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Review

Cellular separations: A review of new challenges in analytical chemistry

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ABSTRACT

The ability to generate a sample of cells of a given phenotype is a prerequisite for many cellular assays. In response to this growing need, numerous methods for cell separation have been developed in recent years. This Review covers recent progress in the field of cell separations and cell chromatography. Cell separation principles—such as size and affinity capture—are discussed, as well as conventional methods such as fluorescence-activated cell sorting and magnetic sorting. Planar flow cell arrays, dielectrophoresis, field-flow methods, and column separation devices are reviewed, as well as applications of these methods to medicine and biotechnology. Cell attachment and adhesion strategies and a comparison of techniques are also presented.

Contents

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1. Introduction

The isolation, enumeration and sorting of cells by phenotype is important in both clinical diagnostics and basic research. Sorting cells, either for subsequent culture or to purify one cell phenotype from a complex sample, has evolved into several chemical, magnetic and mechanical methods. Currently, the majority of cell separation systems are based on flow cytometers or other expensive and complicated instrumentation such as large bench top sorters or microfluidic sorters. While these instruments have powerful measurement capabilities and have successfully sorted cells based on a variety of parameters [1], the cost and complexity of the instruments and required infrastructure (facilities, personnel, reagents) preclude their use in some cases. In addition, many cell separation methods use immunofluorescence as the triggering signal or readout. This necessitates the use of fluorescent antibodies, which adds to the analysis cost and may not be amenable to all studies (e.g. reculturing cells). This is especially important when one considers that immunofluorescence imaging requires higher quality optics, excitation sources, and cameras than white light or dark field imaging detection. As a result of these and other limitations, there has been considerable effort over the past few decades to separate cells by chemical or other mechanical means.

Cell separations have progressed dramatically in recent years, fueled in part by technological advances as well as a growing need for new capabilities in research and clinical settings. The explosion of microfluidic formats has triggered a resurgence in cell separations. In addition, multiplexed capillary or column separations have allowed for the rapid detection of multiple phenotypes from a small sample volume. Novel chemical approaches such as aptamers have found niche applications relative to the massively popular antibody capture format. Also, the need for rapid, inexpensive cytometric measurements worldwide has been a major driving force for many cell separation applications. The ability to detect, identify, and enumerate cells in a single device based on phenotype has proven to be invaluable in the fields of medicine, food science, military applications, and basic and clinical research. New cell separation capabilities, combined with recent advances in the laboratory, have redefined the field of cell separations in the last decade.

Since the landmark review by Sharma and Mehendroo [2] there has been considerable progress in the field of cell separation. The field is varied by application as well as by approach. Traditional methods of chromatographic analysis as well as new approaches have been developed and applied to cell separations. Applications include bacterial identification, blood cell isolation, CD4+ T lymphocyte counting, capture of rare cells and the detection of cancer cells circulating in blood. These and other applications are reviewed here, as well as the techniques used to separate cells. In this Review, we focus on recent progress in the area of cell separations rather than an exhaustive literature review.

2. Cell separation overview

2.1. *Fundamental principles*

Cell separations are largely based differences in physical (dielectrophoresis, field-flow fractionation, etc.) or chemical (primarily affinity) methods. For separations involving the former, there must be a significant difference between the size, dielectric potential, buoyancy or other parameter. For example, cellular sieving methods take advantage of the size,

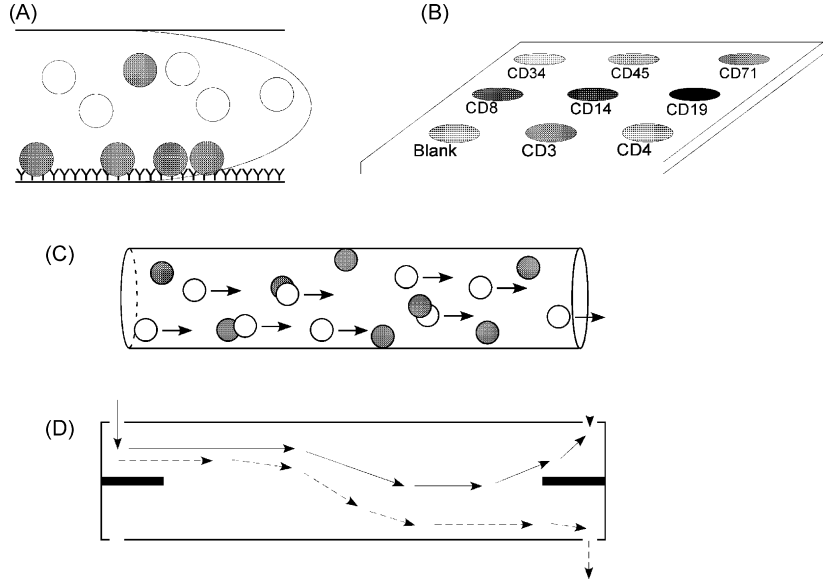

Fig. 1 – Overview of common cell separation strategies. Affinity capture separations rely on the adhesion of cells to a matching antibody (A). Unretained cells are washed to waste. The affinity format can be applied to array formats (B), where more than one capture molecule is attached to a surface. Here, antibodies corresponding to different CD antigens are used to separate blood cells. Affinity separations can also occur in a column (C), facilitating elution and collection. Cells can also be separated by mechanical methods, such as the SPLITT method (D, redrawn from [53]).