Possible Application of Polyamine Graft Copolymer to Targeting Drug Delivery

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Polystyrene microspheres were coated with polyamine graft copolymer and mixed with rat lymphocyte suspension. In the mixture of T and B cells, the microspheres formed large aggregates, while they were fairly well dispersed in the T cell suspension. This result was understood to be the result of preferential adsorption of B cells to the microspheres, indicating it would be possible to deliver drugs to B cells alone with these copolymer-coated microspheres.

Keywords polyamine graft copolymer; cell separation; microsphere; targeting drug delivery; lymphocyte

In view of developing cell separators, Maruyama *et al.* have recently prepared novel biofunctional copolymers which could distinguish rat lymphocyte subpopulations from each other.[1] They coated glass beads with these copolymers and made a tiny column packed with the copolymer-coated beads. Rat lymphocyte suspension was then passed through this column, and it was found that more than 90% of B cells were trapped by this column while almost all T cells were eluted.[2] Although the exact mechanism of this separation is not yet fully understood, the fact that this copolymer can separate rat lymphocyte subpopulations indicates that it could be used for constructing a targeting drug delivery system, and this report describes the possible utilization in this way. Since the glass beads are too big for a model of a drug carrier, we prepared polystyrene microspheres and coated them with the copolymer.

When the copolymer-coated microspheres were added to the rat lymphocyte suspension in Hank's balanced salt solution (pH 7.0), considerably large aggregates were seen and some cells were found adsorbed to the microspheres (Fig. 2). It is difficult to say which cell is a B or T cell under microscopic observation, but the adsorbed cells are sure to be B cells. Next, after the lymphocyte suspension was passed through a copolymer-coated glass bead column, the microspheres were put into an eluted cell suspension, composed primarily of T cells. Few aggregates and adsorbed cells were in evidence (Fig. 3).

From these observations it is safe to say that the copolymer-coated microspheres preferentially adsorb B cells in a similar manner as the copolymer-coated glass beads. The exact mechanism underlying these interactions is not yet fully understood but an electrical interaction between the cells and the copolymer-coated beads is suggested to play a key role.[3,4] Aside from the mechanism, the results obtained are quite encouraging since, if some drugs are loaded in the microspheres (preferably smaller microspheres), it will be possible to deliver a drug to B cells alone.

To be able to lower the dosage and reduce the side effects of a drug, delivery of the drug to specific tissues is highly desirable and keenly awaited. The current ideas of targeting drug delivery are almost all based on utilizing such biological specificities as antigen–antibody, ligand–receptor, enzyme–substrate, *etc.* Although the data shown here are preliminary and qualitative and were found for rat lymphocytes *in vitro*, the fact that the synthetic copolymer

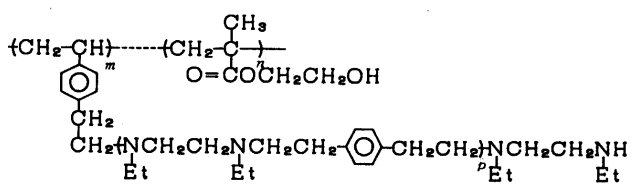


Fig. 1. Structural Formula of Polyamine Graft Copolymer

Polyamine macromers were copolymerized with 2-hydroxyethyl-methacrylate. *m* and *n* show the degree of polymerization, the exact numbers of which are not specified, but the polyamine macromer was found to hold 13% of the copolymer by nuclear magnetic resonance (NMR), electronspectroscopy for chemical analysis and elemental analysis[5] and *p* was 13.6 (average).

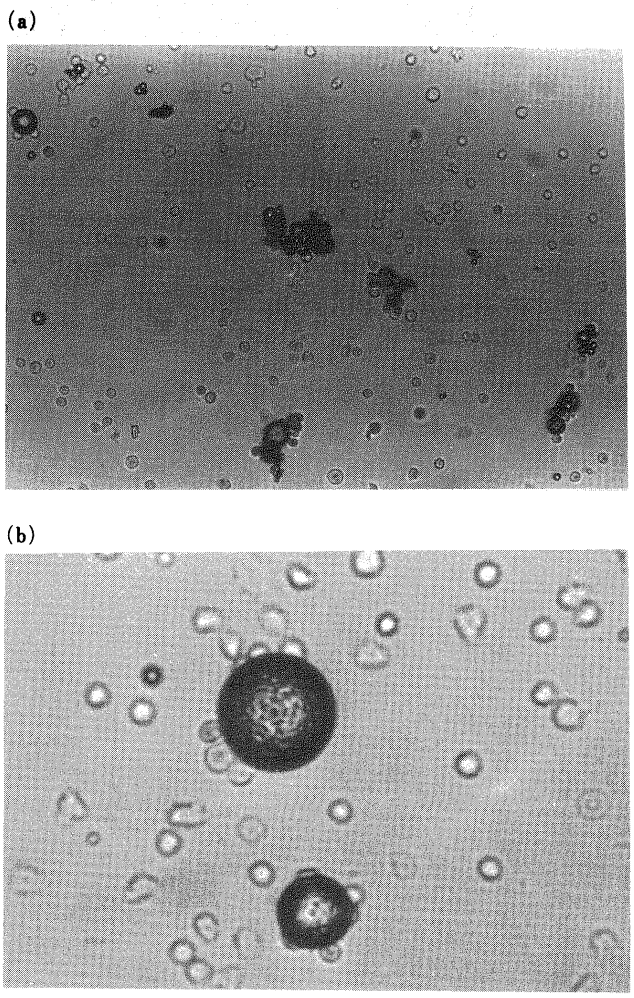
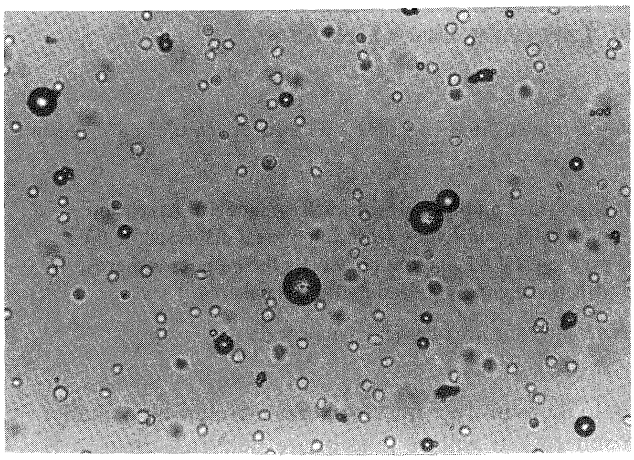


Fig. 2. Optical Microphotographs of the Mixture of Copolymer-Coated Polystyrene Microspheres and Rat Lymphocyte Suspension (T and B Cells)

(a) ×150, (b) ×600.

(a)



(b)

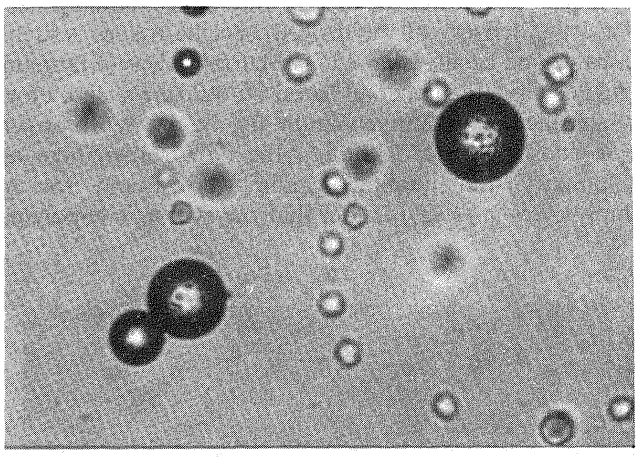


Fig. 3. Optical Microphotographs of the Mixture of Copolymer-Coated Polystyrene Microspheres and Rat Lymphocyte Suspension (T Cells Alone)
(a) ×150, (b) ×600.

is also capable of recognizing specific cell populations paves the way for constructing targeting media other than those of biological origin.

Experimental

One gram of polystyrene (Aldrich, molecular weight unspecified) was dissolved in 10 ml of methylene dichloride. This solution was emulsified in water with the aid of a mixture of 2% (w/v) polyvinyl alcohol and 1% (v/v) polyoxyethylene (20) sorbitan monolaurate, and stirring was continued until all methylene dichloride had evaporated. The resultant polystyrene microspheres were washed several times with ethanol on a centrifuge and finally dried *in vacuo*; their mean diameter was measured with a particle sizer (Malvern 3601) and found to be 37 μm. The polyamine graft copolymer (amine content was 13% and average molecular weight of the polyamine macromer was 6600), which was the kind gift of Dr. Y. Nabeshima of Science University of Tokyo, was dissolved into ethanol to give approximately 0.05% (w/v) solution. The structural formula of the copolymer used is illustrated in Fig. 1. To this solution was added 1 g of the polystyrene microspheres and the suspension was then filtered through a membrane filter. Microspheres thus obtained were dried *in vacuo*.

Rat lymphocyte suspension was obtained from mesenteric lymph nodes of a Wistar male rat (5 weeks old). The lymph nodes were squeezed between glass plates in Hank's balanced salt solution (pH 7.0) to give a lymphocyte suspension. Viability of the cells was measured by trypan blue exclusion method and was found more than 95%. Separation of T cells from the lymphocyte suspension was done using the copolymer coated glass bead column according to the method by Maruyama *et al.*[2] To the T cell suspension (1×10^7 cells/ml, counted by a Coulter Counter) or mixture (1×10^7 cells/ml) of T and B cells in Hank's balanced salt solution was added an appropriate amount of polyamine-coated polystyrene microspheres. The mixture was incubated for 15 min at room temperature and a small portion was observed under a microscope.

References

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