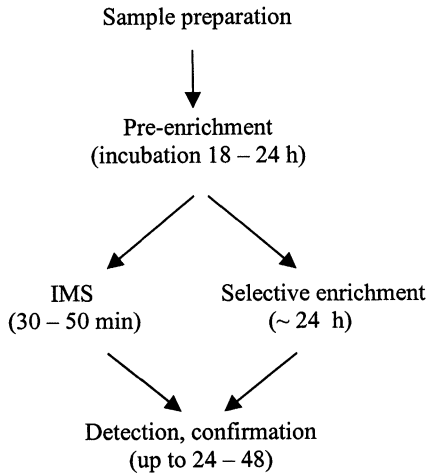

FIGURE 8 A scheme of immunomagnetic separation (IMS) and a standard cultivation procedure for the isolation and detection of microbial pathogens.

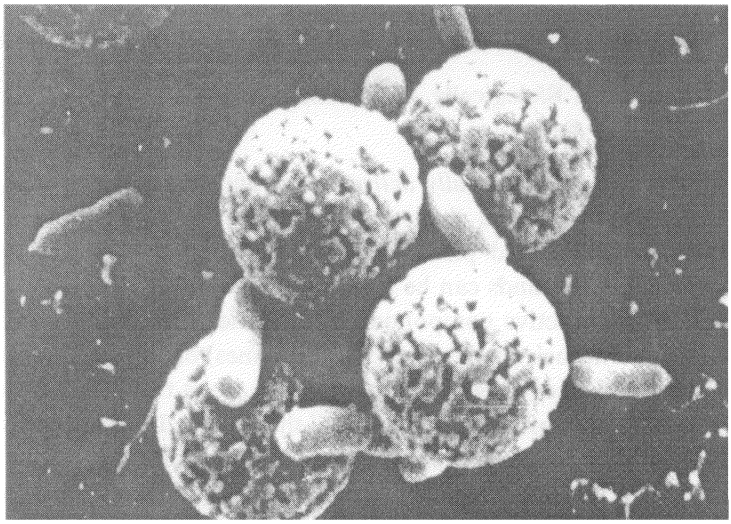

FIGURE 9 An electron micrograph showing *Escherichia coli* O157 attached to immunomagnetic particles Dynabeads (size 2.8 μm). Reproduced, with permission, from materials provided by Dynal, Norway.

in the course of heavy metal ions and radionuclides removal from water [31].

Parasitology

Although in the area of parasitology there have not been so many examples of magnetically separated protozoan parasites, *Cryptosporidium* and *Giardia* are the parasites where IMS is the technique of interest. The occurrence of *Cryptosporidium* and *Giardia* outbreaks as a result of their presence in drinking water have brought about an increased need for their detection at levels necessary to protect human health. Therefore also US EPA recommended IMS to determine *Cryptosporidium* and *Giardia* at low concentrations (Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA, April, 1999). Two kits (Dynal, Norway; Clear Water Diagnostics, USA) are currently commercially available for this purpose. In very low turbidity samples IMS demonstrated significantly better results than the standard procedures, nevertheless in turbid water the recovery efficiency of IMS diminished.

MAGNETIC MODIFICATION AND ASSAYS

Immobilisation of Biologically Active Compounds, Xenobiotics and Cells

Immobilisation of *biologically active* compounds such as enzymes on magnetic carriers allows their rapid removal from the system or targeting to the desired place by using an external magnetic field. Immobilized compounds can be used for the isolation of complementary biologically active compounds, xenobiotics, cell organelles and cells, to perform their specific activities (enzymes) or to be transported to the desired place (drugs). There are many ways to immobilize the compounds of interest and practically all the standard procedures used in affinity chromatography can be used for this purpose. Many of commercially available magnetic particles can serve as carriers for the immobilization. Alternatively, magnetoliposomes have been used for the immobilization of membrane-bound enzymes, antibodies or for entrapment of various drugs. Also magnetic microspheres prepared from various biopolymers, such as albumin or chitosan, or synthetic polymers such as poly(L-lactic acid) have been used for the entrapment of drugs.

Microbial cells immobilized on magnetic carriers have been used as biocatalysts in some applications. For example magnetically immobilized cells of *Mycobacterium* sp. were used for the side chain degradation of cholesterol [32] and *Sacharomyces cerevisiae* cells immobilised in magnetic alginate gel [33] or adsorbed on surface-modified magnetite [34] were used for the production of ethanol. Unidentified microorganisms, immobilised on magnetic particles, were used for batch and continuous mode removal of phenol from contaminated water [35].

Immunomagnetic Assays and Magnetic Immunoassays

Immunoassays mediated by immunological antibody-antigen reactions are currently used to determine the concentration of analytes useful in medical diagnostics. Antibodies or antigens are typically immobilized onto a solid phase, which traditionally has included filters, tubes, wells or plastic beads. The use of magnetic microspheres

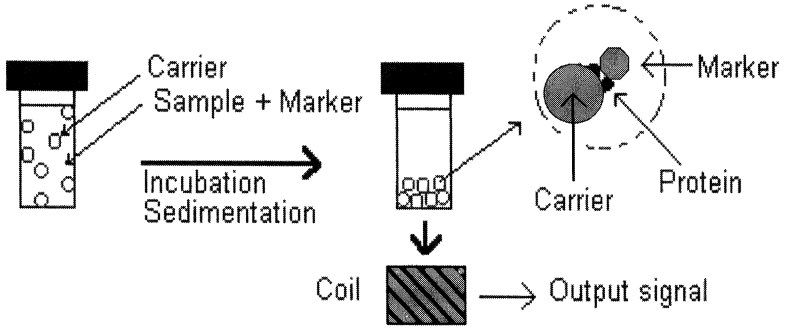

FIGURE 10 The principle of magnetic immunoassay. Reproduced, with permission, from materials provided by EURIS, Sweden.

as the solid phase has revolutionised the field of clinical biochemistry by facilitating the development of more sensitive higher-throughput automated immunoassays. *Immunomagnetic assays* are usually faster and more reproducible and many today's automated immunoassay systems rely on magnetic separation [36]. For the detection standard enzymes, radioisotopes, fluorescent substances or chemiluminiscence can be used. For the purpose of environmental analysis sets for the determination of more than 15 pesticides, polyaromatic hydrocarbons, trinitrotoluene, polychlorinated biphenyls and BTEX (benzene, toluene, ethylbenzene, xylene) in water, soil and food are commercially available (SDI Europe, UK).

Recently also a magneto binding assay based on the magnetic permeability determination of the magnetically labelled targets was introduced as a new type of biologically active compound assays [37]. The principle of *magnetic immunoassay* is based on standard immunoassays but on the contrary to them the targets are labelled with superparamagnetic particles or colloids and a magnetic transducer is used for the detection and quantification of magnetically labelled targets (Fig. 10).

Examples of Other Magnetic Modifications

Other types of magnetic modification have not been used often, nevertheless they can be used to change the properties of studied

biologically active compounds as shown in the following examples. Magnetic derivatives of polyethylene glycol were conjugated with selected enzymes; such conjugates exhibited magnetic properties and formed stable dispersions in both organic solvents and aqueous solutions [38]. Antibodies modified in a similar manner can be used to bind to cancer cells; such modified antibodies are expected to be applicable clinically as a therapeutic agent for the induction of hyperthermia [39]. Magnetic particles with immobilized acetylcholinesterase have been used for the determination of organophosphate and carbamate pesticides using modified flow injection analysis technique [40]. Enzymes immobilized on magnetic membranes have been used for the construction of enzyme electrodes for the determination of specific amino acids and sugars [41]. A procedure called DIANA (Detection of Immobilized Amplified Nucleic Acids), employing magnetic particles with immobilized streptavidin, has been developed for the colorimetric detection and quantification of amplified DNA produced during a PCR procedure [42]. Magnetic carriers with immobilized N-terminal radiolabelled peptide substrate or magnetic derivatives of dye-stained gelatine have been used as insoluble substrates for the determination of proteolytic activity [43, 44].

MAGNETIC TECHNIQUES IN BIOMEDICAL AND CLINICAL APPLICATIONS

The development of suitable magnetically responsive nano- (micro)-spheres and a possibility of their *in vivo* medical applications (usually connected to cancer therapy) are another specific and intensively studied tasks. If we do not take into account immunomagnetic separation of a variety of human cells for research and diagnostic purposes, the biomedical research has been focused on a few specific problems, namely immunomagnetic isolation of human cells from bone marrow and whole blood, use of magnetic nanoparticles as magnetic resonance imaging contrast agents, target delivery of drugs and radionuclides and magnetic fluid hyperthermia. The stage of the research ranges, according to the particular subjects, from the basic experiments to a preclinical phase. To overcome many difficulties and

to answer numerous questions interdisciplinary collaborative effort of many different professions is necessary.

Isolation and Detection of Cells from Bone Marrow and Blood

The underlying processes which control the development and progression of carcinomas are a major area of medical research. There are two demands for selective separation of tumour cells. First, the presence of circulating tumour cells in the blood has the potential of being markers of metastatic disease and secondly, tumour cells have to be removed from bone marrow prior its autologous transplantation.

The detection of isolated tumour cells in the bone marrow and the blood from patients is usually accomplished by a few standard methods. However, these methods are cumbersome if large numbers of cells (*i.e.*, $> 1 \times 10^7$) are to be analysed. Considering that patients may have only 1 to 2 cancer cells per 2×10^6 mononuclear cells it becomes evident that a large number of cells (10^7 or 10^8) have to be screened in order to detect the rare cancer cells. Therefore the possibility of selective *enrichment of cancer cells* from bone marrow and peripheral blood of patients using immunomagnetic separation has been studied. For example positive and negative IMS was used for enrichment and detection of viable breast carcinoma cells with significant increase in the number of tumour cells detected [45]. Also to increase the sensitivity of standard detection techniques (flow cytometry, fluorescence microscopy, or immunocytochemistry) high-gradient magnetic cell sorting (MACS) was used [46].

The possibility of use of IMS for the selective *separation of cancer cells* from bone marrow and peripheral blood of patients has been studied since the turn of 1970s and 1980s. The idea was to remove selectively cancer cells from bone marrow prior bone marrow autologous transplantation after applied chemotherapy. In a few last years the technique has been clinically tested with more than a hundred patients. For example immunomagnetic purging of lymphoma and leukemia cells was evaluated in autologous bone marrow grafts. Although the levels of tumour cells in bone marrow were significantly reduced, small but for a patient significant amount of residual cancer cells could still be detected in the selected grafts [47, 48].

Due to the demand of complete removal of cancer cells from bone marrow (blood) that could be hardly achieved with hundred-per-cent certainty there is another approach to autologous (or allogenic) transplantation. Immunomagnetic separation is used for the selective *isolation of stem CD 34+ cells* that should then be applied back to a patient treated with chemotherapy. Recently two commercial automated systems have been available for clinical use: Isolex 300i Magnetic Cell Selection System (Nexel, USA), and CliniMACS (Miltenyi Biotec, Germany, Fig. 11). Currently the systems are under clinical verification in different countries and ready for the clinical trial phase.

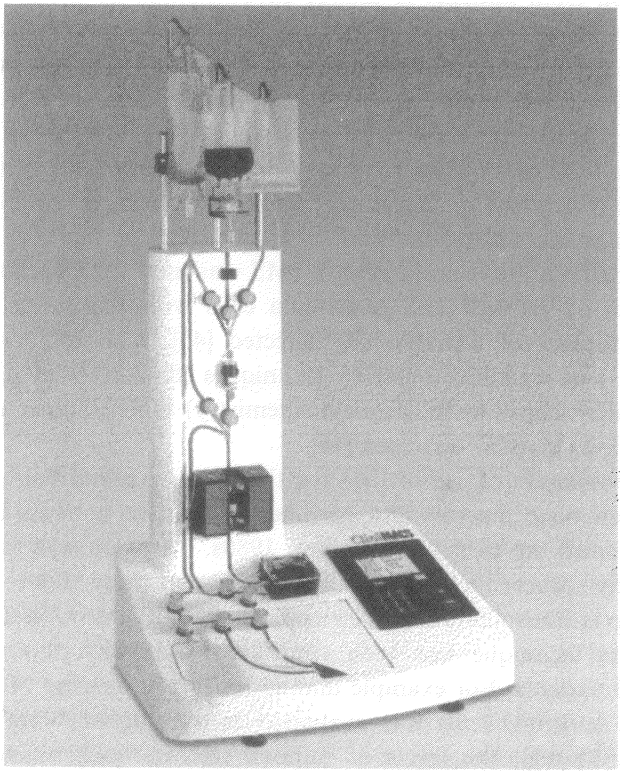

FIGURE 11 The automated system for clinical isolation of human cell subsets CliniMACS. Reproduced, with permission, from materials provided by Miltenyi Biotec, Germany.

Magnetic Resonance Imaging

Currently magnetic resonance imaging (MRI) belongs to standard medical examination methods. 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 is dependent on the proton density and magnetic relaxation times so as MRI contrast depends on the chemical and molecular structure of the tissue and is usually manipulated by adjusting the instrumental parameters [49]. In early 1980s it was recognised that target-specific superparamagnetic nanoparticles can serve as a dramatic source of exogenous contrast and have rapidly become an important and indispensable tool for the non-invasive study of biologic processes with MRI. Superparamagnetic magnetite-dextran nanoparticles are usually used when the particle size varies widely and influences their physicochemical and pharmacokinetic properties. Clinical experiments with more than a thousand patients showed that target-specific superparamagnetic contrast agents may allow the localization of specific tissues such as tumours by magnetic resonance imaging. Their main present and future applications by the parenteral route are the imaging of gastrointestinal tract, liver, spleen and lymph nodes and they are expected to be further extended to heart, kidneys, and other organs. Ultrasmall superparamagnetic iron oxide particles are also blood pool agents which could be used for perfusion imaging (*i.e.*, brain or myocardial ischemic diseases) as well as for imaging of vessels in Magnetic Resonance Angiography. These agents open up an important field of research leading to more specific agents adapted to clinicians' needs in diagnostic imaging.

Drug and Radionuclide Targeting

Although site-specific direction of drugs within the organism would benefit a patient in many diseases, the active drug targeting has clinically not yet been possible. Conventional treatment regiments are not able to achieve significant drug concentration in diseased compartments without distributing drug throughout most other (healthy) body parts. Therefore magnetic biodegradable (biocompatible) particles or magnetoliposomes have been used for the

immobilisation or encapsulation of drugs or radionuclides and the transport to a target place using an external magnetic field has been studied. The goal of the research work of many scientists of many different scientific disciplines is to develop a system able to deliver, concentrate, release and bound a specific drug (or radiopharmaceutical) at the target tissue with the possibility of lowering the amount of the toxic (often cytotoxic) drug applied. Thus significant reduction of harmful effects of most conventional anticancer drugs to the healthy tissues could be achieved.

Although many experiments were done, all of them were performed with small animals (mostly rats). In 1995 there were some clinical experiments with a few selected patients with localised solid tumours when anticancer drugs reversibly bound to ferrofluids were concentrated in locally advanced tumours by magnetic field created by magnets arranged at the tumour surface outside of the organism [50]. Nevertheless magnetically controlled target chemotherapy or radiotherapy are expected to be still at the level of basic research in the next years.

Magnetic Fluid Hyperthermia

Hyperthermia is another promising approach to cancer therapy based on the heating of the target tissue to the temperature between 42°C and 46°C that generally reduces the viability of cancer cells and increases their sensitivity to chemotherapy and radiation. Although various methods are employed in hyperthermia each of them has its limitation. The inevitable technical problem is the difficulty of the uniform heating of only the tumour region up to the required temperature without damaging normal tissue. The use of magnetic nanoparticles or magnetoliposomes for the localised non-invasive hyperthermia has been therefore studied [51]. Using magnetic fluid hyperthermia (MFH) a particulate ferromagnetic or ferrimagnetic material is confined within the treatment area and is further exposed to an external alternative electromagnetic field. The confined magnetic particles dissipate the energy of the field in the form of heat through various kinds of energy losses and therefore cause hyperthermia in the area of their confinement. Although MFH has been studied for about thirty years and numerous reports have been published the

experimental results obtained only demonstrated the physical potential and the biological efficacy *in vitro* and *in vivo*. Currently there are still many difficulties that must be overcome, many tasks to be solved and a large number of questions to be answered (likewise *e.g.*, in drug targeting) before the techniques can be established in clinical use.

CONCLUSIONS

Biomagnetic techniques have already shown their potential in various scientific disciplines. The application of magnetic carriers, adsorbents, modifiers and labels is an innovative and efficient way of applying magnetic separation processes to non-magnetic (diamagnetic) targets of biological origin, both at the molecular and cellular level. As a result of specific properties of the affinity magnetic particles (labels) magnetic separation can also be performed in situations where other procedures are complicated or fail. Many ready-to-use products are available and the basic equipment for standard work is relatively inexpensive. The non-separation biomagnetic techniques, especially those used in medicine such as MRI, drug targeting and hyperthermia, represent another very important and promising area of research and applications. In these applications, however, quite a lot of work has still to be done.

The magnetic separation techniques can be automated and miniaturized. Fully automated systems for the detection and determination of important clinical markers are available and new ones are under development. Also portable assay systems can be constructed for the determination of environmental contaminants directly on site or for the near-patient analysis of various disease markers. On the other hand, large-scale applications will probably also be developed and used for the isolation of rare biologically active compounds directly from the crude culture media, wastes from food industry *etc.* Of course, prices of magnetic carriers have to be lowered, and special types of low-cost, biotechnology-applicable magnetic carriers prepared by simple and cheap procedures have to become available.

The scope of possible applications of the discussed techniques is very broad and many new procedures in various fields of biosciences

and biotechnologies will undoubtedly be developed in the near future. Biomagnetic techniques could thus be the techniques for the 21st century.

Acknowledgement

The work was supported by the grant No. 203/98/1145 from the Grant Agency of the Czech Republic.

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