

Novel methods of cell separation using molecular and nanometer-scale magnetic reagents

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We have developed a number of magnetic cell analytical and preparative techniques based on molecular and nanometer-scale magnetic particles which provide the necessary resolving power and throughput for detection and separation of cells in large volumes. The advantages of using soluble magnetic reagents are fast reaction kinetics and sensitivity to the level of expression of cell surface antigen. At various stages of the project, we have tested lanthanide ions (erbium), ferritin and a reconstituted ferritin (magnetoferritin), and commercial ferrofluids (iron dextran colloids) as the soluble magnetic label.

The challenge associated with using submicrometer magnetic labels is the low magnetic moment, even at high fields. This requires novel designs of the magnetic analytical and preparative instruments. In particular, we focused on continuously sorting devices, in which the global sorting parameters, such as the resolving power and throughput, depend on the local equilibrium of the magnetic and viscous forces acting on the cell. This required a higher level of engineering sophistication than in the design of the batch-wise high-gradient magnetic separator (HGMS) columns. The proof-of-principle experiments were conducted on human peripheral lymphocytes, bone marrow, cord blood, a mononuclear cell fraction from patients mobilized for apheresis, and culture cell lines.

The well-controlled conditions of the magnetic cell sorting in a flow enabled us to separate cells based on the cell surface expression level. This is not possible with the current, commercial HGMS columns. Three types of the magnetic flow separation instruments were developed: the dipole flow sorter, the quadrupole flow sorter, and an analytical cell deposition apparatus. The dipole flow fractionator separated cells into ten fractions, from the least magnetic (low antigen expression) to the most magnetic (high antigen expression). The quadrupole flow sorter has the potential for sorting speed (throughput) of 10^7 cells per second, from the initial fractional target cell concentration (purity) of 0.5% to final purity of 80%, at the recovery of the target cells of 80%. For detection of rare cells, a unique technique of depositing the magnetically-labeled cells directly on the microscope slide was developed. It provided convenient means for rapidly scanning the cell sample for cells of unusual phenotype, morphology, and genetic makeup. In a model cell mixture of peripheral lymphocytes and MCF-7 breast carcinoma cell line, we were able to selectively deposit and enrich 100,000-fold the MCF-7 cells from 1 to 1,000,000 mixtures with the lymphocytes.

The molecular and nanometer-scale magnetic probes may play a similar role in magnetic flow sorting to that of the fluorescent reagents in fluorescence-activated cell sorting (FACS) techniques. In addition, the magnetic probes provide a means of cell sorting based on antigen expression level using simpler equipment than the FACS instruments. They may provide means for rapid, high resolution cell separation for analytical and preparative applications.