MOLECULAR DESIGN OF MATERIALS HAVING AN ABILITY
TO DIFFERENTIATE LYMPHOCYTE SUBPOPULATIONS

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INTRODUCTION

Development of effective methods for cell separation is one of the major objects in the field of biomedical science. Separation of blood cells has become increasingly practical and more widely used in research and in the clinical field. Especially, there is widespread utility of separating B and T cells, the two major subpopulations of lymphocytes. In the clinical field, their separation is important in therapy and diagnosis of immunodiseases as well as in assessing the matching of donor-recipient pairs in transplantation. In the field of fundamental and applied biology, preparative separation of lymphocyte subpopulations is essential process for producing high-value biological substances, including interferon, lymphokine, and mono-clonal antibodies.

Adhesion chromatography, one of the promising methods for cell separation, is a method which utilizes differences in the adhesive properties of the cells with matrix surfaces as a basis for their separation. Efficiency of adhesion chromatography is strongly dependent on the ability of matrix surfaces to differentiate cell populations in terms of adhesivity without undesirable perturbation or activation of separating cells. To develop effective column matrices, efforts should be made to clarify the effect of chemical and physical properties of materials on the behavior of adhering cells.

Although many studies have been carried out to describe the behavior of cells at interfaces based on results obtained by macroscopic observations, including wettability measurements and electrophoresis, a generalized theory which clearly explains the behavior
of adhering cells has not been established yet. The plasma membrane of cells has a highly heterogeneous or mosaic structure composed of different types of molecules. Changes in this mosaic structure caused by a change in molecular assemblage of these molecules will strongly affect the shape and function of adhering cells. This suggests that microscopic features of material surfaces should be taken into account for explaining the behavior of adhering cells and designing new matrices for adhesion chromatography.

In the course of our studies done to clarify the effect of surface charge of materials on cell adhesion, we have found that the shape and adhesivity of platelets were suppressed on microphase separated surfaces of polystyrene/polyamine comb-type copolymer (SA copolymer), suggesting that undesirable contact activation of platelets was effectively suppressed on polymer surfaces having appropriate microdomain structures. To explain this unique behavior of SA copolymer, we have presented a hypothesis of "capping control" in which the microphase separated structure of the copolymer was assumed to regulate the shapes and adhesivity of platelets through its effect on the redistribution of membrane components (proteins and/or lipids) of the platelets. Further, we have pointed out the feasibility of SA copolymer as a new column matrix for adhesion chromatography, because non-specific adhesion due to the contact activation could be effectively neglected on this copolymer surfaces.

This paper describes the utility of microphase separated SA copolymer as a new column matrix for adhesion chromatography of B and T cells, and discusses the role of microphase separated structure in their differentiation.

EXPERIMENTAL

Materials. Preparation of polystyrene/polyamine comb-type copolymer (SA copolymer), polystyrene (PSt), poly(p-diethylaminoethyl-styrene) (PEAS), and random copolymer of styrene and p-diethylamino-ethylstyrene (P(St-EAS)) were reported elsewhere. The structural formulas of these polymers are shown in Figure 1. A thin film casted on carbon-coated copper grid was stained by osmium tetroxide (OsO₄) vapor for 24 hrs. The microstructures of the film surface were observed by a transmission electron microscope.

Column preparation. Glass beads (40-60 mesh) were coated with one of the above-mentioned polymers by solvent evaporation technique under dry nitrogen atmosphere. A definite weight of polymer-coated beads was closely packed in the poly(vinyl chloride) tubing (3 mm ID) fitted with Nylon mesh column supports and a stopcock. The column primed with physiological saline was filled with 0.2 M phosphate buffer solution of rat serum albumin (0.09 g/dl), and was in-