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# Perspective on Microfluidic Cell Separation: A Solved Problem?

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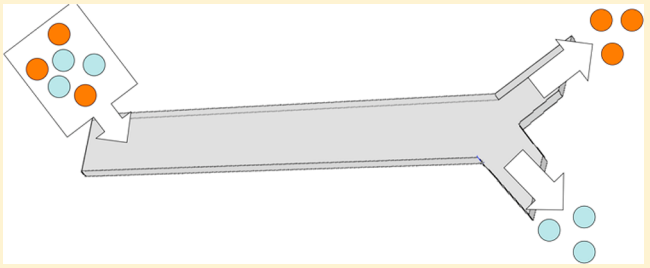
**ABSTRACT:** The purification and sorting of cells using microfluidic methodologies has been a remarkably active area of research over the past decade. Much of the scientific and technological work associated with microfluidic cell separation has been driven by needs in clinical diagnostics and therapeutic monitoring, most notably in the context of circulating tumor cells. The last several years have seen advances in a broad range of separation modalities ranging from miniaturized analogs of established techniques such as fluorescence- and magnetic-activated cell sorting (FACS and MACS, respectively), to more specialized approaches based on affinity, dielectrophoretic mobility, and inertial properties of cells. With several of these technologies nearing commercialization, there is a sense that the field of microfluidic cell separation has achieved a high level of maturity over an unusually short span of time. In this Perspective, we set the stage by describing major scientific and technological advances in this field and ask what the future holds. While many scientific questions remain unanswered and new compelling questions will undoubtedly arise, the relative maturity of this field poses some unique challenges.



The history of mammalian cell separation dates back to the 1960s, when parameters that could be exploited for target cell isolation were beginning to emerge. In 1968, Böyum published his seminal paper on Ficoll-density gradients for the isolation of lymphocytes from whole blood based on density differences among blood cell populations.[1] The 1970s saw a rapid advance in cell separation techniques, spawning a new preprocess step for cell analyses. Panning techniques[2] and rosette-based[3] platforms further increased efficiencies of blood separation. Herzenberg and co-workers in 1972[4] introduced a fluorescent-based separation method known as fluorescence-activated cell sorting (FACS). In FACS, the cells are segregated on the basis of their unique membrane or intracellular protein expression patterns, via tagging through the cell receptor and fluorescent ligand interactions. Later, Rembaum and co-workers (1977)[5] developed an immunomagnetic technique, now known as magnetic-activated cell sorting (MACS), based on specific labeling of cells with magnetic beads for separation. Although some of the old techniques are becoming obsolete, most of these traditional separation techniques remain standard practice in the laboratory. However, the more bulk-like separations, larger benchtop instruments, do not address many of the current questions in biological or clinical research due to a lack of limited sample handling capability and low target cell concentrations on one hand and the need for higher throughput analyses on the other. Many of today's state-of-the-art separation tools have throughputs in $10^5$–$10^7$ cells per hour and fail to isolate cells with high purity and recover rare cell populations (<1% of the total cell content). Today, FACS and MACS remain the most widely utilized methods, but limited sample amounts coupled with requirements of high sensitivity have spawned the development of a broad range of microfluidic cell separation methods. With the vast number of diagnostic and analytical tests now available, samples need to be divided among platforms and today's separation platforms need to adapt to an ever-smaller sample amount. We realize that in some cases larger volumes are required due to sampling statistics but, overall, microfluidics has proven to be the next step in the separation of small volumes.

The distant origins of microfluidics lie in the field of analytical chemistry[6] (gas-phase chromatography, high-pressure liquid chromatography, and capillary electrophoresis) and today see applications in physics, chemistry, biology, and energy. Specifically, the microscale laminar flow in these platforms has allowed for significant advances in controlled cellular manipulation; to date, over 3500 research papers in microfluidic cell separation have been published.[7] Microfluidic isolation can be generally divided into two broad categories of enrichment modalities, either isolation based on the cell physical characteristics (e.g., size and density) or cell biochemistry (e.g., antigen expressions).[8] The evolution of physical and biological separation has been well described in several recent review articles.[9−13] As illustrated in Table 1, there are several microfluidic devices that have been developed for separation based on cell size, shape, and density, including inertial microfluidics[14] and deterministic lateral displacement.[15] Microfluidic techniques such as optical force separation, dielectrophoresis, and acoustophoresis probe physical properties like refractive index, dielectric properties, and compressibility, respectively.[10,11,16] Conversely, biochemical or affinity-based

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**Table 1. Descriptions and Comparisons Among Different Cell Separation Techniques and Applications Best Suited for Each Technique**

| method | discrimination parameters | advantages | limitation | suggested primary applications |
|---|---|---|---|---|
| inertial microfluidics | (1) cell size; (2) cell shape | (1) label-free; (2) high throughput (>$10^{10}$ cells/h) | (1) physical and biological differences can be too subtle; (2) diluted samples | (1) tissue engineering; (2) regenerative medicine; (3) "-omic" analyses |
| deterministic lateral displacement | (1) cell size; (2) cell shape | (1) label-free; (2) high throughput (>$10^{10}$ cells/h) | (1) physical and biological differences can be too subtle; (2) requires significant dilution of samples | |
| hydrodynamic sorting | (1) cell size; (2) cell shape | (1) label-free; (2) high throughput (>$10^{9}$ cells/h) | (1) physical and biological differences can be too subtle; (2) requires precision machining and precise flow control | |
| filters | (1) cell size; (2) cell deformability | (1) label-free; (2) well understood mechanism; (3) trapping of cells in device | (1) prone to clogging; (2) very precise control of the channel geometry (nm range); (3) very low throughputs (>$10^{4}$ cells/h) | |
| optical microfluidic separation (non-FACS) | (1) cell size; (2) intrinsic optical characteristics | (1) label-free; (2) high sample resolution; (3) trapping of cells in device | (1) low optical characterization of most cells; (2) very low throughput ($10^{3}$–$10^{4}$ cells/h) | |
| acoustophoresis | (1) contract factor (cell density and compressibility) | (1) label-free; (2) gentle on cells | (1) most cells have contrast factors of the same sign; (2) medium to high throughput (>$10^{7}$–$10^{8}$ cells/h) | (1) "-omic" analyses |
| dielectrophoresis | (1) cell dielectric properties; (2) cell size; (3) electric field parameters | (1) label-free; (2) size independent | (1) biological basis underexplored; (2) potential differences can be too subtle; (3) low to medium throughput ($10^{6}$–$10^{7}$ cells/h) | |
| adhesion-based microfluidics | (1) cell surface marker expression; (2) antibody/ligand binding kinetics; (3) cell interaction with surface | (1) highly specific separations; (2) allows for control of fluidic shear forces; (3) trapping of cells in device | (1) requires cell-specific marker; (2) dependent on antibody-ligand specificity; (3) lack of a standard detachment method; (4) very low throughputs ($10^{5}$–$10^{6}$ cells/h) | (1) tissue engineering; (2) diagnostics and therapeutic monitoring |
| fluorescence-activated cell sorting (FACS) | (1) optical signal intensity and/or morphological features | (1) gives spatially specific information; (2) identifies complex/subtle phenotypes | (1) often requires exogenous labeling; (2) trade-off between speed and resolution; (3) time consuming labeling; (4) low throughput $10^{7}$ cells/h | |
| magnetic-activated cell sorting (MACS) | (1) magnetic field strength; (2) cell surface marker expression; (3) magnetic label binding kinetics | (1) can be highly specific depending on target ligands | (1) often requires exogenous labeling; (2) time consuming labeling; (3) medium throughput $10^{9}$ cells/h | (1) diagnostics and therapeutic monitoring; (2) "-omics" analyses |



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isolation platforms generally take advantage of unique antigen expression patterns on cells to effectively separate.[12,16,17] It is well-known that cell populations each have a unique "fingerprint" that can be essentially used as a way to identify it within a heterogeneous suspension. Techniques like FACS and MACS label cells with either fluorescent or magnet tags, respectively, to allow for separation. Adhesion-based techniques use the advantageous surface-to-volume ratios of microfluidics to adhere cells, via the same antibody−antigen links as FACS and MACS, within the channel.[18] More details on these techniques will be covered in the next section, but it is clear that researchers now have several tailored tools and methods to separate a desired cell population.

In our view, the first application in which microfluidics has and will make a large impact will be as a preprocessing step in the workflow of numerous biological assays. Proteomics,[19] genomics,[19,20] tissue engineering,[21] and induced pluripotent stem cell research[22] are adopting microfluidics as a means of isolating and probing key rare cell populations. As microfluidic cell separation continues to illustrate its utility, application areas are predicted to continue to grow in both number and complexity. In the next five years, as the efficiencies of microfluidic cell separation devices with biologically relevant samples (e.g., blood, lymphatic tissue, tissue digestates) increase, whole cell diagnostics and therapeutic monitoring will start to emerge in the clinic. On the other hand, in ten years, with higher purity separations in microfluidic devices, the availability of adult stem cells for regenerative medicine and autologous stem cell transplants will likely increase. Finally, 20 years from now, we predict that home-based therapeutic monitoring and diagnostics will become a reality as packaging and miniaturization of these technologies improves.

The remainder of this Perspective article discusses how emerging approaches will enable more application-specific isolation methods. Below, we briefly survey the relatively large range of current microfluidic cell separation technologies to illustrate that there is no "one size fits all" isolation method and that microfluidic platforms need be tailored to companion techniques and sample types. We also discuss prospects of commercializing microfluidic cell separators and discuss future opportunities as well as challenges.

## ■ ADVANTAGES AND LIMITATIONS OF THE CURRENT STATE OF THE ART

As introduced previously, cell isolation in microfluidics is generally confined to either technologies based on physical differences or biochemical differences in a heterogeneous cell suspension. Each specific modality has characteristic advantages and shortcomings as outlined in Table 1. Although no specific standard has been established to define a "good" microfluidic separation platform, the best microfluidic devices to date have illustrated high recovery of the target cells while minimizing nontarget, or interference, cells form the collected stream. We have broadly characterized these metrics via qualitative metrics, rather than quantitative evaluation. For a more quantitative assessment, there are a number of excellent reviews on microfluidic cell separation that provide examples of high recovery and high purity platforms.[10−12,16,18] Another metric that is important, especially in cases of larger volume samples, is the ability to maintain a high throughput, thus reducing the time necessary for the isolation. Microfluidic devices, based on their inherent micrometer dimension, have very low overall volumes (nL−$\mu$L) and thus require high throughputs in order to process high volume samples. This fact is most evident for rare cells, where samples like blood with >$10^9$ cells per mL need to handle several milliliters of concentrated samples in a minimal time. Much of the current literature in rare cell handling, particularly in circulating tumor cells (CTCs), has illustrated >100 $\mu$L/min flow rate ($10^8$−$10^{10}$ cells/h) throughputs, and thus, milliliter handling is no longer a constraint in rare cells. That being said, there are several approaches (adhesion-based, dielectrophoresis, optical/FACS) that have inherently low ceilings with respect to throughputs based on fundamental limits imposed by the either the chemistry and/or physics of the platform. As noted in Table 1, the approaches that have the high throughputs are MACS and many of the physical isolators, with trade-offs in other metrics. As introduced in Table 1, the weight of the advantage and limitations results in specific modalities of isolation being suited for key application types. As an example, the attachment of magnetic beads to the target cells of interest is not a disadvantage in diagnostics, but it is a potential barrier in regenerative medicine. Separations based on physical features, such as size, density or, dielectric properties, are universally limited by the need for the target cells to possess a distinguishing parameter versus background, nontarget cells. For example, diluted whole blood is an excellent candidate for most size-based microfluidic separation of red blood cells from nonred blood cells, as the erythrocytes have unique shape and size,[23] but to separate rare cell types within the nonred blood cell population, no passive technique is a viable methodology, as there is almost always an overlap in the characteristic size, shape, dielectric properties, or compressibility of the white blood cells and the rare target cells. Due to the crowding and elastic collisions in dense biological samples like blood, 5−100× dilutions are generally needed to accomplish efficient separation metrics; the one exception is the separation of blood based on the natural tendencies of red blood cells to marginate from white blood cells in microchannels.[24] Davis et al. noted that with whole blood in deterministic lateral displacement there is hydrodynamic coupling of the cells to each other, entrapping a small cell in a larger cell's flow field and preventing separation of the small one from the large one and entropic effects by which larger cells are moved by depletion forces to the outside of a channel.[23] In cases where the target cell can be individually selected on the basis of size, density, shape, or other physical parameter, these platforms are preferred where high purity is desired.[11,25] Due to the variability in cell characteristics and limitations on separation resolution, i.e., the smallest difference in parameters possible to isolate the cell, efficiencies are generally less than 90%,[26] defined as the number of target cells captured over the total target cell number in the cell suspension. These platforms are also label-free and tend to provide higher throughput enrichments (>$10^8$ cells/h).[11] There are numerous examples of physical-based cell separation platforms that illustrate poor purities, but we believe that the technique of physical isolation works well to achieve high purity purification.[27−29] However, the application (and selection of parameters) of a physical constrained enrichment is very often poorly implemented. There are have been many reports of size-based separation of CTCs from whole blood, but it has been well noted that several CTCs are of similar diameter to white blood cells, thus raising the issue of false estimations of CTC counts blood and causing erroneous prognosticative results.[30]

In contrast to separations based on physical cell dynamics, biochemical or affinity-based approaches are considered size


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and shape independent; although in the case of MACS, drag forces do play a role in the isolation.[31] Affinity-based enrichment distinguished cells are based on specific antigen expression methodologies, where the target cells are either labeled with a fluorescent/magnetic bead tag or directly captured on a stationary substrate. This approach allows for much higher efficiencies (<95%) than label-free approaches but necessitates a way of removing the cells from the bound tags or cell release from substrates. To date, there remains a lack of standardized tag removal processes from cell surfaces[32] although multiple methods have recently been developed to release captured cells from adhesive surfaces. Traditionally, fluidic forces and enzymatic treatment for cell detachment is the most common. The process of shearing the cells off the surface poses the issue of possible cell damage and subsequent reduction in viability. Enzymes digest extracellular matrix (ECM) proteins thus causing cells to be released. This treatment can be invasive as cell surface proteins (ion channels, receptors, cell-to-cell junction proteins, etc.) are also digested. Alternatively, temperature induced cell detachment methods, based on the fact that ECM generally adheres to a hydrophobic surface rather than a highly hydrophilic surface, have been engineered. Although the microfluidic community understands the need for noninvasive cell harvesting methods, only a few works in electrically induced,[33,34] pH change-induced,[35,36] degradable hydrogel coatings,[37−40] and light-induced methods[41,42] have attempted to address this issue. An excellent review by Zheng et al. describes these approaches in detail.[43] Recently, work in aptamer designs has also illustrated some promise as an alternative approach to releasable functionalities.[44]

Furthermore, to ensure high fidelity isolation, the selection of the capture ligand is paramount to target the cells of interest.[18] Looking once again at the example of CTC separation, most literature reports have used EpCAM as the capture and/or labeling antibody.[8,45] Although EpCAM does serves as an excellent identifier of an epithelial cell (or carcinoma cell) in the bloodstream, CTCs upon detachment from the primary tumor undergo an epithelial-mesenchymal transition (EMT).[8,45] EMT results in a loss of EpCAM expression and reduction in CTC enumeration and diagnostic efficacy. Therefore, CTC separation presents a clear example of why there is no "one size fits all" isolation method and that microfluidic platforms need be specifically tailored to the overall objective. By selecting a marker for a specific tumor type, such as PSMA for prostate cancer,[46,47] affinity-based microfluidic platforms have shown excellent promise. On the other hand, cancers with a family of different receptor expressions, such as breast cancers with Her2+ and Her2− populations or the various lung cancer phenotypes,[48] are best isolated using size-based separation. Again re-enforcing the claim of no "one size fits all" isolation method.

Finally, an additional limitation to affinity-based platforms is the lower throughput due to receptor−ligand kinetics and/or instrument limitations (e.g., magnetic strength and fluorescent interrogation constraints). A concern related to the binding kinetics is the effective coating of these adhesion ligands (i.e., antibodies, peptides, aptamers) to the substrate. In addition to reaction rates, the number of binding events directly influences the enhancement of the affinity-based cell sorting. Compounding the challenge is the randomness of biomolecule orientation; for IgG antibodies, there are two binding regions located on the molecule, but positioning these regions in such a fashion as to minimize steric hindrance is difficult. As described by Lauffenburger and co-workers,[49,50] ligand coupling to a surface also results in a loss in the protein activity. When coating substrates (beads or surfaces) with antibodies, specifically, it has been shown that no more than one antibody-active site is available and within those accessible sites only 20−50% of those sites are actually active binding ligands. Overall, this can result in low binding event efficiencies and large amounts of unused and costly proteins.

The above brief review illustrates why no "killer" separation platform has been demonstrated and we believe, on the basis of the current literature, that a truly universal cell separation device will likely never be engineered. As one example, devices that aim to separate cells directly from small volumes of whole blood must factor in that blood behaves as a non-Newtonian fluid.[51] Such a device would not work as efficiently in the separation of lymphocyte populations from ophthalmologic vitreous biopsy samples, which tend to have volumes >20 mL and are mainly composed of Newtonian buffer wash solution.[52] The unique constraints of the myriad of applications where cell separation plays a role along with metrics unique to each discipline make it difficult to design truly universal separation systems. Furthermore, even with the new advances in multicomponent cell separation instruments, as described later, there are still no approaches that we see addressing the various biological fluids, throughputs, and conditions necessary to make the holy grail of cell separators.

## MICROFLUIDIC CELL SEPARATION AS PREPROCESSING TOOLS TO ADVANCE COMPANION TECHNOLOGIES

As mentioned earlier, microfluidic cell separation techniques stand to play important roles in analytical (e.g., proteomics, genomics) and clinical applications (e.g., diagnostics, tissue engineering, and stem cell therapeutics). We term the operative technologies associated with these applications as "companion technologies". In the clinical realm, there have been a number of recent diagnostic assays developed on the basis of specific target cell populations such as CTCs for metastatic cancer,[30,53] mature endothelial and endothelial progenitor cells for cardiovascular disease,[54,55] fetal maternal red blood cells,[56] and malaria diagnostics.[57] A fact unique to diagnostics and therapeutic monitoring is that purity can and is generally secondary to efficiency. Still, unfortunately, most of the current diagnostic platforms have failed to meet all the efficiency requirements for clinically relevant separation and enumeration tools. As an example, the clinically defined threshold for CTC enumeration as a biomarker for therapeutic monitoring is 5 cells/mL whole blood.[53] This essentially equates to finding 1 target cell in a billion nontarget cells. Here, it is imperative that every cell is isolated from the sample, but in these cases, purity is not as important. This is because, to date, all clinically used platforms use a detailed fluorescent staining protocol to identify the CTCs in the isolate; thus, nontarget cells can be easily excluded from the biomarker assay. The sacrifice of a high purity in favor of extremely high recoveries will continue to be imperative for microfluidic separation to succeed in the clinic. On the other hand, one of the key challenges associated with tissue engineering and regenerative medicine is isolation of pure populations of cells, where efficiency is considered secondary. Importantly, minimization of nontarget cell populations is critical for tissue cultures that are intended for implantation. In addition, the presence of surrounding nonspecific cell

types may influence the cell differentiation path; thus, pure populations are necessary for controlled differentiation.[58] High purity isolations are also significant in -omic analyses (e.g., genomics and proteomics), and fundamental biological assays as nontarget cells result in unwanted background noise or interference.[59] On the basis of the previous section and Table 1, platforms based on physical characteristics can be tuned to provide high purity isolations but have not yet reached efficiencies capable of a clinical diagnostic. Biochemical affinity-based approaches, based on highly specific antigen targets, can yield high efficiency separations but are inherently limited by the interference of the labeling moieties, i.e., the receptor−ligand binding itself can perturb the cell.[60,61]

Thus, the selection of a microfluidic separator should be specifically tuned to the intended application. The preferred devices for most fundamental biological assays, including proteomics and genomics, are label-free to minimize labeling interference, but in -omic analysis, because cells can be lysed after purification, magnetic bead labels can be easily separated from the lysate. Conversely, in both FACS and affinity-based separation, it is a significant challenge to separate the bound species (receptor−ligand) from the unbound solution species. This inherent limitation is also true of other fundamental biological assays based on lysed cells, where the binding chemistries cause interference in the analyses.

Of the many separation platforms, magnetic beads can successfully provide high purity isolation but the beads pose a challenge in regenerative and tissue engineering applications due to generation of reactive oxygen species (ROS)[62−64] and gene expression changes.[65] When cells are exposed to metallic nanoparticles, ROS are produced with high chemical reactivity. High ROS levels can damage cells by peroxidizing lipids, disrupting DNA, modulating gene transcription, altering proteins, and resulting in a decline in physiological function and cell apoptosis/death. On the other hand, FACS and affinity-chromatography moieties can be internalized and thus do not perturb the viability and/or function of the cells. It should be noted that the presence of bound species could result in a signaling cascade that will change the cells. This limitation also goes for passive separations due to high physical forces, dielectric stimulation, and even magnetic influences on some cells of interest. This presents a chicken-and-egg problem of sorts wherein to separate a cell type one must change the cell environment, at least minimally, but such a change in the environment may affect the cell undesirably from the standpoint of the final application. Two recent papers by Vickers and co-workers[60,66] have illustrated this principle, where the authors show that a brief shear exposure can change the receptor expression pattern of a cell[60] and that the act of labeling cells with a ligand can cause receptor recycling as well as expression changes.[66] Without flow or receptor tagging, cells can generally not be effectively separated; however, the above studies illustrate the need to verify or control for changes in key cell properties, such as the receptor expression profile or even overall phenotype.

In diagnostics and therapeutic monitoring, the number of cells indicates the presence, change, or remission of a disease state. High efficiency (i.e., every cell must be accounted for) is needed. In most cases, staining[67] or impedance[68] can be used to enumerate the cells of interest, but all the cells of interest must be isolated and concentrated for this to be of value. Therefore, affinity-based techniques that provide high efficiencies are ideal candidates for diagnostics. Techniques like adhesion-based separation[38] and FACS[4] also allow for the identification of cells based on multiple marker expressions, such as distinguishing mature endothelial cells from endothelial progenitor cells based on KDR+ expression on all endothelial cells and expression of CD34+ only on stem cell populations.[38] Separation of cells can be from small volumes of sample (blood drop or biopsy punch) or from very large samples (vitreous humor samples). Therefore, there is a need to be able to concentrate the samples down to a feasible volume for interfacing with companion technologies (LC/MS, PCR, culture plates, etc.). Postseparation, several additional treatments may be needed thus presenting additional challenges to marrying microfluidics to larger instrumentation. Proper buffers and postseparation conditions for accurate analyses will be needed as instrumentation interfacing progresses. Techniques that can trap the cell within the microchannel (filters, optical trapping, and adhesion) or within solution (MACS) are excellent candidates for concentrating, buffer exchanging, and postanalysis treatments. Of course, these statements are contingent on a clean and viable release of the cells or effective collection of lysed cellular products, both challenges in their own right.

Overall, in the arena of supporting and advancing companion technologies, we believe that new microfluidic sample processing tools will emerge with better purity and efficiency capabilities as well as better integration with the companion technology instrumentation. Second, these new platforms will expectedly be tuned to both the companion technology type and the sample type.

## AVENUES AND BARRIERS IN COMMERCIALIZATION OF MICROFLUIDICS-BASED DEVICES

It is clear that microfluidics has a high potential as an enabling technology for multiplexed, preprocessing in a number of key biological areas. Over the past two decades, several attempts from both start-up and larger corporations have emerged to commercialize microfluidic cell separation platforms. These pioneering companies, mostly in the diagnostics field, have argued that microfluidics provides a better methodology for isolation of key cell populations by reducing the device footprint and making the instrumentation more mobile, providing a higher throughput versus the state of the art, and reducing the overall cost of manufacturing. Although all these points are definitely well founded, only a limited number of products have been delivered to date.[69,70] As the need for higher efficiencies and better purities has accelerated, so has the complexity of many of the devices. These overly intricate approaches only confound commercialization strategies, presenting harder manufacturing parameters and lowering the reliability of operation. A second factor that has limited research in microfluidics and, in turn, commercialization has been the funding of platforms that meet these requirements of the industry. Approaches that are simple and robust are often not considered sufficiently "innovative" from the standpoint of bodies that fund academic research. This trend has, in our opinion, led to a growing glut of new technologies that are simply too complex and ineffective to cross the gap between academic laboratories and the realm of robust, affordable, manufacturable, and useful devices.

Currently, end users are facing difficulties interfacing and synchronizing the microfluidic components with existing hardware, and these new techniques challenge current lab workflows, making adoption of these platforms difficult. We believe by approaching microfluidic cell separation from an

interdisciplinary perspective, interfacing of techniques will begin to receive more attention and adoption by the end-users will increase. Overall, as microfluidics becomes more and more commonplace in lab, clinical, and industry settings, these hurdles will naturally diminish, but only time will tell when cell separation will move from tabletop FACS instruments to hand-held sample processing tools.

Analogous to continual improvements being made in conventional FACS technologies and methods, we expect continued evolution in miniaturized analogs of FACS as well as MACS. These miniaturized analogs have the advantage of posing lower barriers of entry to potential users and therefore draw greater levels of activity in both academia and industry. As mentioned in Table 1, a major limitation of both of these techniques is the time and effort involved in tagging specific cell types in a sample with either fluorescent or magnetic tags. Coupling microscale analogs of FACS and MACS with on-chip tagging is a relatively simple step for which approaches have already been developed.[71] Such coupling not only will automate the tagging step but also has the potential to significantly reduce the typical 30 min incubation time requirement. Beyond such comparatively incremental improvements, it is our prediction that major, paradigm-changing innovations in FACS and MACS will be rare.

It has been well noted that material selection plays a large role in commercialization.[72,73] In most academic laboratories, which universally contribute new technology in industry and manufacturing, poly(dimethylsiloxane) (PDMS) is the polymer of choice for research due to its ease of use and cost at the research scale. Researchers commonly require only few to a hundred devices for experiments, whereby industry standards will minimally require hundreds to thousands of devices. Unfortunately, PDMS is not a commercially scalable material and thus can pose barriers to translation, depending on the separation modality of choice. A notable exception, however, are the elastomeric properties of PDMS make it an excellent material for fluid controls via displacement valving and pumping. Rigid polymers, like cyclic olefins and poly(methyl methacrylate) (PMMA), and rigid ceramics, like silica and glass, require high capital cost and complex equipment for fabrication, thus making implementation at the initial research level more prohibitive. It should be noted that, once translated, the cost per device related to rigid polymer fabrication is low and the production line is in place and well established by the DVD/CD industry. Furthermore, chemical treatments and functionalization strategies have been proven for these rigid polymers, thus posing no further barriers.[74] Also, the initial capital cost is not the only metric for commercial translation; one must also consider unit production cost and production rate, as well as production compliance, which rigid plastics can provide via the existing DVD pipeline.

The hydrophobicity of PDMS makes it an excellent candidate for many size-based and adhesion-based techniques as the material properties mitigate nonspecific binding. Materials like glass and silica, on the other hand, have a high tendency for nonspecific binding and require treatments to lower inefficiencies. Other characteristics that need to be accounted for are the electrical and optical properties of the substrate, specifically for dielectrophoresis and optics-based separations. Autofluorescence can interfere with techniques that require probing such as microfluidic FACS. With these few examples, we anticipate the development of new elastic polymer candidates for microfluidics, which have better overall properties and the scalability necessary for manufacturing. We also envision the influence of 3D printing of polymers and ceramics to play a role in advancing microfluidics, as layering and interfacing of numerous materials is possible, thus allowing for tuned substrate characteristics.

As first noted by Adams and Soh,[75] the integration of multiple separation forces gives one the ability to precisely control the cell separation dynamics and can allow for new modalities of separation. For example, several groups have harnessed sized-based separation platforms to effectively remove red blood cells from whole blood starting samples in order to enhance purity and recovery metrics,[76−78] and others have used combinations of other modalities, such as multistage adhesion-based separation.[38] Overall, relative to single stage isolation methods, cooperative platforms that utilize multiple separation modalities can typically provide superior recovery and purity. We propose that, as the need for lower cell numbers and more accurate separations are needed, approaches combining more than one separation modality will be the optimal means to achieve these metrics. We anticipate a greater focus on microfluidic separation platforms that combine two or more separation techniques, offering the opportunity for new scientific principles to be established in addition to new technology.

Within the technological realm, integration of the non-microfluidic elements of cell separation systems remains a vexing challenge; however, a trend has been established with recent journal publications describing the design and assembly of an integrated "box", i.e., a console which accepts the starting sample, performs a microfluidic cell separation or sorting process, including all sample and reagent transfer steps, and reports a result or delivers a purified cell subset.[79,80] While this growing trend addresses questions that inevitably arise about translational potential when any new microfluidic cell separation methodology is developed, it worth pausing to ask if such work belongs within the scientific domain as opposed to that of commercial product development.

## CONTINUED EVOLUTION OF SEPARATION SCIENCE IN MICROFLUIDIC CELL SEPARATION

The evolution of separation science, including that of cell separation, has been guided by the emergence of analytical challenges and companion technologies in a broad range of fields such as clinical medicine, biology, proteomics, and genomics, to name only a few. However, a considerable gap exists between research communities in microfluidic cell separation and our counterparts in these other disciplines. It is our view that major advances in cell separation technologies will require a much closer interaction between these communities. In the area of proteomics and genomics, for instance, major opportunities exist for the miniaturization and integration of sample preparation steps in order to handle limited amounts of clinical samples. Microfluidic cell separation methods can serve as the true front-end of such integrated sequences provided they are integrated effectively with downstream processing steps.

For example, as stratification of patients for customized therapeutic regimens becomes more common, proteomic profiling of specific cell types in a biopsy may be critical. Achieving such capability will require innovations in both technology and manufacturing. Challenges such as concentrating the samples without sample losses, subsequent cell lysis, and interfacing with mass spectrometry instrumentation will all have to be overcome in a manner that, once again, is tailored to a

particular disease or therapy. Furthermore, with the ever-increasing push for fast, cheaper, and smaller -omic platforms, microfluidic components will inevitably provide the foundation of these techniques both on the sample (front) end and in intermediate steps that relate more to the companion -omic instrumentation.

On the basic science side, while single cell genomic technology has advanced significantly with regard to sequencing and informatics, there is currently no directly interfaced cell procurement and purification. As the need for specific cell analyses progresses, not only will microfluidics be required to individually entrap single cells but also a robust targeted separation method must be integrated with current technologies. This poses a challenge in the context of a broader "smaller, faster, and cheaper" mindset in necessitating a multidisciplinary development scheme. Finally, we also believe that insights into new and atypical cell separation modalities may be revealed from the perspective of biology, for example, separations based on cellular secretions, intracellular signaling activity, and in vivo cell migration processes such as chemotaxis.

## FUTURE MICROFLUIDIC CELL SEPARATORS

Microfluidic systems that handle and separate cells have a unique ability to probe scientific questions outside the domain of separation science. The combination of predictable fluid shear properties, surface topography and chemistry, and microchannel geometry have, for example, been utilized in studies of hematopoiesis[81] and angiogenesis,[82] to name only two examples. Combining microfluidic interrogation of single cells using approaches described in the preceding sections offers opportunities to apply big data principles in collecting and handling information collected by integrated microfluidic cell separation systems. Thus, we anticipate that major advances in the science of microfluidic cell separation will occur concurrently with advances in companion areas. As more and more such interdisciplinary studies are performed and described in journals spanning many disciplines, however, there will be an increasing risk of "reinventing the wheel", in other words performing studies and building devices that are similar to those already described.

## CONCLUSIONS

While microfluidic cell separation has reached a point of relative maturity, major challenges and significant barriers to the broad adoption of existing techniques continue to exist. It is our prediction that activity in this field will shift from the focus on individual modes of separation demonstrated in highly specific contexts to multimodal microfluidic cell separators that are well-integrated with companion analytical technologies and downstream applications in the clinical and analytical realms.

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### Notes

The authors declare no competing financial interest.

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