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Assay Development

Paramagnetic microparticles for optimized biological separations

Polymer-based particles with highly irregular surfaces enhance the existing advantages of the technology through their large surface area and high density.

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Magnetically responsive particles have been utilized for a variety of laboratory applications, including nucleic acid separation, protein purification, cell isolation, therapeutics, and diagnostics. Their use can greatly simplify biological separations and reduce assay times. Often, they eliminate the need for column chromatography or centrifugation.

In addition, magnetic separation procedures are usually scalable, both up and down, and often allow the establishment of systems coupling fully automated, high-throughput separation with molecule detection. As with most biological separation procedures, the issues of binding capacity and specificity are critical. Higher binding capacities characteristic of magnetic microparticles are usually associated with increased sensitivity and lower cost per assay. And magnetic carriers have recently been finding employment in clinical and biomedical applications in the area of targeted drug delivery.

This article reviews applications for magnetic-particle-based technology, with a focus on the enhanced characteristics of irregular, high-surface-area magnetic particles.

Magnetic Particle Anatomy

Magnetic microparticles are composed primarily of ferrimagnetic materials. In cell separations, the magnetic labels most commonly used are ferrimagnetic magnetite (Fe3O4) and maghemite (g-Fe2O3). Although the particles under discussion are often referred to as magnetic—and for ease they will be here—they are, strictly speaking, superparamagnetic. This term designates particles that will be responsive to a magnetic field but that will not themselves become magnetized. Such

particles disperse easily once they are removed from the magnetic field.

Some magnetic particles are polymer based. In these, the magnetically responsive material is added to a polymer matrix by one of two processes: either it is entrapped during the polymerization process, or it is attached to the particle after polymerization. The particles are then usually overgrown in either case, in order to encapsulate the magnetic material and provide functional groups on the surface.

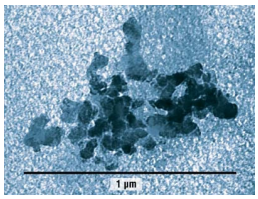

Figure 1. A BioMag superparamagnetic particle is a crystalline aggregate of magnetite encapsulated by aminosilane and, as shown by this transmission electron micrograph, has a highly irregular shape. Its surface area relative to mass is more than 100 m2/g (click to enlarge).

Magnetic particles can also be composed of crystalline aggregates that are encapsulated by any of a variety of polymers such as polystyrene, dextran, or silanes. With this type of particle, as with polymer-based particles, it is important that the magnetic material be completely encapsulated. This is to prevent leaching of the iron complexes, which can cause problems in biological systems.

Practical Advantages

The introduction of magnetic particles in biological applications such as immunology, cell separation, molecular biology, and diagnostics has made processes for separating cells, DNA, and proteins faster and easier.

In cell separation applications, a particular type of cell can be isolated from a complex mixture by numerous means, among them fluorescence-assisted cell sorting via flow cytometry; density-gradient-based methods; and magnetic- particle-based methods. Cell isolations based on magnetically responsive particle technology offer many advantages over other procedures. The main one is that they are relatively simple to perform. Target cells can be isolated from fairly crude mixtures, including blood, tissue homogenates, stool, soil, microbial culture media, water, and food, among others. Also, the procedures are gentle, can be scaled up or down as necessary, and can replace centrifugal, filtration, or chromatographic separation. These attractive properties allow the development of automated cell isolation systems.

Magnetic particles are used also in the study of biological processes using whole cells, where the goal may be simply to detect the presence of pathogens such as Escherichia coli, Salmonella, and Staphylococcus in clinical, food, or environmental samples.[1-3] Another objective may be to detect the expression of a eukaryotic cell surface marker.[4,5] Researchers may also find it useful to isolate a particular cell type in order to study it in a controlled manner. In converse fashion, they may deplete a cell type by means of magnetic-particle-based separation in order, by its absence, to study its influence on biological processes.

For the most part, cell separations are performed by using an antibody– magnetic-particle complex; however, antigens can also be attached for the capture of antigen-specific cells such as antibody-producing cells from hybridoma cultures and antigen-specific B-cells, and also for the biopanning of antibody phage-display libraries.[6,7] Lectins as well can be attached to the surfaces of

magnetic particles and used as capture molecules for polysaccharides and glycoproteins expressed on the surfaces of eukaryotic and prokaryotic cells. In the reverse approach, magnetic particles with attached oligosaccharides can be used to isolate lectin-expressing cells.[7]

Cell separation has been used in clinical applications for the identification and isolation of circulating tumor cells in peripheral blood.[8] Another use has been the depletion of T-cells from donor bone marrow in order to combat graft-versus-host disease in patients receiving bone marrow transplants.[9]

Magnetic particles are also employed extensively to purify nucleic acids, proteins, and cellular organelles. While these types of separations can be affinity based, like cell separations, they can also rely largely on the principles of conventional chromatographic separations. Magnetic-particle-based separations of nucleic acids have been performed by means of ion-exchange functionalized particles, silica-coated particles, nonspecific absorption, and sequence-specific oligonucleotide capture. Several manufacturers provide products suitable for protein purification via magnetic particles functionalized with capture molecules. Particles functionalized with nickel or glutathione molecules can be used to purify recombinant proteins containing 6X His or GST affinity tags; protein A or protein G for immunoglobulin purification; and particles containing secondary or primary antibodies for magnetic immunoprecipitation.

A Mobile Surface Area

Magnetic separations are somewhat analogous to chromatographic separations in that an increase in particle surface area usually results in larger binding capacity. The major difference, of course, is that, in the former, the solid phase is mobile and responsive to magnetic fields.

One manufacturer, Polysciences Inc. (Warrington, PA), has developed superparamagnetic particles that have an irregular, bumpy surface, which dramatically increases the surface area of the particle. Trade named BioMag, these particles are made of a crystalline magnetite formulation encapsulated with aminosilane (see Figure 1). Their irregular surface provides 20 to 30 times more surface area than similar-sized spherical particles. An irregular magnetic particle 1 µm in breadth has a surface area in excess of 100 m2/g, as opposed to 4–8 m2/g for a 1-µm-diameter spherical particle.

The greater surface area of the irregular particles translates into greater binding capacity. When compared with a similar-sized spherical particle in experiments, BioMag oligo (dT) particles bound significantly larger amounts of polyadenylated messenger RNA (mRNA) (see Figure 2). In another experiment, BioMag streptavidin particles and two other magnetic streptavidin particles that were spherical were used to bind radiolabeled biotinylated oligonucleotides. The irregular particles bound approximately twice as much as one type of spherical particle and more than eight times the amount bound by the other spherical particle (see Figure 3).

Particle size can influence cell separation results, as well. Experiments were conducted to compare equal masses of 1-µm irregular magnetic particles with 1.8-µm irregular magnetic particles for depletion of CD45 positive white blood cells. Use of the smaller particles resulted in levels of depletion approximately 15% greater at the highest volume of particles tested (see Figure 4). Again, the higher capture rates of the smaller (BioMagPlus) particles are attributable to their higher ratio of surface area to mass.

The size of magnetic particles used in biological separations can vary dramatically. As might be expected, the size of the particle in large part determines how it will behave in solution and what type of magnetic separation device will have to be used. Magnetic particles can be broadly grouped as large particles (0.75 to about 5 µm) and small particles (less than 0.75 µm, and mainly 10–200

nm).

All three sizes are used in cell and antigen capture applications. In the case of the colloidal labels, cell labeling kinetics are quite rapid; little or no mixing is required.7 The effects of Brownian motion on larger particles is minimal, making mixing of these particles necessary for efficient antigen capture.

Size is a factor to consider not only in regard to mixing kinetics; the effect of magnetic-particle size on magnetic responsiveness also is important. While there are some exceptions, in general, the smaller the magnetic particle, the stronger the magnetic field needed for effective separation. This can be confounded, however, by differences in the amount of magnetite or maghemite contained in the particles. For example, when larger particles containing small amounts of magnetically responsive material are used, longer separation times or more-powerful magnetic fields are required.

Shape, Density, and Hydrodynamics

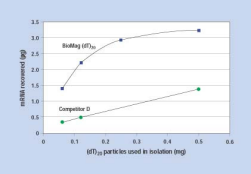

Figure 2. The isolation of polyadenylated mRNA by irregularly shaped magnetic oligo (dT)20 (BioMag) and by spherical (Competitor D) magnetic oligo (dT)20 particles was compared experimentally over a range of particle amounts. The irregular particles isolated significantly more mRNA at all levels, this greater binding capacity reflecting the greater surface area (click to enlarge).

The relationship of the hydrodynamic motion of magnetic particles to the capture of bacteria has been recently described.[1,2] In these studies, the motion of the particles in an aqueous suspension was considered to be controlled by gravity, buoyancy, and friction. The authors used the mass of the particle, the density of the solution, the density of the particle, gravitational acceleration, the viscosity of the solution, and the radius of the particle to predict mathematically the total volume of solution traversed by the particles.

An important distinction must be noted: whereas the hydrodynamic radius of spheres is the same as the radius of the sphere, the hydrodynamic radius of nonspherical particles is determined primarily by their shape.[1] The quantitative expression developed by the researchers predicts that particles with larger mass, higher density, and a nonspherical shape favor bacterial capture.

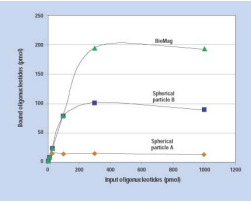

Figure 3. Irregularly shaped streptavidin-conjugated BioMag particles and two different spherical polymer streptavidin-conjugated magnetic particles were compared for their ability to bind radiolabeled biotinylated oligonucleotides. The maximum binding for the irregular particles was just under 200 pmol, compared with approximately 100 and 15 pmol for the other particles. These results reflect, in part, differences in surface area among the three types of particles (click to enlarge).

Streptavidin-conjugated irregular particles with a surface area of 1000 cm2/mg and a density of

2.5 g/ml were compared with an equal mass of spherical magnetic streptavidin-conjugated polymer particles having a surface area of 80 cm2/g and density of 1.3 g/ml, using biotinylated antibodies to E. coli O157. In this comparison, capture of E. coli was two to three times greater when particles of higher density were used, and capture required less mixing time with these higher-density particles.[1] Additionally, when the capture efficiencies of high-density smaller particles (1-µm particles) and high-density larger particles (18-µm particles) with the same antibody content were compared, the larger particles exhibited more than 40 times the capture of E. coli O157 after 60 minutes of incubation.[2] This agrees with the mathematical model the researchers developed, which predicted that the larger particles would have a total sedimentation volume more than 100,000 times that of the smaller particles.

In other words, the larger and more dense the particle, the more volume the particle will be exposed to during the mixing and capture phase of the assay. Therefore, according to these studies, when antibody concentration is held constant, the rate-limiting step controlling bacterial capture is the frequency of the bacteria-particle collision, which is favored when particles of greater density and size are used.

Applications

Many reviews have been published on the use of magnetic-particle technology in genomics and proteomics, drug discovery, biomedicine, and clinical applications.[10-13] Biological and biomedical applications of magnetic-particle technology have been employed widely for DNA, RNA, protein, and cell separations in genomic, proteomic, and immunological research. The ability to automate magnetic-particle separations and obtain high-purity products while reducing reagent costs, eliminating laborious and time-intensive steps, and minimizing the mechanical stress to which products are exposed makes the technology attractive for high-throughput applications such as sample preparation. And in drug discovery, the screening of large numbers of compounds to identify potential drug candidates is another application for which automated high-throughput magnetic-particle technology is well suited.

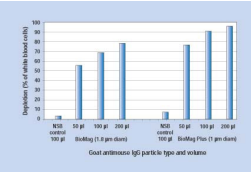

Figure 4. Results of the experimental depletion of CD45 positive white blood cells by irregularly shaped BioMag (1.8-µm) and BioMagPlus (1-µm) amine particles conjugated to anti-CD45 antibodies. For both particles, the greater the volume of particles, the greater the depletion percentage. BioMag Plus, with a smaller average diameter, presents an increased surface area, which is reflected in greater capture rates (click to enlarge).

Magnetic-particle-based technology has also been used for clinical screening and diagnostic applications. Their ability to covalently couple with proteins, enzymes, antibodies, and other ligands makes magnetic particles suitable for direct use in bioassays or as affinity ligands for the capture of target molecules and cells.[10] The ability of these particles to bind protein molecules without significantly changing the biochemical activity of the proteins is the basis for magnetic-particle-based immunoassays. Magnetic particles coated with leukocyte-specific antibodies have been used to detect and remove tumor cells from whole blood samples, bone marrow, and prepared mononuclear cells, and have enabled subsequent diagnosis.[11]

In addition to the analysis of clinical samples, magnetic particles have been used for the detection of pathogenic organisms in food and environmental samples. Particles are coated with antibodies targeted to surface-specific proteins on the microorganism, then testing to identify the organism is

conducted.
The relative simplicity and speed of procedures based on magnetic-particle technology well suits the design of rapid diagnostic tests. Because target molecules can be isolated from fairly crude mixtures, sample preparation is minimized. Also, magnetic-particle-based technology allows target molecules to be concentrated in order to increase signal intensities, thus enhancing detection.

In addition to magnetic-particle technology lending itself well to automation for high-throughput sample preparation for biological and biomedical research, the use of magnetic particles in drug targeting and delivery for clinical applications is also being actively investigated. Magnetic particles bound with established drugs can be employed for site-specific drug targeting in the human body using a magnetic field.[13] Site-specific targeting can increase the amount of drug delivered directly to the target area, while at the same time minimizing systemic distribution or exposure to normal cells. More-localized targeting of a drug may also make possible reduction in the dosage.[12] Many types of targeted drug delivery systems are being investigated, including magnetoliposomes, magnetic nanoparticles, and biodegradable magnetic particles.[13]

For magnetic particles to be effective for drug targeting, they must be smaller than, or comparable in size to, a cell, virus, protein, or gene so that they can get near the target of interest.[12] In performing targeted drug delivery, biocompatible ferrofluids or colloidal suspensions of magnetic particles in a liquid carrier are injected into the body. A strong magnetic field gradient is produced over the target site outside the body, drawing the particles to the target region.

The first reports of magnetic-particle targeting of sarcoma tumors in rats with a cytotoxic drug (doxorubicin) were encouraging.[12] This has led the way to continued investigation and, more recently, preliminary human trials. In addition to treating tumors and cancerous cells, magnetically controlled drug targeting may be used to deliver other types of drugs, such as antiinflammatories, steroids, antibiotics, and any others that can be bound reversibly to ferrofluids.

The future holds promise of increased use of magnetic-particle technology and delivery systems. One potential application is the transfer of genes into cultured cells or bacteria for the production of pharmacological biological products.[12] Magnetic particles might also serve as detection probes to replace current radioactive, fluorescent, or chemiluminescent modes of detection.[10] And specifically targeted magnetic particles could be used to initiate a biochemical reaction within a cell.[12]

Conclusion

The various uses of magnetic particles for biological separations speak to the many benefits they confer. Biomagnetic separations are simple, robust, scalable, and amenable to automation. Furthermore, many options exist with regard to magnetic-particle characteristics, allowing selection of a type of particle that suits an application well.

Success in biomagnetic separation procedures is highly dependent on the specificity and avidity of the capture molecule, the abundance of target, and other factors. Particle morphology, size, and density are important for the significant influence they can have on separation effectiveness. For example, particle diameter being constant, particles having irregular shape will present more surface area to the separation and demonstrate better capture of target analytes. Additionally, recent studies have shown that factors that influence the hydrodynamics of mixing can strongly affect the efficiency of pathogen capture, with particles of greater density and larger diameter exhibiting higher levels of pathogen capture.

The wide use of magnetic particles in biological separations has led to interest and research in their potential for clinical applications. The ability to couple specific drugs to magnetic microparticles small enough to enter the body has led to investigation into the use of magnetic particles as

targeted drug-delivery systems, as well as studies of their utility for gene transfer and in vivo tracking. The specificity and sensitivity of magnetic-particle technology promises to stimulate the development of a range of future applications.

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