Magnetic Separation News, 1984, Vol. 1, pp. 103–109
0731-3632/84/0102-0103 $25.00/0

Printed in the United Kingdom

THE USE OF MAGNETIC MICROSPHERES FOR THE REMOVAL OF TUMOUR CELLS FROM BONE MARROW

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Abstract A technique for the removal of tumour cells from bone marrow is described. This involves rendering the tumour cells 'magnetic' by attaching them to magnetite-containing microspheres by a double layer of antibody. Bone marrow containing these 'magnetic' tumour cells is then passed through a system of magnets so that the tumour cells are drawn out of the bone marrow. Treated bone marrow may then be reinfused into the patient from which it was taken.

Many malignant diseases initially respond well to chemotherapy and/or radiotherapy, but may subsequently recur. When treated by further drug therapy in conventional doses they may become resistant, thus making a cure for the disease very unlikely. It is therefore desirable in some cases of relapsed disease to use very high dose chemotherapy combined with radiotherapy to bring about tumour regression, but the agents used will affect not only the tumour cells present in the body, but also normal dividing cells, in particular bone marrow stem cells. Over the last few years autologous bone marrow transplantation following high dose chemotherapy and/or radiotherapy has become an accepted form of treatment for patients suffering from some malignant diseases[1-3]. This mode of therapy removes the very chemo- and radiosensitive bone marrow stem cells from the body while therapy is being given,

thus protecting them, and they may be reinfused into the patient once therapy is completed. The stem cells then reengraft in the bone marrow compartment. Because they are the patient's own cells, the problems associated with receiving bone marrow from a donor (allogenic transplantation), such as graft versus host disease, are avoided.

This form of therapy is, however, limited by the fact that bone marrow cells removed for autologous transplantation may be contaminated with tumour cells. If this is the case, retransfusion of the bone marrow following therapy may lead to re-seeding and engraftment of the tumour cells along with the normal bone marrow cells. Several methods of removing tumour cells from bone marrow prior to reinfusion have been described. These include the use of monoclonal antibodies and complement to kill cells, the use of toxic substances such as ricin linked to monoclonal antibodies, or the use of the fluorescence-activated cell sorter[4-10]. We are developing a new technique for tumour cell removal, involving the use of monoclonal antibodies directed against the contaminating tumour cells. These are used to target magnetic microspheres to the surface of the malignant cells, and the treated marrow may then be passed over a series of magnets prior to reinfusion into the patient, to remove any 'magnetic' tumour cells present.

The bone marrow to be treated is collected into tissue culture medium and is heparinised to prevent clotting. We aim to treated approximately 3×10^8 cells per kilogram body weight of the patient, and for a child weighing 20 kilograms, this will normally result in about 400 mls of bone marrow diluted with peripheral blood being collected. The bone marrow and blood are then centrifuged, and the plasma and layer containing the nucleated cells (the buffy layer) is collected. The red cells may be discarded. A panel of monoclonal antibodies directed against the tumour from which the patient is suffering is added

to the buffy layer and plasma, and an incubation period of 30 minutes follows. The antibodies used are highly specific to the tumour cells and are selected so that they will not bind to normal bone marrow cells. Several antibodies are used to treat each bone marrow, so that if there is any variation in the antigens expressed on the tumour cell surface, it is likely that they will be recognised by at least some of the antibodies present.

When the bone marrow has been incubated with the monoclonal antibodies, it is washed to remove any free antibody and added to the microspheres which have been pre-coated with sheep anti-mouse immunoglobulin. Because the monoclonal antibodies against the tumour cells have been raised in mice, the sheep anti-mouse immunoglobulin coating the microspheres binds to the monoclonals. The tumour cells are therefore attached to the microspheres by a double layer of antibody (Figure 1). The magnetic microspheres used in this procedure are made of polystyrene and are monodisperse. They are exactly the same size, 3 microns in diameter, and have a pitted surface (Figure 2). They contain magnetite in a concentration of 20% weight for volume, to render them magnetic. The surface area of each bead is greatly increased by virtue of the fact that it is pitted; there are approximately 10^9 beads per milligram, and a gram of beads has a surface area of 100-150m^2. As polystyrene adsorbs protein, it is possible to couple an antibody onto the surface of the microspheres: 10 mg of beads will adsorb 700 µg of sheep anti-mouse immunoglobulin. This is accomplished by rotating the beads with the antibody for 12 hours. Once this has been done, the beads are washed to remove any unbound antibody.

The microspheres coated with the sheep anti-mouse antibody are incubated with the bone marrow containing the panel of monoclonal antibodies against the tumour cells for 2 hours, during which time they are rotated in the cold. After this period, the bone marrow containing the microspheres is passed slowly through

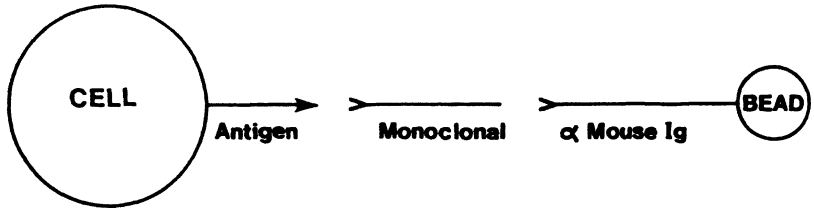

FIGURE 1 The tumour cell is attached to the microspheres by a double layer of antibody.

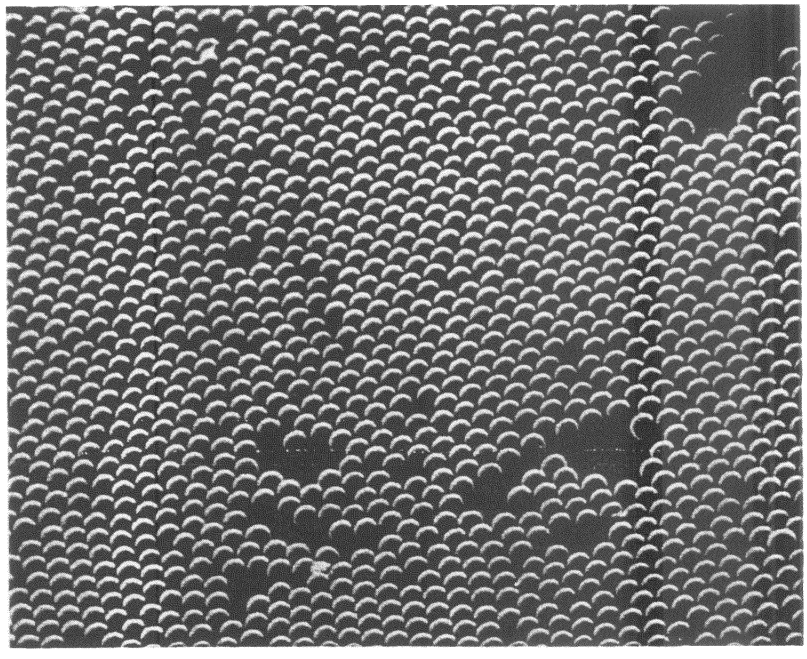

FIGURE 2 Electron micrograph showing the microspheres to be all the same size.

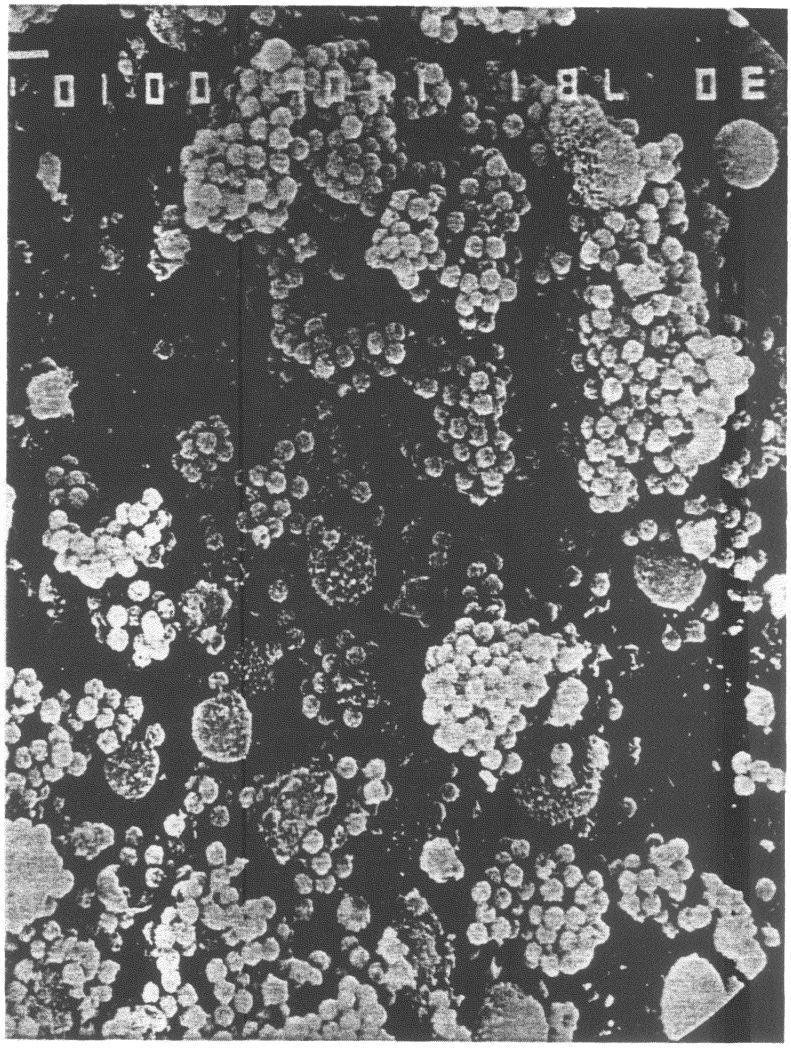

FIGURE 3 Scanning electron micrograph showing the beads attached to the tumor cells.

a series of chambers backed by samarium cobalt magnets. Any cells attached to the microspheres are drawn into the magnetic fields (Figure 3), while cells not coated with beads pass on to be collected, ready for retransfusion. After the bone marrow has passed through the chambers these are flushed through with saline to remove any normal cells which may have become trapped in the chambers.

The entire procedure is conducted under strict sterile conditions, and at each stage an assay is carried out designed to assess the viability of the normal bone marrow cells. Once the procedure is completed, the 'cleaned' marrow may be either retransfused into the patient immediately, or, if indicated, frozen and stored in liquid nitrogen.

To date we have used this method for 'cleaning' the bone marrow of patients suffering from neuroblastoma, a particularly malignant tumour of childhood. However, the technique could be used for removing any tumour cells against which monoclonal antibodies are available. We have shown that a 99.9% depletion of tumour cells from bone marrow is possible when 5×10^9 cells in total are present at the beginning of the procedure. We have not yet used the procedure on bone marrow heavily contaminated with tumour cells, but with a 2% infiltrate of tumour cells, this level of efficiency of tumour cell removal is obtained. Efficiency of the cleaning technique is checked after the procedure by incubating the treated bone marrow with anti-neuroblastoma monoclonal antibodies raised in mice. A further incubation of the marrow with fluorescene-labelled sheep anti-mouse immunoglobulin follows, after which any positive cells are clearly visible when the bone marrow is inspected under ultraviolet light.

While other methods for tumour cell removal are as effective as this, some, for example the fluorescence activated cell sorter, cannot deal with the large volume of material involved in treating

bone marrow cells in sufficient number to support a bone marrow graft. The method therefore has potential for the removal of many different types of tumour cells from bone marrow, and may have application as a general method for cell sorting.

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This work has been supported by SINTEF and the Imperial Cancer Research Fund.