Theory and Practice of Centrifugal Elutriation (CE)
Factors Influencing the Separation of Human Blood Cells

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Received September 8, 1982; Accepted March 30, 1983

Abstract

Centrifugal elutriation (CE) is currently a widely used preparative cell separation technique. In order to optimize the separation of cells that show only small differences in sedimentation velocity, several conditions that might influence the resolution capacity, such as rotor speed, counterflow, jetstream, cell load, density, and viscosity of the elutriation medium, were analyzed.

Experiments carried out with human red blood cells (rbc) indicated that a selectivity losses of rbc from the rotor caused by the jetstream, could be prevented if the separations were carried out at high rotor speeds, as predicted by the theory. In addition, high cell loads \( (5 \times 10^8 \text{ rbc}) \) resulted in better separations than low cell loads \( (5 \times 10^7 \text{ rbc}) \).

Human monocytes were separated into subpopulations that differed only about 0.003 g/mL in density, but have virtually the same size. The separation was carried out either by increasing the density or viscosity of the elutriation medium or by decreasing the rotor speed. In all cases similar results were obtained.

These results indicated that under optimal conditions CE can be applied for the separation of cells that differ only slightly in sedimentation velocity.

Index Entries: Centrifugal elutriation, of red blood cells; human blood cells, centrifugal elutriation of; monocytes, centrifugal elutriation of; theory, of centrifugal elutriation; cell separation, by centrifugal elutriation; resolution, of cell populations by centrifugal elutriation; elutriation, centrifugal; separation, of cells by centrifugal elutriation.

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Introduction

At present centrifugal elutriation (CE) is one of the most powerful methods to separate cells according to sedimentation velocity. However, until now most reports concerning CE dealt with the separation of cells that differed markedly in size [for review see (1)] or density (Beckman manual) and therefore in their sedimentation velocity. In the present communication we investigated whether the CE technique could be optimized in such a way that it could be applied to the purification of cells that differed only slightly in their sedimentation velocity. It has already been demonstrated theoretically that the shape of the separation chamber can influence the resolution (2). Here it is demonstrated that if the various factors that affect the resolution are handled optimally subpopulations of human red blood cells and of human peripheral blood monocytes can be isolated that differ slightly in size and/or density.

Materials and Methods

Media and Reagents

Phosphate buffered saline (PBS) supplemented with 0.14% bovine serum albumin (BSA) (fr. V, Sigma, St. Louis, USA) was used as standard CE medium. To prevent the aggregation of monocytes and platelets, introduction of the cell sample and collection of the first two fractions was carried out in CE medium containing 1% autologous plasma instead of BSA. Elutriation with medium of a higher density (CE-P medium) was achieved by mixing the standard CE medium with CE medium containing 12.5% Percoll corresponding to the relatively low concentration of silica sol of about 3% (Pharmacia, Uppsala, Sweden) or with CE medium supplemented with 0.07% polyethylene oxide (PEO), mol. wt. 600,000 (BDH, Poole, UK, code WSR-205). Details of the procedure were published elsewhere (3).

Measurement of Viscosity

Viscosities, \( \eta \), were calculated from the times, \( t \), required for two liquids to flow through a capillary. The equation

\[
\frac{\eta_1}{\eta_2} = \frac{d_1 t_1}{d_2 t_2}
\]

was used where \( d = \) density.

Cell Preparations

Mononuclear cells were prepared without exposure to body foreign substances as described previously (3). Briefly,uffy coat (BC-1) cells prepared from 500 mL blood containing acid citrate dextrose (ACD), were obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. A seconduffy coat (BC-2) was prepared in a specially designed blood component