CELL SEPARATION METHODS AND APPLICATIONS

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Magnetic Cell Sorting with Colloidal Superparamagnetic Particles

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I. INTRODUCTION

Magnetic cell sorting (MACS) is increasing in popularity in both the clinic and the research laboratory. In this chapter, we focus on the application of small immunomagnetic particles that can be combined with a ferromagnetic matrix and high-gradient fields to simply and efficiently isolate specific subpopulations of cells from heterogeneous mixtures such as peripheral blood or bone marrow. This powerful technique allows large numbers of viable cells to be isolated with high purity and yield. The purified cells are immediately suitable for flow cytometry, molecular biology, culture, and functional transfer into animals, including humans. We review the principles of MACS and discuss applications involving multiparameter separations, rare-cell isolation, and processing of very large numbers of cells for therapeutic purposes.

II. PRINCIPLES OF MAGNETIC CELL SORTING

A. Overview

The general separation process is outlined in Figure 1. The cell-preparation and labeling methods are similar to those used in flow cytometry (1). A monodisperse suspension of viable cells is incubated with a magnetic reagent, which typically consists of a monoclonal antibody (mAb), specific for the cells of interest, covalently coupled to magnetic microbeads 20–100 nm in diameter. The cells are washed to remove excess reagent and resuspended in buffered saline containing protein such as 1% bovine serum albumin (BSA). The suspension is then passed over a column containing a ferromagnetic matrix

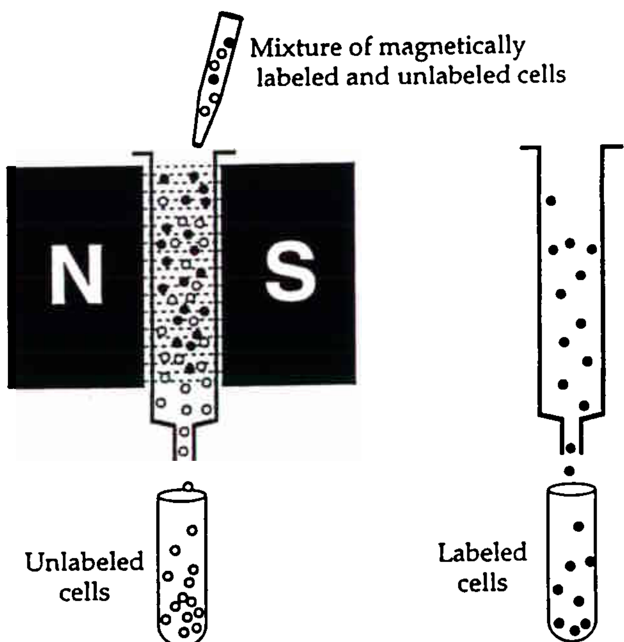


Figure 1 Isolation of magnetically labeled cells. A mixture of magnetically labeled and unlabeled cells is loaded onto a ferromagnetic matrix–separation column placed between the poles of a magnet. Magnetized cells are retained on the column while unlabeled cells pass through. Labeled cells are recovered after demagnetization of the column by removing it from the magnetic field.

placed in a strong external magnetic field. The matrix, which creates a strong magnetic field gradient, acts as a specific cell filter: magnetically labeled cells are retained on the column, while unlabeled cells flow through. After sufficient washing, the matrix and bound microbeads are demagnetized by removing the column from the magnetic field, and the retained cells are easily eluted. No harsh chemical or physical treatments are needed to remove the cells. Both the enriched (labeled) and depleted (nonlabeled) fractions are suitable for further uses.

Alternative magnetic cell separation methods use large (0.5–5 μm) magnetic spheres, with large magnetic moments, and low-strength magnets. While simple in principle, this approach has several disadvantages for the cells. The cell-bead reaction is slower with the larger particles and often requires multiple or prolonged incubations. Aggregation of cells and particles can lead to nonspecific trapping of unlabeled cells and, consequently, low purity of the selected cells. The large magnetic particles can adversely affect the viability and optical properties of the labeled cells, making this method more suitable for depletion than for enrichment. Release of the magnetic particles is often required before the cells can be used.

B. Reagents

Superparamagnetic Microbeads

There are two key properties of the particles. First, they are small enough (20–100 nm) to be colloidal, i.e., they remain dispersed due to the random molecular bombardment of Brownian motion. Second, they are superparamagnetic. The magnetically susceptible core is small enough that the particles do not retain any residual magnetism when removed from the magnetic field. These properties facilitate both the preparation of antigen (Ag)-specific reagents and separation of specific cell types.

Several types of colloidal magnetic microbeads have been used to label and separate cells, including magnetic ferritin (2), coprecipitates of protein and either iron (3) or cobalt (4) species, coprecipitates of iron oxide and methacrylate (5), and coprecipitates of iron oxide and polysaccharide (6). It is the latter class of particles that is most widely used and that has been the focus of our work at Miltenyi Biotec (Bergisch Giadbach, Germany) and AmCell (Sunnyvale, CA).

Production of iron dextran particles was described originally by Molday and MacKenzie (6) and Molday and Molday (7) and has been modified by Miltenyi et al. (8). Briefly, base is slowly added to a mixture of dextran (40 kDa), ferric and ferrous chloride in an aqueous medium to form a dispersion

of particulates of mixed iron oxides (magnetite) embedded in a dextran shell. The magnetic particles are sized and purified by filtration and washing in a high-gradient magnetic field. The particle concentration of the resultant brown suspension is conveniently monitored by the optical density at 450 nm. The material, which is stable at 4°C for several months, is suitable for chemical modification. The chemical composition and physical properties of the microbeads are summarized in Table 1. The particles, which are more than 50% iron oxide, are 20–100 nm in diameter and contain embedded iron oxide particles approximately 10 nm in diameter.

Chemical Modification of Magnetic Microbeads

The microbeads can be modified by a variety of carbohydrate chemistries provided care is taken not to dissociate the iron core or aggregate the particles. Biotin (8), avidin (9), various haptens and fluorochromes, and specific mAbs have all been covalently attached to magnetic microbeads.

Molday and MacKenzie (6) originally used periodate oxidation/sodium borohydride reduction to couple protein A directly to the carbohydrate. Rela-

Table 1 Properties of Iron-Dextran Superparamagnetic Colloidal Reagents[a]

Component	% w/w
Chemical composition	
Iron oxide (as Fe_2O_3)	55–59
Dextran	35–39[b]
Antibody	2–10[c]
Physical Properties	
Size (diameter, nm)	20–100
Iron oxide core (approx. diameter, nm)	10
Density (g/mL)	2.5
Magnetization per cell (gauss μm^3)	30–500
Microbeads/cell	1000–20,000

[a]Properties are presented for a "typical" antibody (Ab) conjugate and typical staining. Various extents of substitution of the iron dextran are optimized for each Ab.
[b]The composition of the particles varies according to the degree of Ab substitution.
[c]Assuming a diameter of 30 nm for the magnetic bead, there are typically 10–200 Ab molecules/particle.

tively mild conditions were used to avoid oxidation of the iron core and aggregation of the conjugates.

A favored means to activate the microbeads is with cyanogen bromide or similar reagents and, subsequently, to introduce amino groups in the form of diaminohexane (8). Introduction of amino groups enables addition of a vast array of functional moieties using isothiocyanates, N-hydroxy-succinimide esters, and other chemistries. Heterobifunctional cross-linking agents are used to couple proteins containing sulfhydryl groups to the amino-modified microbeads. For Abs, native sulfhydryl groups in the hinge region can be exposed by mild reduction or free sulfhydryl groups can be introduced chemically. Typically, 1–20 μg of Ab or other protein is conjugated to 1 OD_{450} mL of microbeads. This corresponds to approximately 10–200 Ab molecules per microbead, assuming a bead diameter of 30 nm. The optimal amount of Ab must be determined for each Ab-Ag pair. Microbeads with low amounts of high-affinity Ab are preferred to minimize nonspecific and Fc receptor–mediated binding to irrelevant cells. The magnetic properties of the beads are retained throughout the chemical modification and are exploited to remove unreacted components at various stages by washing the particles on iron-matrix columns in a magnetic field. The final reagent is stable at 4°C for up to 1 year.

C. Ferromagnetic Columns

Paul and coworkers introduced the use of a random array steel wire (steel wool) as a magnetic filter for the isolation of red blood cells made paramagnetic by the deoxygenation of hemoglobin (10,11). When the loosely packed column is placed in between the poles of an external magnet, a high magnetic gradient is generated only in the immediate vicinity of the wire (12,13). For a permanent magnet of 0.5–1 Tesla (T) and wire with a diameter of 50–100 μm, magnetic forces of approximately 10^4 T/m can be generated compared with only 10 T/m using conventional geometries. Any microbead-labeled cell is attracted and moves toward the wire provided that the magnetic attraction exceeds the viscous force from the liquid flow in the system. Distances between the wires are only 100–200 μm, rather than the 1–20 mm for nonmatrix systems, so magnetized cells only need travel a short distance to be trapped. Nonmagnetized cells simply pass through the loosely packed matrix.

Molday and Molday (7) used similar filters for immunomagnetic cell separation. Coating the wires with a thin, biocompatible plastic polymer layer prevents corrosion and any possible damage to the cells through direct

contact with the wire (8). The coated steel-wool matrix is ideally suited for cell depletion.

Ordered arrays of wire mesh (14) or iron spheres improve the uniformity of flow through the columns. At Miltenyi Biotec and AmCell, we use a closely packed matrix of ferromagnetic spheres coated with a biocompatible polymer for optimal positive selection. This permeable matrix affords uniform fluid flow throughout the column.

When the iron-sphere matrix column is placed between the poles of an external magnet, a high magnetic gradient is generated in the immediate vicinity of the spheres. Optimizing the size of the spheres is a matter of balancing the magnetic force, which is inversely proportional to the radius, with the need to allow cells to pass easily through the spaces between the spheres. Spheres of 0.05–2 mm in a field of 0.5–1 T are appropriate for cell separations. The maximum attractive potential is at the poles of each sphere, while there is a repelling force at the equator. Thus, magnetically labeled cells are focused in particular regions of the column and nonmagnetically labeled cells flow through.

Both wire and iron-sphere matrix columns can be prepared in a range of sizes. Current columns are appropriate for separating up to approximately 10^{11} total cells, with as many as 10^9 being magnetically labeled and retained on the column. This scaling feature makes it possible to process small (e.g., 1 mL blood) and large (e.g., a leukapheresis harvest) samples in the same amount of time, within 30 minutes after labeling. This is in striking contrast to fluorescence-activated cell sorting (FACS), where cells are processed one at a time. Assuming a rate of 4000 cells/second in a conventional sorter, it would require 0.7 hour to process 10^7 cells and 700 hours to process a leukapheresis pack with 10^{10} cells.

D. Cell-Isolation Strategies

High-gradient MACS with colloidal superparamagnetic particles is a flexible technique that affords both enrichment (i.e., positive selection) and depletion (i.e., negative selection) of specific cell populations. Like other cell-separation procedures, the strategy of choice is dependent upon the availability of specific reagents, the characteristics of the cell mixture to be separated, the relative proportion of desired cell population within the mixture, and full consideration of how the recovered cells are to be used, including any restrictions regarding purity and activation state.

Advantages and Disadvantages of Depletion and Enrichment

Depletion methods ensure target cells remain unlabeled, and this may be advantageous for functional studies such as T-cell activation. It is often cumbersome, however, to remove all nontarget cells, especially if the cells of interest, such as hematopoietic stem cells, represent only a minor fraction of the starting population. A depletion approach would require a complete panel of Abs directed against markers on all other cells. Moreover, as the separation column must be large enough to retain nearly the entire sample, the process is not well suited for bulky samples such as leukapheresis packs. In contrast to positive selection, the resultant cells are diluted during the separation process because of the extensive washing needed. Taken together, these factors suggest that depletion strategies are of limited use in isolating rare cell populations.

Positive enrichment is particularly well suited for isolating rare cell populations from bulky cell mixtures. The iron-sphere columns are optimized for this approach and need only be large enough to hold the desired cells. Consequently, as with other types of affinity chromatography, the selected cells can be effectively concentrated on the column. For the isolation of rare populations, nonspecific cell trapping may be equal or exceed the number of target cells. It is possible, however, to improve the cell purity by eluting the magnetically labeled cells and redepositing them on a magnetic column. Cells labeled with the small magnetic particles can be used in flow cytometry and other cellular and molecular assays. They can be readily transferred into animals without toxicity (15). In fact, dextran-stabilized iron oxide has been used since the 1960s for the treatment of iron deficiencies (16,17). The magnetically enriched cells have been shown to be suitable for cell therapy without removal of the microbeads.

Multiparameter Magnetic Separations

Combinations of positive and negative selection make isolation of cell populations not defined by a single Ag possible. There are several different strategies. First, magnetic separation can be used as a preenrichment for FACS sorting. The iron-dextran microbeads do not interfere with the fluorescent and scatter properties of the cells. Use of FACS for the second step allows for separations based on finer quantitative distinctions in Ag density than is practical in magnetic separations. Second, negative depletion can be used before positive selection. The nonretained cells from the first separation are simply labeled with a second magnetic microbead reagent and enriched on

a second, usually smaller, column. Success depends on efficient retention of the labeled cells in the first separation. Finally, positive magnetic selection can be done before either a second positive selection or negative depletion, provided the magnetic labels can be released from the cells. This can be accomplished by specific competition or biochemical removal of the microbeads. Success depends on efficient release of the magnetic reagent after the first separation. Examples of multiparameter separations are given in the following applications section.

Cell-Labeling Strategies

Labeling the cells with immunomagnetic reagents is relatively straightforward. Both direct (one step) and indirect (two or more steps) methods are used with appropriate incubations and washes. A general advantage of the colloidal magnetic beads is the faster binding reaction compared with larger particles. Incubation times of 15–30 minutes are typical.

It is important to titrate each magnetic bead reagent for optimal cell separation. Cells typically are suspended at a concentration of 10^8/mL, and microbeads are typically used at a concentration of 0.05–5 OD_{450}. In general, the optimal microbead concentration depends on the affinity of the Ab, the level of Ab/microbead, and the nature of the Ag. Too much reagent may lead to retention of nonspecific cells on the column.

Titrations of two anti–T-cell magnetic bead reagents directed to different antigens are shown in Figure 2. The percentage of T cells not retained on the column is plotted as a function of microbead concentration. The anti-CD3 and anti-CD5 conjugates have approximately the same amount of Ab per microbead. The anti-CD5 reagent is substantially more effective in capturing T cells. In general, magnetic bead reagents are used below saturating levels, and it is still possible to label the target Ag with fluorescent reagents for flow cytometry. Higher amounts of reagent may lead to nonspecific and Fc receptor–mediated binding to irrelevant cells. We estimate that there are typically 10^2–10^4 microbeads/cell for efficient cell separation.

One-step reagents, where the mAb is directly coupled to the microbeads, are significantly more convenient to use than two-step reagents because the labeling time is consequently reduced. While substantial effort may be required to prepare several direct conjugates, their commercial availability for specific leukocyte Ags from humans and mice has made them the favored class of reagents. For many purposes, direct reagents provide good magnetic labeling.

Two-step staining is advantageous when a one-step magnetic bead

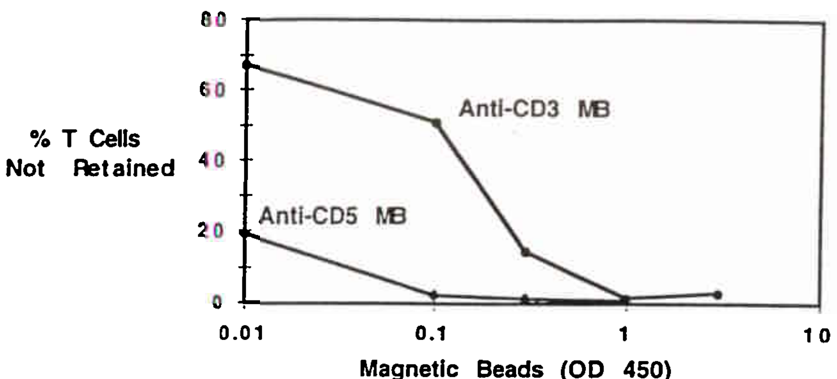


Figure 2 Titration of anti–T-cell reagents. Magnetic beads (MB) conjugated to either anti-CD3 or anti-CD5 mAbs were titrated using 10^7 peripheral blood mononuclear cells (PBMCs) at a concentration of 10^8 cells/mL. The beads have similar levels of antibody. The OD_{450} is a measure of the reagent concentration. The separation is monitored as the fraction of target cells not retained on the column.

reagent is unavailable, when multiple populations are targeted, and when the primary Ab does not work well directly conjugated to magnetic beads because of low affinity, difficulty with the coupling reaction, or steric hindrance. Four combinations of primary and secondary reagent pairs are in common use: unmodified primary Ab and anti-immunoglobulin–magnetic microbeads; primary tetrameric Ab complexes, consisting of an anti-dextran Ab coupled to an anti-target cell Ab, and unmodified iron-dextran microbeads (18); biotinylated primary Abs and avidin-magnetic beads; and hapten-conjugated (e.g., fluorescein or digoxigenin) primary Ab and anti-hapten magnetic microbeads.

Monitoring the Cell Separation

Magnetically labeled cells can be labeled with fluorescent Abs and analyzed by flow cytometry and/or microscopy. The small magnetic particles do not interfere with the light scattering or fluorescence properties of the cells. Analysis of the original and resultant fractions allows the separation process to be readily analyzed for yield, purity, and viability of the target cells. Since the magnetic bead reagents are usually used far below saturating concentrations, even fluorescent recognition of the same epitope can be used. Specific contaminating populations of interest can also be enumerated.

III. EXAMPLES OF MAGNETIC-CELL SEPARATIONS

The following examples focus on recent applications of MACS: multiparameter sorting, sorting based on an intracellular Ag, and instrument-mediated magnetic purification for potential therapeutic purposes.

A. Multiparameter Magnetic Cell Sorting

Negative Selection Followed by Positive Selection

There is no commonly accepted surface Ag to define human peripheral blood dendritic cells (19,20). However, a combination of positive and negative selection strategies can be used to isolate the small (0.5%) population of this cell type (Fig. 3). Peripheral blood dendritic cells express CD4 (21), which is also present on a subset of T cells, monocytes, and natural killer (NK) cells. These nondendritic cells are first removed from the blood peripheral blood mononuclear cells (PBMCs) by magnetic depletion using a two-step labeling process with hapten-conjugated anti-CD3, CD11b, and CD16 as the primary cocktail, followed by anti-hapten Ab conjugated to microbeads as the secondary reagent. The cells are depleted on a wire mesh column to enrich the dendritic cells to approximtely 16% of the resultant population. $CD4^+$ blood dendritic cells are then directly labeled using anti-CD4–conjugated microbeads and enriched using a iron-sphere column. Final dendritic cell purity of > 95% and yield of approximately 70% can be achieved with this approach.

Nucleated red blood cells from maternal blood are a potential source of fetal cells for the diagnosis of genetic disease (22). This population, which contains both maternal-derived and fetal-derived cells, is present at about 0.1% of peripheral blood nucleated cells. The cells can be enriched with a combination of negative and positive selection (23). An example is shown in Figure 4. The majority of leukocytes are depleted on a wire-mesh column using two-step magnetic staining with biotin–anti-CD45 as the primary reagent and avidin microbeads as the secondary reagent. The target cells, as identified by staining with anti-CD71 and glycophorin A, are enriched to 10–20% purity in this step with a yield of 50–70%. The $CD45^-$ cells are then labeled with anti-CD71 magnetic beads and the nucleated red blood cells further enriched on an iron sphere column. The resultant cells are typically 70–90% pure.

For double separations, it is important that the cells with the magnetic label are efficiently retained on the first column. Any labeled cells that pass through may be captured on the second column and contaminate the final population. This potential problem is minimized by using a wire matrix

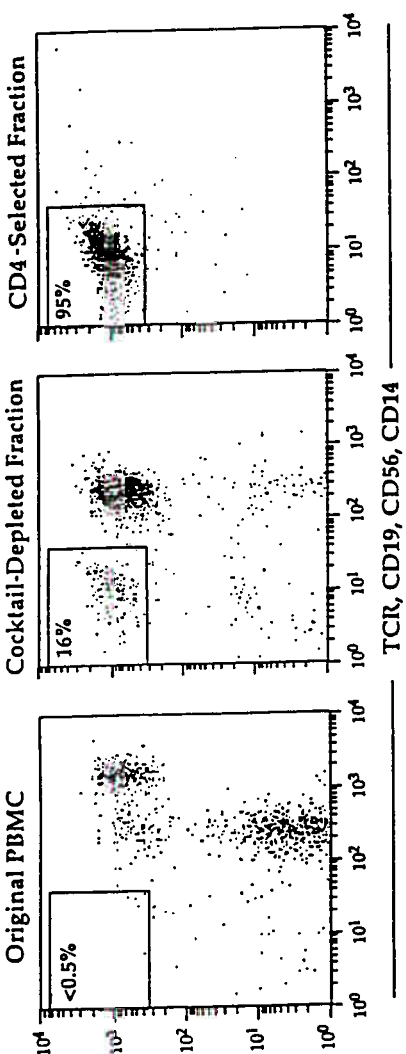


Figure 3 Enrichment of human peripheral blood dendritic cells. Dendritic cells were isolated from peripheral blood mononuclear cells (PBMCs) by depleting T cells, monocytes, and natural killer (NK) cells using a cocktail of hapten-conjugated anti-CD3, CD11b, and CD16 antibodies (Abs) followed by antihapten microbeads and a wire array column. Dendritic cells then were further enriched using anti-CD4 microbeads and an iron-sphere column. Fluorescence-activated cell sorting (FACS) analysis shows dendritic cells, which express the HLA class II DR molecule [but not markers for T cells (TCR), B cells (CD19), NK cells (CD56), or monocytes (CD14)] comprised < 0.5% of starting population. The depletion step increased the frequency to 16% and the positive selection further increased the purity to 95%.

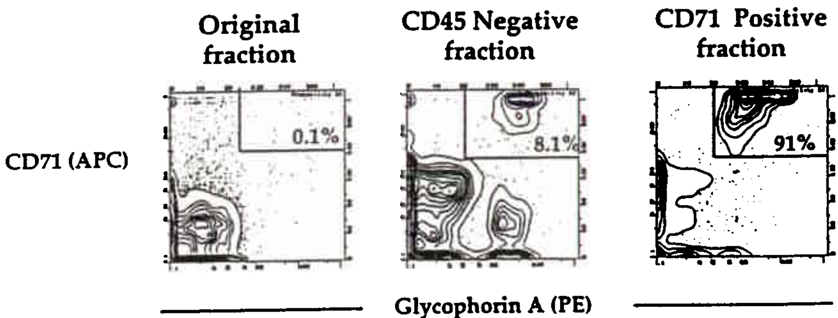


Figure 4 Isolation of human peripheral blood nucleated red blood cells. Leukocytes were removed using biotin anti-CD45, streptavidin microbeads, and a wire column. The $CD45^-$ fraction was then labeled with anti-CD71 microbeads and enriched on an iron-sphere column.

column, designed to have a stronger magnetic field gradient, for the initial depletion step and an iron-sphere matrix for the subsequent positive selection.

Double-Positive Selection

Human B-1 cells express low levels of the "pan T cell" Ag CD5 and represent approximately 2% of all PBMCs (24). They can be isolated by two magnetic separation strategies: T-cell depletion followed by anti-CD5 positive selection or sequential positive selection with anti-CD19 and CD5 using bead-release technology. Data from the double-positive method are shown in Figure 5. B cells are first labeled with anti-CD19 microbeads and enriched to > 99% purity with an iron-sphere column. The magnetic microbeads then are released from the enriched B cells using a biochemical reaction. Since CD5 is expressed at low levels on B cells, the cells are labeled with a two-step procedure, hapten-conjugated anti-CD5 primary reagent and anti-hapten Ab conjugated to magnetic beads as the secondary reagent. The B-1 cells are positively selected on a second column to a purity of > 90%. The contaminating cells are mostly $CD5^-$ B cells. This is a result of incomplete release of the first-step magnetic beads. It is often possible to remove cells with residual magnetic beads by passing the released cells over a magnetic column before labeling with the enrichment reagents.

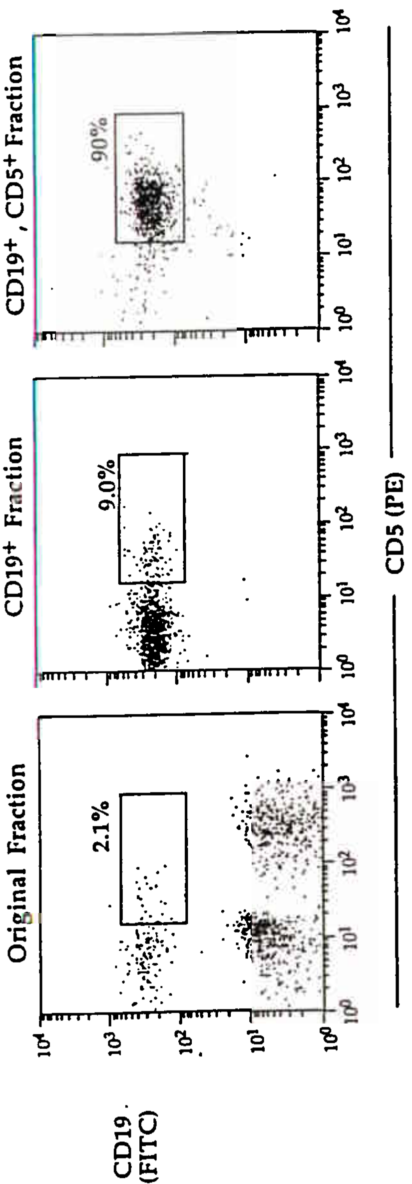


Figure 5 Isolation of peripheral blood B-1 cells. CD19⁺, CD5⁺ cells were isolated from peripheral blood mononuclear cells (PBMCs). Before separation, fluorescence-activated cell sorting (FACS) analyses showed that 2.1% of PBMCs were CD19⁺ and CD5⁺. PBMCs were labeled with anti-CD19 microbeads and the B-1 cells enriched to 9% purity using an iron-sphere selection column. The beads were released enzymatically, the cells magnetically labeled with fluorescein isothiocyanate (FITC)-anti-CD5 and anti-FITC microbeads, and CD19⁺, CD5⁺ cells purified to > 90% on a second column.

B. Positive Selection of Epithelial Tumor Cells Based on an Intracellular Marker

Detection and quantitation of epithelial tumor cells that have metastasized to the peripheral blood or marrow are potentially important for staging disease and evaluating cell preparations for autologous transplantation after myeloablative chemotherapy. The cytokeratins are well accepted as the most specific marker for epithelial cells, with essentially no background expression on blood leukocytes or erythrocytes (25). This is essential since the target cells are present at extremely low levels ($< 10^{-6}$) even in patients with late-stage disease. Permeabilization of the cells allows anticytokeratin magnetic beads to bind the Ag residing just below the plasma membrane. Griwatz et al. (26) have used preformed mouse anticytokeratin/rat-antimouse immunoglobulin magnetic beads and Siewert and coworkers (27,28) and Qian et al. (29) have used direct-conjugate anticytokeratin magnetic beads.

Briefly, blood samples are permeabilized with a mild detergent in a procedure that also lyses the red blood cells, fixed, reacted with the magnetic bead reagent, washed, and separated on iron-sphere columns. In contrast to separations based on cell-surface Ags, the cells are not viable after the permeabilization and fixation procedure. However, they are suitable for enumeration by flow cytometry, immunocytochemistry, and polymerase chain reaction (PCR).

An example is shown in Figure 6. T47D epithelial tumor cells were spiked into buffy coat preparations at 1000 cells/10^8 leukocytes. No tumor cells were detected by flow cytometry in the original fraction. After enrichment, a distinct cytokeratin positive, $CD45^-$ population is observed. The cells are enriched with a purity of 77% and a yield of 56%. Staining with anti-epithelial surface Ag-Ab confirms that these cells are epithelial.

C. Clinical Applications

The large-scale isolation of sterile, viable, clonogenic hematopoietic cells from human leukapheresis and bone marrow harvests is becoming increasingly important for therapeutic transplantation and gene therapy. Clinical-grade MACS are facilitated by the computer-controlled Cell Selector developed and manufactured by AmCell Corporation and introduced in clinical trials for autologous CD34 transplantation by Amgen Inc. (Thousand Oaks, CA).

The instrument and tubing set are shown in Figure 7. Bags containing magnetically labeled cells and buffer are connected to the separation column by sterile tubing. Two iron-sphere matrix columns are used. The first acts as a

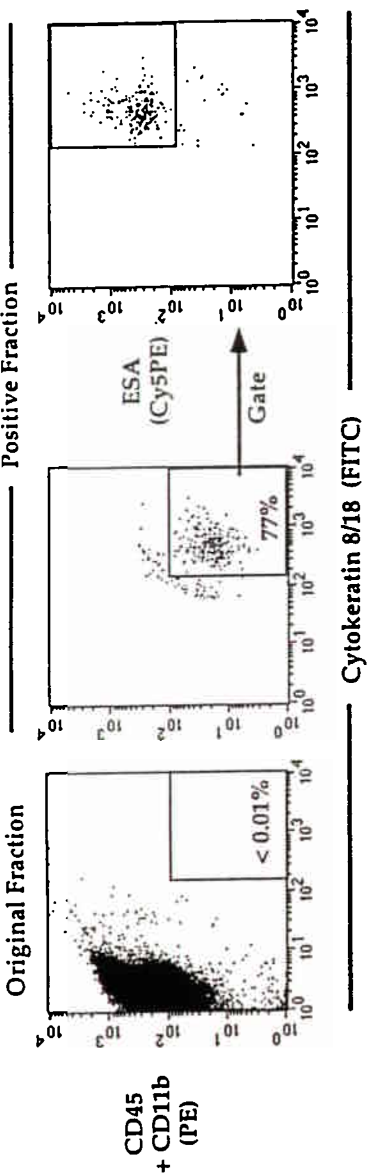


Figure 6 Isolation of epithelial tumor cells from peripheral blood. T47D epithelial tumor cells were spiked into peripheral blood mononuclear cells (PBMCs) at 1000 cells/10^8. Cells were permeabilized, fixed, and then incubated with anticytokeratin 8/18 magnetic beads. The magnetically enriched cells were analyzed by fluorescence-activated cell sorting (FACS) for cytokeratin, the epithelial marker ESA, and CD45 + CD11b.

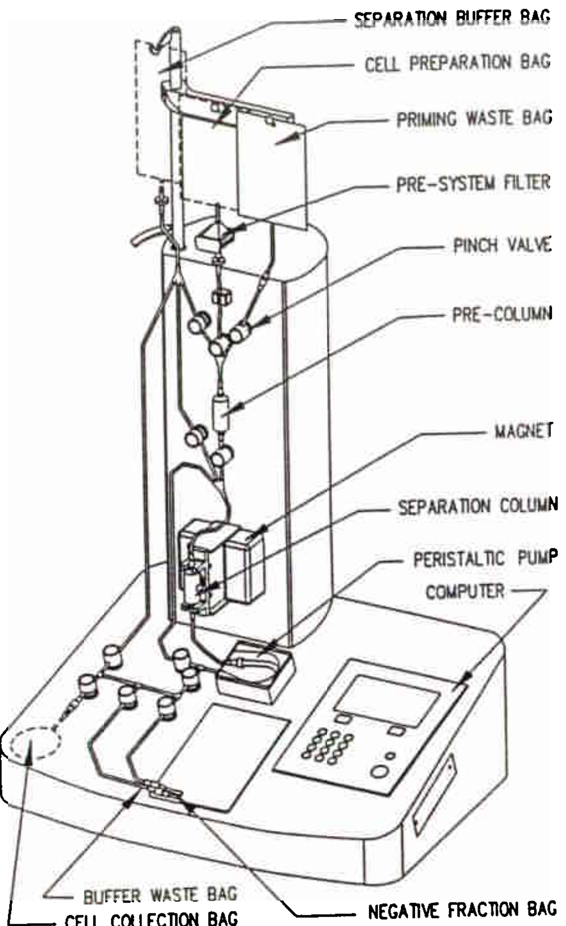


Figure 7 The Cell Selector instrument. The instrument is shown with the tubing set, including the iron-sphere ferromagnetic columns installed. Key features are identified in the diagram.

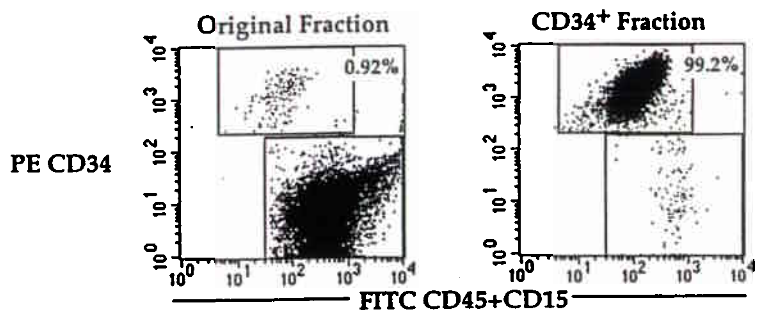
prefilter to remove nonspecific "sticky" cells, and the second is for magnetic retention of selected cells. The computer controls the placement of the column in the magnetic field and fluid flow through a pump and a series of valves. Each step in the cell-separation process is automated: cell deposition, washing, elution, redeposition, washing, and elution of the final product into a sterile collection bag. Two depositions on the magnetic separation column typically are used to improve cell purity.

The $CD34^+$ cell population contains progenitor cells required for hematopoietic reconstitution after myeloablation during high-dose cytotoxic chemotherapy. A number of groups have reported success using MACS with microbeads to purify $CD34^+$ cells (9,30–33), including some with the automated Cell Selector (34,35).

Figure 8A shows a sample FACS analysis of the enrichment of CD34 cells with the Cell Selector. The sample is a leukapheresis harvest from a donor who has been stimulated with Filgrastim (r-metHuG-CSF) to mobilize progenitor cells into the peripheral circulation. The preparations typically have $> 10^{10}$ leukocytes and a relatively high proportion of CD34 cells—typically 1%. Cells were labeled in the collection bag with a direct conjugate, anti-CD34 magnetic bead reagent, then washed in-bag by centrifugation, and attached to the tubing set. The resulting CD34 cells were 99% pure. Table 2 summarizes the results from 10 separations. Average purity and yield were 95 and 92%, respectively.

For autologous or allogenic transplantation, it is usually necessary to monitor the removal of specific contaminating populations, such as cancer cells or T cells. This removal is usually discussed in terms of log depletion. For example, a log depletion of 3 corresponds to a 1000-fold reduction in the number of a specific population. The value depends on the number of cells in the final fraction, the original fraction, and the nonspecific retention of the cell type in the Cell Selector. Table 2 includes log-depletion data from the CD34 cell isolations from the stimulated leukapheresis samples. T cells are depleted the very effectively, by an average of 4.5 logs. B cells and monocytes are removed by about 4 logs. These results suggest that cancer cells can be effectively removed from patient leukapheresis harvests, assuming they behave similarly to T cells.

T cells have been isolated with the Cell Selector. In an experiment designed to test the capacity and versatility of the system, T cells were isolated from a leukapheresis harvest with anti-CD5 direct-conjugate magnetic microbeads (Fig. 8B). The sample is from an unstimulated donor and contained 55% $CD3^+$ T cells among all the nucleated cells. A total of 2.4×10^9 T cells were retained on the column and isolated with excellent purity (99.6%) and good yield (78%).



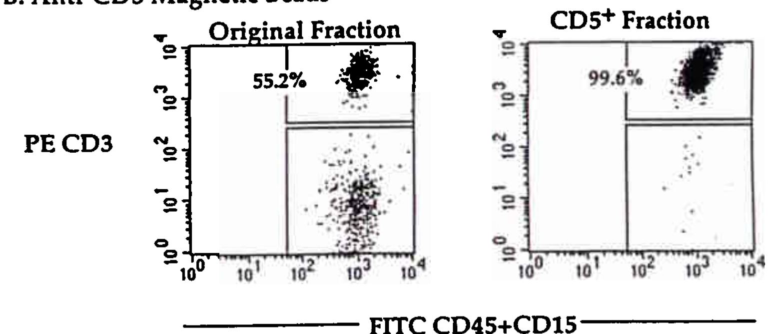


Figure 8 Clinical scale isolation of hematopoietic cells from leukapheresis harvests. (A) $CD34^+$ cells were isolated using a direct anti-CD34 microbead reagent and the Cell Selector. Fluorescence-activated cell sorting (FACS) analysis indicates the $CD34^+$ cells are enriched to high purity and yield. (B) T cells were enriched with anti-CD5 magnetic microbeads. A total of 2.4×10^9 T cells were enriched to high purity. The yield was only 78% because limitations in the column capacity were reached.

IV. SUMMARY

The applications of MACS using colloidal superparamagnetic particles are growing as both an exclusive cell-separation step or as a complement to other isolation protocols such as FACS. The most exciting areas include the isolation of human cells for therapeutic transplantation, such as with CD34 progenitor cells, and potential uses for clinical diagnosis such as with breast cancer cells.

Table 2 Therapeutic Scale Isolation of CD34 Progenitor Cells from Leukapheresis Harvest of Filgrastim-Stimulated Patients

	CD34 cells[a]			Log Depletion		
	Original	Purity	Yield	CD3 T cells	CD14 monocytes	CD20 B cells
Average	0.8%	95%	92%	4.5	3.9	4.1
SD	0.3	4	5	0.2	0.2	0.3
Range	0.4–1.3%	88–99%	84–99%	4.1–4.7	3.4–4.4	3.6–4.7

[a] $n = 10$ for CD34 cells data and 5 for log depletion data.

Future developments in MACS technology will almost certainly increase its utility in both the clinic and the research laboratory.

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REFERENCES

1. Kantor AB, Roederer M. FACS analysis of leukocytes. In: *Handbook of Experimental Immunology*, 5th ed., Edinburgh: Blackwell Scientific, 1996, 49.1–49.13.
2. Owen CS, Lindsay JG. Ferritin as a label for high-gradient magnetic separation. *Biophys J* 1983; 42:145–150.
3. Owen CS, Sykes NL. Magnetic labeling and cell sorting. *J Immunol Methods* 1984; 73:41–48.
4. Poynton CH, Dicke KA, Culbert S, Frankel LS, Jagannath S, Reading CL. Immunomagnetic removal of CALLA positive cells from human bone marrow. *Lancet* 1983; 1:524.
5. Molday RS, Yen SP, Rembaum A. Application of magnetic microspheres in labelling and separation of cells. *Nature* 1977; 268:437–438.
6. Molday RS, MacKenzie D. Immunospecific ferromagnetic iron-dextran reagents

for the labeling and magnetic separation of cells. *J Immunol Methods* 1982; 52:353–367.
7. Molday RS, Molday LL. Separation of cells labeled with immunospecific iron dextran microspheres using high gradient magnetic chromatography. *FEBS Lett* 1984; 170:232–238.
8. Miltenyi S, Muller W, Weichel W, Radbruch A. High gradient magnetic cell separation with MACS. *Cytometry* 1990; 11:231–238.
9. Kato K, Radbruch A. Isolation and characterization of CD34+ hematopoietic stem cells from human peripheral blood by high-gradient magnetic cell sorting. *Cytometry* 1993; 14:384–392.
10. Paul F, Roath S, Melville D. Differential blood cell separation using a high gradient magnetic field. *Br J Haematol* 1978; 38:273–280.
11. Melville D, Paul F, Roath S. Direct magnetic separation of red cells from whole blood. *Nature* 1975; 255:706–708.
12. Kolm HH. The large-scale manipulation of small particles. *IEEE Trans Magnet* 1975; 11:1567.
13. Oder RR. High gradient magnetic separation theory and applications. *IEEE Trans Magnet* 1976; 12:428.
14. Thomas TE, Lansdorp PM. Selective separation of cells using magnetic colloids. *Prog Clin Biol Res* 1994; 389:65–77.
15. Schmitz J, Thiel A, Kuhn R, et al. Induction of interleukin 4 (IL-4) expression in T helper (Th) cells is not dependent on IL-4 from non-Th cells. *J Exp Med* 1994; 179:1349–1353.
16. Groden BM, Whitelaw J, Will G. The treatment of iron-deficiency anemia by iron dextran infusion, with special reference to the effect on blood-grouping, coagulation, sedimentation and haemolysis. *Postgrad Med J* 1968; 44:433.
17. Slade IJ, Iosefa RN. Intravenous iron-dextran (Imferon) in the treatment of iron deficiency anemia. *J Med Assoc Georgia* 1968; 57:137–139.
18. Thomas TE, Abraham SJ, Otter AJ, Blackmore EW, Lansdorp PM. High gradient magnetic separation of cells on the basis of expression levels of cell surface antigens. *J Immunol Methods* 1992; 154:245–252.
19. Thomas R, Davis LS, Lipsky PE. Isolation and characterization of human peripheral blood dendritic cells. *J Immunol* 1993; 150:821.
20. Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* 1991; 9:271–296.
21. O'Doherty U, Steinman RM, Peng M, et al. Dendritic cells freshly isolated from human blood express CD4 and mature into typical immunostimulatory dendritic cells after culture in monocyte-conditioned medium. *J Exp Med* 1993; 178:1067–1076.
22. Bianchi DW, Flint AF, Pizzimenti MF, Knoll JH, Latt SA. Isolation of fetal DNA from nucleated erythrocytes in maternal blood. *Proc Natl Acad Sci USA* 1990; 87:3279–3283.
23. Busch J, Huber P, Pfluger E, Miltenyi S, Holtz J, Radbruch A. Enrichment of fetal

cells from maternal blood by high gradient magnetic cell sorting (double MACS) for PCR-based genetic analysis. *Prenat Diagn* 1994; 14:1129–1140.

24. Bhat NM, Mithal A, Bieber MM, Herzenberg LA, Teng NN. Human CD5+ B lymphocytes (B-1 cells) decrease in peripheral blood during pregnancy. *J Reprod Immunol* 1995; 28:53–60.
25. Pantel K, Felber E, Schlimok G. Detection and characterization of residual disease in breast cancer. *J Hematother* 1994; 3:315–322.
26. Griwatz C, Brandt B, Assmann G, Zanker KS. An immunological enrichment method for epithelial cells from peripheral blood. *J Immunol Methods* 1995; 183:251–265.
27. Siewert C, Martin VM, Miltenyi S, Schmitz J. Enrichment of micrometastic carcinoma cells from hematopoietic cell samples by high-gradient magnetic cell sorting (MACS). *Mulhouse Manual* 1996.
28. Martin VM, Siewert C, Miltenyi S, Schmitz J. Enrichment of epithelial tumor cells from peripheral blood of breast and prostate carcinoma patients by high gradient magnetic cell sorting (MACS). ISHAGE Conference, 1996.
29. Qian F, Kantor AB, Gibbons I, Chen W, Recktenwald D. A sensitive carcinoma cell detection assay. ISHAGE Conference, 1996.
30. Martin-Henao GA, Ingles-Estevee J, Cancelas JA, Garcia J. Isolation of CD34+ hematopoietic progenitor cells in chronic myeloid leukemia by magnetic cell sorting (MACS). *Bone Marrow Transplant* 1996; 18:603–609.
31. Dinicola M, Siena S, Corradini P, et al. Elimination of follicular non-Hodgkin-lymphoma (F-NHL) cells and high recovery of $CD34^+$ cells from blood-cell transplants by the Miltenyi cell sorting system. *J Exp Hematol* 1996; 24:696.
32. Briddell R, Zilm K, Kern B, et al. Depletion of T-lymphocyte subsets in peripheral blood apheresis harvests from normal donors by purification of cells expressing the CD34 antigen. *Blood* 1995; 86:918.
33. Guth S, Koster M, Thiel A, et al. Immunomagnetic isolation of subpopulations of CD34+ human hematopoetic progenitor cells using releasable superparamagnetic microbeads. *Exp Hematol* 1995; 23:812.
34. McNiece I, Briddell R, Stoney G, Kern B, Recktenwald D, Miltenyi S. CD34 cell isolation using the Amgen Cell Selection Device. *Exp Hematol* 1996; 24:95.
35. McNiece I, Briddell R, Recktenwald D, et al. Large scale isolation of CD34 expressing cells using the Amgen Cell-Separation Device results in high levels of purity and recovery. *Exp Hematol* 1995; 85.