

## CELL LABELING WITH MAGNETIC AND NON MAGNETIC IMMUNOMICROSPHERES FOR SEPARATION AND DIAGNOSIS

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**Abstract** -The investigations of labeled cells with small polymeric spherical particles carrying antibodies on the surface yielded information on membrane receptors by Scanning electron microscopy and led to the identification and separation of cell subpopulations. The separation of various types of cells could be achieved by altering the electrophoretic mobility or by application of magnetic microspheres. The latter were also used to eliminate neuroblastoma cells from human bone marrow. There is good evidence that the magnetic method constitutes a major step toward a successful therapy of some forms of cancer.

During the last ten years chemical techniques have been developed to label specific groups of cells with polymeric reagents which have antibodies bound to their surface. Suitable polymeric microspheres for cell labeling, cell separation etc. could be synthesized by various polymerization methods e.g. emulsion, radiation, aldol condensation and radiation grafting. Hydrophilic and hydrophobic monomers were used to prepare spheres of different composition and size with or without functional groups. The latter were used to bind antibodies covalently to the surface of the spheres. Synthetic polymeric microspheres coupled with specific antibodies form reagents known as immunomicrospheres. Immunomicrospheres containing magnetite were recently successfully used to remove neuroblastoma cells from patients bone marrow.

Synthesis of immunomicrospheres. In order to obtain the required properties which vary according to the desired applications, immunomicrospheres were synthesized by different methods. Two classical methods of colloid and surface chemistry i.e. emulsion and ionizing radiation polymerization were utilized to produce polystyrene, poly 2-hydroxyethyl methacrylate, polyvinyl pyridine, polyvinyl toluene, polyacrylamide or copolymeric microspheres varying in hydrophilicity, density, porosity or size (1). Hydrophilic and crosslinked (and therefore insoluble) functional microspheres ranging in diameter from approximately 0.01 to 8 $\mu$ m can be conveniently synthesized by cobalt gamma radiation polymerization (2, 6). The main advantage of the ionizing radiation technique is that relatively pure microspheres can be obtained since the synthesis is generally achieved in absence of a free radical initiator or emulsifier. The particles are preferably formed in presence of small amounts of polyethylene oxide (PEO, 0.2 to 0.4 wt%), which acts as a steric stabilizer and is probably grafted to a small extent onto the spheres under the influence of radiation. It was possible to copolymerize a variety of water-soluble monomers with 2-hydroxyethyl methacrylate, a known biocompatible monomer used in the manufacture of contact lenses (2-4).

Copolymerization with methacrylic acid and acrylamide yielded carboxyl and amide functional groups that can be bonded to antibodies by means of carbodiimide or glutaraldehyde methods (5). Copolymerization with isomeric vinylpyridines or 2-dimethylaminoethylmethacrylate yielded weakly or strongly basic groups, respectively, capable of binding with acids. In order to start from a homogeneous system a water-soluble crosslinking agent was used. By choosing suitable comonomers, it was possible to incorporate hydroxyl, carboxyl, amido, and dimethylamino functional groups into the particles. For a number of applications it was desirable to tag the particles with fluorescent dyes. To ensure permanent attachment of the fluorescent molecules to the latex spheres, we have designed fluorescent monomers and copolymerized them with nonfluorescent acrylic monomers (2). The fluorescent tagging and the introduction of other functional groups could also be achieved by reacting the polymeric microspheres first with diamino alkanes through the cyanogen bromide procedure and subsequently with fluorescent compounds. When covalently bound to antibodies or lectins, these reagents were successfully used to label murine and human (cells). In order to

produce immunomicrospheres in which the antibody is bound covalently, a functional group on the surface of the microsphere must be available. Hydroxyl, carboxyl or aldehyde functionality is particularly desirable. The covalent bond ensures that no leakage of antibody will occur during reactions of immunomicrospheres with antigens on cell membranes or elsewhere. However it was recently discovered that high surface area hydrophobic spheres may adsorb antibodies with sufficient binding force to be successfully applied to a variety of purposes. Of particular interest are polyacrolein microspheres (6) and polyacrolein on the surface of commercial latices (7). Both types react with antibodies by means of simple procedures.

#### Applications

- (1) Radioactivity: If immunomicrospheres are tagged with a radioactive substance, the technique permits specific cells to be qualitatively and quantitatively identified through their assumed radioactivity. If the specific cells to which the radioactive immunomicrospheres become attached are malignant then the attached radioactivity could serve to selectively kill the malignant cells while leaving nearby healthy cells alone.
- (2) Fluorescence: If immunomicrospheres are labeled with a fluorescent substance, the procedure permits specific cells to be qualitatively and quantitatively identified, and to be separated from other cells by their flowing through a fluorescence-activated cell-sorting instrument.
- (3) Electrophoresis: Immunomicrospheres can be prepared that carry an electrical charge on their surface. If such electrically-charged immunomicrospheres are specifically attached to human red blood cells, the mobility of such labeled cells in an electric field changes compared to the mobility of untreated human red blood cells. This alteration can be used in subtle cell separations. It turns out that turkey red cells have the same electrical mobility as do human red blood cells. Thus, a mixture of human and turkey red blood cells, cannot be separated by the application of an electrical field. But, if electrically charged-immunomicrospheres that attach specifically to human red blood cells are added to a mixture of human and turkey red blood cells and an electric field is applied, the labeled, slower-moving human red blood cells can easily be separated from the turkey cells. This experiment is illustrative of a general cell-separation technique (8).
- (4) Electron opacity: If immunomicrospheres contain an electron-opaque metal, such as gold or platinum, the technique permits better spatial resolution of cell surfaces as viewed with the electron microscope.
- (5) Drug delivery: If immunomicrospheres are filled with a drug that can alter or destroy the specific cells to which the immunomicrospheres attach themselves, the technique permits the treatment or destruction of the specific cells. Thus, in their selective destruction of cancer cells, immunomicrospheres can be thought of as "smart missiles" in which the antibody-portion is the guided missile and the drug-filled microsphere is the explosive warhead. In research with isolated cultures of cancer cells, where no guiding antibody is needed, polyglutaraldehyde microspheres to which adriamycin is attached were shown to destroy the cancer cells. Adriamycin is an antibiotic that also is a potent anti-tumor agent.
- (6) Magnetism: If immunomicrospheres contain magnetic iron oxide ( $\text{Fe}_3\text{O}_4$ ), then a magnetic field can be used to separate the labeled cells from all others. This has already important uses in cancer therapy. The first therapy by means of magnetic immunomicrospheres of a cancer known as neuroblastoma was carried out successfully on a patient in April 1983 in London (England). By January 1984 twenty-five cancer patients were treated with most encouraging results. The procedure used was as follows:  
A portion of the bone marrow containing 0.3 to 3% of cancer cells removed from the patient was treated with magnetic immunomicrospheres ( $3\mu\text{m}$  O.D. microspheres coated with an antibody). The immunomicrospheres attached themselves specifically to neuroblastoma cells leaving normal cells free. A flow system was then employed using permanent magnets and an electromagnet to effect the rapid and efficient removal of magnetic tumor cells from bone marrow. Before reinfusion of "cleansed bone marrow" the patients received lethal chemotherapy and total body radiation (10).  
In view of results observed so far one may conclude that the above described magnetic method may well constitute a major step towards the successful therapy of cancer. Furthermore the applications described here may be extended to other areas of biology as well as microbiology, agriculture, veterinary medicine and to industrial uses e.g. to immobilized enzymes. In addition it seems fairly obvious that future investigations of immunomicrospheres will produce dramatic improvements in diagnosis and therapy.

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