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Advances in microfluidic cell separation and manipulation

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Abstract

Cellular separations are required in many contexts in biochemical and biomedical applications for the identification, isolation, and analysis of phenotypes or samples of interest. Microfluidics is uniquely suited for handling biological samples, and emerging technologies have become increasingly accessible tools for researchers and clinicians. Here, we review advances in the last few years in techniques for microfluidic cell separation and manipulation. Applications such as high-throughput cell and organism phenotypic screening, purification of heterogeneous stem cell populations, separation of blood components, and isolation of rare cells in patients highlight some of the areas in which these technologies show great potential. Continued advances in separation mechanisms and understanding of cellular systems will yield further improvements in the throughput, resolution, and robustness of techniques.

Introduction

Manipulation of cells is essential in biological research, clinical diagnostics, and cellular therapies. In a research context, separating heterogeneous cell samples into subpopulations enables the identification of phenotypes of interest and subsequent physical and biochemical analyses. In clinical diagnostics, blood samples must be separated before analysis of biochemical or cellular components. Emerging diagnostic tests require the isolation of rare cells, such as circulating tumor cells, from blood samples for disease diagnosis, monitoring, and treatment. In regenerative medicine, cell samples must be characterized and purified before transplantation to ensure the safety and efficacy of treatment. In each of these contexts, current technologies have several bottlenecks in sample volume, throughput, robustness, sensitivity, and cost.

Microfluidics has emerged as an important tool set for specialty separations in the last couple of decades. Separation has long been a central theme in microfluidics research starting with the development of miniaturization of DNA separation devices in the early 1990s. A major motivation has been to reduce the size, cost, and complexity of technologies, making them more accessible to scientists and clinicians, while taking advantage of physical phenomena that are prominently manifested at the micro scale [1]. Many microfluidic platforms have taken advantage of these novel techniques for separating cells based on various physical and biochemical properties. In general, microfluidic devices are uniquely suited for manipulating biomolecules, cells, and organisms: low Reynolds number, laminar flow allows highly controllable, fast fluid manipulation; channels on the order of 10–100 μm enable use of small sample volumes; and fabrication from poly-dimethyl-siloxane (PDMS) makes devices simple to make, cheap, and amendable to imaging [2–4].

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This article focuses on advances in the last few years in microfluidics-based technologies for the manipulation of cells. We present advances in principles of microfluidic separation and manipulation mechanisms (Table 1) and highlight notable applications of these methods in research and clinical diagnostics; for some well established techniques, please also refer to other reviews [3,5–7].

Advances in principles of separation and manipulation mechanisms

Fluorescence-based and imaging-based separation

Fluorescence activated cell sorting (FACS) is widely used for analysis and sorting of cell populations. The miniaturized, microfluidic version of this technology—known as μFACS—uses the same principles: a cell suspension is hydrodynamically focused within a channel, interrogated with a laser beam, and sorted based on fluorescence intensity or wavelength [8]. The main challenge μFACS technologies face is replicating the throughput of conventional FACS while maintaining spatial resolution. Recent advances in μFACS have been primarily in the use of image acquisition and real-time digital image processing techniques that allow sensitive detection at high flow rates [9,10••]. The integration of optical waveguides in devices provides high excitation efficiency for multi-point detection of samples [9]; this is important for detection of low fluorescent signals. To obtain blur-free images with preserved spatial resolution at high speeds, ultra-fast imaging technology has been coupled with real-time image processing [10••]. Other recent advances in μFACS include the development of systems for sorting larger and irregularly shaped objects such as cell aggregates [11], embryos [12], and organisms such as the nematode *Caenorhabditis elegans* [13••,14]. The major contribution of these approaches is that information is spatially specific (e.g. tissue-specific or cell-specific expression of genes of interest or subcellular localization of proteins of interest) and therefore reveals greater details of the systems of interest. Separations therefore can be performed based on more specific, quantitative, and complex information. An extension of this approach, valuable but relatively unexplored, is in the sorting of cells and organisms based on complex and/or subtle phenotypes. For example, genetic screens may yield novel phenotypes that are difficult or impossible to distinguish by eye. To identify such phenotypic differences, image processing, machine vision, and machine learning techniques [15–17] can be used in conjunction with a flow-based sorting system, as demonstrated in Crane *et al.* [13••].

Magnetic separation

Parallel to μFACS, microfluidic magnetic-based separation devices borrow principles from conventional magnetic activated cell sorting (MACS) technologies [18]. To separate a cell type of interest, a sample is first incubated with magnetic microparticles or nanoparticles functionalized with a cell-specific antibody. When the sample is flowed through a microfluidic channel, magnetized surfaces or an applied magnetic field can be used to trap [19] or deflect [20–23] bound cells [Figure 1]. The advantage of MACS is that driving the separation is relatively simple and inexpensive, therefore making this technique field-deployable. However, in applying MACS, one would need to, in most cases, use an antibody-based labeling approach to attach a magnetic tag to the objects of interest, adding complexity to the separation. To date, microfluidic magnetic separation devices have largely been proof-of-concept, with a few exceptions such as the CellSearch system (Janssen Diagnostics, Raritan, NJ, USA)—a commercialized immunomagnetic separation system for isolating circulating tumor cells. Future development in increasing tagging efficiency and throughput will make this technique more broadly applicable.

Dielectrophoretic separation

Dielectrophoretic (DEP) separation of cells is an intrinsic, label-free technique based on polarizability of cells. Particles including cells experience a DEP force in a non-uniform electric field [24]. In conjunction with field flow fractionation (FFF), cells can be differentially positioned within a channel, allowing collection of the distinct subpopulations at the channel outlet in a continuous mode [25–27] [Figure 1]. One challenge is that dielectric differences between cell types are often subtle and below the resolution of separation techniques. To improve DEP separation based on size differences, a temporally [28] or spatially [25] pulsed DEP force can be applied (as opposed to a continuous DEP force). Alternatively, to perform non-size-dependent separations, isodielectric separation (IDS) can be used, [29,30••] where cells are flowed through a solution with a conductivity gradient in an electrical field gradient. This directs cells to positions in the conductivity gradient where their net polarization charge vanishes (the isodielectric point). The advantage of IDS is that it is based solely on cell dielectric properties and not on size, and hence allows researchers to examine effects of gene knockouts on the dielectric property change in cells. A challenge of designing DEP-based separations is that the biological basis of cell dielectric properties is not well known, making it difficult to predict cell behavior in DEP fields. Recent efforts have identified genes whose functions modulate intrinsic electrical properties [30••] and morphological characteristics [31•] of cells. DEP is likely to become a specialty technique where one would need to know *a priori* that there is a dielectric property difference in the systems of interest.

Mechanical and hydrodynamic separation

Filtration is the simplest method of mechanical separation. Devices can be fabricated with arrays of barriers [32–35] or holes [36] which allow smaller, more deformable cells to pass through while retaining larger, stiffer cells. Filtration is easy to carry out and works well for dilute samples; however, one drawback is that clogging often limits the utility and the throughput of these devices for concentrated or large-volume samples. Size limitations also apply; features that are too small impose fabrication challenges and create high pressure drops in the system.

Hydrodynamics-based cell separation techniques rely on intrinsic physical properties of cells and how these properties determine cell behavior in laminar flow. The hydrodynamic lift force experienced by a cell is dependent on its size, shape, and deformability and causes cell migration across fluid streamlines in a channel. Thus, cells that differ with respect to these mechanical properties will experience different magnitudes of the force and will be positioned at different locations in a channel [37–41] [Figure 1]. Because this is a passive mechanism, it is likely to be generalizable and easier to operate. Hydrodynamic principles are often used in blood separations, as there are significant size differences between the different cellular components of blood [42,43]. Applications include the isolation of circulating tumor cells [37,44] and bacteria [45,46] from blood.

Another class of hydrodynamic separations uses a technique known as deterministic lateral displacement (DLD). In DLD, arrays of obstacles are used to displace particles above a critical size so that they follow a deterministic path through the array, leaving particles below a critical size to follow streamlines and travel in the direction of the average fluid flow [47,48]. This technique is good for separating cell populations with characteristic sizes, and has been successfully implemented for separating blood components [49], leukocytes from blood [50], and epithelial cells from fibroblasts [51].

A significant advantage of these mechanical-based and hydrodynamics-based techniques is that they rely on intrinsic properties of cells, making them easy to operate so long as cell

subpopulations have distinct physical properties. This is in contrast to label-based separation techniques, the efficiency of which depends both on cell properties and the reliability of the labeling technique. However, the challenge is that typically the difference between the cell populations of interest and the background population is limited and may not be large enough to be resolved using a single stage of separation or a single technique. It is likely that for different applications either multistage or multimodal separations will be the best solution.

Separation based on molecular adhesion or molecular surface interactions

Another class of separation techniques relies on molecular interactions such as cell surface receptors to ligands and antibody recognition of antigens. An example of such a separation technique is miniaturized affinity chromatography, where cells uniquely identified by the expression of one or more surface molecules can be captured in a microfluidic device by surface-tethered antibodies [Figure 1]. The advantages of this class of techniques are that it is highly specific and that it can be used to separate cells with similar or identical physical properties. Molecular interaction-based separations have been successfully implemented in applications such as the isolation of circulating tumor cells (CTCs) from patient blood samples [52,53•,54] and the separation of stem cell populations [55,56]. Recent work has focused on enhancing cell-surface interactions within microfluidic devices in order to improve the efficiency of cell capture. This has been achieved with channel features that promote mixing [57], pillar arrays that control cell trajectories [53•], porous channels that direct fluid flow to the surface [58•], and shallow channels on the order of cell size [59]. One subset of techniques uses cell rolling as a means of separation. Inspired by the *in vivo* phenomena of leukocyte rolling to sites of injury, devices use patterned substrates and/or surface grooves to separate cells based on adhesive and stiffness properties as they interact with the surface [60– 62]. Besides antibodies or protein/peptide ligands, another emerging class of techniques uses aptamers [63,64], which are selected in an *in vitro* process from a pool of RNA or DNA candidates [63]. This directed evolution approach can allow aptamers of specific properties to be evolved; however, currently generating aptamers in practice is less convenient, so the utility of aptamers in separations is somewhat limited. In all adhesion-based separations, the major challenges lie in the fact that separation performance is highly dependent on the specificity and binding kinetics of the antibody/ligand/aptamer used, and purchasing or developing optimized antibodies/ligands/aptamers can be costly. Most development in microfluidics in this area will likely be in clever uses (and combinations) of these techniques to achieve high throughput while maintaining separation efficiency.

Other separation techniques

In addition to the mechanisms mentioned above, acoustic forces can be used to separate cells based on size, density, and compressibility. In the presence of a standing ultrasonic wave, an acoustic force perpendicular to fluid flow drives cells to different positions in the channel cross-section [65–67]. Similarly, optical forces can also be used to trap and sort cells, in which a strongly focused beam of light produces an electric dipole moment in cells, which causes them to move up the optical gradient and become trapped at the focal point [68–70]. The advantage of both of these techniques is that they are label-free, using intrinsic properties of the samples as the basis for separation. However, future research should be directed toward making these techniques more efficient, as the variability of these physical properties of the biological samples are limited. By coupling with (affinity) labeling, one could generate larger differences in optical or acoustic signatures, which could be a good way to enhance contrasts and enhance separation (with the caveat of no longer being ‘label-free’). It is likely that the best use of acoustic-based and optical-based separations is in tandem with other techniques in order to enhance the overall separations.

Droplet microfluidics as an emerging manipulation technique for many applications

Most of microfluidic devices in the biological realm handle spatially continuous flows, for example to culture cells or tissues, or to perform separations. Microfluidic droplet generation and manipulation in contrast handles samples in discrete packets; it enables high-throughput manipulation of biological and chemical reactions. Droplets generated in microfluidic devices are small (on the order of femtoliters to nanoliters in volume) and can easily be mixed, merged, and sorted. Droplet-based platforms are ideal for compartmentalizing cells and reagents in self-contained reactions, enabling combinatorial and high-throughput screening studies [71,72]. Technologies have been developed for the manipulation of bacteria, yeast, mammalian cells, and *C. elegans*. Studies have demonstrated fluorescence-based sorting, cell transformation and transfection, DNA/RNA analysis, and genome sequencing. The major limitation of microfluidic droplet-based platforms is the level of robustness in their operation: for uniform and reproducible droplet formation and manipulation, parameters such as surface tension (often the type of surfactant is critical) and flow rates must be precisely controlled. While for some applications throughput is important, for many it is not as critical as robustness; new methods to produce droplets of well-defined dimensions in a well-controlled manner, independent of specific surfactants used, would move this field forward; a recent development in passive droplet formation by Dangla *et al.* [73,74] is a good step toward the design goal of some applications. Other areas for further development include continued integration of manipulation and analysis steps in single devices and automation of devices, which will enable many great transformations of current methodologies for doing molecular screens, genetic and drug screens, and process screens.

Examples of applications of separation and manipulation technologies

There are many uses of the techniques that we review here for various biotechnological and biomedical applications. We showcase two examples to illustrate how frontiers of technologies have been pushed in the last few years. For further examples, we refer our readers to a number of extensive review articles on these topics [3,6,7,75].

Separation of rare cells in diagnostics

In clinical diagnostics, analysis of blood cellular components can yield information about disease state and progression, as well as patient response to treatment. Microfluidic platforms have been developed for the separation of platelets, erythrocytes, and leukocytes based on size and deformability [42,76]. These technologies have potential for use in point-of-care settings when small amounts of blood are tested. However, in all blood separations, handling of large amounts of undiluted whole blood remains challenging, as devices are prone to issues like clogging after prolonged usage.

In cancer diagnosis and treatment, isolation and analysis of circulating tumor cells (CTCs) yields valuable information, yet is challenging due to the scarcity of these cells (1–100 mL^{-1}). Separation technologies must be able to process large volumes of blood, identify CTCs very specifically, and preserve cell viability for analysis. These requirements limit the utility of size- and deformability-based separations; rather, use of antibodies, ligands, or aptamers for CTC-specific markers has shown more potential [5,52–54,77••]. Examples of microfluidic technologies demonstrating isolation of CTCs from patient samples and clinical monitoring of cancer progression include the commercially available CellSearch system (Janssen Diagnostics, Raritan, NJ, USA) and the devices developed by Nagrath *et al.* [54] and Gleghorn *et al.* [53•]. Despite the demonstrated functionality of these technologies, a major challenge lies in preserving the viability of CTCs for further analysis; both immunomagnetic and adhesion-based separation techniques make it hard to retrieve cells

without stressing them or removing cell surface proteins. Additional challenges remain in identifying markers specific to each cancer type and reducing false positive or negative rates, as well as handling large amounts of patient blood through the microfluidic systems. It is likely that combinations of multiple separation techniques (e.g. hydrodynamic-based removal of some blood cell components, followed by CTC isolation) or multiple separation stages will improve the performance of these devices in clinical applications.

Organs-on-chips

One way in which microfluidic cell sorting and manipulation can be used in larger-scale research and toward clinical biological contexts is in the development of so-called 'organs-on-chips'. These microfluidic-based three-dimensional cell culture models attempt to recapitulate the cellular, mechanical, and biochemical components of human organ microenvironments [78]. They can serve as accessible substitutes for *in vivo* organ models and allow investigations of human physiology and disease, as well as models for drug screens and therapeutics screens [79–82].

Outlook

The last two decades of research has shown that miniaturization provides numerous advantages for cellular separation and manipulation in research and clinical contexts. For analytical types of applications, many of the techniques have been well characterized and specialized; future development resides in combining different modalities, continuing to improve the resolution, efficiency, and robustness of these separation techniques, and automating and controlling the analytical procedures. Related to this are the challenges associated with various fabrication steps and materials used for different separation techniques, as well as the interface between the macro and the micro worlds. Some development will also be in the areas of making these analyses inexpensive and field-adaptable for point-of-care applications, particularly in resource-poor settings. For preparatory purposes, these techniques will need to be able to handle larger throughput and operate robustly for long periods of time. Because microfluidic chips have small volumes, for preparatory applications, separation techniques that are operated in a continuous mode will be more useful than those in batch mode because of throughput requirements. Additionally, some techniques, such as image-based techniques, will greatly rely on other fields in engineering such as computer-science (for image data processing, statistical learning/pattern recognition) and robust systems control, to be able to make real impacts on both basic research (e.g. genetics and cell biology) and biomedical sciences (e.g. diagnostics in cancer or infection).

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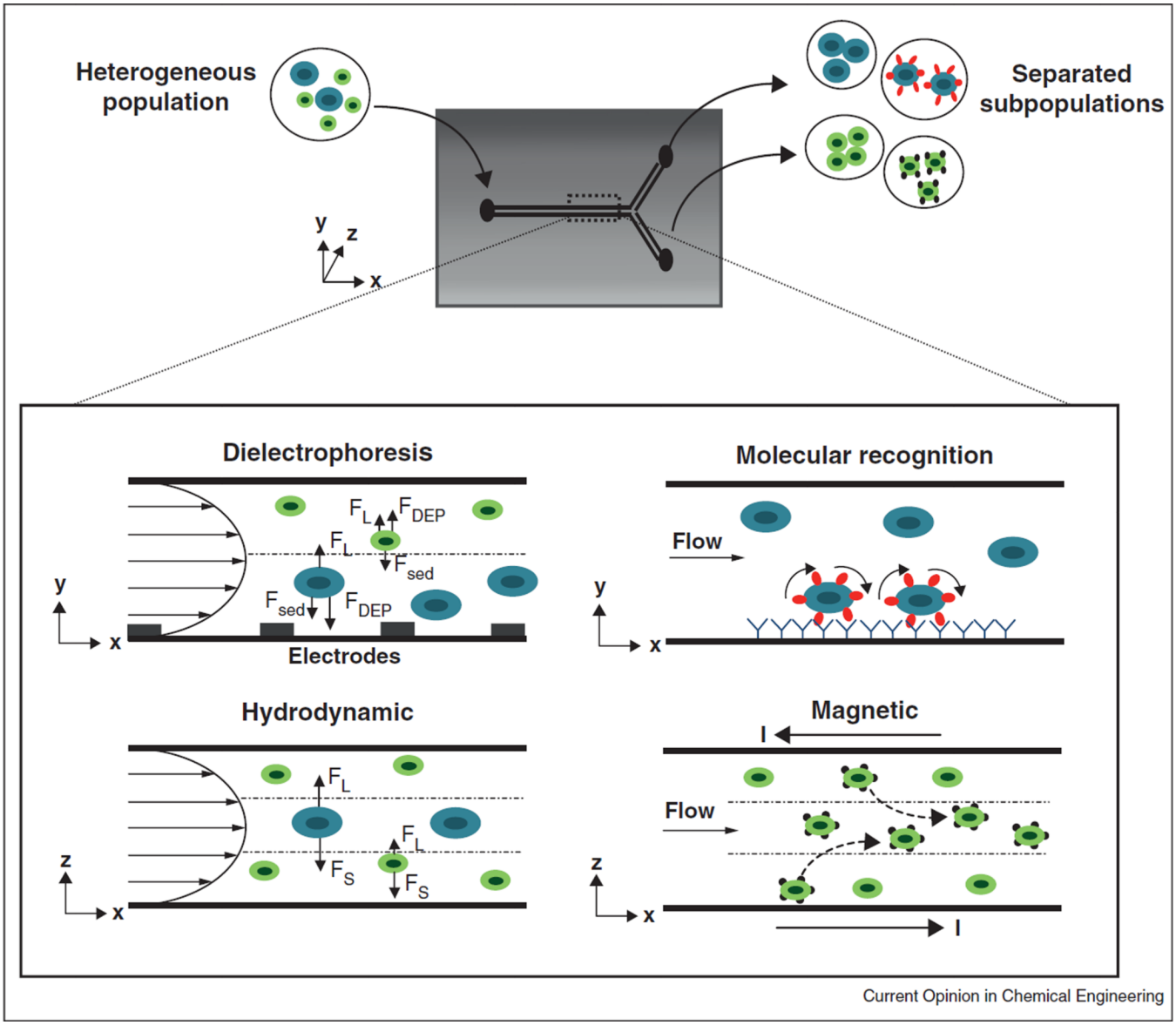

Figure 1.

Heterogeneous cell populations can be microfluidically separated based on intrinsic and extrinsic characteristics. In dielectrophoresis (DEP)-based techniques, cells are exposed to a non-uniform electric field and differentially positioned along the height of the channel, depending on the relative magnitudes of the DEP force (FDEP) and the sedimentation force (F_{sed}) due to cell weight. In hydrodynamic-based separations, a competing hydrodynamic lift force (F_L) and a shear-gradient lift force (F_S) act to position cells laterally. Molecular recognition-based techniques depend on the interaction of cell surface molecules (red) with antibodies or other ligands (blue) functionalized on the channel surface; cells are captured on the surface and can exhibit rolling behavior. In magnetic-based separations, an applied magnetic field is used to deflect and focus cells labeled with magnetic particles (black).

Table 1

Comparisons among different separation techniques

Technique	Key parameters	Advantages	Disadvantages
Imaging- and optical signal-based	Optical signal intensity or morphological features	Gives spatially specific information	Often requires exogenous labeling
			Trade-off between speed and resolution
		Identifies complex/subtle phenotypes	
Magnetic	Magnetic field strength	Can be highly specific	Low to medium throughput
	Cell surface marker expression		
	Magnetic label binding kinetics		
Dielectrophoresis	Cell dielectric properties	No exogenous labeling	Biological basis underexplored
	Cell size	Size independent (IDS)	DEP differences can be too subtle
	Electric field parameters		
Mechanical/hydrodynamic	Cell size	No exogenous labeling	Problems with clogging
	Cell shape		Physical differences can be too subtle
	Cell deformability		Damage to cells
Molecular recognition	Cell surface marker expression	Highly specific	Requires cell-specific marker
	Antibody/ligand binding kinetics		Dependent on antibody–ligand specificity
	Cell interaction with surface		