Josie L. Duncan[1,2]
Rafael V. Davalos[1,2]

[1]Bioelectromechanical Systems Laboratory, Department of Mechanical Engineering, Virginia Tech, Blacksburg, Virginia, USA
[2]Bioelectromechanical Systems Laboratory, Wake Forest School of Biomedical Engineering and Sciences, Virginia Tech, Blacksburg, Virginia, USA

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Review

A review: Dielectrophoresis for characterizing and separating similar cell subpopulations based on bioelectric property changes due to disease progression and therapy assessment

This paper reviews the use of dielectrophoresis for high-fidelity separations and characterizations of subpopulations to highlight the recent advances in the electrokinetic field as well as provide insight into its progress toward commercialization. The role of cell subpopulations in heterogeneous clinical samples has been studied to deduce their role in disease progression and therapy resistance for instances such as cancer, tissue regeneration, and bacterial infection. Dielectrophoresis (DEP), a label-free electrokinetic technique, has been used to characterize and separate target subpopulations from mixed samples to determine disease severity, cell stemness, and drug efficacy. Despite its high sensitivity to characterize similar or related cells based on their differing bioelectric signatures, DEP has been slowly adopted both commercially and clinically. This review addresses the use of dielectrophoresis for the identification of target cell subtypes in stem cells, cancer cells, blood cells, and bacterial cells dependent on cell state and therapy exposure and addresses commercialization efforts in light of its sensitivity and future perspectives of the technology, both commercially and academically.

Keywords:
Blood cells / Cancer cells / Dielectrophoresis / Stem cells / Subpopulation
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1 Introduction

Heterogeneity of clinical samples has required technology adept for identification, separation, and concentration of target subpopulations for *in vitro* testing, diagnostics, drug assessment, and prognoses. One example is the heterogeneity of the tumor microenvironment or the gut microbiome; the isolation of target components will enhance the understanding of disease progression and allow for drug assessment of fast-developing diseases. To effectively understand all components that make up a sample, it is useful to study each component in isolation; thus, separation methods are necessary for physiological samples.

Laboratory cell sorting has been primarily performed using centrifugation and flow cytometry methods. Centrifugation methods are predominantly size- or density-based separations which lack specificity for cells similar in size. Traditional flow cytometry techniques such as fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) require labeling of the cells by a fluorescent or magnetic antibody that is specific to the effect by which the cells are desired to be sorted. While these methods are useful for the identification and separation of similar cell types due to the use of labels that bind to specified surface antigens or intracellular components, there are several disadvantages for downstream applications and cell viability. In response to traditional methods, the cell undergoes high levels of stress, often resulting in a stark decrease in viability, and is modified with notable changes due to the marker, limiting the possibility of downstream culture and accurate subsequent

Correspondence: Josie L. Duncan, Bioelectromechanical Systems Laboratory, Virginia Tech – Wake Forest School of Biomedical Engineering and Sciences, Virginia Tech, Blacksburg, VA, USA.
Email: josied@vt.edu

Abbreviations: **cDEP**, contactless dielectrophoresis; **CSC**, cancer stem-like cell; **CTC**, circulating tumor cell; **DEP-FFF**, dielectrophoresis field flow fractionation; **DEPIM**, dielectrophoretic impedance measurement; **DFF**, Dean Flow Fractionation; **eDEP**, electrode-based dielectrophoresis; **eROT**, electrorotation; **FACS**, fluorescence activated cell sorting; **FFPE**, formalin-fixed paraffin-embedded; **hMSC**, human mesenchymal stem cell; **HTCD**, highly toxigenic Clostridium\penalty -\@M difficile; **iDEP**, insulator-based dielectrophoresis; **IDP**, isodielectric position; **IDS**, isodielectric separation; **MACS**, magnetic-activated cell sorting; **nDEP**, negative dielectrophoresis; **NSPC**, neural stem/progenitor cell; **NTCD**, nontoxigenic Clostridium difficile; **PBMCs**, peripheral blood mononuclear cells; **pDEP**, positive dielectrophoresis; **PMN**, polymorphonuclear neutrophils; **TIC**, tumor initiating cell; **tw-DEP**, traveling-wave dielectrophoresis

Color online: See article online to view **Figs. 1–4** in color.

studies [1]. While traditional labels used to differentiate cell types are often highly specific, it can be difficult to determine new biomarkers and subsequent labels for sorting or characterization. Cell separation and characterization techniques requiring labels such as FACS and MACS are disadvantageous when there are limited or altogether lack of available biomarkers, such is the case for glioblastoma, neural, and colorectal cancer stem cells [2–4]. Further, labels may also induce phenotypic changes or have cytotoxic effects on the cell, as seen with the detection of bacterial persistence and cancer cell isolation [5,6]. As a result of label-induced changes and biomarker identification, development, and availability required for identification and separation using traditional methods, electrokinetic techniques that exploit label-free technologies have been explored as alternative methods.

Dielectrophoresis (DEP) is an electrokinetic technique that has been used primarily for cell characterization and separation in microfluidic devices. In this technique, a neutral particle becomes polarized in a non-uniform electric field induced by applied potentials. Dielectrophoresis is advantageous when generated from an alternating current (AC) signal in that the frequency-dependent induced polarization effect capitalizes on the bioelectric properties of the cell membrane and cytoplasm (low-frequency) or intracellular properties (high-frequency) of the particle of interest. DEP is not limited to AC signals, however. Direct current (DC) signals can also be used to vary the field gradient magnitude to differentiate cell types. In addition to harboring advantages such as low-cost and rapid detection of target bioparticles, DEP is a label-free technique, requiring no dyes or biomarkers for identification.

Several reviews cover DEP applications in regard to live/dead cell separations, subcellular/nanoscale characterization [7,8], and even rare cell isolation from heterogeneous samples [9–14], spanning over several organisms from cancer to bacteria to proteins. These applications, however, do not exploit the high-sensitivity potential of dielectrophoresis and its ability to detect minute intrinsic differences between similar or related cells for clinical applications. Additionally, several reviews provide an excellent characterization of DEP as it compares to traditional and microfluidic methods of cell sorting and characterization [15,16]. This review focuses on the use of dielectrophoresis (DEP) for identification, characterization, and separation of similar or related subpopulations obtained from blood, cancer, bacteria, and stem cell populations; exploring changes in the bioelectric properties due to disease progression, therapy application, and cell state over the past 25 years. Here, we review recent advances in applications of DEP for similar/related subtypes to highlight the sensitive capabilities of this technology in contrast to its commercialization patterns that focus on dissimilar cell separations.

2 DEP Theory

Dielectrophoresis is the induced motion in response to a force exerted on a neutral particle in a non-uniform electric field. The dielectrophoretic force is defined as:

$$\vec{F}_{DEP} = 2\pi r^3 \epsilon_m Re[K(\omega)] \nabla \left(\vec{E} \cdot \vec{E}\right) \quad (1)$$

where r is the radius of the particle, ϵ_m is the permittivity of the media, $\vec{E}$ is the applied electric field, and $Re\,[K(\omega)]$ is the real part of the Clausius–Mossotti factor as a function of frequency (ω) defined as:

$$K(\omega) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (2)$$

where $\varepsilon_j{}^*$ is the complex permittivity of particle, p, or the media, m, and is defined as

$$\epsilon_j^* = \epsilon_j - i\frac{\sigma}{\omega} \quad (3)$$

where ϵ_j is the baseline permittivity, σ is the conductivity, and i is the complex number $\sqrt{-1}$.

The Clausius–Mossotti factor is a frequency-dependent quantity that determines the direction of the DEP force. If the particle of interest is more polarizable than the surrounding media, the particle will move towards the area of the highest electric field gradient, experiencing positive DEP (pDEP). Conversely, if the media is more polarizable than the particle, the particle will experience negative DEP (nDEP) and be repelled from the areas of the highest electric field gradient (Figure 1A).

From Equation 2, the two frequencies where the particle experiences a DEP force of zero, in transition between nDEP and pDEP, are known as crossover frequencies (Figure 1C). The first crossover frequency (f_{xo}), typically <100kHz for cells, can be used to determine differences in the membrane properties between cell types. The second crossover frequency (f_{x1}), typically >10MHz for cells, can be used to determine differences in the cytoplasm and intracellular components [17,18]. Within a heterogeneous sample, similar cells can be differentiated using the DEP crossover frequency method; that is, determining the frequency where one subpopulation will experience pDEP while the other experiences nDEP. Because DEP is able to detect intrinsic properties of cells, there are various factors that contribute to shifts in the DEP spectra of cells, particularly similar subpopulations. At low frequencies, minute changes in membrane capacitance and cytoplasmic conductivity due to changes in viability, membrane composition, membrane surface area, nucleus to cytoplasm ratio, and ion transfer across the cell membrane are detectable by DEP. At high frequencies, DEP is useful for distinguishing similar cell types due to changes in the nuclear conductivity and permittivity. That is, if cells differ in membrane capacity and/or cytoplasmic conductivity at low frequencies, or intracellular properties such as nuclear membrane capacitance or nucleoplasm conductivity at high frequencies, one cell might experience pDEP while a similar cell with varying properties will experience nDEP allowing for separation as a result of bioelectric signatures.

While there are a variety of methods to induce dielectrophoretic forces on bioparticles in a microfluidic device, the main types of dielectrophoretic devices are electrode-based

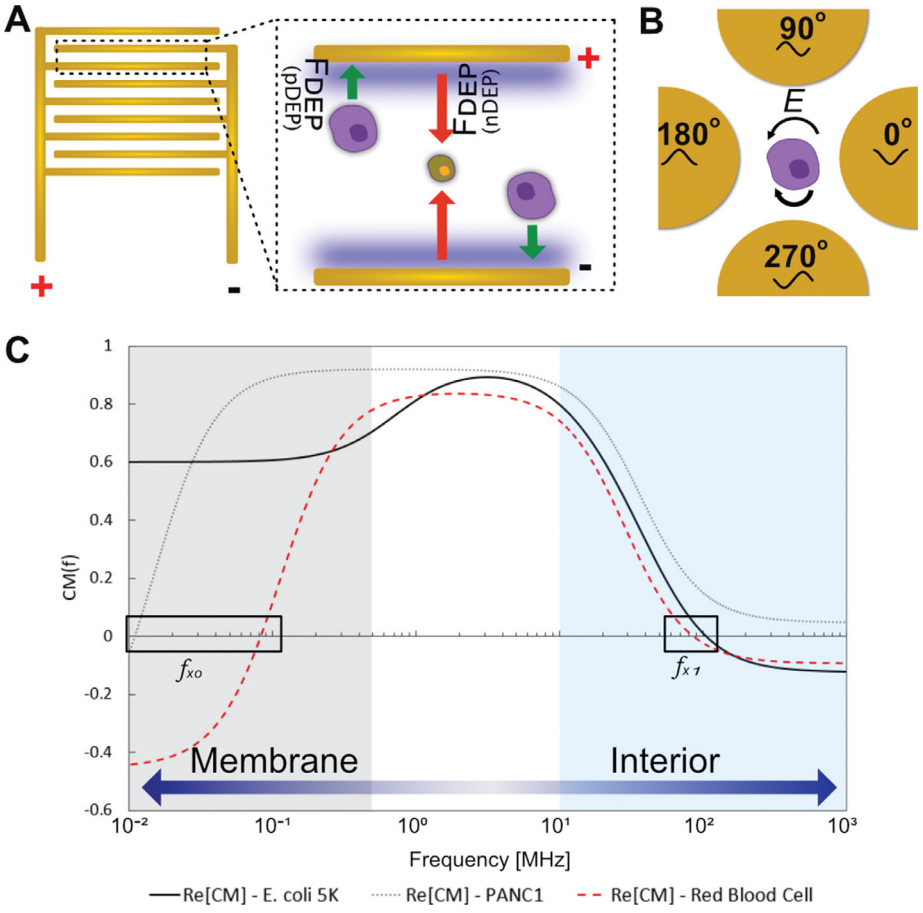

Figure 1. Schematic of (A) pDEP and nDEP, (B) eROT, and (C) representative frequency-dependent Clausius–Mossotti curve for bacteria, a cancer cell, and a blood cell at a media conductivity of 120 μS/cm [19].

DEP (eDEP), insulator-based DEP (iDEP), and electrorotation (eROT). eDEP devices typically contain planar or 3D electrodes made of a conductive material such as precious metals like gold or platinum. This type of DEP is also known as conventional DEP. Often, these electrodes are fabricated in an interdigitated electrode array (IDE) allowing non-uniformities in the field to concentrate between the electrode fingers. Device evolution and applications specific to eDEP technology can be found in other works [20–22]. iDEP uses conductive electrodes to create a uniform electric field across a microfluidic channel containing insulating structures, often posts, to induce areas of a high-field gradient. It is important to note that recently it was discovered that the induced motion of a particle by iDEP generated from a DC signal (DC-iDEP) is predominantly a result of second-order electrophoresis on the particle, rather than dielectrophoresis [23,24]. Various recent applications of iDEP have been highlighted in other works [25,26]. eROT is a single-cell analysis technique that employs quadrupole electrodes energized by four out-of-phase sinusoidal signals that cause the cell of interest to rotate (Figure 1B). The rotation speed, dictated by the field and the imaginary part of the Clausius–Mossotti factor, can then be used to calculate the electrical properties of the cell such as cytoplasm conductivity and membrane capacitance for characterization purposes. eROT is particularly useful in low-throughput cell characterization, while separation experiments often are operated under flow conditions to balance the DEP force and the sedimentary force, also known as DEP field-flow fractionation (DEP-FFF). Similarly, isodielectric separation (IDS) is often used to capitalize solely on the dielectric properties of the membrane capacitance and cytoplasmic conductivity irrespective of size or density, by inducing flow of a gradient of media increasing in conductivity over planar electrodes traversing the gradient at an angle. When a particle reaches dielectric equilibrium with the corresponding media conductivity, the cells exit the planar electrodes and continue flowing downstream. The location where the particle reaches equilibrium and exits the array is the isodielectric position (IDP), a metric useful for quantification of this method. While this review will highlight certain applications of eROT, the scope of this review will be limited to the aforementioned methods of DEP used for higher-throughput cell analysis translatable to the commercial market.

3 Applications

Here, we present a variety of biomedical applications that highlight the sensitivity of dielectrophoresis for characterization and/or separation of similar cell subpopulations such

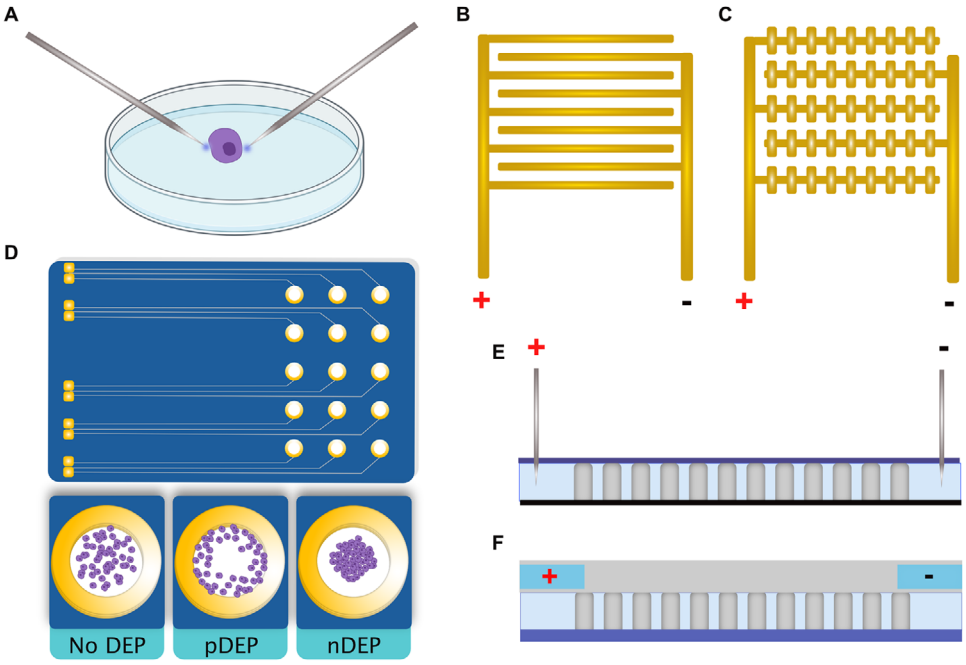

Figure 2. Representative electrode configurations and experimental setups. (A) Needle electrode in a dish. Created with BioRender.com. (B) interdigitated electrode array (IDE), (C) castellated electrode array, (D) 3DEP microwell system, (E) iDEP, (F) cDEP.

as stem cells, cancer cells, blood cells, and bacteria. Figure 2 shows representative electrode configurations commonly used in the following applications.

Each study is summarized to display the value it has added to the dielectrophoresis field in Table 1 to illustrate the extensive use of DEP to separate closely related and similar subpopulations . Table 1 also includes sensitivity metrics of each study when available. Where a sensitivity metric is not directly reported, experimentally determined cell properties such as cytoplasmic conductivity and membrane capacitance are included in the table to highlight the minute differences between electrical properties of cells successfully detected by dielectrophoresis.

3.1 Stem cells

Stem cells have the ability to self-renew and produce daughter stem cells or differentiate into cells with specialized functions. Since their discovery in 1960, stem cells have been studied to better understand disease development and have been hypothesized to be useful in regenerative medicine and tissue engineering [27]. In order to reliably study disease and regeneration therapies, it is necessary to predict and sort stem cells in different cell states from the same lineage. Many studies have focused on developing markers for flow cytometry of stem cells (primarily hematopoietic stem cells) [28]. These markers, however, often lack specificity between different stem cells from the same origin [3]. Recently, dielectrophoresis has been used to characterize differentiated cells from the same undifferentiated parent as well as stem cells based on their differentiation potential prior to differentiation, providing a solution to the unavailability of distinct biomarkers.

3.1.1 Neural stem cells

Following the first studies of DEP for stem cell separation, prior to 1997, where $CD34^+$ peripheral blood stem cells were separated without the use of biomarkers, Flanagan et al. used an interdigitated electrode array to determine a shift in frequencies where each cell type was fully trapped for neural stem/progenitor cells (NSPCs) based on their differentiation potential [3,29,30]. A subsequent study used eDEP to characterize NSPCs by correlating their membrane capacitance to their differentiation potential [31]. This study found that NSPCs exhibit DEP behavior similar to that which they are biased to differentiate: lower frequencies for astrocytes and higher frequencies for neurons. In 2014, Nourse et al. found that plasma membrane properties are sufficient for the detection of neural stem cell differentiation bias. Additionally, N-glycans on the surface of the membrane are indicators of whether an NSPC will differentiate into an astrocyte or a neuron [32]. Recently, a two-element microfluidic device with a hydrophoresis module upstream of a DEP module has been developed by Jiang et al. for the enrichment of astrocyte-biased progenitor cells from murine and human NSPCs (Figure 3A) [33,34]. In 2019, Liu et al. characterized and separated NSPCs using DC-iDEP with fields ranging from 10^8 to 10^9 V/m^2, concentrated on sawtooth electrode geometries based on their fate–bias by quantifying the ratio of electrokinetic mobility to DEP mobility [35].

3.1.2 Skeletal and mesenchymal stem cells

While neural stem cells have been of particular interest, other stem cell populations have also used DEP for the separation

Table 1. Summary of experimental parameters

Cell Type	Cell	Electrode description	Media conductivity [S/m]	Voltage	Frequency	Experiment Description	Sensitivity measurement	Reference
Bacteria	*S. epidermidis*	Platinum needle electrodes	0.001–0.002	20 Vpp	1 Hz–20 MHz	Drug sensitive vs resistant strains	σ_{wall}: Untreated JD140: 0.3 S/m Untreated JD141: 0.275 S/m Treated JD140: 0.01 S/m Treated JD141: 0.25 S/m	[64]
Bacteria	*E. coli, B. subtilis, B. cereus, and B. megaterium*	DC-iDEP	0.022, 0.104	50–75 V/mm	N/A	Gram-negative and -positive	N/A	[118]
Bacteria	*E. coli, hns, hha, & hha hns* derivatives	eDEP - castellated electrodes	10^{-3}–0.13	6–10 Vpp	10 kHz–200 MHz	Isogenic strains with one mutant allele	σ_{cyto}: *E. coli 5K*: 0.48 S/m Hha-3 *hns*: 0.33 S/m Hha-3: 0.48 S/m 5K *hns*: 0.31 S/m	[65]
Bacteria	M. smegmatis	eDEP - IDE	0.0005, 0.001	0.1–1.5 V	1 kHz–40 kHz	Drug assessment	σ_{cyto}: Treated: 0.98 S/m Untreated: 0.3 S/m	[119]
Bacteria	PA14 & mutants; *S. mitis* SF100 and PS344	DC-iDEP	0.01	10 V/mm	N/A	Mutant characterization	N/A	[66]
Bacteria	*M. smegmatis*	eDEP - 3D carbon electrodes	0.05	20 Vpp	7MHz	Drug assessment	90% enrichment of intact cells with 99% purity	[5]
Bacteria	*E. coli* - three strains	DC-iDEP - sawtooth electrodes	0.0055, 0.0343	160–470 V/cm	E_{onset}: O6:K1:H1: 163, 259, 427 V/cm for 27, 90, and 300 μm gates; O55:H7: 290, 470 V/cm for 27 and 90 μm gates; O157:H7: 324 V/cm for 27 μm	Strain characterization	N/A	[67]
Bacteria	*C. difficile* – toxigenic strains	3DEP, eROT	0.005	3DEP: 10 Vpp	3DEP: 50 kHz–45 MHz; eROT: 175 kHz (HTCD in HTCD-S), 250 kHz (HTCD in NTCD-S)	Strain characterization by toxigenicity	σ_{cyto}: BHI: 0.48 S/m HTCD-S: 0.5 S/m NTCD-S: 0.52 S/m NTCD: 0.6 S/m C_{mem}: BHI: 0.9716 mF/m^2 HTCD-S: 0.9933 mF/m^2 NTCD-S: 0.6879 mF/m^2 NTCD: 0.3409 mF/m^2	[69]

(*Continued*)

Table 1. (Continued)

Cell Type	Cell	Electrode description	Media conductivity [S/m]	Voltage	Frequency	Experiment Description	Sensitivity measurement	Reference
Bacteria	*C. difficile* – toxigenic strains	iDEP	0.105	<300 V/cm	Sweep: 50 kHz–5 MHz; f_{xo}: 300 kHz (HTCD), 500 kHz (LTCD), 900 kHz (NYCD). After treatment 600kHz (HTCD), 300kHz (LTCD), 600kHz (NTCD)	Strain characterization by toxigenicity and drug assessment	N/A	[68]
Blood	Erythrocyte	eROT, eDEP: Polynomial electrodes with two out of phase signals	0.056	eROT: N/A DEP: 3–8 Vpp	eROT: 1kHz–100MHz, co-field rotation: 260kHz (normal), 310kHz (infected) DEP: f_{xo}: 20kHz	Characterization of malarial infected cells	C_{mem}: Infected: 5.7 mF/m^2 Healthy: 11.8 mF/m^2	[82]
Blood	T-lymphocytes, Jurkat E6-1	eDEP - IDE	0.04	N/A	50–500kHz; f_{xo}: 147.8kHz (control), 192.7kHz (activated)	Characterization by activation state	C_{mem}: Activated: 10.26 mF/m^2 Control: 13.24 mF/m^2	[72]
Blood	Erythrocyte	eDEP - wire electrodes	N/A	0.025 Vpp/μm	1MHz	Blood typing for ABO+ antigen expression	N/A	[77]
Blood	Erythrocyte	DC-iDEP	0.052–0.91	68.5 V/cm (>51.4 V/cm)	N/A	Blood typing by ABO antigen expression	N/A	[78]
Blood	Erythrocyte	Quadrupole electrodes	0.9	0.01 Vpp/μm	Range: 100kHz–80MHz, f_{x1}: 17–47MHz for ABO types, enzyme-modified f_{x1}: 29–41MHz, f_{x2}:70–80MHz	Blood typing by ABO antigen expression	N/A	[79]
Blood	Neutrophils	DEP-spring	0.89	0–10 V	Range: 0.1MHz–12.8MHz Optimal: 6.4 MHz	Activation state	N/A	[73]
Blood	Erythrocyte (RBCs)	DC-iDEP	0.052	inlet: 10 V, outlet: 0/6 V	N/A	Enrichment of Babesia-infected RBCs	N/A	[84]
Blood	U937 Monocytes & U937-differentiated Macrophages	eDEP - 3D carbon electrodes, cell deformation	0.002	20 Vpp (Mono) 10 Vpp (Macro)	Range: 50kHz–1MHz, f_{xo}: 140kHz (Monocyte), 50kHz (Macrophages), Optimal: 300–400kHz	Deformation of macrophages differentiated from monocytes	C_{mem}: Macrophage: 13 mF/m^2 Monocyte: 16 mF/m^2	[76]

(*Continued*)

Table 1. (Continued)

Cell Type	Cell	Electrode description	Media conductivity [S/m]	Voltage	Frequency	Experiment Description	Sensitivity measurement	Reference
Blood	U937 Monocytes & U937-differentiated Macrophages	eDEP - 3D Carbon DEP	0.002	10 Vpp	f_{xo}: 17kHz (monocytes), 30kHz (macrophages), Optimal: 30kHz	Enrichment of undifferentiated monocytes and differentiated macrophages	70% enrichment of monocytes to macrophages	[75]
Blood	Erythrocyte	tw-DEP, DEP-FFF	0.02–0.055	5 Vpp	200kHz	Separation of malarial infected cells	95% blood cells trapped and 90% infected cells untrapped	[83]
Cancer	K562 & K562AR	eDEP - Needle electrodes	0.0025	20 Vpp	Range: 10kHz–20MHz, Differences: 2–3MHz (K562), 8–10MHz (K562AR)	Characterization of multidrug resistant subtype	σ_{cyto}: K562: 0.23 S/m K562AR: 0.5 S/m C_{mem}: K562:8.2 mF/m^2 K562AR: 7.6 mF/m^2	[52]
Cancer	K562	eDEP - Needle electrodes	0.0025	20 Vpp	Range: 10kHz–20MHz, Detection: 2–3MHz (K562)	Viability assessment	σ_{cyto}: K562: 0.4 S/m K562 (apoptotic): 0.05 S/m	[53]
Cancer	H357 (OSCC) and UP (HPV-16 transformed keratinocyte)	eDEP - Needle electrodes	0.005	20 Vpp	Range: 1kHz–20MHz, Differences: <5kHz (only H357), 10kHz–16MHz (UP)	Characterization of malignant and "more normal" cells	σ_{cyto}: UP: 0.45 S/m H357: 0.3 S/m C_{mem}: UP: 11.3 mF/m^2 H357: 17.7 mF/m^2	[41]
Cancer	B16F10	eDEP - castellated electrodes	0.174	7 Vpp	Range: 5kHz–50MHz, Separation at 2MHz	Separation of electroporated cells	N/A	[60]
Cancer	MCF7,MCF-7TaxR,MCF-7DoxR,MCF-7MDR1	eDEP - Needle electrodes	0.0025	20 Vpp	Range: 5kHz–20MHz	Characterization of drug resistant derivatives	σ_{cyto}: MCF-7: 0.23 S/m MCF-7TaxR: 0.14 S/m MCF-7DoxR: 0.4 S/m MCF-7MDR1: 0.27 S/m C_{mem}: MCF-7: 12.4 mF/m^2 MCF-7TaxR: 20.6 mF/m^2 MCF-7DoxR: 12.4 mF/m^2 MCF-7MDR1: 12.6 mF/m^2	[55]
Cancer	B16F10	eDEP - castellated electrodes	0.16	5 Vpp	Range: 200–400kHz, Clone 1 difference: 300kHz	Separation of 2 malignant clones from the same origin	N/A	[47]

(*Continued*)

Table 1. (Continued)

Cell Type	Cell	Electrode description	Media conductivity [S/m]	Voltage	Frequency	Experiment Description	Sensitivity measurement	Reference
Cancer	MCF10A, MCF7, MDA-MB-231	cDEP	0.01	20–50 V_{RMS}	MDA-MB-231 separation: 180–210kHz (20 V_{RMS}), 155–175kHz (30 V_{RMS})	Separation of early/late stage cancer	N/A	[43]
Cancer	OSCC: HOK, DOK, H357, H157	eDEP - DEPWell System	0.005	10 Vpp	Range: 4kHz–20MHz	Characterization of cancer, pre-cancer, and normal cells	σ_{cyto}: HOK: 0.71 S/m DOK: 0.42 S/m H357: 0.26 S/m H157: 0.25 S/m C_{mem}: HOK: 0.69 mF/m^2 DOK: 1.09 mF/m^2 H357: 1.51 mF/m^2 H157: 1.43 mF/m^2	[42]
Cancer	PC3 ALDH+ and ALDH-	cDEP	0.01	300 V_{RMS}, 280 V_{RMS}, 150 V_{RMS}	600kHz	Isolation of TICs from Non-TICs	N/A	[49]
Cancer	MOSE - E,E/I,I,L	cDEP	0.0094–0.0107	200 V_{RMS}	Range: 5kHz–70kHz, f_{xo}: 20.14kHz (E), 16.73kHz (E/I), 16.28kHz (I), 11.90kHz (L)	Characterization of different stages of cancer cells	C_{mem}: MOSE-E: 15.39 mF/m^2 MOSE-E/I: 19.7 mF/m^2 MOSE-I: 18.33 mF/m^2 MOSE-L: 26.42 mF/m^2	[44]
Cancer	K562 & K562AR	eDEP - 3D coated electrodes	0.0025	<9 Vpp	f_{xo}: 48.64MHz (sensitive cells)	Drug sensitive vs resistant separation	N/A	[54]
Cancer	NSCLC: HCC1833	eDEP - IDE	0.05	8 Vpp	Range: 100kHz–100MHz, Collection increase: 1MHz	Apoptosis progression after drug exposure	σ_{cyto}: Viable: 0.8 S/m 6h: 0.96 S/m 10h: 0.98 S/m 24h: 0.98 S/m C_{mem}: Viable: 12 mF/m^2 6h: 15.6 mF/m^2 10h: 16 mF/m^2 24h: 16 mF/m^2	[57]
Cancer	EM CTC from metastatic breast cancer	DEPArray		N/A	N/A	Identification of CTC subpopulations	N/A	[48]

(*Continued*)

Table 1. (Continued)

Cell Type	Cell	Electrode description	Media conductivity [S/m]	Voltage	Frequency	Experiment Description	Sensitivity measurement	Reference
Cancer	Jurkat, HeLa	DEPTech, 3DEP - microwells	0.01	N/A	Range: 10kHz–20MHz	Characterization of apoptosis progression	σ_{cyto}: Control 1: 0.45 S/m Control 2: 0.07 S/m DOX: 0.12 S/m DOX Fragments: 0.02 S/m C_{mem}: Control 1: 9.96 mF/m^2 Control 2: 7.75 mF/m^2 DOX: 6.64 mF/^{2} DOX Fragments: 7.75 mF/m^2	[58]
Cancer	LCC1, LCC9	OπDEP, iDEP with passivated electrodes	0.0113	80 Vpp	Range: 50kHz–1MHz, f_{xo}: 700kHz (LCC1), 100kHz (LCC9)	Characterization of breast cancer and drug response treatment	N/A	[59]
Cancer	U251-MG, SF-U251	cDEP	0.011–0.014	70–350 V_{RMS}	20–80kHz	Separation of cancer cells with increased stemness	N/A	[2]
Cancer	MB-CSCs: DAOY, D341, D283 & MS types	HF-DEP - Quadrupole electrodes		N/A	Range: 50–350MHz; f_{xo}: 213MHz (D341), 153MHz (D341-MS), 151MHz (D283), 110MHz (D283-MS), 108MHz (DAOY), 89MHz (DAOY-MS)	Characterization of CSCs from cancer population	N/A	[50]
Cancer	MOSE-L (highly malignant, fast-developing), MOSE-LTICv (slow-developing)	cDEP	0.011–0.012	300–350 V_{RMS}, Optimal: 350 V_{RMS}	Range: 20–40kHz, Optimal: 30kHz	Separation of cancer subpopulations	N/A	[46]
Cancer	U87-MG, LN18	eDEP - Quadrupole electrodes	0.026	2–4 Vpp	U87-MG: 120 MHz (NN), 91 MHz (DN); LN18: 128 MHz (NN), 76 MHz (DN)	Characterization of glioblastoma undifferentiated cells	N/A	[17]
Other	Cumulus-oocyte-complexes (COCs)	eDEP - castellated electrodes	0.01283	3 V	1MHz	Selection of oocytes based on developmental potential		[85]

(*Continued*)

Table 1. (Continued)

Cell Type	Cell	Electrode description	Media conductivity [S/m]	Voltage	Frequency	Experiment Description	Sensitivity measurement	Reference
Other	CHO	DEP-FFF, IDE	0.027	6 Vpp	Range: 35–110kHz, Difference: 65kHz	Differentiation of electroporated cells	N/A	[87]
Other	WRL-68	DEP microarray dot electrode	0.01	20 Vpp	Range: 1kHz–5MHz, f_{xo}: 220kHz (normal), 140kHz (infected)	Differentiation of virus infected cells	N/A	[86]
Other	CHO	eDEP - IDE	0.17	7 Vpp	10MHz	Differentiation of electroporated cells	N/A	[88]
Other	T-cells, anti-CD3 and anti-CD28	eDEP - IDE, two signals 180 degrees out of phase		Amplified voltage	6.8 MHz	Separation of T-cells by activation state	97.1% recoverable separation	[74]
Other	MRC-5 (human lung fibroblast) wild-type. transfected with GFP-fused histone	eDEP - electrodes along channel	1.4	20 Vpp	10MHz	Characterization of cells based on intracellular components	N/A	[89]
Other	HEK, MEF	eDEP and iDEP	0.15	3DEP: 10 Vpp, iDEP: 100 V/cm	3DEP Range: 100kHz–45MHz, iDEP: 0.5MHz	Characterization based on mitochondrial dynamics	σ_{cyto}: HEK-shDrp: 0.52 S/m HEK-shScramble: 0.30 S/m Drp-KO-MEF: 0.56 S/m Mfn-KO-MEF: 0.35 S/m	[90]
Stem	NSPCs	eDEP - IDE	0.015	8 V	Range: 25kHz–10MHz, Optimal: 300kHz (Astrocytes), 1MHz (NSPCs), 5MHz (Neurons)	Characterization of differentiation bias of stem cells	N/A	[3]
Stem	huNSPCs, mNSPCs	eDEP - microwells	0.01	10 Vpp	Range: 2kHz–20MHz Optimal: 500kHz (neurons), 75kHz (astrocytes)	Characterization and correlation of bioelectric properties to differentiation potential	C_{mem}: huNSPCs: 5–13 pF muNSPCs: 9–13 pF	[31]

(*Continued*)

Table 1. (Continued)

Cell Type	Cell	Electrode description	Media conductivity [S/m]	Voltage	Frequency	Experiment Description	Sensitivity measurement	Reference
Stem	MG-63, SAOS-2, STRO-1	eDEP - DEPWell System	0.01	10 Vpp	Range: 1.6–20MHz	Characterization of different stages of bone cell populations	σ_{cyto}: MG-63: 0.23 S/m SAOS-2: 0.52 S/m STRO-1: 0.34 S/m C_{mem}: MG63: 16 mF/m^2 SAOS1: 13.6 mF/m^2 STRO1: 10.7 mF/m^2	[36]
Stem	mNSPCs	DACs - castellated electrodes	0.011	16 Vpp	f_{xo}: 48kHz (NP), 35.1 (AP)	Characterization of differentiation bias and N-glycans on membrane	C_{mem}: NP-biased: 8.2 mF/m^2 AP-biased: 10.7 mF/m^2	[32]
Stem	hMSCs	eDEP - Quadrupole	0.03, 0.1	10 Vpp	Range: 0.01–35MHz, f_{xo}: 1–10MHz	Characterization of heterogeneity in MSCs	σ_{cyto}: Pre-treatment: 0.5 S/m Post-treatment: 0.0063 S/m	[37]
Stem	huNSPCs	eDEP - mircowells, chevron planar electrodes	0.01	6 Vpp	Range: 25kHz–1000kHz, Optimal: 200kHz	Sorting of astrocyte-biased hNSPCs	N/A	[34]
Stem	mNSPCs	eDEP - chevron planar electrodes	0.01	6 Vpp	Range: 10–1000kHz, Optimal: 184kHz	Sorting of astrocyte-biased muNSPCs	N/A	[33]
Stem	mMSCs	eDEP - microwells	0.06	8 Vpp	f_{xo}: 98.6kHz (control), 134kHz (3d control), 175 kHz (3d treatments)	Characterization of differentiation and undifferentiated	σ_{cyto}: Control: 0.88 S/m No treatment: 0.88 S/m Treatment: 0.82 S/m	[39]
Stem	hMSCs, osteoblasts	eDEP – angled IDE		7.2 Vpp, 15.4 Vpp; On-off cycled AC signal	Optimal: 3MHz	Separation of hMSCs and their progeny (osteoblasts)	N/A	[38]
Stem	NSPCs	DC-iDEP, sawtooth electrodes		1.3×10^8–1.3×10^9 V/m^2	N/A	Separation and characterization of neural stem cells	N/A	[35]
Stem/Blood	CD34+ cells from peripheral blood stem cells	eDEP - castellated electrodes		6 Vpp	Range: 5–500kHz, Optimal: 10–50kHz	Enrichment of CD34+ stem cells from blood	N/A	[30]

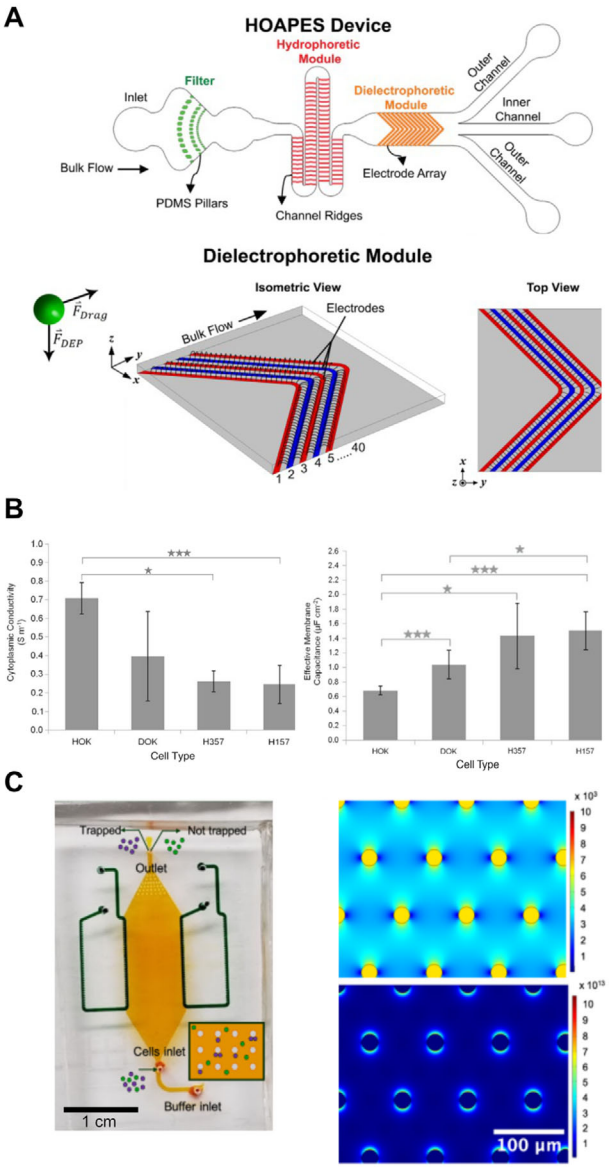

Figure 3. (A) Chevron electrodes preceded a hydrophoresis module dividing the inlet into two focused streams to aid in the separation of stem cells and reduce cell-cell interactions. Adapted from [33], copyright (2019) AIP Publishing B) Differences in cytoplasmic conductivity and membrane capacitance of oral cells of varying malignancy can be detected using dielectrophoresis. Adapted from [42], copyright (2011) Springer Nature. C) cDEP device and generated gradient of electric field for the separation of U251 cells and their spheroid forming derivative based on increased stemness. Adapted from [2], copyright (2019) John Wiley and Sons.

of subpopulations from a heterogeneous mixture of similar cells. Ismail et al. used the DEPWell System to characterize MG-63, SAOS-2, and STRO-1 to represent early and mature skeletal stem cell populations as a feasibility study for potential isolation and separation of skeletal stem and mature cells based on their bioelectric properties [36]. Human Mesenchymal Stem Cells (hMSCs) have been assessed for heterogeneity when compared to a sample treated with a polymer to standardize morphology and limit sample heterogeneity. This study suggests that even within a single sample, heterogeneity is not negligible in regards to changes of the DEP crossover frequency, providing insight into just how sensitive DEP might be for subpopulation detection [37]. Song et al. used an angled IDE device to separate hMSCs from their progeny osteoblasts. This device, in particular, introduces a continuous flow separation system that uses an AC signal cyclically triggered on and off to expose the sample to DEP forces but avoid trapping or slowing of the cells. When the field is on, the progeny osteoblasts are moved away from the original streamline of the sample while the hMSCs are undeterred [38]. Giduthuri et al. used microwells to characterize and identify tenogenically differentiating murine mesenchymal stem cells as early as 3 days into differentiation [39]. According to this study, dielectrophoresis has the potential to inform researchers of phenotypical changes of a stem cell early in the differentiation process, creating a more complete understanding of stem cells. In all, dielectrophoresis can be used to characterize and separate stem cells based on their differentiation bias and has a high sensitivity that is capable of detecting heterogeneity in a single sample.

3.2 Cancer

Several reviews cover studies focused on dielectrophoresis for characterization and separation of cancer cells from other cells [11,40]. Most notably, dielectrophoresis has been extensively studied for the isolation of rare cancer cells, circulating tumor cells (CTCs), from the blood for early diagnostics [9,10]. This review, however, focuses on cancer cell subtypes such as cancer cells with different stages of metastasis, drug resistance, and therapy assessment.

3.2.1 Disease progression

Tumors are inherently heterogeneous and identification of the make-up of the tumor can lead to more effective, personalized treatments and early detection of metastatic cancer. Traditional methods to monitor disease progression such as pathology, blood chemistry studies, or immunophenotyping require either highly trained personnel or detectable target markers to be present. Dielectrophoresis, on the other hand, provides a unique ability to detect small changes in a sample beyond that of which there are developed surface-markers. Dielectrophoresis can provide insight into the true heterogeneity of a sample and thus better inform clinicians of the composition of a tumor or blood sample to advise prognosis and treatment by providing a new perspective to the cellular properties from an electrokinetic perspective.

Dielectrophoresis has been used to successfully characterize the dielectric behavior of normal, precancer, and malignant oral cells [41,42]. Figure 3B shows the cytoplasmic conductivity and membrane capacitance of oral cells of

differing malignancy, detectable by DEP. Not only can cancer cells be separated from the blood, but dielectrophoresis has the sensitivity to decipher different stages of cancer from a similar origin. More recently, contactless dielectrophoresis (cDEP), a type of DEP that uses insulating structures and isolated electrodes to eliminate electrochemical changes to the membrane of the cell and maintain sterility of the sample, was used to decipher early, intermediate, and late-stage mouse ovarian cancer cells [43,44]. A similar study used cDEP to differentiate mouse ovarian cancer cells based on benign to malignant phenotypic expression, as well as differentiated macrophages and fibroblasts, demonstrating the sensitivity of cDEP for subpopulation detection based on phenotypic changes and differentiation [45]. A related study from 2017 by Douglas et al. used a similar cDEP device to separate highly-malignant murine ovarian cancer cells (MOSE-L) from a slow-developing type of the same disease MOSE-L_TICv by balancing the flowrate-induced drag force and the DEP force acting on the cells at a frequency of 30kHz [46]. Similar malignant cells, B16F10 mouse melanoma clones derived from the same origin, were characterized on a castellated electrode array to show the feasibility of detectable differences of cancer cells from a similar origin [47]. Bulfoni et al. showed dielectrophoresis can be used to detect cells based on surface markers without a label. This group used the DEPArray microwells to detect subpopulations of CTCs in epithelial-to-mesenchymal transition, one with only mesenchymal protein expression and another void of any other biomarkers. This study can be translated clinically as the presence of the epithelial and mesenchymal markers in a subpopulation can be correlated to a poorer prognosis for patients with metastatic breast cancer [48].

3.2.2 Cancer stem-like cells

Recently, cancer stem-like cells (CSCs) have become an interesting field of study to provide more information about cancer progression and metastasis. These malignant cells have been shown to have self-renewing capabilities and are often resistant to various drug therapies. Many studies have explored potential biomarkers for identification and separation, but the variability in surface markers and sorting techniques have led to inconclusive results [4]. Dielectrophoresis, however, offers the ability to detect changes in bioelectrical properties within a population, which might be particularly useful in instances where there is not a specific biomarker to label. With the heterogeneity and, oftentimes, the mystery surrounding available CSC biomarkers and subpopulation properties, using a technique such as DEP to interact directly with the intrinsic properties of each subpopulation can be helpful for clinical applications such as diagnostics and prognoses.

Select studies have shown that dielectrophoresis can be useful in isolating CSCs from a heterogeneous sample. Salmanzadeh et al. used cDEP to characterize and separate prostate CSCs (then denoted as tumor-initiating cells (TICs) from non-TICs. At intermediate frequencies, 600kHz, $ALDH^+$ and $ALDH^-$ PC3 cells were distinguished and sorted based on stemness, or the ability to self-renew or propagate new tumors [49]. In 2019, Manczak et al. used ultra-high frequency DEP (>50MHz) with a quadrupole electrode configuration to characterize two glioblastoma cell lines: U87-MG and LN18 in distinct culture conditions to control their phenotypes. This setup was able to detect differences of these undifferentiated cell lines at lower crossover frequencies than their differentiated counterparts [17]. Alinezhadbalalami et al. also used cDEP to show feasibility for separation of spheroid-forming U251 glioblastoma cells from U251 cells based on their increased "stem-ness" as evidenced by nestin, a biomarker that indicates stemness [2]. A recent study used quadrupole electrodes to characterize a decrease in the second crossover frequencies for medullosphere forming medulloblastoma cells with increased stemness from their wild-type [50]. As shown by these studies, DEP is a feasible option for identifying and separating CSCs from other cancer cells based on their increased stem-like properties.

3.2.3 Drug resistance and assessment

Dielectrophoresis has been extensively studied as an *in vitro* method for toxicant and drug assessment monitoring for cancer cells. Several studies in particular focus on the feasibility of dielectrophoresis to quantify drug assessment to detect membrane changes as a result of apoptosis or necrosis after drug exposure when compared to other methods such as staining. Wang et al. studied the feasibility of using DEP to monitor apoptosis progression after exposing HL-60 leukemia cells to genistein (GEN). An interdigitated electrode array was used to determine the crossover frequencies for normal (80–130 kHz), apoptotic (130–200 kHz), and necrotic (>10 MHz) cells within as little as one hour post-treatment [51]. In 2003, needle electrodes were used to characterize leukemic cells, K562, and their multi-drug resistant derivative, K652AR, before and after modulator treatment. This study found that there is a mildly detectable difference between the subtypes citing a difference in their cytoplasm conductivity affecting the frequency where cell collection begins to decrease from full trapping of each cell type (3 MHz for K562 and 8 MHz for K562R). There is not a detectable difference that correlates modulator treatment to dielectric signature [52]. Using a similar setup to the one described previously, Chin et al. detected changes of cytoplasm conductivity dependent on ion concentration within 30 minutes of exposure to staurosporine. In particular, this study found that the decline in trapping of the treated cells had two plateaus signaling subpopulations in their treated sample that might behave differently to the treatment [53]. Following the study from 2003, Demircan et al. found that K562 cells and their multidrug-resistant counterpart, K562AR, are separable at 48.64 MHz using a 3D electrode array where K562AR experience pDEP and the drug-sensitive cells are overcome by the drag force [54]. Labeed et al. used needle electrodes in a petri dish to characterize breast cancer cells and

multidrug-resistant derivatives show that an increase in drug resistance is correlated with an increase in cytoplasmic conductivity, detectable by DEP [55].

As evidenced by the previous studies, dielectrophoresis has been used thoroughly for the characterization of drug-sensitive and drug-resistant cells, particularly in the assessment of cancer cell response to drug treatment, but more recent studies have focused on the separation of drug-resistant subtypes using DEP. In 2018, Chu et al. used optically induced dielectrophoresis to sort and separate representative cells of drug-resistant and drug-sensitive populations. Notably, not only was this platform useful for separating Dx5 (drug-resistant) and MES-SA (drug-sensitive) cells, but it was able to sort subpopulations of the sample undergoing different levels of apoptosis [56]. An interdigitated electrode array has been used to detect apoptosis progression after exposure to ABT-263 of non-small cell lung cancer as early as 2 h post-treatment [57]. Henslee et al. used the 3DEP microwell technology to characterize and quantify the number of cells in various stages of apoptosis progression of Jurkat and HeLa cells after toxicant exposure. This method, while similar to other studies that characterize apoptosis progression, provides validation for the use of the 3DEP system for the accurate quantification of subpopulations within a sample exposed to apoptosis inducers [58]. In 2016, an iDEP device with passivated electrodes was used to monitor the response of closely related breast cancer cells (LCC1, LCC9) derived from the MCF-7 cell line to obatoclax. Both cell lines were differentiable exhibiting different crossover frequencies (LCC1: 700 kHz, LCC9: 100 kHz), and the crossover frequencies for both cell lines were shifted post-treatment with obatoclax [59]. These studies have shown that dielectrophoresis is a feasible, rapid *in vitro* drug-assessment tool that can monitor drug-resistance or membrane changes in less than a few hours. As the technology advances, DEP can be used as a useful clinical tool for *in vitro* monitoring of drug efficacy for personalized cancer treatments.

3.2.4 Treatment assessment

While DEP has been studied for its use in drug assessment, other treatment methods have benefited from efficacy monitoring using dielectrophoresis. Oblak et al. used castellated electrodes to separate electroporated cells, or cells with disrupted membranes due to high voltage electrical pulses, from non-electroporated cells based not only on their change in cytoplasmic conductivity, but also as an indication for significant changes in the membrane properties after electroporation [60]. This study suggests that DEP is a feasible method for determining the precise effect alternative treatments, such as electrical or mechanical stimulants, have on cell properties. Dielectrophoresis can potentially be used in tandem with other technologies to quantify treatment efficacy and, potentially, contribute to treatment planning.

3.3 Bacteria

Bacteria is another popular area of differentiation for applications such as clinical sepsis detection, tumor microenvironment make-up, and antibiotic resistance identification. Traditional methods of bacterial identification from clinical samples often require a multi-day culture followed by fluorescent *in situ* hybridization, phenotypic assays for identification and antibiotic resistance assessment, polymerase chain reaction (PCR), or mass spectrometry. Not only do these methods have limitations based on the required medium, but they are generally time-consuming: requiring multi-day cultures to generate an adequate amount of bacterial cells for detection [61]. Dielectrophoresis offers the capability of identification of bacteria from the same genus as well as characterization of the drug-resistance profile of each strain, mitigating the sample processing time and culturing requirements associated with the gold standard techniques. Dielectrophoresis has been used to isolate bacteria of varying families or genera as well as isolating bacteria from blood [62,63]. This section, however, will focus on the characterization and separation of bacteria on the strain level to highlight the sensitivity of DEP and the life-cycle progression after antibiotic treatments to demonstrate the clinical applications of DEP for infection treatment assessment.

3.3.1 Antibiotic-treated bacteria

In 2003, Johari et al. used needle electrodes to characterize the dielectrophoretic behavior of antibiotic-sensitive and resistant strains of the *Staphylococcus epidermidis* from 1 Hz to 20 MHz. The sensitive and resistant strains had similar signatures prior to treatment with antibiotics. After antibiotic treatments, the sensitive strain was unable to be trapped completely under frequencies below 200kHz 2 days following treatment. Separation of antibiotic-sensitive and resistant strains is possible using dielectrophoresis after treatment [64]. In all, experiments that employ DEP for bacteria subtype characterization are predominantly focused on changes in membrane properties as a result of antibiotic treatment. From this, there is an opportunity to use DEP to study the dielectric behavior of bacteria dependent on intracellular properties. A specific type of eDEP using 3D carbon electrodes has shown to be a high-throughput method for assessment of antibiotics on bacteria, particularly *Mycobacterium smegmatis*, often requiring techniques capable of detecting the target bacteria in fractions as low as 10^{-6} in a mixed population. Elitas et al. used this platform to separate antibiotic-treated bacteria from untreated bacteria at a frequency of 7MHz [5]. Dielectrophoresis is a promising *in vitro* technique for the assessment of antibiotic treatments on bacteria that could be used for developing new antibiotics, infection treatments, and isolating targets of resistant strains for on-chip treatment exposure.

3.3.2 Strain-level bacteria

Varying isogenic mutant derivatives of *Escherichia coli* 5K parent strain were examined for differences in their crossover frequencies based on the presence of one mutant allele. The first cross-over frequency of the parent strain shows noticeably different behavior from the *hns*, *hha*, and *hns & hna* derivatives. At the second crossover frequency, the parent strain was not differentiable from the *hha* mutant, while the *hns* and double mutant have a much lower crossover frequency [65]. Braff et al. characterized six mutant strains and a parent strain of *Pseudomonas aeruginosa* (PA14) using DC-iDEP. Mutant strains from the same parent strain showed differences in dielectric behavior that can be correlated to differences in polarizability of the membrane among isogenic strains [66]. Jones et al. characterized three strains of *E. coli*: O157:H7, O55:H7, O6:K1:H1 using DC-iDEP with a sawtooth geometry with varying distance between sawtooth points to alter the gradient along the channel. After applying potentials ranging from 160 to 470 V/cm, the strains were differentiated based on the field magnitudes where the onset of trapping occurred relative to the gate width between sawtooth points [67]. This study shows the feasibility of DC-iDEP for the characterization of bioelectrical differences among bacteria of the same genus (Figure 4A). Su et al. used iDEP with potentials <300 V/cm to characterize mixed samples of *Clostridium difficile* strains with different levels of toxigenicity and antibiotic efficacy [68]. A follow-up study used 3DEP and eROT for characterization of *Clostridium difficile* based on toxigenicity and culture conditions that contribute to highly-toxigenic (HTCD) strains. While the DEP spectra showed light trends, eROT detected stark differences in peak frequencies for HTCD cultured in HTCD supernatant (175 kHz) as opposed to HTCD cultured in nontoxigenic supernatant (NTCD) (250 kHz), suggesting that toxigenicity is induced by extracellular factors [69].

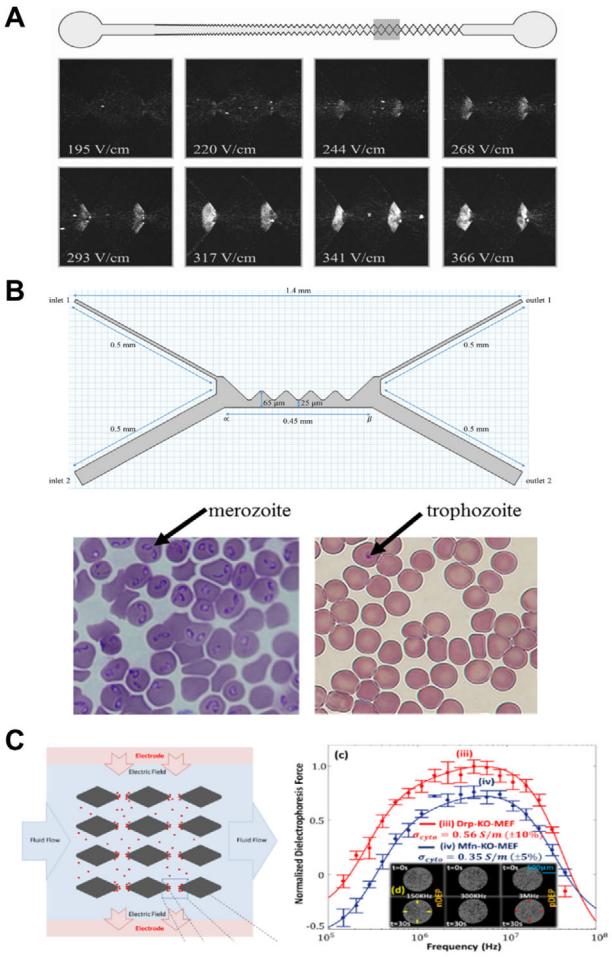

Figure 4. (A) Trapping of *E. coli* mutant O6:K1:H1 only apparent after onset voltage value achieved. Adapted from [67], copyright (2013) Springer Nature. (B) Sawtooth iDEP device used to separate red blood cells infected with *Babesia* protozoan parasites from normal blood cells. Adapted from [84], copyright (2016) AIP Publishing. C) iDEP device and 3DEP characterization of cells based on mitochondrial structure. Adapted from [90], copyright (2017).

3.4 Blood cells

Separation of subpopulations within blood cells is almost exclusively performed using density gradient or cell lysis methods. Density gradient separation uses centrifugation and a density-varying medium to stratify subpopulations of a single blood sample, while selective cell lysis allows for the rupture of unwanted cells that leaves behind debris and eliminates cell types that might be desired downstream. These techniques, however, have low specificity and can only be used for blood cells of different sizes and densities or cell types (e.g., elutriation, red blood cell lysis). Dielectrophoresis provides a label-free method that goes beyond size-based separations as well as a rapid detection method for diagnostics. Several studies have used dielectrophoresis for the separation of leukocytes or platelets from RBCs [70,71]. This review will focus on identifying and separating blood cell subpopulations and blood cell infection.

3.4.1 Blood cell subtypes

Other blood cells characterization using dielectrophoresis capitalize on the membrane differences between cells based on activation state or surface antigen expression. T-lymphocytes were characterized based on induced activation state where the mean crossover frequency of the activated cells was higher than that of the control cells as a result of changes to membrane topography and configuration following activation [72]. In addition to the activation state of T-lymphocytes, Su et al. characterized neutrophils by activation state using a DEP spring to quantify the DEP and drag forces acting on the cells. Similar to the previously described experiment, DEP was able to discern the differences in electrical signature between activated and inactivated neutrophils at frequencies greater than 6.4 MHz [73]. Han et al. used an

interdigitated electrode array with two signals 180° out of phase and found that a frequency of 6.8 MHz was optimal for separating activated T-cells (treated with anti-CD3 and anti-CD28) from naïve T-cells.

Dielectrophoresis for immune response monitoring has potential applications in disease diagnostics and prognoses [74]. Dielectrophoretic force measurements using 3D carbon electrodes were used to characterize undifferentiated U937 monocytes and U937 macrophages differentiated from the same lineage [75,76]. DEP-induced deformation can be used as an "electromechanical biomarker" for characterizing differentiated subtypes from their progenitor.

Dielectrophoresis has also been used for blood typing by analyzing the dielectric signatures of erythrocytes based on the ABO antigen present on the membrane of the cell [77–79]. Following a study by Prieto et al. showing that isodielectric separation can be used to detect differences in resting and activated polymorphonuclear neutrophils (PMN), Jundi et al. used a similar platform to exploit these differences in PMNs for sepsis progression monitoring for clinical applications [80,81]. Clinically relevant studies, such as the one performed by Jundi et al., give a unique insight into the usefulness of dielectrophoresis platforms in clinical settings such as a quick approach to sepsis detection.

3.4.2 Infected blood cells

In 1997, Gascoyne et al. discovered changes in membrane capacitance and conductance of erythrocytes due to malarial infection using eROT and eDEP using two out-of-phase sinusoidal techniques [82]. A follow-up study in 2002 used traveling-wave dielectrophoresis (tw-DEP) and DEP field flow fractionation (DEP-FFF) to separate 5% infected cells from normal erythrocytes [83]. This characterization of membrane properties of normal and infected blood cells suggests that cells with intracellular differences might exhibit membrane changes that are detectable by dielectrophoresis for characterization and separation applications. More recently, Adekambi et al. used DC-iDEP to enrich erythrocytes infected with *Babesia* protozoan parasites that cause liver failure, anemia, and kidney failure (Figure 4B). This is a feasibility study for point-of-care devices for intracellular infections of the blood [84].

3.5 Other cells

While this review has primarily focused on stem cells, cancer cells, and blood cells, there are select studies that used dielectrophoresis to discriminate between similar cells outside of the aforementioned categories. For example, Choi et al. used dielectrophoresis to select oocytes based on their potential for viable fertilization in an *in vitro* fertilization setting [85]. Planar microarray dot electrodes were used to determine the crossover frequency discrepancy between normal hepatic cells (WRL-68) and WRL-68 cells infected with the dengue virus [86]. Changes in electrical properties of Chinese hamster ovarian (CHO) cells induced by electroporation have been studied using dielectrophoresis. Čemažar and Kotnik used DEP field-flow fractionation (DEP-FFF) to separate electroporated cells and non-electroporated cells at an optimal frequency of 65 kHz [87]. A more recent study used a similar approach to characterize electroporated CHO cells based on changes in the internal conductivity at a higher frequency of 10MHz on an interdigitated electrode array [88]. As the understanding of cell complexity grows, characterization and separation of cells based on intracellular organelles and structure has become increasingly popular. One subpopulation of interest includes the characterization of wild-type and GFP-Histone-infected human lung fibroblasts (MRC-5). Yao et al. used digitated electrodes along the length of a microfluidic channel to characterize MCR-5 cells based on their intracellular contents. With an AC signal of 20 Vpp and 10 MHz, the velocities of the wild-type and the GFP-Histone transfected population were discernibly different [89]. Rohani et al. used the DEPTech 3DEP and iDEP to characterize the mitochondrial structure of the human embryonic kidney cells (HEK) and murine embryonic fibroblasts (MEF) (Figure 4C). These results show that an intact mitochondrial structure increases the internal conductivity of the cell and experiences enhanced pDEP at intermediate frequencies of 0.5–15 MHz in this setup [90].

4 Dielectrophoresis to market

Since DEP was discovered in the 1960s, the optimistic revelations about its sensitivity for cell detection, small sample requirement, and label-free differentiation, have hardly left the laboratory bench. While dielectrophoresis has been shown to be a feasible cell characterization and separation technique for research questions that require high sensitivity, downstream assays, and heightened cell viability, this label-free electrokinetic technique is still primarily used academically. One of the principal advantages of dielectrophoresis, its sensitivity, which does not require extensive adjunct biomarker development studies, appears to be a promising solution not met by other cell-sorting technologies. Dielectrophoresis might not prove to be overly advantageous in the industry among other cell-sorting techniques for select applications of cells being sorted based on size or cell type (i.e., cancer cells from healthy cells, bacteria from blood), but its selectivity based on intrinsic properties of cells otherwise difficult to distinguish could provide researchers in academia and industry a solution not readily available.

In order to discuss the future of DEP, this section will detail current efforts in commercializing dielectrophoresis as well as other label-free technologies that will be direct competitors as DEP continues to enter the market. It will also highlight the future directions of commercializing DEP and the opportunity for highly sensitive cell-sorting techniques in the industry.

4.1 Commercialization of DEP

In the past 20 years, few companies have commercialized dielectrophoresis. Namely, Menarini Silicon Biosystems, DEPTech, and Precision for Medicine (formerly Apocell) have dominated the commercialization of this technology.

Menarini Silicon Biosystems is a biotechnology company specializing in image-based cell sorting. Their product, the DEPArray™, uses DEP to create field cages for individual cells that can then be automated to move select cages to a recovery zone for downstream analysis. The primary applications for the DEPArray™ are forensic science and oncology research, notably formalin-fixed paraffin-embedded (FFPE) tissue samples and CTCs from blood [91–95]. This technology, however, does not capitalize on the sensitivity of DEP, but uses the technique for manipulation. The DEPArray™ requires fluorescent markers to identify the target cells and uses DEP to manipulate cells expressing a certain marker independent of the cell electrical properties.

DEPTech, recently joined by DEParator, provides two technologies for characterization and sorting using dielectrophoresis. The DEPTech 3DEP system characterizes up to 20 000 cells in 10s by determining the DEP spectra for a cell sample loaded onto microwell electrodes. This device records the normalized DEP force acting on the cell in response to an AC signal spanning from 1 kHz to 45 MHz [96–99]. The 3DEP system has been used for determining the DEP spectra and estimating the crossover frequency of stem cells, antibiotic-resistant bacteria, and cancer cells in different stages [55,100,101]. While this technology is useful for characterizing cell samples, there is no opportunity for isolation and separation using this system, nor is there a way to differentiate between cell subtypes within a sample. The DEParator, on the other hand, is a label-free sorting system that uses dielectrophoresis. The sample flows through a microwell electrode energized by a user-defined frequency AC signal allowing for target cells to experience pDEP and the rest of the sample to flow through the well [102,103].

Precision for Medicine, formerly known as Apocell, markets the Apostream™, which is a cell sorting device optimized for separating CTCs from blood. This system capitalizes on the differing bioelectric signatures of peripheral blood mononuclear cells (PBMCs) using DEP-FFF, allowing for the isolation of rare metastatic cancer cells [104,105]. While the Apostream™ is primarily promoted as a label-free sorter for CTCs, other applications include stem cells and immune cells. To date, the Apostream™ is the only commercially available product that capitalizes on the selectivity of DEP for label-free cell separations under flow conditions.

Panasonic produced the Bacteria Counter as a rapid detection method for evaluation of oral hygiene by quantifying the number of bacteria present in the oral cavity with comparable accuracy to that of traditional culture methods [106]. This apparatus employs dielectrophoretic impedance measurement (DEPIM), or the concentration of a population using positive dielectrophoresis for subsequent impedance measurement of a concentrated sample as a result of capacitance and conductance between electrodes due to pearl chaining [107].

The primary use of dielectrophoresis both academically and commercially is the characterization of cell lines and separations of dissimilar cells. The sensitivity of dielectrophoresis, however, has not been fully realized in a clinical or commercial setting. Further, studies within the academic regime capitalize on the sensitivity of dielectrophoresis for characterization or identification, but devices that employ both the sensitivity and separation capabilities are lacking when it comes to the isolation of similar cells within a heterogeneous population. There remains a wide market for the commercialization of dielectrophoresis with a heightened sensitivity that is optimized or readily tunable for subtype detection and separation within a single sample.

4.2 Non-DEP competitors

Generally, dielectrophoresis is still an emerging technology not widely implemented in the industry; therefore, it must compete with other cell-sorting technologies widely used in the current market. Coincidentally, most of the market is comprised of biomarker sorting technologies. Of course, when entering the market of cell sorting, dielectrophoresis must set itself apart from other label-free cell sorting companies.

LumaCyte and their product Radiance® combine microfluidics and optics to identify sort cells. The sample will flow through a microfluidic channel, inducing a drag force, and a laser is used to apply an optical force to the sample. The image acquisition system then sorts the cells based on their deformation. The Radiance® is optimal for the study of viral titer, vaccine development, and cell therapy assessment [108,109].

The Beacon® Optofluidic Platform from Berkeley Lights advertises cell sorting for cell line and therapy development and biopharmaceuticals. Optical forces are used to manipulate single cells into tiny chambers called NanoPens. In these chambers, single-cell cloning and synthetic biology development are made possible through media profusion for on-chip culturing while providing information on cell properties such as size, phenotype, RNA expression, and cell-cell interactions to name a few. Optical forces are then used to move the cells/clones out of each NanoPen™ for export.

Clearbridge Biomedics produces the ClearCell® FX1 System for enriching CTCs from blood enabling downstream analyses [110,111]. From a clinical blood sample with red blood cells chemically lysed, the modified sample is loaded into the CTChip®, a disposable microfluidics chip using Dean Flow Fraction (DFF), an on-chip centrifugal technique, to enrich CTCs in less than 1h from 7.5 mL of patient blood. By using microfluidic hydrodynamic forces for the separation of CTCs, the target cells remain label free and can be collected for downstream immunofluorescence, protein analysis, and *in vitro* tumor models or co-culturing.

Biosyntagma aims to map the heterogeneity that contributes to the therapy resistance of complex biological samples. Their product, mPrint Mind™ , identifies areas of tissue that acquire drug-resistant properties and employs artificial intelligence to recommend treatment plans to address the heterogeneous sample. The mPrint Mind™ is most useful for the oncology sector by providing methods for biomarker discovery and personalized treatment recommendations.

The CellCelector™ from Automated Lab Solutions allows for the manual or fully automated selection of cells from a sample using an inverted microscope and imaging. The identification of the target cell can be performed automatically by setting size or morphology gates as well as positive/negative fluorescent staining. For a manual approach, the user can select the cell(s) of interest. Once the target cell(s) are identified, a micro-manipulation robot retrieves the cell(s) and places it in a designated location. This system can select for single cells, adherent colonies, and semi-solid cultures (e.g., spheroids). This system is advertised as beneficial to several applications including stem cell research, cell line development, cell cloning, and CTCs from blood [112–114].

While the aforementioned products have certainly introduced new ways for label-free cell sorting that have advantages over FACS, dielectrophoresis has the potential to separate cells based on their intrinsic bioelectric signature. Further, many of these technologies adopt superficial methods of distinguishing cells that lower the sensitivity of the separation or require user identification, fluorescent staining, or long culture times to be successful. Dielectrophoresis, on the other hand, provides another label-free alternative that capitalizes on the electric phenotypes of cells under an applied field, which, in theory, does not require extensive knowledge from the user to detect distinguishable heterogeneities in a single sample.

5 Future perspectives

While the studies discussed in Section 3 show the sensitivity of DEP to separate similar/related cell types to monitor therapy assessment, disease progression, and cell state, this particular application of DEP to differentiate similar cell subtypes has not been realized commercially. Several works highlight the trajectory of device design and the overall progress of the field [115,116]; this review will serve as insight into the possible directions of dielectrophoresis as it relates to similar/related cells and therapy assessment.

5.1 Commercialization

Commercialization of label-free cell sorting technologies, while available in many forms including dielectrophoresis, have not quite taken over the cell sorting market despite their advantages over traditional methods. Continued commercialization of dielectrophoresis is an attainable goal that can serve as a benchmark for the progression and evolution of the technology. Difficulties in commercializing DEP can be attributed to many factors such as difficult scale-up of the microfluidic manufacturing processes and unavailability of easy access to established protocols and characterization data from experimental set-ups in the literature. Further, more research should be done comparing DEP metrics such as specificity, sensitivity, and viability of the sample post-processing, to that of the golden standards in cell sorting to clearly and accurately communicate the advantages.

As mentioned, dielectrophoresis has been largely bound to the laboratory benches of the researchers studying the technology. This is generally due to the extensive sample preparation requiring the sample to be suspended in a low conductivity buffer as well as the expertise required to perform a successful dielectrophoresis experiment, particularly those that require induced flow (e.g., DEP-FFF) for separating subpopulations. Requiring an "expert experimentalist" not only keeps it out of the hands of the general user, but it makes the process difficult to automate.

Menarini, Precision Medicine, and DEPTech have been successful in creating apparatuses for cell characterization, manipulation, and physical separation of dissimilar cells, but these systems require the user to be well-versed in DEP to make them effectively tunable for varying needs. The hallmark of DEP is largely its sensitivity, but it lacks the automation needed to make it useful for general users wanting to separate similar cells as these frequency ranges are much more narrow than separations of dissimilar cells. While DEP is certainly capable of capitalizing on these narrow regimes for separation, minute detections in heterogeneity within a population could result in an undesirable hyper-sensitivity and generating difficulty in commercialization and automation efforts. Nonetheless, dielectrophoresis has been used for dissimilar cell separations successfully with modifications to device design and applied field parameters; thus insinuating a tunable sensitivity for clinical or commercial applications.

For automation to occur, however, established DEP protocols and an accessible library of collected cell electrical properties and DEP analyses should be readily available. It is important to note that some works are working towards standardizing dielectrophoresis. One example, in particular, is the Clausius–Mossotti simulation software, myDEP. This free software allows researchers to not only model the Clausius–Mossotti curve(s) relevant to their study, but it also allows for researchers to contribute to an accessible database after publishing on new cell properties [19]. Software such as myDEP has the potential to reduce variability in reported electrical properties of cell subpopulations, while also providing a standardized source and accessible location for cell lines characterized by DEP. Further, Hyler et al. has recently introduced a new low conductivity buffer that has been shown to reduce noise in dielectrophoretic spectra on the 3DEP and maintain cell viability and normal metabolic behavior [117]. Creating a standardized buffer for both researchers and end-users that maintains cell satisfaction will ultimately reduce the variation of cell characterization via DEP and improve the integrity of downstream culturing of sorted samples.

5.2 Direction of DEP technology

Dielectrophoresis has been extensively studied at low and intermediate frequency ranges (1 kHz–50 MHz) to identify cells based on the properties of their membrane; very few, however, have explored the ultra-high frequency range (>50 MHz) [17]. While several cell lines have been well-characterized based on their membrane capacitance, the sensitivity of DEP could be enhanced past detection of differences in surface expression and towards intracellular differences due to organelle content, cytoplasmic changes, or nuclear characteristics. The commercialized label-free methods, DEP and others, have yet to provide cell sorting based on intrinsic intracellular differences which are theoretically detectable in this frequency range. Increasing the range of DEP studies will provide a larger picture of the bioelectric signals that distinguish similar subpopulations. While heterogeneity within subpopulations is common, it is possible the sample could be more heterogeneous than previously thought if the internal structure of the cell was analyzed. The ultra-high frequency range (>50 MHz) is not extensively studied and could expand the potential applications of DEP as well as the commercialization possibilities. Similarly, the absolute resolution for dielectrophoresis remains unclear. While the aforementioned studies clearly show the capability of DEP to distinguish between similar subtypes, there is no conclusive measurement that defines precisely how sensitive this technology is. The development of standardization in evaluating the efficacy of DEP will not only provide a method for effectively drawing conclusions from various publications, but will also aid in characterizing the resolution and sensitivity of DEP.

The literature is rich with characterization of the dielectric behavior highlighting the sensitivity of DEP and its ability to separate similar cells without biomarkers. The next step, however, is to use the well-documented dielectric signatures of cells of different subtypes in devices that maintain high sensitivity under flow conditions for separation applications. This can be performed for similar cell separations by employing techniques like DEP-FFF active separation of target cells from a heterogeneous population. Current characterization methods are less helpful because without isolation, there is no potential for downstream retrieval and analysis, limiting the information collected from a single sample.

Dielectrophoresis, while shown to have high-fidelity detection capabilities for similar subpopulations, has remained bound primarily to laboratory benches for technology development or cell characterization. As the technology continues to advance, it is worthwhile not to forgo cell characterization and feasibility studies, but to in tandem pursue clinical applications where DEP will succeed. Dielectrophoresis does not need to perform superior to or replace every method of cell sorting and characterization currently being used, but its sensitive, label-free capabilities would be highly instrumental in clinical settings.

5.3 Specific biological applications

Dielectrophoresis has a myriad of potential applications, both in tandem with other technologies and on its own; some highlighted in previous sections. Research surrounding cancer and bacterial infection are two recently thriving fields where current and future DEP research could be highly beneficial.

Recently, the role of the tumor microenvironment in disease progression, metastasis, immune system evasion, and drug resistance, has been prominently studied for increased understanding as a whole as well as opportunities for new therapies. Similarly, the gut microbiome and the factors that influence it as it relates to immunity and the overall health of an individual has been another quickly emerging topic among researchers. Studying the tumor microenvironment and gut microbiome, both areas of high heterogeneity, require cell identification and separation techniques that are capable of distinguishing between hyper-similar cells, such as CSCs in different cell states or antibiotic-resistance of microbiota within the same genus, all with the intent for downstream assays and culturing. The use of dielectrophoretic techniques in these booming fields could provide the necessary viability of these precious samples, while maintaining desirable selectivity. In turn, a heightened understanding of these areas at a cellular or subpopulation level could lead to better platforms for *in vitro* treatment planning and personalized medicine.

In a related manner, dielectrophoresis has been shown to be useful in identifying subtypes of bacteria from the same genus. Particularly, gram-negative bacteria, with their affinity of acquiring drug-resistant genes, are associated with severe cases of sepsis, particularly bacteria from the genera of *Enterobacteriaceae* and *Staphylococcus* [61]. The feasibility of DEP as a method for rapid detection of strains within these genera has been shown through the aforementioned studies. Further, one current method for identification of bacterial infection in the blood is performing PCR following at least a 12h culture. While PCR offers the sensitivity required for infection detection in blood, this method cannot distinguish between live and dead cells. Additional techniques such as mass spectrometry, fluorescent *in situ* hybridization, or phenotype assays have been used to perform diagnostics in tandem with PCR for an accurate description [61]. Dielectrophoresis, as shown, has not only the capability to distinguish between similar strains of bacteria, but can also separate minute amounts of bacteria from blood cells and determine the antibiotic resistance of subpopulations. The establishment of dielectrophoresis as a feasible method for rapid diagnostics could be a method to perform multiple processes on a single microchip or multiple microchips in series.

6 Concluding Remarks

In the first 35 years following the introduction of dielectrophoresis, researchers have harnessed its ability to

characterize, manipulate, and separate differing cell populations. More recently, dielectrophoresis has been studied as a high-sensitivity tool for the identification and separation of similar/related subpopulations. This review summarizes the evolution of dielectrophoresis towards highly sensitive applications including distinguishing stem cells, cancer cells, blood cells, and bacteria for more complete profiles useful for describing differentiation potential, metastasis and stemness, and drug-resistance/-sensitivity. These studies, however, primarily focus on the low-frequency regime of DEP and capitalize on the membrane and cytoplasmic properties, while a full profile might be gained by exploring cell behavior at high frequencies. Despite the abundant literature highlighting the unique capabilities of DEP for characterizations and separations requiring high fidelity, current commercialization efforts of DEP perform best for dissimilar cells and still primarily remain on lab benches away from clinical settings. With recent advances in dielectrophoretic technology for subpopulations and similar cell types, there is an opportunity to develop standardized protocols and evaluation metrics, as well as high-sensitivity, user-friendly commercialization platforms created for the end-user.

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Rafael V. Davalos has patents in the area which have been licensed to a startup company.

Data availability statement

Data availability not applicable as no datasets were generated or analyzed for this article. Further permissions related to the material excerpted can be found in the corresponding references.

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