

A Mini Summary of Affinity-based Separation Methods for Sample Preparation

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Abstract. Over the years of development, affinity-based separation has become one of the main technologies of cell separation. These methods have met the requirements of high yield, purity, extensibility, and sterility. The techniques for separation of particles and cell purification which are applied in sample preparation in the field of basic scientific research and diagnostic or therapeutic purposes have become highly developed over years. In this paper, the common modern methods of affinity-based separation are discussed and compared, including their principles, fields of application, and limitations.

Keywords: Affinity-based, Sterility, Sample preparation, Cell separation.

1. Introduction

The development of cell purification technology is essential to improve the availability of viable cells used for therapeutic, diagnostic and research applications. There are many kinds of techniques used for cell separation, including non-affinity ones such as filtration, density gradient centrifugation, and dielectrophoresis, and affinity-based methods such as chromatography, magnetic or fluorescence assisted cell sorting, and two-phase partitioning. In most cases, the required purity of cells is only achievable through modern affinity-based methods, due to its characteristic of high target-specificity.

In common affinity-based separation methods, sorts of medium substances, or ligands, are utilized. The common medium substances involved include protein and certain types of antibodies, which trap wanted or unwanted cells by (covalent) bonds and absorption. In contrast, the target cells, or otherwise, are filtered out with or without their reaction with the ligands. Nowadays, the techniques have had the ability to separate particles with similar physical and chemical characteristics, which may include biological cells or biochemical substance including proteins. The different types of substance to be separated vary the method of separation, mainly in the aspect of the design of microfluidic device [1].

Summaries in this field are needed to summarize and classify these diverse and complex methods, also helpful for further investigation in this field. A concise summary is presented here, focusing on affinity-based methods for cell purification, on several chromatographic separation methods with their unique characteristics, more specifically.

2. Typical Affinity-based Cell Separation Methods

In this section, the major methods used to separate cells based on their affinity with certain medium molecules are introduced.

2.1. Inertial Microfluidic Bead Sorting and Multiplexed Binding

The inertial microfluidic bead sorting technique is a fast, multiplexing but inexpensive, affinity-based method for protein and cell separation using a size-coded mixture of multiple affinity capture micro-beads and inertial microfluidic particle sorters. While functioning, all kinds of target cells and proteins construct bonds with microfluidic beads with different sizes in a single binding step, and then they are sorted by a spiral microfluidic channel. Durable yet rapid, continuous-flow, high-throughput isolation of milligram-level protein samples or millions of cells is enabled by the device. The process is completely spontaneous, which is another crucial advantage of the device.

The device works on the principle of dean flow fractionation (DFF). As shown in Figure 1, through the spiral channel in DFF, particles exceeding a certain size threshold can be separated into different flows due to the superposition of inertial lift forces and drag forces depending on the sizes of the particles. The height of the device, the particle size, and the location of the outlets can be specialized to make a single outlet to each flow containing a single sized target bead. Thus, the particles in the fluid, together with different sizes of beads, can be separated successfully [2].

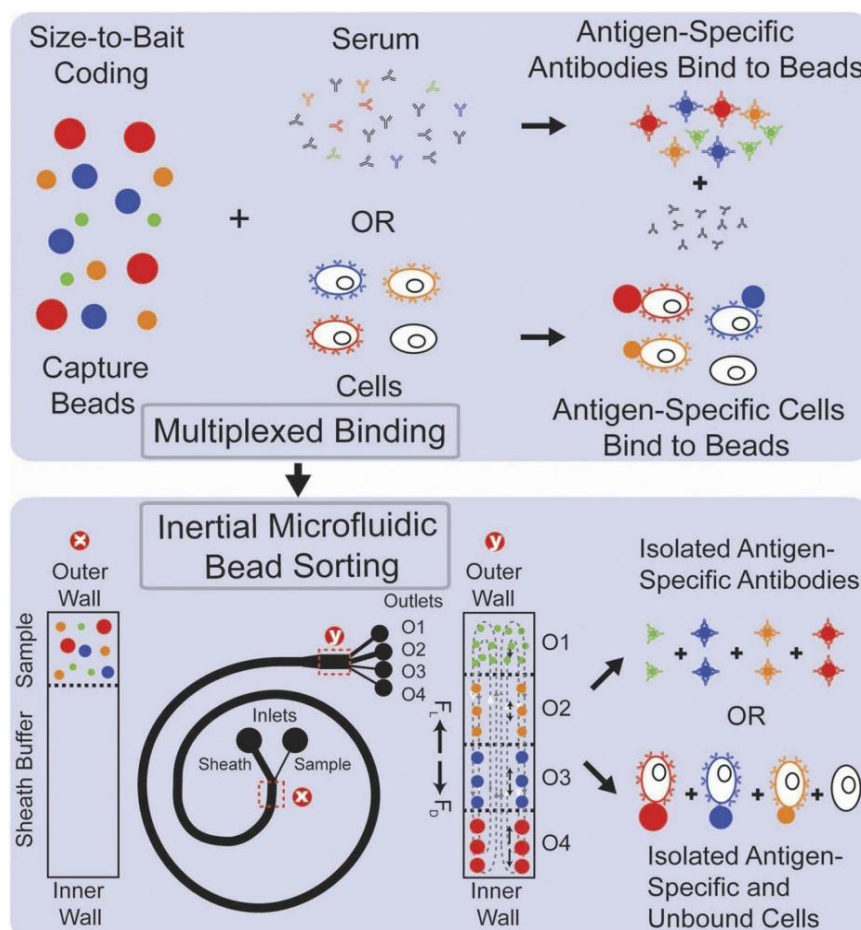


Figure 1. The principle of multiplexed protein and cell sorting using a DFF device.

It is repeatedly checked through experiments that the design provides enough throughput (about 10^4 to 10^7 beads per second) in a single unit, enough for supporting most applications. For protein separation, the precision reaches milligram-scale; for cell separation, the speed approaches 1 to 5 million cells processed per unit device in less than 10 minutes. The accuracy of separation of antibody is proved to be always higher than 80%.

The technique has a bright future: the accuracy of separation can be further improved by connecting several units of device in series, and the efficiency can be further improved by a parallel design. Moreover, by varying the size of beads and particles in a certain range, the device could be used in separation of a larger range of particles.

2.2. Immuno-affinity-based Separation of Mitochondria

Immuno-affinity, meaning using antibodies as affinity ligands, has been one of the conventional affinity ligands for cell separation. It has been widely applied in the field of cell purification. Its popularity is due to the binding selectivity of the antibodies and their indispensable ability to work under various physiological conditions. However, the strength of interaction between the target particles and the ligant, the antibody, requires demanding elution conditions, which may have a crucial effect on cell viability. The problem is always addressed by elution techniques including cleavable linkers and competitive elution.

Immuno-affinity chromatography (IAC) combines the use of Liquid Chromatography (LC) with the specific binding of antibodies or related agents. The methods developed can be used for the analysis of a particular target and for purification and concentration of the analytes before they are further examined by other methods [3]. In recent cases, more financially affordable antibody fragments are utilized instead of the whole antibody, due to their similar ability of processing binding activities.

In a specific example [4], a method to separate mitochondria from mammalian cells after microscale cell-destruction is proposed. The device to approach this is constructed by linear microchannels cast in polydimethylsiloxane (PDMS) as shown in Figure 2. The wanted mitochondria can be captured in the channels, and the remnants of cell lysates flow unimpeded out of the chip. The capture of mitochondria can be directly viewed on the chip. It is observed that from 0 to 5 mL per minute, after fluorescent staining, mitochondria covered approximately 3% of the channel surface. Mitochondria are distinctly spherical, approximately 0.8-1.2 μm in diameter. Further validation of the microfluidic device using untreated cell lysates was performed at 2 mL per minute. The immobilized mitochondria were small, with a diameter of about 490 nm. The surface coverage is about 4%.

Immuno-affinity methods have the feature of biologically available ligands. Like what is described in the example, the specific proteins or antibody utilized as ligands can be prepared through biochemical purification. However, there are always great challenges for purification of these recombinant and therapeutic proteins, because there is a lack of general structural elements for these proteins to be captured through common affinity chromatography methods.

One major shortage of the technique is that the crucial content of the device, the resins, have a limited lifetime, which is highly dependent on the type of antibody used. The usage of non-specialized antibodies as ligands could cause a limited shelf life of the device in real application, along with reasons including degradation and ligand leakage.

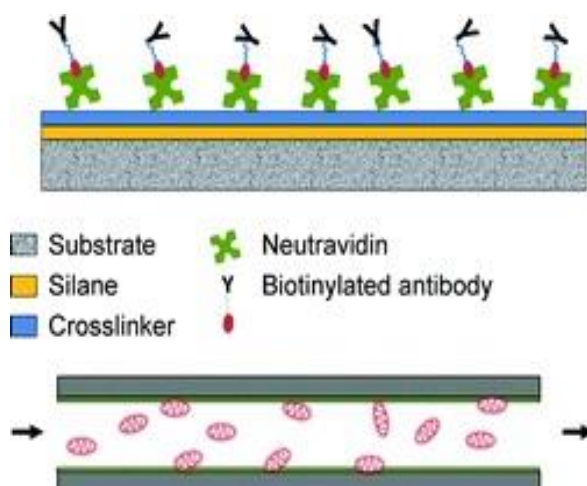


Figure 2. The principle of immuno-affinity-based separation of mitochondria from mammalian cells.

2.3. Rosetting and Avidin-biotin Affinity

A method will be introduced in this subsection, which is the first affinity-based method to combine affinity with conventional density-gradient separation methods. In this technique, antigen-specific cells are incubated with antigen-coated red blood cells, and they form aggregates with red blood cells called rosettes, while non-rosette cells were separated out from them through gradient centrifugation. At first, it was used to separate two distinct mouse immune cell populations using red blood cells gathered from sheep. Also, further studies have shown that by avidin-biotin affinity, rosette-forming antigen-specific cells could be gathered at a higher efficiency and purity, and gradient-density centrifugation and the combination with magnetic field could also have similar effects. Rosetting

method, nowadays, is commonly used in purifying B and T lymphocytes and stem cells, providing considerable purity and recovery.

Over the years, the technology of avidin-biotin has become more and more financially viable for various clinical, industrial, or scientific research purposes. Avidin-biotin complex was proposed for affinity-based separation as a kind of medium from the outset as shown in Figure 3. However, it was a long process for this medium to gain acceptance, partly due to its disadvantage that the strength of binding between avidin and biotin sometimes cannot suit the affinity-based methods, attracting target particles more or less [5].

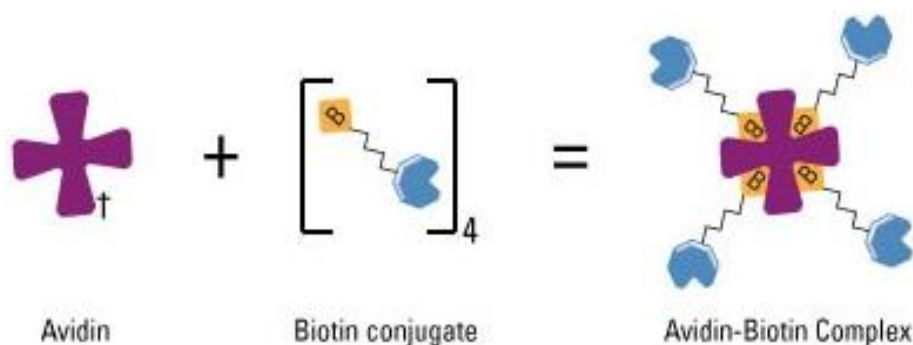


Figure 3. Schematic of the Avidin-biotin interaction [6].

Recent advances in understanding the technical details of preparation of biotin might lead to the invention of special proteins resembling to avidin, possessing recognition characteristics, fulfilling the desired conditions appropriate for Avidin-biotin affinity-based separation.

2.4. Non-chromatographic Affinity-based Separation Methods

Over the years, various non-chromatographic separation methods have also been developed for a lot, as a relatively new concept from traditional chromatographic ones. Several names of typical examples of the technology includes magnetic-activated cell sorting and two-phase separations. In the former technology, a given kind of ligant enables separation, and connections are built between them and all target cells; the latter utilize polymetric materials to make the selection of target cells into an aqueous phase. The main common ground for the two methods is that they both separate target and non-target cells into bulk groups, in some conditions contributing to the upstream separation process.

An important disadvantage of, for example, two-phase separations, is the occurrence of the indelible impurities in the product phase during experiments and real applications [7]. To really make the process of cell separation scalable, an improvement on the technology is required to be done to a top clean phase.

3. Conclusion

The techniques for cell separation are constantly developing to meet the numerous requirements of basic research, diagnostic or therapeutic field applications, and becoming increasingly quantitative, efficient, and dependable. Among these methods, affinity-based methods are still the most highly utilized ones, due to the purity of separation output they can achieve. Microfluidic devices and technologies are a representation of the next frontier of battery manufacturing, because they provide the indispensable ability to perform multiple tasks in a single device unit. Also, as aforementioned, the throughput and the limitation of sample volume processed of a single device could be improved when made in scale and in a parallel structure. To conclude, a wide range of affinity-based separation methods are currently highly available, each of which has its unique advantages and disadvantages. The paper focuses on several mainstream types of separation methods based on affinity principles, including microfluidic bead sorting and multiplexed binding, immuno-affinity separation, and rosetting. During application, the advantage and disadvantage, these sides of a specific method need

to be carefully considered, and the advantages of other parallel methods should be compared when a method is chosen.

On the front of the field of biorecognition, affinity-based separations are increasingly using synthetic ligands instead of conventional protein or antibody ligands. The disadvantage of taking protein and antibody as ligands, now discovered, is the high cost and exceeding binding strength they have. By contrast, synthetic ligands can be produced at a considerable scale with a relatively low cost and without a "batch-to-batch variability". Meanwhile, the demand for better cell elution conditions stimulates the stimuli-responsive ligands, which means that the binding activities with target particles can be controlled by external signals.

It is expected that through experiments or modeling, or both, future studies can focus on the development of various materials able to be utilized as synthetic ligands, fulfilling the requirement of low manufacturing cost, stability, and high efficiency. At the same time, further studies are needed to stabilize the technologies of stimuli-responsive ligands, as another focus of equal importance.

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