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## Rare cell capture in microfluidic devices

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### ABSTRACT

This article reviews existing methods for the isolation, fractionation, or capture of rare cells in microfluidic devices. Rare cell capture devices face the challenge of maintaining the efficiency standard of traditional bulk separation methods such as flow cytometers and immunomagnetic separators while requiring very high purity of the target cell population, which is typically already at very low starting concentrations. Two major classifications of rare cell capture approaches are covered: (1) non-electrokinetic methods (e.g., immobilization via antibody or aptamer chemistry, size-based sorting, and sheath flow and streamline sorting) are discussed for applications using blood cells, cancer cells, and other mammalian cells, and (2) electrokinetic (primarily dielectrophoretic) methods using both electrode-based and insulative geometries are presented with a view towards pathogen detection, blood fractionation, and cancer cell isolation. The included methods were evaluated based on performance criteria including cell type modeled and used, number of steps/stages, cell viability, and enrichment, efficiency, and/or purity. Major areas for improvement are increasing viability and capture efficiency/purity of directly processed biological samples, as a majority of current studies only process spiked cell lines or pre-diluted/lysed samples. Despite these current challenges, multiple advances have been made in the development of devices for rare cell capture and the subsequent elucidation of new biological phenomena; this article serves to highlight this progress as well as the electrokinetic and non-electrokinetic methods that can potentially be combined to improve performance in future studies.

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### 1. Introduction

The isolation, fractionation, and capture of cells from suspensions has a wide range of applications, from the detection of bacteria (Liu et al., 2007a; Wu et al., 2009) to the enumeration of cancer cells (Gascoyne et al., 2009; Chen and Du, 2006; Nagrath et al., 2007). The benefits and limitations of flow cytometers, immunomagnetic separators, and other macro-sized sorting equipment have been studied extensively in experimentation and in review (Pappas and Wang, 2007; Chen et al., 2008; Kulrattanarak et al., 2008) when compared to the abilities of microdevices. This article focuses on devices and techniques with potential to analyze cells that are typically found at low concentrations in suspension; such devices are currently used, or have the potential to be used, for applications in environmental pathogen detection (Lapizco-Encinas et al., 2005; Yang et al., 2006) and cancer cell isolation from patient blood samples (Gleghorn et al., 2010). The discussion is divided into sections that detail two major classifications of microfluidic approaches,

non-electrokinetic and electrokinetic, followed by a summary of performance criteria by which these methods are evaluated; studies that focused on quantifying these performance specifications are highlighted in tables at the end of the article. While rare cell capture is the ultimate motivation of this paper, many of the described methods exist only as proof-of-concept studies. Thus, this article serves to highlight both the progress made in using microfluidic devices for rare cell capture and the techniques that may contribute to rare cell capture in the near future.

### 2. Non-electrokinetic methods

This section focuses upon non-electrokinetic methods of cell isolation, capture, or fractionation from a suspension. As such, it lends itself naturally to organization by sorting technique: antibody capture, size-selective sorting, streamline focusing, and sheath flow. Each sorting methodology is further subdivided into cell separations of interest: blood cell fractionation, cancer cells, other mammalian cells, and prokaryotes and viruses.

Blood cell fractionation, as defined here, focuses on isolation of cell types native to circulation. Most of the studies described here revolve around the capture or elimination of white blood cells

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(WBCs). WBCs are of value in many diagnostic assays and studies of disease progression, but they must first be separated from the bulk blood suspension. However, WBC concentrations are low as compared to red blood cells (RBCs), roughly 1 to 1000 (Murthy et al., 2004; VanDelinder and Groisman, 2007). Conversely, for the purpose of leukemia treatments, blood transfusions, etc. it is vital to eliminate WBCs as a source of contamination (Sethu et al., 2005).

Studies for the isolation of cancer cells focus on capturing circulating tumor cells (CTCs) or approximating them with model cell lines. CTCs can be found in the circulation of cancer patients (Nagrath et al., 2007; Gleghorn et al., 2010; Stott et al., 2010) and have been used as prognostic indicators of patient survival (Danila et al., 2007) as well as representative tissue for genetic analyses (Stott et al., 2010). CTCs are  $10^6$  rarer than WBCs, making their capture particularly challenging (Nagrath et al., 2007; Adams et al., 2008; Gleghorn et al., 2010).

Non-electrokinetic microfluidic techniques have also been applied to study other mammalian cells. Applications are quite disparate, ranging from sorting of cells based on stages of cell cycle (Choi et al., 2009) to isolation of fetal nucleated red blood cells (nRBCs) from maternal blood (Huang et al., 2008; Mohamed et al., 2004, 2007).

### 2.1. Immobilization via antibody or aptamer chemistry

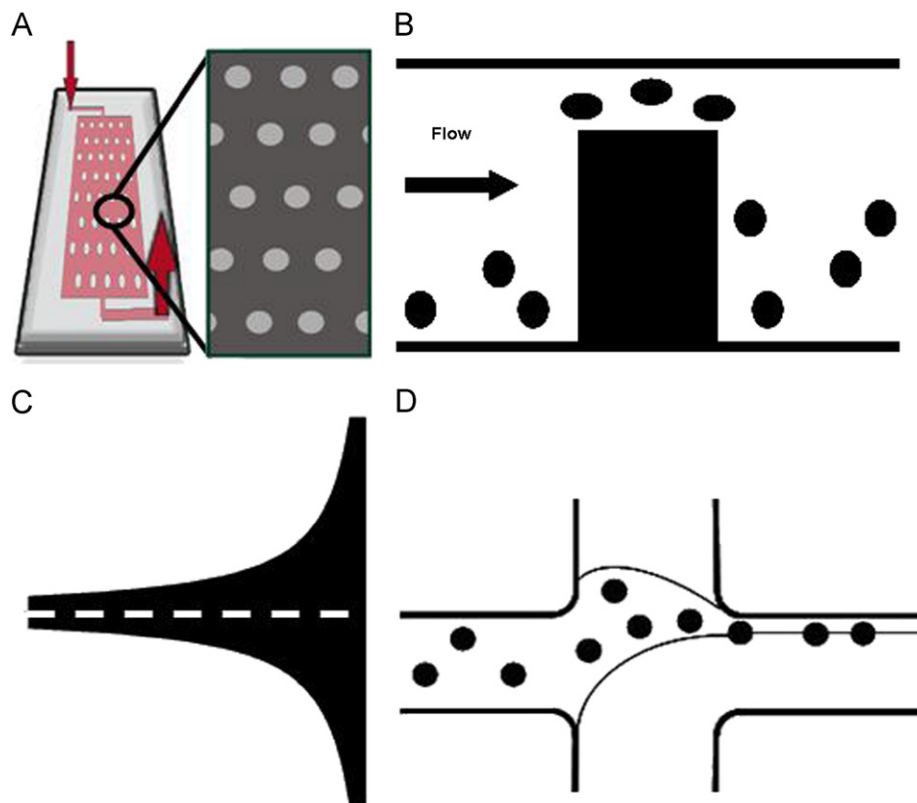
The microfluidic devices discussed in this section take advantage of biochemical interactions to enhance rare cell capture or fractionation. Immunocapture is a technique frequently used in the extraction of cells, viruses, and proteins from suspension. It employs anti-sera to target biological agents of interest. In rare cell isolation, immunocapture presents an

opportunity to separate cells with extremely high specificity from a suspension, in a viable state. In practice, this technique is analogous to microscale affinity chromatography for cells possessing unique markers or characteristics (Plouffe et al., 2007).

#### 2.1.1. Blood cell fractionation

Chang et al. studied the effect of microfluidic structures on white blood cell (WBC) adhesion using different pillar geometries and orientations. They compared square and rhombic arrays with square and ellipsoidal micropillars, respectively. The micropillars were physisorbed with E-selectin to identify different leukocyte model cell lines (in cell media) via adhesive rolling speeds. Cell rolling velocities were two times as high in rhombic arrays, resulting in 130- to 160-fold enrichment, as opposed to 200-fold enrichment in square arrays. By comparing microarray geometries under identical flow and immunocapture conditions, Chang et al. demonstrated that the type of pillar geometry alone influenced cell adhesion mechanics and, by extension, isolation (Chang et al., 2002).

In contrast, Murthy et al. focused upon the effects of shear stress on leukocyte adhesion mechanics. They studied the effects of shear stress using a Hele–Shaw flow cell with a device geometry that created a linear variation in shear stress along its axis (see Fig. 1C). The researchers used anti-CD5, anti-CD19, and PEG to isolate T- and B-lymphocytes from a heterogeneous PBS suspension. Non-target cells were depleted from heterogeneous mixtures, resulting in suspensions that were 97% pure (Murthy et al., 2004). Sin et al. extended this work to blood, and studied the effects of suspension density on cell binding and the time-scale of cell-antibody kinetics. Within 3 min they obtained 100% and 75% pure suspensions of T-lymphocytes and B-lymphocytes, respectively (Sin et al., 2005). Wang et al. also captured T-lymphocytes using anti-CD3-coated



**Fig. 1.** (A) Schematic of a micro-pillar device's architecture. Adapted from Gleghorn et al. (2010). (B) Schematic of a Weir microfilter's operation. Adapted from Ji et al. (2008). (C) Example of a Hele–Shaw flow cell where the dotted line is the region of linearly increasing shear. (D) Schematic of a sheath-flow based separation system. Adapted from Wu et al. (2009).

micropillars. They surrounded their pillars with segmented curved walls to increase the range of shear stresses experienced by the cells. Using this technique, they were able to isolate T-lymphocytes spiked in blood with 80% efficiency (Wang et al., 2010). These studies, in combination, demonstrate that the efficiency and specificity of cell immobilization can be altered by changing the flow conditions within the microfluidic device.

### 2.1.2. Cancer cells

Many microfluidic devices take advantage of the 3D structure of channels to increase the surface area available to be coated with the antibody or aptamer of choice. Du et al. demonstrated the efficacy of this technique in straight microchannels by differentially capturing human mammary epithelial cells and breast cancer cells by use of epithelial membrane antigen (EMA) and epithelial growth factor receptor (EGFR) (Du et al., 2007). The sensitivity of capture to antibody dilution alone was also demonstrated using the same device geometry. Using this isolation technique, their capture rates from a PBS suspension ranged up to 30%. Xu et al. used DNA aptamers within an S-shaped microfluidic device (Xu et al., 2009) to capture cancer cells from PBS. Using aptamers targeted to various leukemia and lymphoma cell lines, their device efficiencies ranged from 50% to 83% with 88–97% purity. Recent work by Wang et al. on silicon nanopillars (SiNPs) indicated that the topology of the microdevice itself may contribute greatly to the efficiency of rare cell capture. Comparing EpCAM functionalized SiNPs and flat surfaces, there was an approximately 6-fold increase in capture efficiency, from 4–14% to 45–65% (Wang et al., 2009).

Cancer cells have also been captured from blood-based systems. Liu et al. used nickel micro-pillars to immobilize functionalized superparamagnetic beads to create a capture zone within their microfluidic devices. Using magnetic fields, they then immobilized and released an immortalized lung cancer cell line mixed with human RBCs. This method produced 133-fold enrichment with 62–74% capture efficiency (Liu et al., 2007a). Adams et al. observed cell margination along the walls of linear channels when working with whole rabbit blood. They hypothesized that this reduced the rate of cell-antibody interactions in their devices (Adams et al., 2008). This phenomenon was no longer seen when straight-walled channels were exchanged for sinusoidally varying ones. In combination with anti-epithelial growth factor receptor (EpCAM) antibodies, Adams et al. achieved immortalized breast cancer cell capture efficiencies of 97%. The device was translated to the capture of model prostate cancer cells spiked in PBS, using anti-prostate specific membrane antigen (PSMA) aptamers with an efficiency of 90% (Dharmasiri et al., 2009).

While the prior studies worked with model cell lines spiked in buffer solution (Du et al., 2007; Xu et al., 2009; Dharmasiri et al., 2009; Wang et al., 2009) or blood systems (Liu et al., 2007a; Adams et al., 2008; Nagrath et al., 2007; Gleghorn et al., 2010), this method has also been used for cancer patient blood samples (Nagrath et al., 2007; Gleghorn et al., 2010). Nagrath et al. used a dense array of micro-pillars coated in EpCAM to increase the number of cell-antibody interactions for a given suspension volume. Using this approach, they isolated lung, prostate, pancreatic, and other cell lines from blood samples with average efficiency and purity of 65% and 50%, respectively (Nagrath et al., 2007). Recently, Gleghorn et al. used computational modeling to design micro-pillar arrays such that cell-antibody interactions were size-dependent. Using microdevices functionalized with anti-PSMA antibodies, prostate cancer cells were captured at efficiencies of 85–97% with purities of 68% (Gleghorn et al., 2010) (see Fig. 1A).

### 2.1.3. Other mammalian cells

Plouffe et al. used previously discussed microfluidic devices (Murthy et al., 2004; Sin et al., 2005) to selectively isolate endothelial cells (ECs) and smooth muscle cells (SMCs) from suspension. They coated their devices with peptides (REDV and VAPG) targeted to ECs and SMCs and investigated binding to target cells as a function of shear stress. Using these peptide sequences, they differentially isolated ECs and SMCs from homogenous and heterogenous suspensions with purities of 86% and 83%, respectively (Plouffe et al., 2007). Plouffe et al. further demonstrated the feasibility of peptide-based capture systems by using a 3-stage isolation system to deplete heterogenous suspensions of ECs, SMCs and fibroblasts (Plouffe et al., 2008). Using this system, they were able to achieve 96% to 99% depletion of the three different cell types with over 97% viability of non-immobilized cells. Their work agreed with results on shear-dependent cell capture discussed previously (Murthy et al., 2004; Sin et al., 2005), showing this relationship to be true regardless of cell type.

## 2.2. Size-based sorting

Size-based sorting affords the ability to capture target cells without knowledge of the target cell's biochemical characteristics. This is an attractive option if the target cell's size is extreme in relation to its medium and also if the cell's properties are not well understood, as opposed to immunocapture, which can be employed regardless of cell size but requires knowledge of a unique cell trait that can be used as a marker. Both methods have been demonstrated to work successfully on non-pretreated biological samples (Sethu et al., 2005; VanDelinder and Groisman, 2007; Nagrath et al., 2007; Zheng et al., 2007; Adams et al., 2008; Gleghorn et al., 2010). Many approaches have been used to attempt size-sensitive isolation, ranging from size-dependent transport through small geometries to size-dependent particle pathlines in open obstacle arrays (Inglis et al., 2008; VanDelinder and Groisman, 2006, 2007; Sethu et al., 2005; Sin et al., 2005; Davis et al., 2006; Ji et al., 2008).

### 2.2.1. Blood cell fractionation

Much research has been done to develop microfluidic platforms to fractionate blood components, particularly WBCs, based on size. Sethu et al. developed a microfluidic diffusive filter for WBC depletion from whole human blood. The system allowed biconcave red blood cells (RBCs) egress from the main device while larger WBCs were retained. The filtration elements were placed on the sides of the main channel to minimize clogging by distributing RBC egress points along the length of the channel rather than focusing them in one area. To maintain equivalent volumetric flow rates in each segment, they used a flared geometry designed using Hele–Shaw flow approximations. Using this diffusive filter technique, over 97% WBC depletion was achieved (Sethu et al., 2005).

Ji et al. reviewed various other microfluidic filtration techniques for the application of WBC depletion. They found that pillar filters and cross-flow filters had high WBC depletion rates and could be used to process large sample volumes (Ji et al., 2008). VanDelinder et al. also investigated cross-flow filters for WBC depletion, and observed that RBC clogging hindered performance. They subsequently attempted WBC isolation using repeated microfluidic array geometries, achieving 98% WBC retention from human blood with no RBC lysis (VanDelinder and Groisman, 2006, 2007).

Davis et al. and Inglis et al. used microfluidic devices featuring pillars. Rather than using the pillars to create microfluidic slits to obstruct larger cell flow, they used the micropillars to create

particle-size-dependent pathlines such that target cells were sorted into predetermined outlet ports based on size alone (Davis et al., 2006; Inglis et al., 2008). Using this technique, Davis et al. depleted lymphocytes and monocytes from blood with 100% efficiency and Inglis et al. were able to separate lymphocytes from diluted blood suspensions with 73% efficiency.

### 2.2.2. Cancer cells

Zheng et al. developed parylene microfilters for the isolation of immortalized prostate cancer cell lines. Using pressure-driven flow to force cell suspensions through a micro-filter, their cell recoveries ranged from 87% to 89% (Zheng et al., 2007). Cells retained on the microfilters were lysed for genomic analysis. More recently, Hosokawa et al. developed microcavity arrays to select for immortalized lung carcinoma cells based size and deformability. Using negative-pressure from a peristaltic pump to draw cell suspensions through the arrays, they achieved 97% capture efficiency and 98% viability. Hosokawa et al. extended this work to breast, colon, and gastric tumor lines with greater than 80% efficiency. Chen et al. used a combination of experimental results and physical modeling to develop a weir filter to selectively isolate cancer cells based upon their deformability (Chen and Du, 2006) (see Fig. 1B). Using a filter fabricated specifically for their model lung adenocarcinoma cells, they were able to achieve over 99.9% capture efficiency from diluted human blood samples.

### 2.2.3. Other mammalian cells

Mohamed et al. also used pillar filters for the goal of isolating fetal nucleated red blood cells (fNRBCs) from maternal blood (Mohamed et al., 2007). The pillars were placed to create successively narrower channels in the device such that cell capture between pillars was a function of size and deformability. RBCs and fNRBCs were isolated from goose blood and cord blood samples, respectively. Mohamed et al. reported no significant clogging using this staged pillar technique; however, blood samples were diluted pre-isolation. Huang et al. separated NRBCs based on size-dependent pathlines as described previously (Davis et al., 2006; Inglis et al., 2008). Their device successfully eliminated over 99% of RBCs; NRBCs were further purified from contaminating WBCs by use of magnetic separation. Huang et al. successfully enriched NRBCs by a factor of 10–20 more than previously reported techniques (Huang et al., 2008).

## 2.3. Sheath flow & streamline sorting

These devices take advantage of low Reynolds number fluid flow associated with the imposition of certain geometries or parallel fluid flows of different flow rates to passively sort or segregate target cells (see Fig. 1D). This is another label-free and chemistry-free method of cell isolation that is most commonly used when size differences between cells are significant and, unlike size-based sorting techniques, only exerts fluid stresses on the cell rather than physical compression through filter elements. However, dense biological suspensions must be diluted to achieve maximum device performance.

### 2.3.1. Blood cell fractionation

SooHoo et al. used a microfluidics-based aqueous two-phase system (ATPS) to enrich leukocytes from blood suspension. Using one stream of polyethylene glycol (PEG) and one of dextran (DEX), with Zap-oglobin as the lysing agent, they achieved 100% RBC depletion from human blood samples (SooHoo and Walker, 2007). Zheng et al. developed devices based on T-shaped bifurcated channels to separate WBCs from RBCs. By adjusting the length of the T-channel, and the vertical distance between upstream and

downstream side walls, cells were directed to different stream lines based on size alone. They were able to separate WBCs from diluted blood with 97% efficiency. However, they found that RBC orientation heavily influenced the segregation of small WBCs from RBCs (Zheng et al., 2008).

### 2.3.2. Other mammalian cells

Kuntaegowdanahalli et al. used spiral microchannels to segregate cells based on size across the width of their devices. Using a five-loop system, they sorted neuroblastoma cells from glioma cells with 80% efficiency (Kuntaegowdanahalli et al., 2009). The cells were then placed in culture and exhibited 90% viability after sorting. Lin et al. used multiple sheath flows in parallel to sort yeast cells from suspension. They used two streams of unequal flow rate to achieve a focusing effect and were able to separate yeast cells with 87.7% efficiency and 94.1% purity (Lin et al., 2009).

In contrast, Choi et al. used a series of slanted microfluidic channels of periodically varying heights to sort cells by cell-cycle phase. The slanted obstacles generated streamlines that diverted cells transverse to the flow, towards the wall of the device. There, the cell-obstacle interactions diverted larger cells out of the transverse streamlines, keeping them near the wall, while smaller cells diverged from the wall (Choi et al., 2009). They achieved lateral separation of  $G_0/G_1$  phase and  $G_2/M$  phase monocyte model cells with over 4-fold  $G_2/M$  cell enrichment.

### 2.3.3. Prokaryotes and viruses

Wu et al. used sheath flows to sort *E. coli* from blood. High concentrations (greater than  $10^8$  cells/ml) of *E. coli* cells were spiked into diluted human RBCs and were enriched 300-fold over the course of separation. They demonstrated a sorting efficiency of 62% and purity of 99.87%. The bacteria were expanded in culture and exhibited over 95% viability (Wu et al., 2009).

In summary, the devices described above all use varying non-electrokinetic techniques to successfully isolate a broad range of cell types. However, despite a variety of isolation mechanisms and microfluidic designs, there is no single microfluidic device that can produce pure cell populations with high efficiency. For these devices to be used for rigorous biochemical and genetic assays, it is essential that a method of high purity, high efficiency capture be found. An additional challenge is that many rare cells of interest (e.g., leukocytes, CTCs, yeast, bacteria) are found in the blood, a dense suspension that often hinders characterization of device performance. For microfluidic devices to reach their full potential as rare cell capture platforms, it is essential that these elements be addressed and improved upon.

## 3. Electrokinetic methods

Electrokinetic methods use electric fields to actuate cells. In microfluidic devices, the two most widespread electrokinetic techniques for manipulating cells are electrophoresis and dielectrophoresis. Electrophoresis refers to net migration due to the action of an electric field on the net free charge of a particle. This technique has been used to study cells at the membrane level (Mehrishi and Bauer, 2002), and methods such as capillary electrophoresis and microfluidic free-flow electrophoresis have been developed to separate different populations of biomolecules, viruses, bacteria, and eukaryotic cells (Kremser et al., 2004; Turgeon and Bowser, 2009). However, as the net charge of a cell's electrical phenotype is often not specific enough to distinguish between a mixture of different cells, electrophoresis has been used minimally as a cell separation technique and is not suited for



applications in rare cell capture. Thus, this review will focus primarily on dielectrophoretic techniques.

Dielectrophoresis (DEP) refers to the net migration of polarized particles owing to interactions with an electric field gradient, and depends on cell wall, membrane, and cytoplasmic electrical properties (Jones, 1995; Kirby, 2010). The DEP force is a direct function of these electrical properties as well as cell size, the electrical properties of the fluid medium, and the magnitude and frequency of the applied electric field; the dependence on this wealth of parameters makes DEP an attractive tool for distinguishing between different cell types (Voldman, 2006; Hawkins et al., 2009). DEP response is classified into two regimes: when particles are more polarizable than the medium, positive DEP results and the particles are attracted to stronger field regions; conversely, when particles are less polarizable than the medium, negative DEP results and the particles are repelled from stronger field regions; the frequency at which the DEP force switches from one regime to the other (i.e., when the force is zero) is termed the “crossover frequency” (Jones, 1995; Morgan and Green, 2002). The sign and magnitude of the DEP force provides the basis for DEP cell separation techniques, and this review will cover the most common device geometries used for these techniques. The scope of this review on DEP methods will be limited to those used for capture, separation, or concentration of bulk cell populations; DEP methods for single cell capture or manipulation are covered in other reviews (Voldman, 2006; Hawkins et al., 2009; Bao et al., 2008; Kang and Li, 2009; Zhang et al., 2010). The DEP methods are organized by the type of device geometry used; each section includes a brief description of the physics associated with the technique and a summary of how it is applied to separate different cell types with a view towards pathogen detection, blood fractionation, or cancer cell isolation. Many DEP experiments have used model systems to characterize geometric performance, or as mockups of rare cell capture

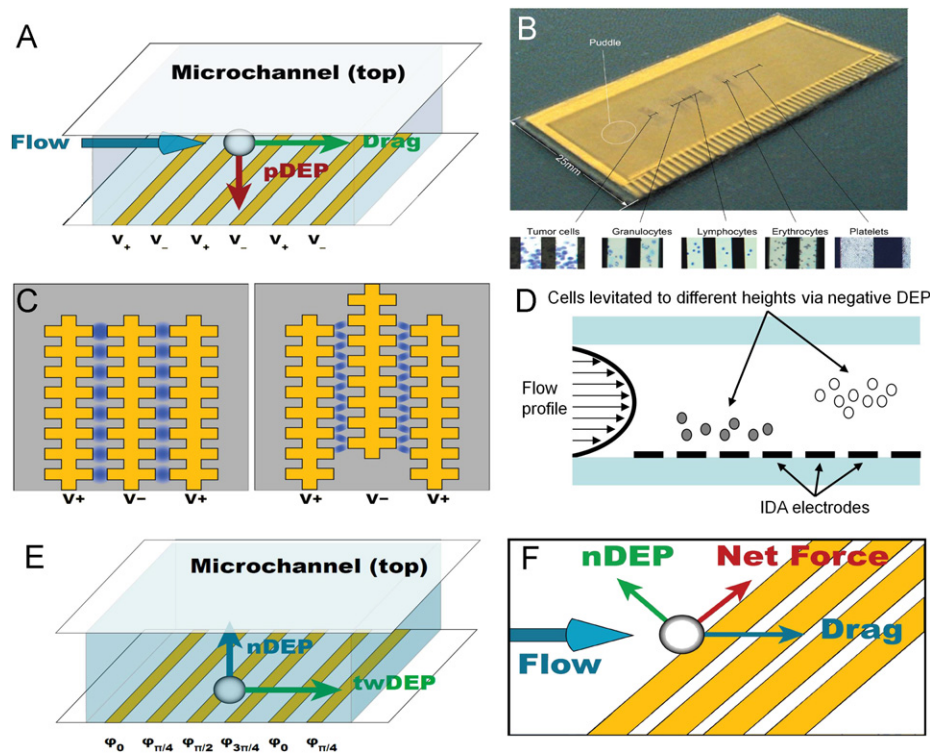
experiments. Thus, this section includes many devices that do not capture rare cells, but whose performance informs the potential for rare cell capture with DEP.

### 3.1. Electrode-based DEP

Microfabricated electrodes are the most common and practical method for creating the non-uniform electric fields necessary for DEP. While potential limitations to the use of electrode-based DEP include fouling and electrolysis at low electric field frequencies as well as increased fabrication time and cost required for more complex electrode configurations, a majority of DEP techniques use microfabricated electrodes owing to their simplicity and flexibility in implementation. The following sections will cover the most common and simple device geometries used for cell separation.

#### 3.1.1. Interdigitated array (IDA) electrodes

Interdigitated arrays consist of spatially alternating sets of grounded and energized electrodes that create non-uniform electric field regions and trap particles against a flow via positive DEP (Fig. 2A). IDA electrodes are one of the most commonly used electrode configurations because they use entails minimal design parameters (electrode length and width, inter-electrode distance, and channel depth) and experimental parameters (flow rate, electric field magnitude and frequency), and yields analytical solutions for electric fields and particle motion (Sun et al., 2007). IDA electrodes are typically used for “binary” cell separation; an electric field is applied to capture the target cells from a mixture of two or more cell types via positive DEP, the non-target cells are minimally affected by the field or repelled via negative DEP and are flushed out of the device, and finally the field is turned off to release the target cells for separate collection. Through DEP



**Fig. 2.** (A) Interdigitated array (IDA) electrodes. (B) Electromembrane slide showing fractionation tumor cells and blood components. Reproduced from Cristofanilli et al. (2008). (C) Castellated IDA electrodes. (D) DEP field-flow fractionation operates by levitating cells against gravity to different heights in the channel via negative DEP, allowing separation to be achieved based on their differing flow velocities. (E) Configuration and forces in a twDEP electrode array. (F) Summation of forces near an angled electrode.

characterization, a frequency regime can be selected in which one cell type is attracted to the electrodes (positive DEP) while another cell type is repelled into the regions separating the electrodes (negative DEP). Rare cell capture requires that all non-target cells be repelled, which can be demanding if the suspension is complex.

IDA electrodes have been used to separate or concentrate bacteria for potential applications in pathogen sensing. Typical cell concentrations used for these studies lie in the range of  $10^5$ – $10^9$  cells/mL. Efforts to detect foodborne pathogens such as those in the genus *Listeria* include separation of live and heat-treated *L. innocua* with 90% efficiency; as the cell membrane becomes permeable upon death, large changes in conductivity can result in differences in the DEP response of live and dead cells (Li and Bashir, 2002). Researchers have also used positive DEP to attract a mixture of *Listeria* and *Escherichia* species to antibody-coated electrodes and selectively capture only *L. monocytogenes* (i.e. immunocapture) with 87–92% efficiency (Yang et al., 2006; Koo et al., 2009). To aid efforts in detecting environmental pathogens, researchers have demonstrated concentration of *Bacillus subtilis* spores (a surrogate bacteria used for research on *Bacillus anthracis*, i.e., anthrax) from airborne environmental samples containing diesel particulate matter with up to 60% purity; appropriate frequency ranges for separation were selected based on crossover frequency measurements (Fatoyinbo et al., 2007). Additionally, Gadish et al. concentrated *B. subtilis* by integration of a chaotic mixer to bring the spores into closer proximity with the IDA electrodes and enrich the sample ninefold (Gadish and Voldman, 2006), and Liu et al. captured *B. anthracis* with 90% efficiency for impedance measurements in order to detect viable spores electrically by their germination (Liu et al., 2007b).

IDA electrodes have also been used for blood fractionation. Cristofanilli et al. used an “electrosmeared” slide that was coated to promote cell adhesion and patterned with IDA electrodes to which different electric field frequencies were applied along the length of the device (Cristofanilli et al., 2008). Near the inlet port, a low frequency was applied to levitate all cells via negative DEP to avoid adhesion to the slide, and as the blood sample (obtained from a murine aspiration biopsy) was flowed further along the device, different constituents of blood as well as biopsied tumor cells (from a cancer line grown in nude mice) were pulled toward and adhered to the electrodes via positive DEP in different regions of the slide, based on their previously characterized dielectric properties (Fig. 2B) (Cristofanilli et al., 2008).

### 3.1.2. Castellated IDA electrodes

Castellated electrode arrays consist of interdigitated electrodes with width variation along their length, which create alternating regions of high and low electric field magnitude at the tips of the electrodes and the regions separating each electrode, respectively (Fig. 2C). The advantage of castellated electrodes is the localization of high electric field regions, which can be used to trap or concentrate flowing cells in the device effectively. The procedure for cell separation using castellated electrodes is the same as that used with straight IDA electrodes; this procedure has been used for binary separation of a mixture of two bacteria types, including yeast, *E. coli*, and *Micrococcus lysodeikticus* (Marks et al., 1994), as well as for separation of viable and non-viable yeast cells (Markx and Pethig, 1995). Optical absorbance of DEP trapping was measured to calculate the effective conductivity of the cells and predict their DEP response.

Castellated IDA electrodes have been used for cell separation between bacteria and blood cells for applications in pathogen

detection, with typical cell concentrations of  $10^6$ – $10^7$  cells/mL; researchers have demonstrated separation of *M. lysodeikticus* from erythrocytes based on their differing dielectric properties (Wang et al., 1993). Isolation of erythrocytes infected with malaria pathogen from healthy erythrocytes was also achieved with 90% efficiency owing to the sharp increase in membrane conductivity of erythrocytes hosting malarial parasites (Gascoyne et al., 2002). In addition, Huang et al. demonstrated simultaneous separation of multiple bacteria (*Bacillus cereus*, *E. coli*, *L. monocytogenes*) from diluted blood with up to 97% efficiency using size-based DEP separation and post-separation PCR analysis (Huang et al., 2003).

Castellated IDA electrodes have also been used for applications in cancer cell isolation. Becker et al. characterized the dielectric parameters of cultured breast cancer cells, lymphocytes, and erythrocytes using particle electrorotation techniques, and subsequently trapped the breast cancer cells from a suspension of diluted blood, demonstrating up to 95% purity in captured cancer cells (Becker et al., 1995). More recently, Tai et al. developed an automatic platform for separation of viable and non-viable cultured human lung cancer cells based on differing dielectric properties with 81–84% efficiency and nucleus collection for nuclear protein extraction (Tai et al., 2007). While castellated IDA electrodes are similar in function and application (e.g., binary sorting) to straight IDA electrodes, their ability to create alternating regions of high and low electric field magnitude makes them better suited for concentrating samples or patterning particles at a specific location than straight IDA electrodes. As is the case for straight IDA electrodes, the challenge associated with castellated IDA electrodes is in finding a frequency or set of frequencies such that only the rare cells are attracted to the electrodes.

### 3.1.3. IDA electrodes for flow-field fractionation

In DEP flow-field fractionation (DEP-FFF), IDA electrodes are fabricated on the bottom of a device channel, and flowing particles of differing dielectric properties are levitated against gravity via negative DEP. The levitated particles equilibrate to different heights in the channel owing to the distinct DEP force on different types of particles, and these differing heights allow separation to be achieved by sequential collection based on different flow velocities due to the parabolic velocity distribution of low-Reynolds-number Poiseuille flow (Fig. 2D). The velocities of different cells can be characterized by measuring cell elution fractograms as a function of frequency (Huang et al., 1999). The main advantage of DEP-FFF is its ability to achieve separation of bioparticles with size and/or dielectric differences under a constantly applied electric field, therefore avoiding the need for activation and deactivation of the field as required by binary sorting devices.

DEP-FFF has been used often as a technique to separate different cell types in blood, with cell concentrations ranging from  $10^5$  to  $10^7$  cells/mL. Researchers have demonstrated separation of erythrocytes from latex beads and characterization of their different levitation heights (Rousselet et al., 1998), as well as binary separation of human leukocyte subpopulations (T-, B-lymphocytes, monocytes, and granulocytes) based on differing membrane dielectric properties with 87–98% purity, which can be used for clinical applications in differential analysis of leukocytes (Yang et al., 2000). More recently, Hashimoto et al. performed selective capture of neutrophils and eosinophils from a mixed leukocyte suspension with 80% efficiency by deflecting the target cells away from the IDA electrodes and toward an antibody-coated layer on the opposite wall (Hashimoto et al., 2009). DEP-FFF also has been used extensively for the separation and isolation of cancer cells. In particular, the Gascoyne research group has

demonstrated separation of cultured human leukemia cells from diluted blood after characterizing the cells by DEP levitation experiments (Huang et al., 1997), separation of cultured human breast cancer cells from whole blood based on measured differences in cell size and membrane capacitance (Yang et al., 1999; Gascoyne et al., 2009), and separation of cultured human breast cancer cells from normal T-lymphocytes and hematopoietic CD34+ stem cells (Huang et al., 1999; Wang et al., 2000), all with efficiencies and/or purities over 90%.

In more recent years, DEP-FFF has been used for a larger variety of applications as well as in different device geometries. These applications include separation of cells with high and low embryogenic potential in suspension cultures of carrot based on their differences in size and cytoplasm density (Falokun et al., 2003), toxicity testing by dielectric characterization of cultured human leukemia cells with membrane dissimilarities due to exposure to various toxic agents (Pui-ock et al., 2008), and enrichment of a progenitor cell population in a mixture of cell debris and erythrocytes from freshly harvested adipose tissue (Vykoukal et al., 2008). Finally, vertical IDA electrodes have been fabricated on the sidewalls of the device channel (as opposed to horizontal electrodes on the bottom of the channel) to achieve lateral separation through separate outlets. This device geometry has been used to separate mammalian cells of different sizes (Wang et al., 2009) and viable from non-viable yeast cells (Braschler et al., 2008), as well as to enrich *Babesia bovis*-infected erythrocytes sevenfold (Braschler et al., 2008). Unlike trapping on straight or castellated IDA electrodes, DEP-FFF allows cells to be separated based on the magnitude of the DEP response rather than just the sign of the response, and rare cell capture can be achieved in theory if the DEP response of a cell can be distinguished within the sensitivity of the device.

#### 3.1.4. IDA electrodes for traveling-wave DEP

IDA electrodes have been used for a technique called traveling-wave DEP (twDEP) to fractionate bioparticles. The electrodes are independently driven with different electric field phases, and particles are levitated against gravity owing to negative DEP (Fig. 2E). Fractionation is achieved by varying the electric field phases to drive the particles transverse to the direction of flow at different velocities. Cui and Morgan detailed the design and fabrication of a twDEP device and demonstrated particle motion using polystyrene latex particles (Cui and Morgan, 2000). The main advantage of twDEP is that fractionation may be achieved based on the particles' differing velocities alone; there is no need to drive fluid flow or to trap or concentrate particles via positive DEP.

Building on the successful implementation of twDEP on polystyrene beads, a number of biological separations have been achieved. Bacteria separation has been demonstrated by use of viable and non-viable yeast cells (Talary et al., 1996; Kua et al., 2007); also, blood fractionation has been demonstrated by separating T-lymphocytes and erythrocytes by applying multiple frequencies to direct the cells to move in opposite directions such that they were collected separately through different outlets (Loire and Mezić, 2003). twDEP has also been used for applications in pathogen detection; a spiral electrode array was characterized and used for a 1000-fold enrichment of malaria-infected erythrocytes from normal erythrocytes with 90% purity (Wang et al., 1997; Gascoyne et al., 2002). Application of the traveling field caused normal erythrocytes to be trapped at the electrode edges via positive DEP, while infected cells were levitated via negative DEP and carried to the center of the spiral (Gascoyne et al., 2002). More recently, Cheng et al. developed a high-throughput 3D twDEP device used for focusing and sorting

particles, and demonstrated its ability to separate bacteria and blood cells based on DEP mobility magnitude as well as direction (Cheng et al., 2009). Other recent studies used twDEP for characterization of cultured lymphoma and myeloma cells for potential applications in rare cell capture (Cen et al., 2004) and the development of a DEP pump for blood delivery in microfluidic devices (Lei et al., 2009).

#### 3.1.5. Angled electrodes

Angled electrodes are most often used for binary separation of bioparticles or to create localized particle pathlines due to the particles' negative DEP mobilities. As the particles approach an electrode, the negative DEP force that acts on them can exceed drag forces, resulting in a net force parallel to the electrodes. Particles then travel along the length of the electrode until drag forces exceed the DEP force, at which point the particles can flow past the electrodes (Fig. 2F). Displacing particles transverse to the direction of flow allows angled electrodes to preferentially direct particles to different outlets or focus them into concentrated streams.

Angled electrodes have been used to sort and concentrate various bacterial samples. Cheng et al. designed a device with 3D electrode gates to focus and separate yeast and *E. coli* into different outlets, after which surface-enhanced Raman scattering was used to detect bacteria concentration and evaluate efficiency (Cheng et al., 2007). Kim et al. tagged *E. coli* with different sized microspheres and used angled electrodes to separate the two target cell types into different outlets, after which capture efficiency and purity was evaluated using flow cytometry (Kim et al., 2008). More recently, a magnetic separation module was incorporated into the device to capture magnetically tagged cells and separate them from unlabeled non-target cells, which improved the device's ability to separate multiple cell types (Kim and Soh, 2009). Vahey and Voldman developed a separation method termed "isodielectric separation," which uses a diffusive mixer to establish an electrical conductivity gradient across the width of a channel containing angled electrodes (Vahey and Voldman, 2008). DEP forces vary along the length of the electrodes, which direct and separate viable and non-viable yeast cells across the device in the direction of decreasing conductivity until they reach their respective isodielectric points, where there is no net force (Vahey and Voldman, 2008).

Angled electrodes have also been used for binary sorting of mammalian and blood cells. To address the need for a noninvasive method for sorting cell populations according to their cell-cycle phase, Kim et al. separated cultured human breast cancer cells based on their differing sizes due to their cell cycle phase (Kim et al., 2007). Angled electrodes were also used to demonstrate a low-stress, size-based, DEP platelet separation technique, separating platelets from diluted whole blood with 95% purity (Pommer et al., 2008).

#### 3.2. Insulative DEP

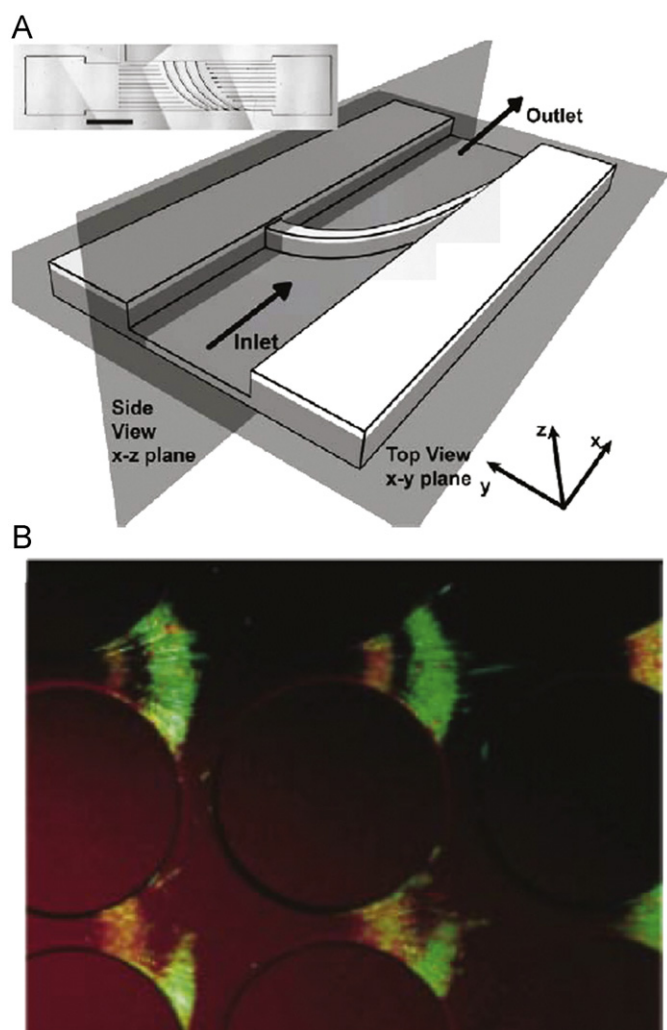
Insulative DEP techniques rely on constrictions or expansions in channel geometry to generate electric field non-uniformities and deflect or trap bioparticles via negative DEP. While this approach places limits on the frequencies and geometries used, the main advantage of insulative DEP is that no internal electrodes are used. This leads to simpler device fabrication, reduced propensity for fouling, and the possibility of using a DC field for electrokinetic particle transport as well as trapping via DEP (Lapizco-Encinas et al., 2004a).



### 3.2.1. Angled and curved constrictions

The simplest geometry in an insulative DEP device is a perpendicular insulative constriction in the device channel. Kang et al. demonstrated size-based separation of live cells by using rectangular constrictions to deflect larger cells (white blood cells and cultured mammalian breast cancer cells) via negative DEP to a different trajectory than smaller blood components (red blood cells, platelets) (Kang et al., 2008). Binary sorting is achieved by fabricating two outlet channels for the separate trajectories.

Extending the basic principles of rectangular constrictions, angled constrictions have also been used to separate and concentrate bioparticles. The DEP force acting perpendicular to the constriction depends on the angle that the constriction forms with the channel. If this DEP force is smaller than the drag force, then particles will flow past the constriction unaffected; if, however, the DEP force exceeds the drag force, then the particles are stopped at (and deflected parallel to) the constriction. Angled constrictions have been used to demonstrate size-based separation of *B. subtilis* from polystyrene particles (Barrett et al., 2005). Curved constrictions, in which the angle of constriction varies continuously across the channel, have also been used to separate different sized particles (Fig. 3A) (Hawkins et al., 2007).



**Fig. 3.** (A) Schematic of curved constriction in channel depth. Inset: top view of device fabricated in Zeonor 1020R polymer substrate. Reproduced from Hawkins et al. (2007). (B) Trapping of live (green) and dead (red) *E. coli* with separation of populations using insulative post array. Reproduced from Lapizco-Encinas et al. (2004a). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.2.2. Post arrays

DEP trapping using an array of insulating posts was reported by Cummings and Singh, who also investigated various geometric variables that affect the electric field, including post shape, distance between posts, and array angle to the applied field (Cummings and Singh, 2003). Using an array of circular posts etched in a glass substrate, researchers at Sandia National Laboratories have demonstrated trapping of polystyrene beads (Mela et al., 2005) and separation of live and dead *E. coli* based on their differing magnitudes in negative DEP response (Fig. 3B) (Lapizco-Encinas et al., 2004a). The group later demonstrated separation and concentration of any two pairs of *E. coli*, *B. subtilis*, *B. cereus*, and *Bacillus megaterium* (Lapizco-Encinas et al., 2004b), as well as tobacco mosaic virus (Lapizco-Encinas et al., 2005). A direct application of this technique is for the detection of microbes in drinking water, which is hindered by current analytical instruments that require significant concentration of microbes in order to detect them (Lapizco-Encinas et al., 2005).

### 3.2.3. Other geometries

A variety of other device geometries have been designed for bioparticle separation and isolation using insulative DEP. Chou et al. used an array of constrictions to trap and concentrate single- and double-stranded DNA (Chou et al., 2002). Pysher et al. designed channel walls with a sawtooth pattern to produce spatially resolved separation of live and dead *E. coli* and *B. subtilis* (Pysher and Hayes, 2007). More recently, Church et al. fabricated a serpentine channel to filter *E. coli* from yeast cells (Church et al., 2009), Cho et al. positioned plastic membranes with honeycomb-shaped pores between electrodes to trap and release *E. coli* in the flow channel (Cho et al., 2009), and Shafiee et al. developed a “contactless” DEP technique to isolate live/dead cultured human leukemia cells by using thin insulating barriers to separate the electrodes used to apply the electric field from the sample channel, thus preventing potential issues such as contamination and bubble formation (Shafiee et al., 2010).

### 3.3. Prospects for DEP rare cell capture

The preceding sections on electrode-based and insulative DEP techniques introduced the most common device geometries that researchers have used to separate different populations of cells. Those studies that focused on quantifying experimental performance criteria such as efficiency, enrichment, and/or purity are summarized in Table 2. Overall, DEP methods are advantageous because they do not require a biochemical labeling step to achieve continuous-flow separation. Additionally, it is possible to achieve DEP cell separation without *a priori* knowledge of the different cells' properties. For binary separation using IDA electrodes, only the frequency range in which the cells experience DEP forces opposite in sign needs to be known; for methods that use angled electrodes or insulative constrictions and techniques such as DEP-FFF and twDEP, only the cells' relative DEP response magnitudes are required to achieve separation of several cell types. As such, DEP offers the ability to isolate single cells (because of its sensitivity to cellular dielectric properties) as well as the possibility for separation of cell populations in which not all cell types have been characterized. In the latter case, DEP potentially can be used to screen for cells with unknown membrane phenotypes, which can facilitate research on bacterial species such as *Mycobacterium tuberculosis*, whose pathogenicity is closely tied to membrane composition (Rhoades et al., 2005).

Using only DEP techniques for rare cell capture in pathogen detection or tumor cell isolation, however, is challenging; studies have reported significant decreases in cell capture efficiency or



purity as target cell concentrations became more dilute (Gascoyne et al., 2009; Fatoyinbo et al., 2007; Gascoyne et al., 2002; Huang et al., 2003). While numerous DEP methods for cell separation of artificial samples have been reviewed in this article, we are not aware of a study that demonstrates strictly dielectrophoretic capture of pathogens from environmental (air or water) samples or capture of viable tumor cells from whole blood of cancer patients. In the future development of rare cell capture microfluidic devices, it may be beneficial to merge DEP methods with techniques such as magnetic-activated cell sorting (Kim et al., 2008; Kim and Soh, 2009) or immunocapture (Yang et al., 2006; Hashimoto et al., 2009). Such hybrid techniques combine the actuation of DEP with the chemical-specificity of immunocapture techniques; a system could be developed in which the applied electric field is tuned low enough to cause no physiological harm to target cells while inducing a strong enough DEP force to cause or prevent interactions with immunocapture surfaces. These synergistic effects have the potential to minimize problems associated with immunocapture techniques (e.g., nonspecific binding) and yield higher performance in rare cell capture efficiency and purity compared to using DEP techniques alone.

#### 4. Performance criteria

In the previous sections, we have described a variety of different methods to isolate a multitude of rare cell types. In this section, we quantitatively compare these disparate studies with a unified set of performance criteria. Comparing the literature systematically identifies the strengths and weaknesses of the field as a whole and provides insights into future research directions. In the following paragraphs, we define performance metrics by which the literature will be evaluated (see Tables 1 and 2) and draw conclusions upon analyzing these criteria. Italics are used to highlight headings in these tables.

When comparing different rare cell capture devices, it is important to distinguish between the *cell type modeled* (e.g., cells obtained from biological samples) and the *cell type used* (often an immortalized cell line). This is imperative when the target cell's biological characteristics are not well understood, e.g., circulating tumor cells. While the use of well-understood model cell lines eases the characterization of device performance, their relations to clinical samples are not always well-defined. Likewise, the *carrier media* used for experimentation is often chosen to simplify device characterization. Many rare cells that are targeted for isolation exist in dense biological suspension when *in vivo*, e.g., blood. However, many such fluids present other cellular material that confound quantification of performance for example, by changing viscous or conductivity properties. For this reason, rare cells are often captured from diluted biological solutions or even buffer solutions. For devices that use DEP methods, the conductivity of the media and the cell concentrations used are also important as these parameters affect the DEP force and capture efficiency or purity, respectively.

A number of quantitative metrics can be used to describe device performance. *Efficiency* is the most commonly used measure of performance in rare cell isolation literature. Efficiency is defined as the fraction of successfully isolated/fractionated cells with respect to the total number of target cells introduced into the device. High-efficiency microfluidic cell isolation devices are often operated at higher volumetric flowrates than high-purity ones, resulting in increased throughput (Gleghorn et al., 2010; Sethu et al., 2005). Another common metric is *enrichment*, the multiplicative factor by which the number of rare cells per unit volume is increased. Depletion, in contrast, operates by capturing

non-target cells within the device, leaving a more pure subpopulation at the outlet (Plouffe et al., 2008, 2007). *Purity* is the number of target cells captured divided by the total captured cell population. Purity is an important metric for measuring the selectivity of a device, but its optimization usually results in lower efficiencies and throughputs. However, high purity samples are desirable for a variety of biomolecular assays and tools. Equally important is the *viability* of cells post-capture. Some devices define viability as the percentage of cells left in a functional state post-capture and others post-culture *ex-vitro*. When comparing results from different methods, it is also important to compare the *number of steps/stages* involved. The possibility of increased performance with multi-stage processing versus the simplicity of device operation are major concerns for devices designed for clinical applications. However, the number of steps/stages was not included for devices that employ DEP methods, as a majority of those listed in Table 1 had similar procedures that include dielectric characterization, cell staining, on-chip capture or fractionation, and post-process cell counting. Given the data in Tables 1 and 2 organized under the headings described in previous paragraphs, we can make a number of observations about rare cell capture in microdevices.

#### 5. Discussion and conclusion

Multiple strides have been made in the enrichment, fractionation, and capture of rare cells. The devices outlined in this review have been successfully used for applications ranging from the enrichment of bacteria to the genetic analyses of cancer cells (Wu et al., 2009; Stott et al., 2010). Microfluidic devices for rare cell capture have elucidated new biological phenomena and afforded multiple avenues of further scientific investigation. Current devices have been successfully implemented in the enumeration of rare cells ranging from NRBCs to CTCs (Huang et al., 2008; Nagraath et al., 2007; Gleghorn et al., 2010); however, the lack of a single microfluidic device that can isolate pure cell populations with high efficiency limits the number of molecular and genetic tools that can be used on these populations.

Additionally, few cell capture studies directly process biological samples (Nagraath et al., 2007; Gleghorn et al., 2010; VanDelinder and Groisman, 2006, 2007). In contrast, most devices spike cell lines into buffer solution (Chang et al., 2002; Murthy et al., 2004; Sin et al., 2005; Plouffe et al., 2007; Zheng et al., 2007; Kuntaegowdanahalli et al., 2009; Choi et al., 2009; Dharmasiri et al., 2009; Xu et al., 2009; Plouffe et al., 2008), or pre-diluted/lysed blood samples (Zheng et al., 2008; Davis et al., 2006; Huang et al., 2008; Wu et al., 2009). Importantly, in devices that employ DEP methods, efficiency and purity performance is low when target cell concentrations are dilute (Fatoyinbo et al., 2007; Gascoyne et al., 2002; Huang et al., 2003; Gascoyne et al., 2009), thus making rare cell capture using DEP techniques alone extremely difficult. In addition, many more cell capture devices approximate the *ex vivo* target with a model equivalent (Xu et al., 2009; Dharmasiri et al., 2009; Li and Bashir, 2002; Gadish and Voldman, 2006; Fatoyinbo et al., 2007; Becker et al., 1995; Yang et al., 1999; Huang et al., 1999) rather than capture of the actual *in vivo* target (VanDelinder and Groisman, 2007; Sethu et al., 2005; Yang et al., 2006; Liu et al., 2007b). WBC fractionation is the only technique where undiluted samples are commonly used; a few examples exist for other rare cell types (Nagraath et al., 2007; Huang et al., 2008; Gleghorn et al., 2010).

Similarly, the viability of cells after the capture process is not a well-quantified area, but one that is a crucial performance metric for rare cell capture devices. Mechanical stresses from shear, either from electric- (e.g., DEP forces), contact- (e.g., from pillar

**Table 1**  
Non-electrokinetic cell fractionation/isolation.

Application	Cell type modeled	Cell type used	Carrier media	# Steps/ stages	Off-line processing	Volumetric/linear flowrate	Efficiency	Enrichment	Purity	Viability	Analysis technique	Reference
Blood cell fractionation	B-lymphocytes/ T-lymphocytes	Raji/ Molt-3	PBS	1	staining	$10 \frac{\mu\text{L}}{\text{min}}$ ; na	97%	na	na	na	anti-CD5, anti-CD19, PEG IC	Murthy et al. (2004)
	BC/PC/CC/ lymphoblast	MCF7/ PC3/ HeLa/ Daudi	DMEM, Blood	1	staining, enumeration	0; 0	80%	na	na	na	EpCAM IC	Wang et al. (2010)
	B-lymphocytes/ T-lymphocytes	Raji/ Molt-3	PBS	1	staining, enumeration	$30 \frac{\mu\text{L}}{\text{min}}$ ; na	75%– 100%	na	na	na	anti-CD5, anti-CD19 IC	Sin et al. (2005)
	Myeloid cells	HL-60/ U-937	RPMI-1600	1	labeling, enumeration	na; $700\text{--}1400 \frac{\mu\text{m}}{\text{s}}$	na	$130 \times$ – $200 \times$	na	na	E-selectin IC	Chang et al. (2002)
	Leukocytes	Leukocytes	Whole human blood	2	enumeration, lysing	$5\text{--}12 \frac{\mu\text{L}}{\text{min}}$ ; na	na	na	97%	na	SBS	Sethu et al. (2005)
	Leukocytes	Leukocytes	Whole human blood	1	labeling, enumeration	$0.06 \frac{\mu\text{L}}{\text{min}}$ ; na	98%	na	na	na	SBS	VanDelinder and Groisman (2007)
	Leukocytes	Leukocytes	Diluted human blood	1	enumeration	$10\text{--}50 \frac{\mu\text{L}}{\text{min}}$ ; na	70%	na	na	na	SBS	Ji et al. (2008)
	Leukocytes	Leukocytes	Diluted human blood	1	enumeration	$10 \frac{\mu\text{L}}{\text{min}}$ ; na	72–85%	na	na	na	SBS	Ji et al. (2008)
	Leukocytes	Leukocytes	Diluted human blood	1	enumeration	$20 \frac{\mu\text{L}}{\text{min}}$ ; na	70–95%	na	na	na	SBS	Ji et al. (2008)
	Leukocytes	Leukocytes	Diluted human blood	1	flow cytometry, staining, lysing, enumeration	$1 \frac{\mu\text{L}}{\text{hr}}$ ; na	99.6%	na	na	na	SBS	Davis et al. (2006)
	Lymphocytes/ monocytes	CD4+ cells/ CD14+ cells/ J45 lymphocytes	Diluted human blood	1	labeling, enumeration	na; $1 \frac{\text{mm}}{\text{s}}$	73%	na	na	na	SBS	Inglis et al. (2008)
	Leukocytes	Leukocytes	Diluted human blood	1	lysing, enumeration	$2 \frac{\mu\text{L}}{\text{min}}$ ; na	na	$100 \times$	na	na	ShF	SooHoo and Walker (2007)
	Leukocytes	Leukocytes	Diluted human blood	1	Dilution, enumeration	$0.06 \frac{\mu\text{L}}{\text{min}}$ ; na	97%	na	na	na	StF	Zheng et al. (2008)
Cancer cells	Normal breast cell/ BC	HME/ TTU-1	PBS	1	enumeration, staining	$15 \frac{\mu\text{L}}{\text{min}}$ ; na	30%	na	na	na	EMA/ EGFR IC	Du et al. (2007)
	Leukemia/ lymphoma	CCRF-CEM/ ramos/ toledo	Modified PBS	2	cytometry, staining, enumeration	$300 \frac{\text{nL}}{\text{s}}$ ; na	50–83%	$135 \times$	88– 97%	na	Scg8/ TD05/ Sgd5 IC	Xu et al. (2009)
	BC	MCF7	Whole rabbit blood	3	check	na; $1\text{--}10 \frac{\text{mm}}{\text{s}}$	97%	na	na	na	EpCAM IC	Adams et al. (2008)
	PC	LNcaP	PBS	3	enumeration, staining	na; $2.5 \frac{\text{mm}}{\text{s}}$	90%	na	na	na	PSMA, EpCAM IC	Dharmasiri et al. (2009)
	BC	MCF7	DMEM	1	staining, enumeration, SEM	0; 0	45–60%	na	na	na	EpCAM IC	Wang et al. (2009)
	LC	SPC-A-1	Diluted human blood	1	enumeration	$0.1 \frac{\text{mL}}{\text{hr}}$ ; $22 \frac{\text{mm}}{\text{s}}$	99.9%	na	na	na	SBS	Chen and Du (2006)

Table 1 (continued)

Application	Cell type modeled	Cell type used	Carrier media	# Steps/ stages	Off-line processing	Volumetric/linear flowrate	Efficiency	Enrichment	Purity	Viability	Analysis technique	Reference
	LC	A549	Human RBCs	2	staining, enumeration	0; 0	62–74%	133 ×	na	na	WGA IC	Liu et al. (2007a)
	LC/PC/ pancreatic cancer/ colon cancer	LC/PC/ pancreatic CTC/ colon CTC	Whole human blood	1	staining, enumeration	1–2 $\frac{\text{mL}}{\text{hr}}$ ; na	na	na	42%– 67%	na	EpCAM IC	Nagrath et al. (2007)
	LC/PC/BC, bladder cancer	NCI-H1650/ PC3-9/ SKBr-3/ T-24	PBS	1	staining, enumeration	1 $\frac{\text{mL}}{\text{hr}}$ ; na	> 65%	na	na	na	EpCAM IC	Nagrath et al. (2007)
	LC	NCI-H1650	Whole human blood	1	staining, enumeration	1–2 $\frac{\text{mL}}{\text{hr}}$ ; na	> 60%	na	na	na	EpCAM IC	Nagrath et al. (2007)
	PC	LNCaP	PBS	1	labeling, enumeration	1 $\frac{\text{mL}}{\text{hr}}$ ; na	97%	na	na	na	PSMA IC	Gleghorn et al. (2010)
	PC	LNCaP	Whole human blood	1	labeling, enumeration	1 $\frac{\text{mL}}{\text{hr}}$ ; na	85%	na	68%	na	PSMA IC	Gleghorn et al. (2010)
	PC	PC CTCs	Whole human blood	1	labeling, enumeration	1 $\frac{\text{mL}}{\text{hr}}$ ; na	na	na	62%	na	PSMA IC	Gleghorn et al. (2010)
	LC/GC/ colon cancer / BC	NCI-H358/ AGS/ SNU-1/ SW620/MCF- 7/ Hs578T	Whole human blood	1	labeling, enumeration, SEM, staining	200–1000 $\frac{\mu\text{L}}{\text{hr}}$ ; na	> 80%	na	na	98%	SBS	Hosokawa et al. (2010)
	PC	LNCaP	PBS	1	labeling, enumeration, electrolysis, PCR	Manual; na	87–89%	na	na	na	SBS	Zheng et al. (2007)
	PC	LNCaP	Whole human blood	1	labeling, enumeration, electrolysis, PCR	Manual; na	89%	na	na	na	SBS	Zheng et al. (2007)
Other mammalian cells	Endothelial cells/ smooth muscle cells	H5V/ A7r5	PBS	1	labeling, enumeration, staining	40 $\frac{\mu\text{L}}{\text{hr}}$ ; na	na	na	86%; 83%	na	REDV/VAPG peptide IC	Plouffe et al. (2007)
	Endothelial cells/ smooth muscle cells/ fibroblasts	H5V/ A7r5/ 3T3-6	PBS	2	labeling, enumeration, staining	1 $\frac{\text{mL}}{\text{hr}}$ ; na	na	na	96– 99%*	97%	REDV/VAPG/ RGDS peptide IC	Plouffe et al. (2008)
	Neural stem cells	SH-SY5Y/ C6	PBS	1	staining, enumeration, flow cytometry	TBD	89%	na	na	90%	SBS	Kuntaegowdanahalli et al. (2009)
	G <sub>2</sub> /M myeloid cells	U937	10 mM sodium borate	1	flow cytometry	4 $\frac{\mu\text{L}}{\text{min}}$ ; na	na	4 ×	na	na	StF	Choi et al. (2009)
	Nucleated RBC	Nucleated RBC	Diluted Human Blood	2	filtration, dilution, staining	13 $\frac{\text{mL}}{\text{hr}}$ ; na	na	10 × –20 ×	na	na	StF	Huang et al. (2008)
Prokaryotes & Viruses	<i>E. coli</i>	<i>E. Coli</i>	Diluted Human RBCs	1	staining, enumeration, SDS page	2–18 $\frac{\mu\text{L}}{\text{min}}$ ; na	62%	300 ×	99.87%	95%	ShF	Wu et al. (2009)

IC = immunocapture, SBS = size-based sorting, ShF = sheath flow, StF = streamline focusing, BC = breast cancer, CC = cervical cancer, GC = gastric cancer, LC = lung cancer, PC = prostate cancer.



**Table 2**  
Electrokinetic cell fractionation/isolation.

Application	Cell type modeled	Cell type used	Carrier media	Off-line processing	Experimental parameters	Efficiency	Enrichment	Purity	DEP technique	Reference
Pathogen detection	<i>L. monocytogenes</i>	<i>L. innocua</i>	DI water, 2 $\mu\text{S}/\text{cm}$ ; $10^5$ cells/mL	Cell counting	1 $V_{pp}$ , 50 kHz	90%	–	–	IDA	Li and Bashir (2002)
	<i>L. monocytogenes</i>	<i>L. monocytogenes</i>	DI water, 1–15 $\mu\text{S}/\text{cm}$ ; $10^2$ – $10^3$ cells/mL	Cell counting	0.2 $\mu\text{L}/\text{min}$ , 800 $\mu\text{m}/\text{s}$ ; 20 $V_{pp}$ , 1 MHz	87–92%	–	–	IDA + IC	Yang et al. (2006)
	<i>B. anthracis</i>	<i>B. subtilis</i>	DI water, $5 \times 10^{-4}$ S/m; $3.8 \times 10^6$ cells/mL	Measure absorbance	100 $\mu\text{L}/\text{min}$ ; 40 $V_{pp}$ , 100 kHz	–	$9 \times$	–	IDA	Gadish and Voldman (2006)
	<i>B. anthracis</i>	<i>B. subtilis</i>	DI water, 7.6 mS/m; $9.9 \times 10^7$ spores/mL, $2.1 \times 10^7$ diesel particles/mL	Hema-cytometer	0.5–4 mL/hr, 94 $\mu\text{m}/\text{s}$ ; 10 $V_{pp}$ , 1 MHz	na	na	$\leq 60\%$	IDA	Fatoyinbo et al. (2007)
	<i>B. anthracis</i>	<i>B. anthracis</i>	DI water, 2–3 $\mu\text{S}/\text{cm}$ ; $10^7$ – $10^9$ spores/mL	Cell counting	0.2 $\mu\text{L}/\text{min}$ , 40 cm/min; 20 $V_{pp}$ , 100 kHz	90%	na	na	IDA	Liu et al. (2007b)
	<i>Plasmodium falciparum</i>	Malaria-infected erythrocytes	Sucrose buffer, 22–55 mS/m; $10^7$ cells/mL	Cell counting	5 $V_{pp}$ , 200 kHz	90%	$50$ – $200 \times$	na	Castellated	Gascoyne et al. (2002)
	na	<i>B. cereus</i> , <i>E. coli</i> , <i>L. monocytogenes</i>	Mannitol + PBS, 180 $\mu\text{S}/\text{cm}$ ; 4 $\mu\text{L}$ blood + 1 $\mu\text{L}$ of $10^6$ <i>B. cereus</i> or $7 \times 10^5$ <i>E. coli</i> or $10^6$ <i>L. monocytogenes</i>	PCR amplification	10 $V_{pp}$ , 10 kHz	$\leq 97\%$	na	na	Castellated	Huang et al. (2003)
na	<i>E. coli</i>	DI water, 10–20 $\mu\text{S}/\text{cm}$ ; $10^5$ cells/mL	Cell counting	100 Pa; 2000 V/cm	90+%	$3000 \times$	na	iDEP	Lapizco-Encinas et al. (2005)	
na	<i>E. coli</i>	PBS, 0.5 mS/m; $9.3 \times 10^3$ cells/mL	Cell counting	100 $\mu\text{L}/\text{min}$ ; 128 V/mm, 300 kHz	66%	na	na	iDEP	Cho et al. (2009)	
Cancer cell isolation	Lung cancer	A549-luc-C8	DMEM buffer, 72 $\mu\text{S}/\text{cm}$	Flow cytometry	3 $\mu\text{L}/\text{min}$ ; 15 $V_{pp}$ , 16 MHz	81–84%	na	na	Castellated	Tai et al. (2007)
	Breast cancer	MDA231	Sucrose buffer; $10^7$ malignant, $3 \times 10^7$ normal cells/mL	Cell counting	5 $V_{pp}$ , 200 kHz	na	na	95%	Castellated	Becker et al. (1995)
	Breast cancer	MDA-435	Sucrose buffer, 56 mS/m; $5 \times 10^6$ cells/mL, 2:3 ratio of MDA-435:RBCs	Cell counting	0.5 mL/min, 780 $\mu\text{m}/\text{s}$ ; 1.4 $V_{pp}$ , 5 kHz	na	na	98%	FFF	Yang et al. (1999)
	Breast cancer	MDA-435, -468, -231 cells	Sucrose buffer, 30 mS/m; $10^5$ – $10^6$ cells/mL, 1:1000 ratio of tumor cell to PBMNs	Cell counting	1.5 mL/min; 2.8 $V_{pp}$ , 60 kHz	$\leq 92\%$	na	na	FFF	Gascoyne et al. (2009)
	Breast cancer	MDA-435, CD34+ stem cells	Sucrose buffer, 10 mS/m; $10^6$ cells/mL, 3:2 ratio of CD34+ to MDA-435	Flow cytometry	2 mL/min; 4 $V_{pp}$ , 40 kHz	na	na	96–99%	FFF	Huang et al. (1999)
	Breast cancer	MDA-MB-231	PBS, 100–200 mS/m; $10^6$ cells/mL	Flow cytometry	200–400 $\mu\text{L}/\text{hr}$ ; 20 $V_{pp}$ , 500 kHz	na	$4.4 \times$	96%	Angled electrodes	Kim et al. (2007)
Blood fractionation or enrichment	Leukemia	THP-1	Sucrose buffer, 110–115 $\mu\text{S}/\text{cm}$ ; $10^6$ cells/mL	Cell counting	0.02 mL/hr, 222 $\mu\text{m}/\text{s}$ ; 20–50 $V_{rms}$ , 200–500 kHz	90+%	na	na	Contactless DEP	Shafiee et al. (2010)
	Leukocytes	Leukocytes	Sucrose buffer, 10 mS/m; $2 \times 10^6$ cells/mL, 1:1 ratio	Flow cytometry	2 mL/min; 4 $V_{pp}$ , 20–50 kHz	na	na	87–98%	FFF	Yang et al. (2000)
	Leukocytes	Leukocytes	GIT medium, 13 mS/cm; $5 \times 10^6$ cells/mL	Cell counting	1.5 $\mu\text{L}/\text{min}$ ; 20 $V_{pp}$ , 1 MHz	80%	na	na	FFF + IC	Hashimoto et al. (2009)
	Leukocytes	MDA-435, CD34+ stem cells	Sucrose buffer, 10 mS/m; Separation: $1.2 \times 10^6$ cells/mL, Leukocyte enrichment: $5 \times 10^5$ cells/mL	Flow cytometry	Separation: 2 mL/min, Leukocyte enrichment: 0.5 mL/min; 4 $V_{pp}$ , 40 kHz	55–75%	na	92–99%	FFF	Wang et al. (2000)
	Malaria	Erythrocytes infected with <i>B. bovis</i>	PBS, 60 mS/m	Cell counting	500 $\mu\text{m}/\text{s}$ ; 4.7–9 $V_{rms}$ , 90 kHz–4 MHz	na	$7 \times$	na	FFF	Braschler et al. (2008)
	Malaria	Erythrocytes infected with <i>P. falciparum</i>	Sucrose buffer, 0.055 S/m; 2000 cells with 5% parasitized cells	Cell counting	3 $V_{pp}$ , 2 MHz	na	$1000 \times$	90%	twDEP	Gascoyne et al. (2002)
	Platelets	Concentrated platelets + whole blood	Sucrose buffer, 50 mS/m; $10^7$ cells/mL	Flow cytometry	150 $\mu\text{L}/\text{hr}$ ; 6.6 mm/s; 100 $V_{pp}$ , 1 MHz	na	$5.3 \times$	95%	Angled electrodes	Pommer et al. (2008)

filters) or fluid-induced forces (e.g., obstacle-based arrays) can lead to gene upregulation or even induce an apoptotic response (Wernig et al., 2003; Okahara et al., 1998). Directly tied to cell viability is cell release and culture post-capture. Some attempts have been made to elute rare cells from devices (Xu et al., 2009; Adams et al., 2008; Dharmasiri et al., 2009; Liu et al., 2007a; Zhang et al., 2008; Zheng et al., 2007; Wu et al., 2009), especially those using affinity-based methods (i.e., immunocapture) (Adams et al., 2008; Dharmasiri et al., 2009). Although a majority of devices that employ DEP methods do not quantify post-process viability, other researchers have established that exposure to electric fields from microfabricated electrodes used for DEP techniques often does not alter cell viability (Wang et al., 1999; Ho et al., 2006). Electric field magnitudes and frequencies used for these devices are listed in the *Experimental Parameters* column of Table 2. Ultimately, in situations where the target cell can be as few as 1 per billion non-target cells (e.g., bacteria, viruses, CTCs), cell expansion in culture will be a critical step in obtaining enough material for further experimentation.

For future studies and biological applications, the major areas for improvement are ability to elute cells in an undamaged state, increased cell survivability, and systems capable of delivering both high capture efficiency and purity. The development of such a platform could be facilitated by incorporating both electrokinetic and non-electrokinetic methods to create hybrid systems, as in recent efforts (Yang et al., 2006; Hashimoto et al., 2009; Kim et al., 2008; Kim and Soh, 2009). Combining the sensitivity of DEP cell manipulation with the robustness of immunocapture has the potential to improve rare cell capture efficiency and purity, and such hybrid systems have scientific value and applicability across a variety of biological fields.

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