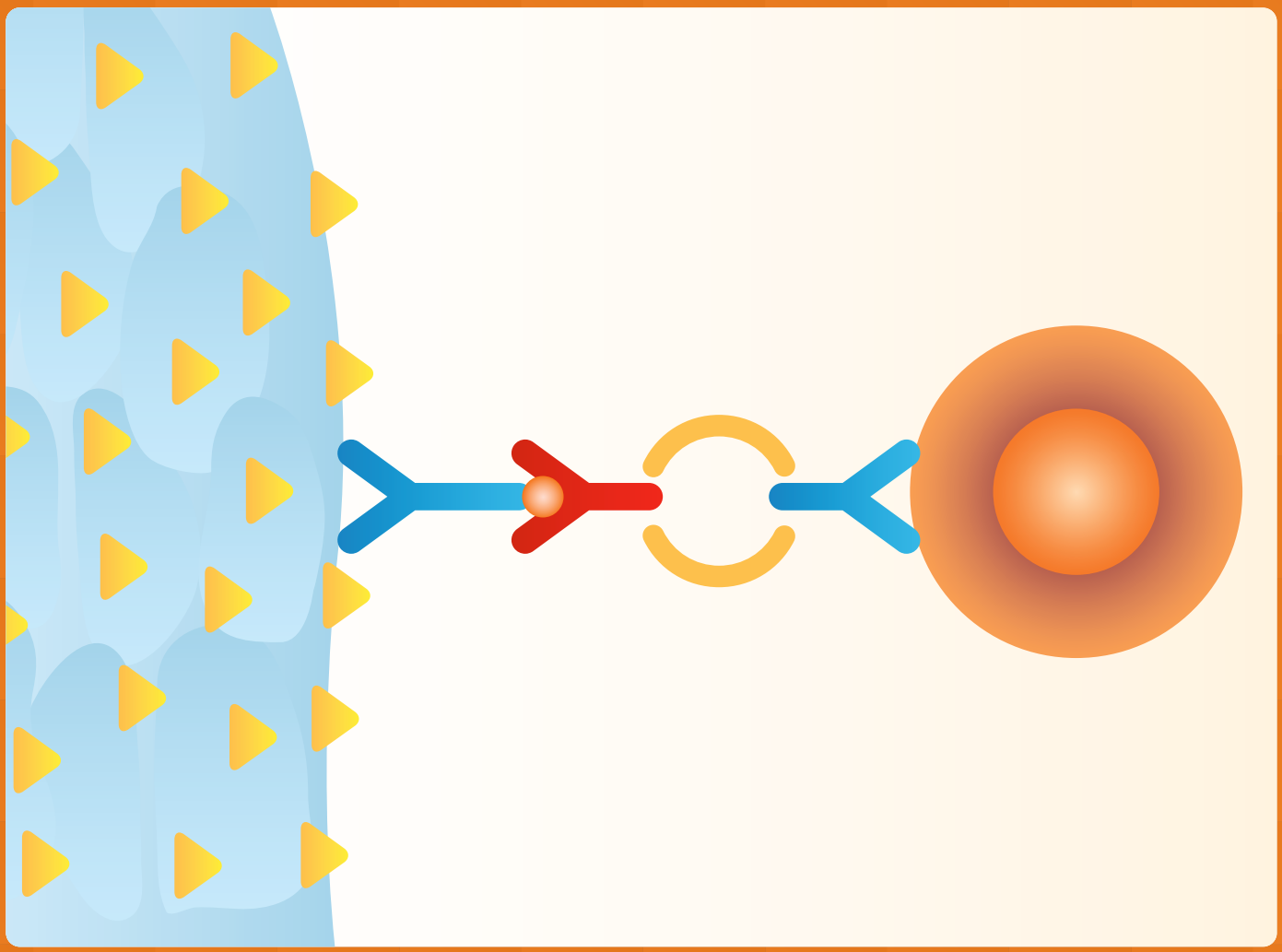
The basic guide to magnetic bead cell separation

Discover how to use biomagnetic separation for an efficient cell isolation



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SUMARY

Chapter I
Introduction: Biomagnetic Bead Cell Separation

Magnetic cell separation entails the action of magnetic force on cellular particles – blood, bone marrow, cultivation media, food, soil, stool, tissue-homogenates, water, and so forth – set in solution. Magnetic force applied to the solution separates treated cells (those with magnetic beads attached) from untreated, encouraging specialized analyses of targeted cells.

Cell separation is a recommended methodology, widely used in research and therapy. Its objective is isolating specific cells from a mixed population, for further evaluation or culture during a biomedical or similar procedure. It enables study of targeted cell types, with exceptionally limited contamination or interference from the total mass of cells in a heterogeneous population.

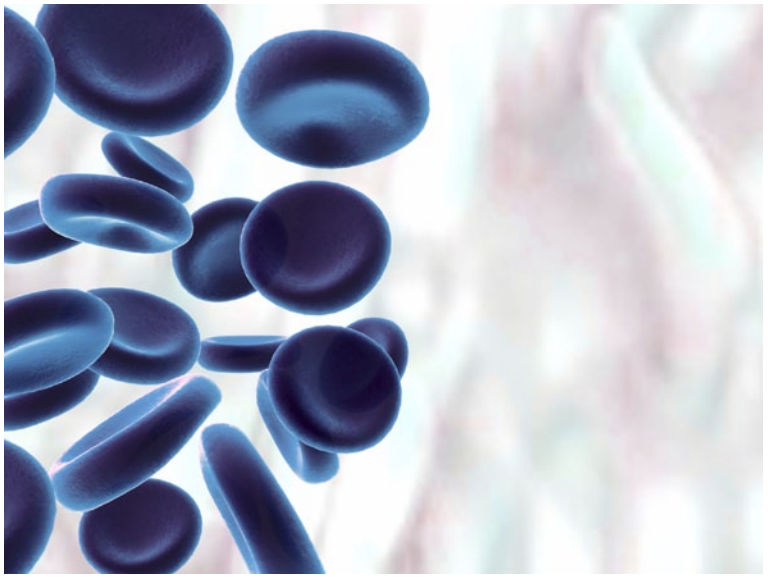
Magnetic bead cell separation is typically implemented in a mixed, batch mode. Larger magnetic beads (>2 µm) are preferred; smaller beads require more complex techniques to effect separation.

Basic Magnetic Bead Cell Separation Technology

Microscopic, synthetic beads provided a core of magnetite or other magnetic material, and coated with a thin polymer-shell, are subjected to chemical modification, facilitating covalent protein-attachment: beads bind and isolate proteins, DNA or RNA. Labeled cells – with beads attached via antibodies, pectin or other substances – are placed with the entire mixed-cell population into a magnetic field. They respond to separation processes through the interaction with space-varying magnetic-force, providing a separation-alternative to centrifugation, columns, filtration, or precipitation.

Within the context of magnetic cell separation technologies, the tube-based method offers distinct advantages to its alternative, passing cells through a dense column-matrix. Tubular cell separation is fully implemented in a single vessel. Magnetic beads are added to a cell-sample, which is incubated. Targeted cells are pulled toward the magnet when its power is applied, effectively separating cells with attached beads. Properly implemented, this process eliminates undue cell stress that can be generated by column-based separation methods or from exposure to iron, significantly diminishing the risk of experimental procedures negatively impacting cell function and phenotype.

Positive separation techniques use primary antibodies, species-specific antibodies, enzymes, lectins or strepavidins to coat beads and attract cells. This procedure positively labels the cells targeted for analysis or culture; the unlabeled cells are discarded. Negative separation methods employ a cocktail of antibodies to coat untreated cells, when species-specific substances are unavailable; in this case, labeled cells are discarded while unlabeled are retained.



Advantages of Cell Separation through Magnetic Bead Sorting (MBS)

Magnetic Bead Sorting generates a simple method for cell separation, as unlabeled cells without attached beads separate from those treated by beads. Appropriately implemented – as, for instance, a pre-enrichment procedure prior to flow cytometry-sorting – MBS can significantly diminish the time required to complete the sorting process, simultaneously reducing the total quantity of cells that need to be sorted. However, if a greater quantity of cells are needed for analytical purposes, cell populations can be augmented more readily than with other cytometric methodologies.

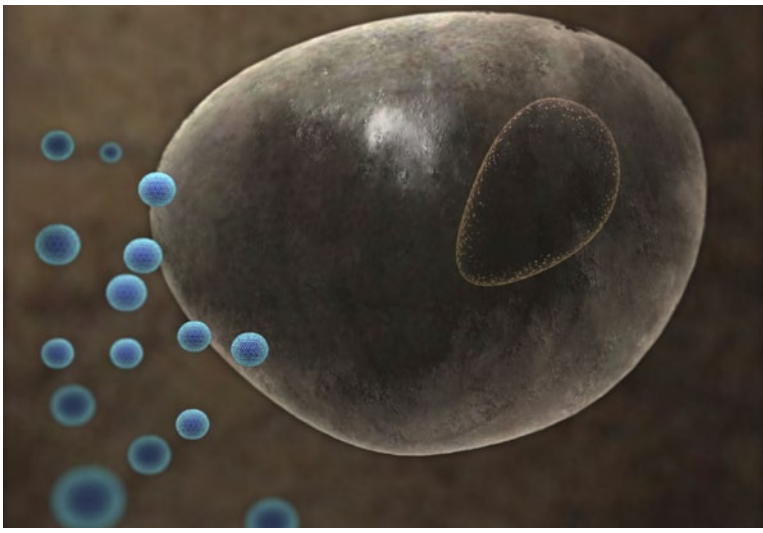
MBS offers higher selectivity of separation without enacting complex protocols or relying on costly lab-equipment. Process and outcomes are more stable than electric-field separation technologies or other methods of separation. MBS generates extended sample enrichment potential if additional analyses – chromotagraphic/electromigratory – are required. Liquid-phase kinetics are enabled, leading to enhanced isolation of targeted cells. Repeated washing processes are unnecessary, yet cell samples are generally pure, unaltered and viable.
Separation of target cells with magnetic beads is compatible with the majority of contemporary life science/biomedical techniques and applications. Tubular cell separation methods are gentler than alternative techniques, offering less threat of contamination from the host cell population or stress to cells caused by more complicated separation regimens. It is very adaptable, effective with small or large cell populations.

Chapter II
Life Sciences: Magnetic Beads Used for Biomedical Applications

Magnetic beads demonstrate many biomedical applications for treatment and research. Their number and range is increasing as understanding and adaptation of the technology for medical purposes grows.

Process Advantages

Beads' magnetic moment facilitates their rapid recovery in solution by magnetic racks, typically within seconds. Magnetically charged beads are darker in color, a condition that improves visibility. The consequent changes in suspension color can be used to monitor the separation, ensuring dependable collection of all appropriately-bound target molecules.



Beyond Cell Separation

Today, the most extended applications of magnetic beads are immunoassays and immunoprecipitation. Similar to cell separation, these isolate selected protein antigens from others within a solution through antibody-specific binding procedures. Magnetic bead techniques implement faster protein binding, with less and gentler sample-handling, aided by less complicated process-automation.

Further contemporary biomedical/life science uses of magnetic bead binding technology include:

- **Isolation of ribonucleic acid (RNA)**: RNA is an essential component of all biological life. Magnetic beads are useful for extracting RNA from cells to analyze cell functions and study various diseases (such as cancer). In addition, isolating RNA is a fundamental procedure of cloning, stem cell research, and the investigation of evolutionary processes.
- **Deoxyribonucleic (DNA) research and genetic exploration**: DNA is the hereditary material of life, human and otherwise. Encoding the genetic instructions required for appropriate development and functioning of all living organism, DNA contains the biological information that renders each species unique. Nucleic acid isolation assisted by magnetic beads has become an important method of extracting DNA for analysis and genetic exploration.
- **Biomicrofluidic technologies**: Representing a multidisciplinary confluence of medicine and biological science, magnetic bead-assisted biomicrofluidic lab-on-a-microchip technologies generate new analytical capabilities for assessment of molecules, cells, tissues and microorganisms.
- **Exosome analysis**: Present in all bodily fluids, exosomes have been associated with cell signaling. They are involved in multiple biological functions. Magnetic beads offer a fast, gentle means of exosome isolation for proteomic analyses and RNA profiling, among other uses.
- **Bioassays**: A method for determining the activity or impact of a substance - drug, hormone, vitamin, etc. - by testing its biological effect or potency on a living organism, subsequently measured in comparison to recognized standards. Used for bioassays, magnetic beads provide high levels of chemical and physical stability for measuring changes of selected particles within a sample, simply and at low cost.
- **Tissue engineering**: Essential to regenerative medicine, tissue engineering leads to the creation of replacement blood vessels, bone, cartilage, heart muscles, nerves and other needed bodily components during medical treatment and healing processes. In clinical cell-based treatments, application of highly selective cell separation technologies enhances the quality of tissue repair and the subsequent clinical outcome. Targeted biomagnetic beads have proven useful for separating cells and effectively manipulating biomolecules to stimulate tissue engineering appropriate to specified biomedical regenerative processes.

Further Uses of Magnetic Beads

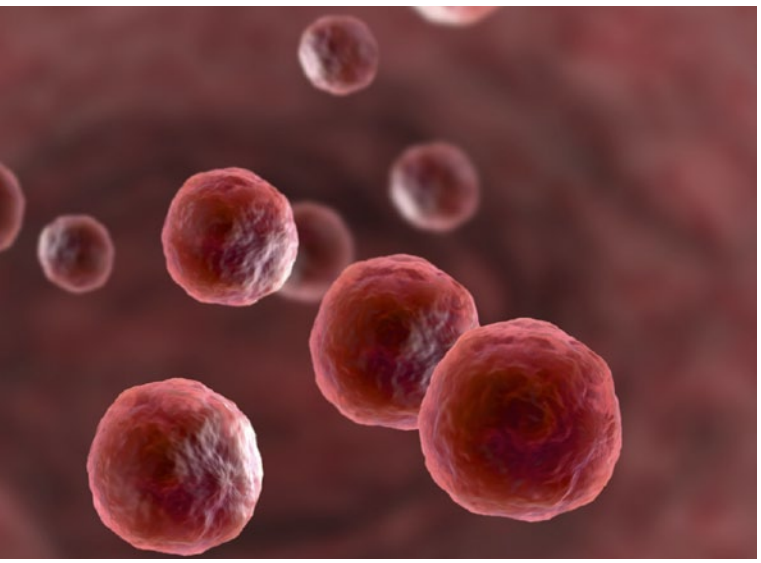
Other current life science uses of magnetic beads are the purification and screening of antibodies and proteins, phosphopeptide enrichment, and modified drug delivery during medical treatment. For instance, biomagnetic cell separation has a significant potential for provisioning biological compounds, including the development of a spectrum of pharmaceuticals. This includes dosing modalities for drug delivery during treatment, as well as development of drug types.

In addition, HIV pathogenesis, advanced cancer research, and emerging **eMedicine applications** have been engendered by magnetic cell separation techniques. **Magnetically mediated Hyperthermia** (MMH) has proven useful in localizing treatment of cancerous tumors within the body, wherein magnetized particles can be heated sufficiently to kill tumor cells. The list of eMedicine applications should only grow in the future.

Chapter III:
The Character and Quality of Magnetic Bead Cell Separation Processes

Cell separation improves understanding of cell function, generating discoveries for improved medical practice and research. Yet, experimental procedures for removing cells from their natural environment can adversely affect their function and expressed physical traits - morphology, or biochemical/physiological properties. Thus, separation processes appropriate to the biomedical or related task at hand are required to assure optimum process efficacy. Magnetic bead cell separation efficiently utilizes the principle of the attractive power of magnetic force on selected particles in liquid solution.

Tube-based magnetic cell separation not only isolates the cells selected for study or use, but also easily removes unwanted cellular-types from the sample, resulting in higher yields of pure, functionally viable cells. Capturing specific cells from a larger population of mixed cell-types stimulates a broadened range of both research exploration and medical diagnoses.



In all cases, it is imperative to determine the desired outcomes of the isolation/separation processes, and their assessment. That is, determining the appropriate measurement of purity, recovery and viability is an overriding factor of process efficiency.

Since magnetic bead separation typically provides the highest quality of these factors, its application can be further improved by identifying the specific properties of targeted cells. Certain procedures need to be enacted to further specify cell separation objectives.

Dividing and Categorizing Cells in Terms of Their Specific Properties

Cell isolation and preparation are fundamental requirements of cell separation. Successful implementation can yield highly enriched cell suspensions, but proceed best when appropriate categorization of targeted cells is enacted, according to their specific properties.

Intracellular Properties: Magnetic bead cell sorting is most effective when a single (one) separation criterion or characteristic is the basis for separation. Of importance is categorizing cells according to their fundamental intracellular properties, in the form of macromolecules: DNA, RNA and proteins.

- **DNA**: DNA-categorization is important because, on its own, it cannot catalyze biological reactions, and thus is applicable to specialized research purposes. DNA is double-stranded, with a double-helix structure and its own internal repair systems, which also affects its uses.
- **RNA**: Single-stranded with a highly complex structure, RNA has no internal repair capabilities. SA-coupling procedures are used frequently for isolation purposes for both RNA/DNA sequencing and binding.
- **Protein molecule interactivity**: Unlike DNA and RNA, proteins cannot encode genetic information and use amino acid rather than nucleotides for building-blocs. However, like RNA, proteins catalyze biological reactions. Like RNA, proteins are single-stranded with a highly complex structure; they also lack internal repair capabilities.

Categorization and division of cells according to their unique properties is necessary to focus cell separation applications. Each of the macromolecules - DNA, RNA and proteins - are sufficiently specialized in form and function to require accurate recognition and individualized processing.

Extracellular Properties: Features such as cell shape (morphology), size and surface protein expression impact the processes needed to effect cell separation.

- **Morphology**: Folds, ruffles and microvilli occurring in the cell membrane may impact the efficiency of microbead applications. Cell surface morphology influences the cell's capacity to hold a magnetic charge, and thus responds to magnetic bead separation technology.
- **Size**: Just as larger microbeads are generally more efficient than nanobeads for most cell separation processes, larger-sized cells typically hold a magnetic charge better than smaller.
- **Surface protein expression**: Within any single cell, protein distribution is predicted by its surface protein expression, which also reflects the quantity of RNA within the cell.

As with intracellular characteristics, categorizing cells according to their extracellular factors provides instructive guidelines for selecting the precise magnetic bead technologies appropriate to a particular cell separation process.

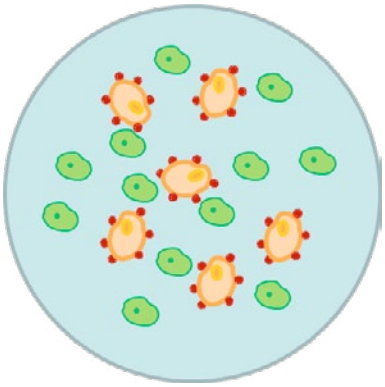
Chapter IV: Microbeads/Nanoparticles for Cell Sorting

Magnetic nanoparticles form the basis for capturing and separating molecules from a sample solution. The particles act as carriers, sequestering target molecules via attachment sites present on their surface, and shuttling them in the direction of an induced magnetic force. The attachment sites are customizable, endowing the particles with specificity and allowing them to be effective for a wide range of applications ranging from investigative to technological and biomedical.

Surface functional groups and biomarkers

In order to serve as carriers, magnetic particles need to be able to recognize their target from a mixed sample and attach to it successfully. For this reason, beads are endowed with a surface functional group that renders them capable of binding a particular type of biomarker. The biomarker, typically an antibody, affords the bead its specificity, allowing it to target cells with the corresponding antigen. A wide range of biomarkers are available, making the repertoire of binding options suitable for many types of processes.

There are essentially two types of surface functional groups: those that bind a biomarker covalently, and those that do so non-covalently. The characteristics and properties of these bindings makes each particularly suited to specific types of applications. **Covalent attachments** are selective and generally more restrictive than their non-covalent counterparts. These types of surface groups recognize and bind a well-defined locus on the target. **Non-covalent attachments**, on the other hand, are inherently less specific and not as stable as covalent attachments. The choice to use one over the other will depend on the overall goals of the process and the properties of the molecule being bound.



Biomarkers are attached to nanoparticles through a process called **coating**. The biomarker directs the particle to the target. Examples of biomarkers include antibodies (monoclonal or polyclonal), peptides, and short oligonucleotides. Specificity is one of the main concerns when deciding which biomarker to utilize, but it's not the only parameter that should be taken into account. The ultimate forces that should drive the selection should be the characteristics of the sample and the target molecule. It's the overall aim of the process that will determine the type of biomarker required.

Nanoparticles vs. microbeads

Magnetic particles are available in a range of sizes, and there are inherent characteristics, dependent on size, that govern a particle's behavior in a magnetic gradient. Particles can be divided into two categories: microbeads and nanoparticles. Microbeads range in size from 0.5 to 500 micrometers, while nanoparticles are smaller, ranging from 5 to 500 nanometers. In general, larger beads tend to be more frequently used, as smaller beads (<200 nm) require more complex techniques during separation.

There are a number of advantages to using larger beads in a protocol. Larger particles are more likely to avoid the types of cellular damage that can be incurred by smaller beads. For instance, smaller particles can cause oxidative damage and may alter a cell's physiology and/or signaling and genetic expression. Also, larger beads are less susceptible to endocytosis, and not likely to interfere with a cell's structure during a process.

On a larger scale, bigger particles have a tendency to interact with each other when a magnetic field is applied. As it would be discussed in next chapters, this significantly reduces the timescale of a process and allows separation to be carried out with a lower gradient. Similarly, larger particles are better able to overcome drag and other inertial forces in a sample.

The methodology being employed will ultimately govern the size and characteristics of the particles utilized in any particular process. That being said, using larger particles will introduce fewer technical challenges, and are therefore often preferable.

Chapter V:
Methods of Magnetic Cell-Sorting

Magnetic cell sorting has demonstrated extreme utility for isolating virtually all cell types from complex biomedical samples and cultured cells. **Antigens** (cell-surface proteins) provide the extracellular characteristics for enriching heterogeneous cell-mixtures typical of magnetic cell sorting. Attachment of target-specific antibodies to beads' surfaces generates sorting, securing intact cells to allow isolation within a complex liquid suspension.

The two essential methods of magnetic cell-sorting are **High Gradient Magnetic Separation** (HGMS) and **Low Gradient Magnetic Separation** (LGMS). The selected method of separation naturally impacts separation workflow, purpose, devices employed, and process results. The size of the particles used during separation will be a relevant factor informing the methodology. Large beads (>00 nm) mutually interact, thereby accelerating the separation process. As such, they can be effectively separated with moderate gradients. In contrast, smaller beads (<100 nm) must be separated individually. They require higher gradients for separation, with gradient-levels increasing as bead size decreases.

It's important to understand that the magnitude of the gradient is directly proportional to the magnetic force when the beads are saturated. When the magnetic particle is in the low field region, the force is then proportional to the gradient of the square of the magnetic field. A magnet generates a force because it produces a field that varies with distance. The gradient itself

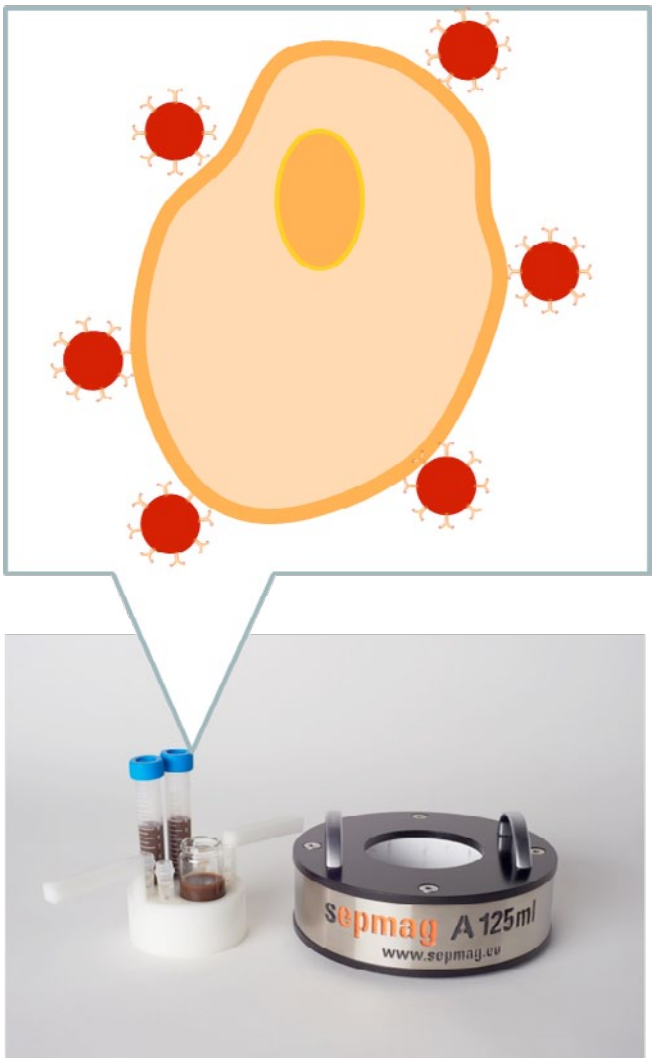
depends on the geometry of the permanent magnet and volume of the sample. While larger-size magnets maintain magnetic field uniformity across larger distances, they are ineffective when separating larger volume solutions; more significant, the magnitude of the magnetic field gradient needs to remain constant. For this reason, the design of the magnetic separation rack is not straightforward. Increasing the size of the magnet will not yield a proportional increase the magnetic field gradient.

High Gradient Magnetic Separation (HGMS)

High gradient magnetic separation has become a favored technique of biomedical cell separation. The magnetic force generated during a HGMS process must be high enough to overcome drag and other forces produced by the flow of the sample. The high gradient necessary to overcome these forces can only be achieved in small-gap systems. Thus, the process requires specialized, low-volume columns. The column is packed with stainless steel beads or wool, which are magnetized when a magnetic field is applied. When a sample containing magnetically labeled particles is pumped through the column, the particles are retained by the induced magnetism of the matrix. The particles themselves are superparamagnetic. Upon removal of the magnetic field, the magnetic moment of the particles becomes zero and they can be collected by elution.

HGMS is most frequently used for small volume processes such as cancer research, immunology, neuroscience, and stem cell research. One of the classical uses of HGMS is an antibody-binding method that utilizes cell-surface antigens as properties for separation. Iron oxide particles labeled with antibody are selectively conjugated to cells with the corresponding antigen. Unlabeled cells within the sample are eluted following placement in a magnetic field.

Selection can either be positive or negative. With positive selection, the supernatant is discarded, while the targeted particles are retained. In this case, labeled cells, those under investigation, remain in the magnetic field until they are detached from it. This is the source of separation. Cells coated with magnetic beads will experience a magnetic force and will



travel in the direction of the magnetic field gradient. In negative selection, the opposite is true. The washed (‘cleaned’) supernatant contains the desired particles, those targeted by the process. The beads/particles captured by the magnetic field are those removed from process consideration.

The outcome of a HGMS process is affected by a number of variables, including the size, shape, and distribution of the magnetic particles utilized. Typically, HGMS designs will be customized for the purposes of each separation protocol. Careful development and execution of unique parameters particular to each separation condition is essential, and will play a role in the ultimate efficiency and integrity of the process.

Low Gradient Magnetic Separation (LGMS)

Unlike HGMS, low gradient magnetic separation does not require specialized, pre-packed columns. LGMS makes use of specific magnetic field patterns that allow the process to be carried out in containers ranging from microtubes for smaller-scale laboratory volumes, to bottles or other containers for larger-scale processes. The magnetic gradient is generated by Permanent Magnet arrays that ensure stable and reproducible conditions.

Magnetic separators for LGMS biotechnology applications employ batched-mode and relatively low field gradients to separate magnetic beads from liquid suspension. Since such opposing forces as sedimentation can retard separation, LGMS requires magnetic force over the beads to be sufficiently large to compel them to move more quickly than the sedimentation or other retarding effects. Beads with enough magnetic moment will experience a dipolar interaction between them, which will accelerate the separation. As such, LGMS relies on a larger critical size of microbeads to capture and retain cells for sorting and separation.

Where conventional **inhomogenous LGMS** processes generate significant force in the retention area, resulting in possible cell membrane stress and lysis, homogenous force systems avoid this problem. Because the magnetic force is constant, all beads experience it equally, traveling at a uniform speed to the retention zone.

Contemporary LGMS systems also avoid complexity issues affecting process scalability because their magnetic force is homogenous and therefore well defined. Thus, scaling up of separation processes is typically far less complicated than inhomogenous force systems. Unlike conventional flow cytometry methods, the selection of specialized cell populations can be scaled up as needed. The well defined conditions are easy to reproduce at the different volumes required at the various levels of process implementation.

There are a number of factors that can affect a separation process, and variables such as particle magnetic content and size are critical considerations to take into account before settling on a particular platform. Ultimately, however, the choice of methodology will be dictated by the process goals, and subsequent protocol design will be aimed at maximizing both yield and efficiency.

Chapter VI: How Magnetic Bead Cell Separation Works

Coated magnetic beads are capable of interacting with and binding to a corresponding target within a sample. Binding specific biomarkers to the surface functional group present on the bead (e.g., streptavidin) ensures that the interaction is limited to specific cells. Recovery of material for further studies is greatly simplified when beads are concentrated from suspension, by means of an external magnet.

Properties of Magnetic Beads

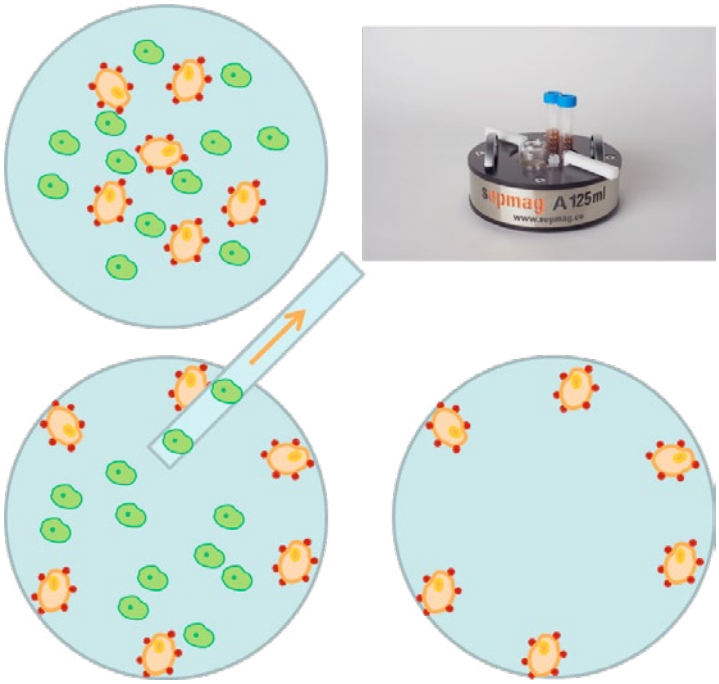
Microbeads can access the minute scale-lengths of biological cells necessary for cell separation. The biomagnetic beads are functionalized with antibodies or proteins, which endow them with specificity. It is possible for a number of beads to attach to a single cell. Each cell is then confronted by the net impact of all beads binding to it. For micron size cells, the number of attached beads is likely to be in the range of tens.

During a biomagnetic separation process, a space varying field is generated from a magnetic source. Magnetizable objects - in this case, **superparamagnetic beads** - will move in the direction of the magnetic field gradient. Cells experience the net influence of the magnetic force over all beads attached to them and are drawn to the magnet. For flow-through applications, for instance, separation is possible by situating the magnetic source at an appropriate angle to the net hydrodynamic flow of the solution and the cells within it.

Utilizing beads of uniformly consistent size and surface adhesion is essential to reduce particle variability and improve the level of reproducibility within and between processes.

Magnetic Force

The magnetic force will determine the separation speed of the magnetic beads. In all cases, the magnetic force is a consequence of field gradients acting on magnetic moments. A magnetic force capable of separating cells without overwhelming the sample or damaging cells during the process is necessary. As such, a **homogenous magnetic gradient** ensures that particles further from the magnet in the solution are subject to sufficient magnetic force to draw them to the magnet, and particles closer to the magnet do not suffer from undue stress due to prolonged exposure to a high magnetic field, which can damage the cells.



For optimal performance, it is necessary to saturate the magnetic beads, ensuring a constant, fixed magnetic moment. Saturated beads will experience a dipolar interaction and will form chains or clusters. These clusters will be less affected by drag forces and will have a higher magnetic moment, allowing them to move faster than solitary beads.

Separation Techniques

Two primary systems can be applied for implementing magnetic bead cell separation: direct and indirect.

In a **direct system**, biomagnetic beads activated with an appropriate surface ligand are circulated directly into the sample containing the cell mixture. Beads bind with targeted cells during incubation, and are subsequently magnetically retrieved.

An **indirect system** is somewhat more complicated. Indirect processes proceed in several steps. Sensitized target cells are incubated with an affinity ligand. Following a wash step, the cells are then incubated with beads activated with a secondary surface ligand.

Selection of a particular system is reliant upon the purpose and ultimate goals of the procedure. Sufficient procedural variety exists to ensure an appropriate methodology can be applied in all cases.

Overall, the success of a magnetic bead cell separation process relies on (1) the selection of suitable biomagnetic beads and (2) the application of an appropriate magnetic force. Choosing the right beads will ensure specificity and reproducibility, while applying the correct magnetic force will increase bead capture, decrease process time, and minimize cell damage. Taken together, these two factors will play a key role in determining a protocol's success, resulting in a separation process that is specific to the needs of a given application.

Chapter VII:
Applying a Magnetic Field for Cell Sorting Processes

Use of magnetic beads provides an efficient and innovative method of harnessing magnetic separation processes to non-magnetic, cellular targets of biological origin. When beads are attached, the ensemble of the cells and beads becomes a magnetizable object.

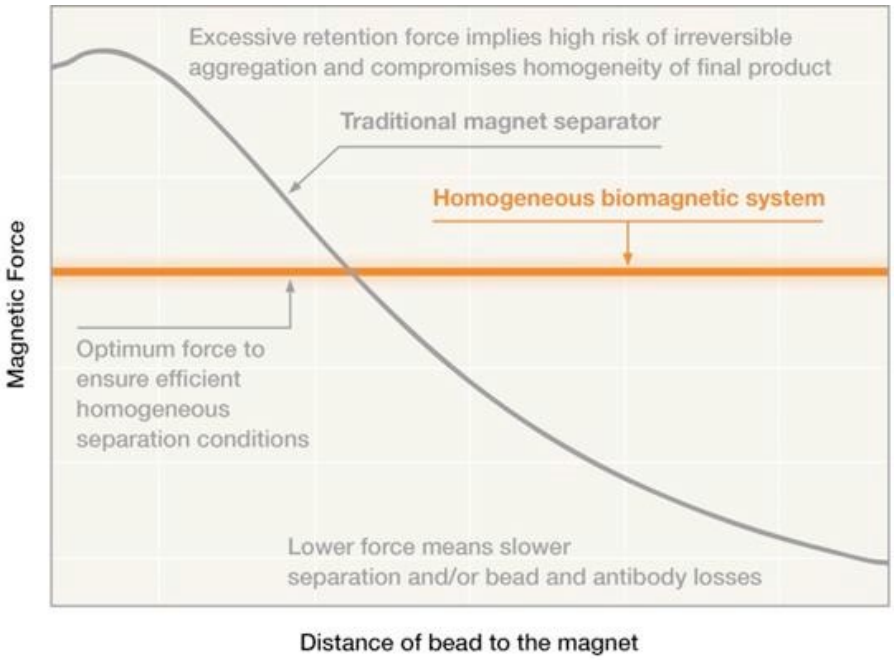
The magnetic beads are superparamagnetic and will therefore display magnetic properties when a magnetic field is applied, but will retain no residual magnetism after the magnetic field is removed. Depending on the concentration and size of the beads, they can be induced to behave independently or to cluster together to form aggregates.

Cooperative magnetophoresis

If the magnetic field is high enough to saturate the beads and the resultant magnetic moment big enough, they will interact with each other to form chains. The aggregates will not only be less susceptible to drag forces, but will also have a higher magnetic moment. As a result, their movement will be accelerated, optimizing their collective power. The size of the magnetic force necessary to induce the beads to form aggregates is dependent on their diameter, and increases as the diameter of the beads decreases.

Cooperative magnetophoresis, the phenomenon in which particles aggregate to form clusters during a separation process, shortens the time necessary to complete a separation protocol by orders of magnitude. In contrast, **non-cooperative magnetophoresis**, in which the beads do not aggregate but rather behave as individual particles, is a slower separation regime. Whether or not the beads aggregate is dependent on the concentration, size, and magnetic content of the particles. The relationship of these factors to the beads' aggregating behavior in a magnetic field is mathematically defined. As such, it is possible to increase the speed and efficiency of a protocol by calculating the parameters required to induce clustering. The key is to induce clustering, while preventing uncontrolled and irreversible aggregation.

In cases where the magnetic force is inhomogenous, the force experienced by the beads will vary depending on the bead's distance from the magnet. Beads that are far away will be unsaturated and thus have a small magnetic moment. This will make clustering less likely, and the beads will be more susceptible to drag and other forces, slowing their rate of motion and increasing the timescale of the separation.



The parameters on which a magnetic field depends are more difficult to define in **inhomogenous systems**. As a result, these processes are more difficult to scale up. Increasing the volume of a process also increases the distance that a bead has to travel, and its distance in relation to the magnet. Thus the forces necessary to maintain a reasonable timescale increase as the sample volume increases.

In **homogenous systems**, the magnetic force does not vary. All the beads in the system are subject to the same force regardless of their position in relation to the magnet, and therefore move at the same speed. The timescale required for a process is a function of the diameter of the separation vessel.

Electromagnet or Permanent Magnets considerations

The decision to use **Permanent Magnets or Electromagnets** will be based on a number of factors, including the sample volume, process goals, and system methodology. Each type of magnet has characteristics that will make them better suited to a particular process. The two classical options are electromagnets and permanent magnets.

Electromagnets are generally more amenable to smaller volumes. The heat generated as a result of resistance is small in these cases, and can be dissipated easily. Larger volumes require larger electromagnets, which can generate excessive amounts of resistive heat, requiring costly cooling systems and power supply maintenance.

Unlike electromagnets, permanent magnets aren't powered by electricity and don't require cooling systems or maintenance. As such, they are more suitable for larger volumes. With large, open permanent magnets, precautions must be taken to avoid stray fields that can be generated, constituting a safety hazard, but modern advanced designs keep safety distances short and the resulting footprint small. Also, unlike the case with an electromagnet where the magnetic field will depend on parameters such as current (and therefore the power supply precision and stability), permanent magnets keep the magnetic field constant during tens of years.

The characteristics inherent to permanent magnets and electromagnets must be taken into account when designing a protocol. There are benefits and drawbacks intrinsic to each type. Ultimately, however, the methodology will be informed by the goals and variables specific to each process.

Chapter VIII: Conclusion

This eBook reviews several aspects of biomagnetic cell sorting, a process which facilitates the quick and targeted removal of specific cells from a heterogeneous solution. Cell sorting is essential for a number of purposes, from technological to investigative and biomedical. By harnessing the properties of magnetic beads, **biomagnetic separation** (BMS) allows cells to be isolated for additional downstream applications, without adversely affecting their form or function.

There are a number of advantages associated with the use of biomagnetic beads for cell sorting. The sensitivity of the beads makes the process suitable to small as well as large target cell populations, while maintaining a high degree of selectivity. The beads do not react with or alter the phenotype of their target, nor do they cause undue stress, as can occur with harsher separation techniques such as centrifugation. What's more, the beads' magnetic properties make them recoverable by magnets, a process which occurs within a rapid timeframe.

The potential for biomagnetic separation extends to a number of applications. In addition to immunoassays and immunoprecipitation, biomedical applications for BMS include isolation of ribonucleic acid (RNA), deoxyribonucleic (DNA) research and genetic exploration, biomicrofluidic-technologies, exosome analysis, bioassays, and tissue engineering. As research expands, the list of potential uses for biomagnetic separation will to continue to grow.

Designing a Biomagnetic Separation Protocol

In order to maximize the yield of a BMS process, it is essential to categorize the cellular targets. The specific properties of a cell form the basis by which it will be sorted. Intracellular properties - e.g., DNA, RNA, and proteins - and extracellular properties - e.g., morphology, size, and protein-expression - all impact the process. Categorizing cellular targets according to these characteristics will result in a focused approach to selecting magnetic beads for a particular process.

Cells are recognized and sequestered by biomarkers on the beads. Biomarkers, in turn, are attached via functional groups present on the beads' surface. These surface functional groups may bind a biomarker covalently or non-covalently. **Covalent binding** confers a higher degree of selectivity, while **non-covalent binding** is more restrictive. The choice to use one type of binding over another will largely depend on the properties of the biomarker.

The size of the bead is another consideration when designing a protocol. Microbeads are generally preferred to smaller nanoparticles. In addition to being less susceptible to endocytosis, microbeads (>300 nm) have a tendency to interact with each other, accelerating the separation process and allowing the use of moderate magnetic gradients. In contrast, nanoparticles (<100 nm) must be separated individually, necessitating the use of higher gradients.

Because of this, the size of the particles will be a relevant factor informing the methodology. The type of method employed - i.e., **High Gradient Magnetic**

Separation (HGMS) or **Low Gradient Magnetic Separation** (LGMS) - will affect several aspects of the process, including the devices employed. The high gradient required for HGMS, for instance, necessitates low-volume columns. LGMS, on the other hand, is carried out in containers ranging from microtubes to bottles or other containers.

During a biomagnetic separation process, the beads experience a magnetic force, drawing them in the direction of an applied magnetic field gradient. The magnetic force is generated as a result of the space varying field. The speed with which the beads are separated is determined by the force.

A sufficiently high magnetic field can saturate the beads. Depending on their concentration and size, it is possible for saturated beads to experience a dipolar interaction and aggregate to form chains or clusters. Clusters of beads are less affected by drag forces from the sample and have a higher magnetic moment. As such, they move faster than individual beads, significantly shortening the length of time required for a separation process.

A **homogenous gradient** ensures a non-varying magnetic force. Magnetic beads experience the same force and therefore move at the same speed, regardless of their distance from the magnet. This is not the case when the magnetic force is inhomogenous. In **inhomogenous gradients**, beads that are situated far from the magnet will be unsaturated, making clustering less likely and increasing the timescale of the separation. In addition, the parameters on which the magnetic field depends are more difficult to define, making inhomogenous systems more difficult to scale up.

A final factor relevant to the outcome of a BMS process is the type of magnet utilized to generate the magnetic field. Electromagnets are generally better suited to working with smaller volumes, as the heat generated by smaller magnets can be dissipated easily. Permanent magnets aren't powered by electricity and therefore don't require cooling systems, making them more suitable for larger volumes. In addition, permanent magnets maintain a magnetic field constant for tens of years. This is not the case for electromagnets, where the magnetic field depends on parameters such as current (and therefore the power supply precision and stability). The type of magnet chosen for a particular process will ultimately depend on the sample volume, process goals, and system methodology.

The success of a BMS protocol is reliant on the use of suitable biomagnetic beads and the application of an appropriate magnetic force. Within these parameters, there are a number of variables that can influence the process and affect the final outcome. When these factors are addressed, biomagnetic separation becomes a reliable and powerful technique for cell sorting and other biomedical aplications, capable of efficiently targeting and sequestering cells without altering their essential characteristics.

How SEPMAG® may help?

Since 2004, we are helping our customers to achieve successful cell isolation processes. Our SEPMAG® Systems have homogenous Biomagnetic Separation conditions. Moreover, the magnetic force values are adapted to optimal performance for most commercial magnetic beads. As we use homogenous conditions, process validation and scale-up are straightforward.

When needed, special force values (gentler or stronger force), can be provided under request. We may help you to experimentally determine the 'optimal' force value for your magnetic beads suspension.

Our QCR hardware and software allows monitor each single biomagnetic separation step, and using the data to ensure the quality of the process.

All these benefits, in an extremely safe environment, with no maintenance nor running cost.

Do you need additional information?

Please, check our Resources for more FREE literature on the subject and check our BLOG for the latest updates on Biomagnetic Separation.

If you wish, you may always contact us.

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Follow us on:

SEPMAG SYSTEMS
contact@sepmag.eu
+34 935 820 161

Parc Tecnològic del Vallès
E-08290 Cerdanyola del Valles. Barcelona