ADVANCES IN DIELECTROPHORESIS ON-A CHIP

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Rezumat. *Biopsia lichidă a apărut ca un instrument inovativ în oncologie, oferind o alternativă neinvazivă la biopsiile tradiţională. Una din abordările considerate utilizează celulele tumorale circulante (CTC) din fluidele biologice, cum ar fi sângele, urina şi saliva, pentru a oferi informaţii despre genetica şi dinamica tumorilor maligne. O metodă pentru izolarea CTC-urilor este utilizarea fenomenului de dielectroforeză folosind platforme microfluidice. Lucrarea de faţă trece în revistă progresele în domeniul dielectroforezei pe cip. Prezentăm mai întâi teoria de bază a dielectroforezei, urmată de principalele principii de funcţionare ale dispozitivelor. Discutăm în continuare câteva dintre tehnicile de separare prin dielectroforeză. În cele din urmă, prezentăm principalele provocări şi observaţiile finale. Traducerea tehnologiei DEP de la scară de laborator în aplicaţii industriale rămâne o provocare din cauza complexităţii proceselor ce creează câmpurile electrice neuniforme la scară largă.*

Abstract. *Liquid biopsy has emerged as a transformative tool in oncology, offering a non-invasive alternative to traditional tissue biopsies. One approach utilizes circulating tumour cells (CTCs) from body fluids such as blood, urine, and saliva to provide insights into tumour genetics and dynamics. A method for isolating CTCs uses the dielectrophoresis phenomenon and microfluidic platforms. The present work reviews the advances in dielectrophoresis on a chip. First, we present the fundamental theory of dielectrophoresis, followed by the main operating principles. We further discuss some of the separation techniques using dielectrophoresis. We finally present the main challenges and concluding remarks. We finally present the main challenges and concluding remarks. Translating DEP technology from lab-scale to industrial applications remains challenging due to the complexities of creating large-scale, non-uniform electric fields*

Keywords: Dielectrophoresis, Microfluidics, Lab-on-a Chip, Microtechnology, BioMEMS

1. Introduction

Microtechnology has revolutionized the field of microbiology by providing solutions for life study at the microscale. This interaction of micro and nanofabrication with biology and medicine implements the principles from

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engineering, physics, and material science for developing miniaturized systems and devices that enable precise manipulation [1-4], measurement [5, 6], and analysis [7-10] of biological processes. In the biomedical field, the application of micro- and nanotechnology has facilitated breakthroughs in cell biology, molecular diagnostics, and tissue engineering. Microfluidic devices, for instance, allow researchers to mimic physiological environments in vitro, enabling the study of cellular behaviour under controlled conditions [11-14]. Similarly, lab-on-a-chip systems have transformed diagnostics by integrating multiple laboratory functions onto a single microchip, enabling rapid and cost-effective analysis of biological samples [15, 16].

Moreover, microelectromechanical systems (MEMS) advancements have led to the development of biosensors and implantable devices that monitor biological signals in real-time, enhancing our understanding of complex physiological processes [17, 18]. These technologies provide unprecedented resolution and accuracy and open new avenues for personalized medicine, drug development, and synthetic biology [19, 20]. As microtechnology continues to evolve, its integration with biology promises to address critical challenges in healthcare, environmental monitoring, and biotechnology, making it a cornerstone of modern scientific innovation.

Dielectrophoresis (DEP), introduced by Herbert Pohl in 1951, refers to electrically polarised particles' movement in an uneven electric field. Early theoretical contributions in the field rose from 1879 when Rudolf Clausius and Ottaviano-Fabrizio Mossotti developed a formula describing the polarizability of dielectric materials, which later became essential for understanding DEP. The actual development of the field began in late 1990 when microfluidic emerged, allowing DEP to be integrated into lab-on-a-chip devices. The progress enabled applications such as cell sorting, characterization, and diagnostics. The development of AC-DEP (Alternating Current DEP) made the manipulation of nanoparticles, cells, and other biological entities possible with greater precision. DEP is now used to isolate rare cells, characterise cancer cells, and detecte pathogens. It also plays a role in drug discovery and personalized medicine. The integration of nanotechnology has pushed DEP to manipulate nanostructures for advanced applications in materials science, biosensing, and electronics. Here we present a comprehensive overview on advances in dielectrophoresis on a chip.

2. Basic theory of DEP

The expression of the dielectrophoretic force can be defined as: [21]

$$\langle \overline{F}(t) \rangle = 2\pi\varepsilon_1 R^3 \left\{ \text{Re}[K]\nabla E_0^2 + 2\,\text{Im}[K](E_{x0}^2\nabla\varphi_x + E_{y0}^2\nabla\varphi_y + E_{z0}^2\nabla\varphi_z \right\} \qquad (1)$$

where E_{i0} and $\varphi_i (i = x; y; z)$ are the magnitude and phase, respectively, of the field components in the principal axis directions. K is the well-known complex Clausius–Mossotti factor, defined as:

$$K = \frac{\varepsilon_2^* - \varepsilon_1^*}{\varepsilon_2^* + 2\varepsilon_1^*}, \quad \varepsilon^* = \varepsilon - j\frac{\sigma}{\omega} \qquad (4)$$

where ε_2^* and ε_1^* are the complex permittivity of the particle and the medium, respectively. The complex permittivity for a dielectric material can be described by its permittivity ε, conductivity σ, and angular frequency ω of the applied electrical field E

The expression (1) contains two terms contributing to the DEP motion. The first term relates to the *real* component of the induced dipole moment in the particle. This force directs the particle towards higher or lower electric field regions, depending on whether the $\text{Re}[K]$ is positive or negative. This is the conventional DEP term. The classical DEP force can be given by:

$$F_{DEP} = 2\pi R^3 \varepsilon_1 \,\text{Re}[K]\nabla E_0^2 \qquad (2)$$

The second term relates to the *induced dipole moment's imaginary component and to the field phase's spatial non-uniformity*. This force pushes the particle against or along the direction of travel of the electric field, depending on whether the phases of the field components is $(\text{Im}[K] > 0)$ or $(\text{Im}[K] < 0)$. This is called Travelling Wave Dielectrophoresis (TWD). The expression can be given by: [21]

$$F_{TWD} = \frac{4\pi^2\varepsilon_m r^3 \,\text{Im}[K(\omega)]E^2}{\lambda} \qquad (3)$$

If $\text{Re}[K] = 0$ or $\text{Im}[K] = 0$, the particle experiences no positive or negative DEP force. The frequency at which the particle shows no DEP force is called the crossover frequency. The crossover frequency depends on the dielectric properties of the particle and medium.

As a result, the DEP force depends on the Clausius-Mossotti factor), which varies with the frequency of the applied electric field. Particles experience **positive DEP** (attracted to high-field regions) or **negative DEP** (repelled from high field

regions) depending on the frequency. The **crossover frequency** is the specific frequency at which the particle experiences no DEP force. This frequency shifts with changes in the permittivity and conductivity of the particles and medium. Different types of particles in a mixture can experience opposite DEP behaviours at carefully selected frequency ranges, enabling separation. Besides DEP force, particles in a fluid are influenced by hydrodynamic, gravitational, electro-hydrodynamic, and Brownian forces (significant for sub-microparticles). Brownian motion poses a major challenge for manipulating sub-microparticles. Effective separation mechanisms require detailed consideration of all forces acting on particles. Different DEP device designs address these forces for specific separation tasks.
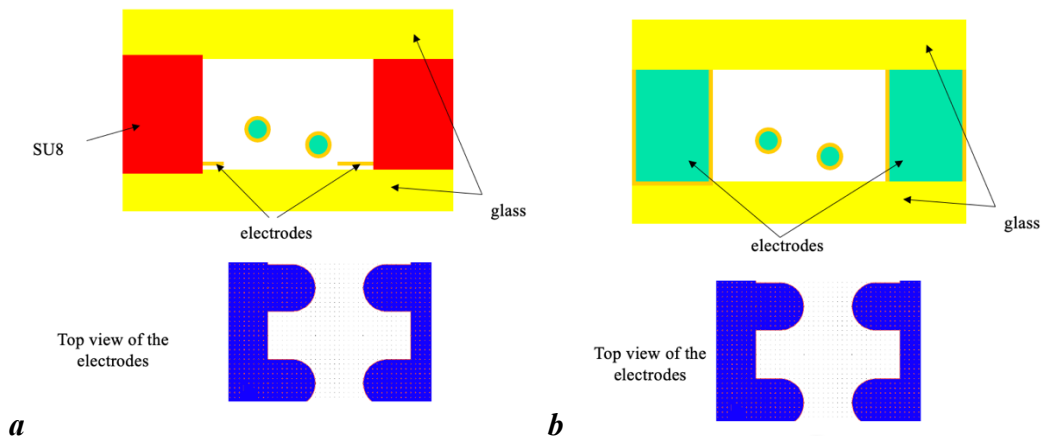
3. DEP devices

Different dielectrophoretic (DEP)-based devices have been designed for biological applications. A critical aspect of generating the DEP force is the generation of a gradient of the electric field. According to the solution for the generation of electric field gradients, the devices can be classified into:

- conventional DEP; [22-24]
- insulating DEP; [25-27]
- travelling wave DEP; [28-30]
- optical DEP. [31-33]

In Conventional DEP, the non-uniformity of the electric field is achieved by changing the electrode shapes, size or positioning in the microfluidic channel. These electrodes fabricated using microtechnology are integrated into a fluidic structure for easy manipulation or separation of the particles. This technique is the most common one. In the simplest version, metallic electrodes such as interdigitated [34], castellated [35]-figure 1a, curved [36, 37], and ring-shaped [38] are patterned using microfabrication technique on a glass plate on a microfluidic PDMS structure. Flowing the particles at a certain distance from the electrode plane requires an increased electric field followed by an increased temperature in the buffer solution -Joule thermal effect- (39). This limitation and the increased temperature can be overcome using 3D electrodes [40, 41]-Figure 1b, 3D asymmetric electrodes [42], or by placing the electrodes on top and bottom of the microfluidic channel [43].

Fig. 1. Conventional DEP devices: a) thin metal film on glass; b) with 3D electrodes

A critical aspect of the conventional DEP is the electric field's gradient distribution across the microfluidic channel's cross-section. While for the thin metal film, these gradients are presented close to the electrode for the devices with 3D electrodes [44], there is a uniform distribution across the cross-section of the microfluidic channel. This effect can be observed in Figure 2 presenting a simulation, where a finite element analysis (showing the electric field distribution) is performed between a planar and a 3D electrode configuration.
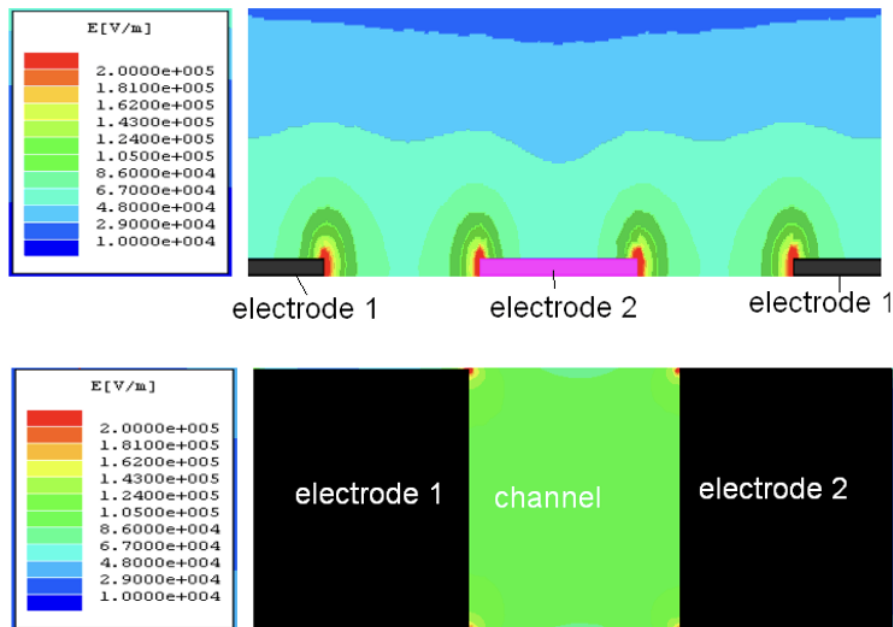
Fig. 2. Simulation of the variation of the electric field for a DEP configuration whit thin film electrodes and 3D electrodes

Figure 3 illustrates the variation of the DEP force for both structures previously analysed. It can be observed that for planar electrodes, the DEP force became very weak at 40 μm from the electrodes plane.
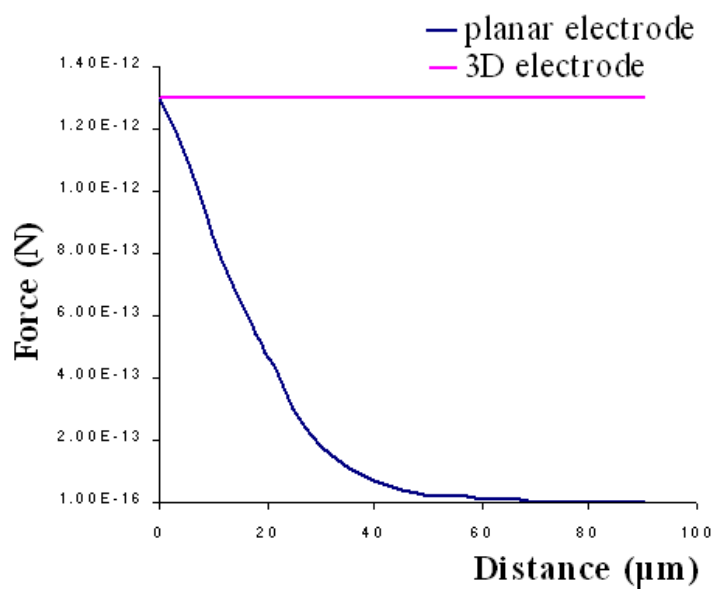

Fig. 3. Variation of the DEP force in vertical direction for planar electrode and 3D electrode

Insulating DEP employs microfluidic platforms to manipulate particles and cells in a non-uniform electric field generated by insulating structures such as posts, ridges, or other geometrical features within the microchannel that act as barriers that distort the electric field. The working principle is presented in Figure 4. These insulating structures can be made from the same material as the microchip or integrated during fabrication. Electrodes are usualy placed at the channel's inlet and outlet to establish the electric field. Unlike traditional dielectrophoresis, electrodes are external and do not require precision patterning within the microchannel. iDEP can operate using a direct current (DC) or alternating current (AC) electric field applied across the device. At the same time, the insulating structures disrupt the uniform field, creating regions of high and low field intensity. iDEP devices offer versatile solutions for applications ranging from biomedical diagnostics to environmental monitoring. The ability to manipulate particles without direct electrode contact reduces costs and enhances device longevity. A recent review on iDEP can be found in [27].
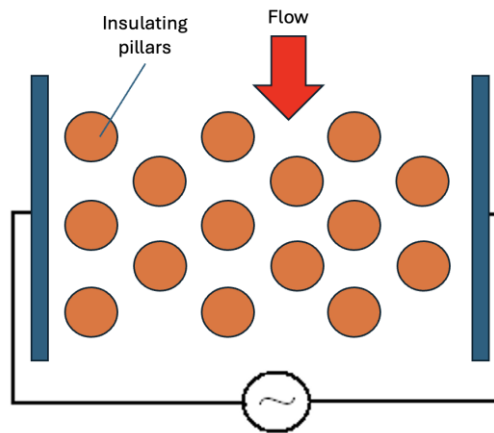
Fig. 4. Schematic presentation of iDEP device

Travel wave dielectrophoresis (twDEP) uses a travelling electric field created by applying AC voltages with phase shifts to multiple electrodes arranged in an array (see Figure 3). This results in a dynamic electric field that propagates spatially, forming a "travelling wave". The force on a particle in twDEP includes two components: (1) a DEP force due to the field gradient (like traditional DEP). (2) A component arises from phase differences in the electric field. This force moves particles along the direction of the travelling wave. The travelling wave force is independent of the direction of the DEP force and enables directional particle transport. An overview of twDEP is presented in [45].
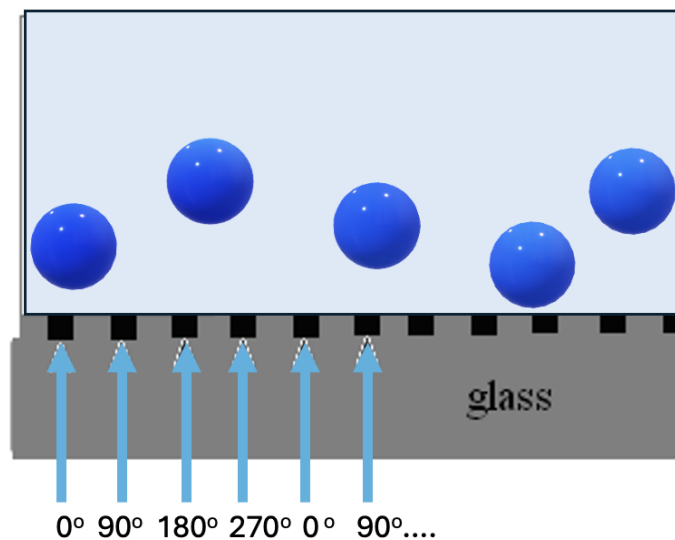
Fig. 5. Working principle of travel wave dielectrophoresis

Optical dielectrophoresis (oDEP) integrates principles of dielectrophoresis and optical forces for the precise manipulation and control of micro- or nanoparticles or cells. It leverages the interaction between a non-uniform electric field and induced dipoles in particles, combined with optically defined patterns, to achieve highly localized and dynamic particle movement. oDEP has some interesting advantages when using light patterns for fine control of the position and movement of particles. These patterns can be fast redefined to adapt to changing experimental needs. Moreover, it is a contactless method with reduced contamination risks or mechanical damage, being at the same time versatile and suitable for an extensive range of particles or cells. A recent overview of oDEP is presented in [46].

4. Cell sorting

Dielectrophoresis (DEP) is a label-free method that leverages cells' dielectric properties to separate them based on their size, membrane capacitance, and internal conductivity. DEP has been increasingly used to rapidly sort cells based on their dielectric properties. For instance, novel DEP-based platforms have improved cancer cell isolation from blood samples, facilitating early diagnosis and personalised medicine. An overview of circulating tumour cell isolation using dielectrophoresis is presented in [47].

Different cell sorting techniques have been developed. Early work was focused on proving the principle of separation, the most common application being a live/dead assay [48]. Iliescu *et al.* [49] report a bidirectional separation method using a DEP chip having a 3D electrode array. The working principle is presented in Figure 6a. The DEP chip is designed with a sandwich structure: glass/silicon/glass layers, as presented in Figure 6b. The top glass layer incorporates two inlets and two outlets, enabling bidirectional fluid input and output. The silicon layer serves as the structural foundation, defining the walls of the microfluidic channels and housing the electrode array, which comprises rows of prismatic pillars. The bottom glass layer includes via holes (one for each pillar) and a metallization layer that connects the silicon pillars in each row to form a single electrode. The 3D electrode structure in this device plays a dual role. First, it generates a uniform dielectrophoretic force across the microfluidic channel, facilitating effective particle manipulation. Second, it creates a fluid velocity gradient within the channel, which results in a variable hydrodynamic force. These combined forces enable precise particle separation and movement control, enhancing the device's performance and efficiency. The functionality of the chip was proved on live/dead yeast cells.
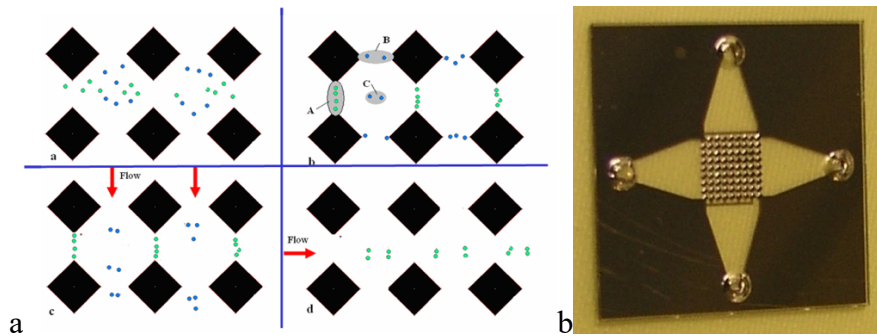
Fig. 6. Bidirectional separation in a DEP device: a) working principle; b) image with the device

Li *et al.* [50] introduced a microfluidic device designed for continuous DEP fractionation and purification of biological cell suspensions. The device comprises three key units: an injector for hydrodynamically focusing cells into a controlled stream, a fractionation region utilising a specialised electric field for cell separation, and two outlets for collecting sorted cells. The injector's behaviour was simulated and experimentally verified, and the device's performance was tested using yeast cells as a model. The device represents progress toward an integrated lab-on-a-chip system capable of performing complete laboratory procedures.

A field-flow separation technique under continuous flow in a DEP chip with 3D electrodes is presented in [51]. The the device's unique design enabled the proposed technique, where the electrodes generate dielectrophoretic forces and define the microfluidic channel's walls . This innovative design creates differences in flow velocity within the channel, a phenomenon exploited to separate two distinct cell populations. In addition to its functional capabilities, the device offers several key advantages. Its completely enclosed structure ensures containment and reduces the risk of contamination. The chip's compact size and small working volume suit applications requiring minimal sample quantities. Furthermore, the use of silicon electrodes eliminates the electrochemical effects typically associated with multi-metal electrode systems, enhancing the device's reliability.

Alazzam *et al.* [52] present a method for the continuous flow separation of MDA231 breast cancer cells from blood using a microfluidic device and dielectrophoresis. The method accurately separated cancer cells from normal blood cells, enabling precise counting, separation, and sub-culturing. Separation was conducted using interdigitated comb-like electrodes arranged in divergent and convergent orientations, with an AC signal of 20 V peak-to-peak and frequencies between 10–50 kHz. The technique relies on the differential response of

malignant and normal cells to dielectrophoretic and hydrodynamic forces at specific frequency bands. The study discusses the experimental setup, including cell preparation, suspension medium, flow conditions, and microchip fabrication.

Kim *et al.* [53] proposed a dielectrophoretic device for efficiently trapping and recovery low-abundant CTCs from large sample volumes, minimizing sample loss during molecular analysis. The device employs dielectrophoresis-based trapping with an attraction zone to control cell positioning and a trap zone to slow flow and capture target cells. It achieved a trapping efficiency of $92 \pm 9\%$ at high inlet flow rates (100 µl/min) and reduced the sample volume by 100-fold for downstream analysis. The system demonstrated its feasibility by successfully trapping CTCs mixed with white blood cells and detecting single nucleotide variants from the recovered cells.

An insulator-based dielectrophoresis (iDEP) device was used in [54] for isolating and enriching low-abundance polystyrene particles and yeast cells. Using mixtures with concentration ratios as low as $1:10^7$, the device achieved over 99% trapping efficiency, capturing particles stably for up to 4 minutes. A four-reservoir system successfully concentrated and redirected rare particles for collection and analysis. The results highlight the capability of iDEP devices for efficiently screening, isolating, and enriching rare particles and cells, making them useful for bio-analytical and clinical applications.

In another study, [55] a field-flow dielectrophoretic (DEP) separation method using a 3D filtering chip, which mimics a capacitor with parallel plate electrodes made of stainless steel mesh and a dielectric medium composed of silica beads in buffer solution (iDEP method). DEP forces, generated by electric field gradients due to non-uniform dielectric media, enable the separation of cell populations based on their dielectric properties. Cells exhibiting positive DEP are trapped near silica bead contact points, while those with negative DEP are repelled and flushed out by hydrodynamic forces. The method was tested on live and dead yeast cells, achieving optimal separation at 150 V, 10–20 kHz frequency, and flow rates of 0.1–0.2 ml/min. Cascading multiple devices can enhance efficiency, making this technique a cost-effective and time-saving tool for continuous cell separation in biopharmaceutical applications.

Wu *et al.* [56] introduced a DEP method using the capture voltage spectrum to measure the dielectric properties of biological cells. The approach involves balancing dielectrophoretic and Stokes drag forces acting on cells within a microfluidic device. The method was applied to human colon cancer cells (HT-29), allowing the real part of the Clausius–Mossotti factor to be determined across

various electric field frequencies. The dielectric properties of the cell interior and membrane were estimated using a single-shell dielectric model. The cell interior's permittivity and conductivity were unaffected by changes in the medium's conductivity, while the membrane's properties increased with higher medium conductivity. Moreover, using DEP, the capture voltage spectrum proved valuable for optimizing conditions to separate HT-29 cells from other cell types, like red blood cells.

In [57] the authors integrated optically induced dielectrophoresis (ODEP) and flow velocity control in a microfluidic system. This system uses four optical filters to separate CTCs from leukocytes based on size differences, enhancing the purity of the isolated cells. The optimal conditions for the system, including light bar width, gap, and flow rate, were determined experimentally. The results show that this method can isolate cancer cells with a purity of 94.9%, achieving a cancer cell recovery rate of 54%.

Yu *et al.* [58] proposed a sequential dielectrophoretic field-flow separation method using a chip with a 3-D electrode structure, where the walls of the microfluidic channels also act as electrodes. This design allows the creation of electric and fluid velocity gradients, to separate two particle populations based on dielectrophoresis (DEP). The method involves four steps: 1) filling the microchannel with a mixture of particles, 2) trapping particles at different locations based on their DEP responses (positive DEP particles at minimum electrode distance and negative DEP particles at maximum distance), 3) increasing flow to sweep out the positive DEP population, and 4) removing the electric field to collect the second population. The method was demonstrated with viable and nonviable yeast cells.

Another study [59] presents an innovative approach to cell isolation and sorting using tunnel dielectrophoresis (TDEP) to manipulate cells by size. The technology demonstrated in this research separates the polystyrene micro-particles with a size difference as small as 1 μm, achieving a separation purity greater than 90%. This method is notable for its ability to perform high-pass, low-pass, and band-pass filtering of a mono-cellular mammalian population with a tunable bandwidth as small as 3 μm. Additionally, the device was successfully used to sort leukocyte subtypes, specifically isolating monocytes from peripheral blood mononuclear cells (PBMCs) in whole blood, achieving a high purity (>85%).

Another field-flow separation method under continuous flow uses a DEP chip with asymmetric electrodes. The DEP device comprises one thick electrode forming the microfluidic channel's walls and one thin electrode. This structure

generates a strong vertical electric field gradient that levitates particles experiencing negative DEP. The separation technique traps one particle population at the bottom of the channel using positive DEP while the other, exhibiting negative DEP, is levitated and flows out. The method was tested using viable and nonviable yeast cells [60].

[61] presented a DEP-based microfluidic system for separating MDA-MB-231 cancer cells from different subtypes of white blood cells (WBCs) emphasing cell viability for post-processing operations like cell culture and genetic analysis. The study explores three sidewall electrode configurations to assess separation performance. Simulation results show that semi-circular electrodes perform best, with a 95% recovery rate under consistent conditions. The applied electric field is lower than the threshold for cell electroporation, and Joule heating studies confirm that the cells remain undamaged.

In [62], the authors presented a new dielectrophoretic-based microfluidic device designed for precise separation of multiple particle and cell types. The device uses two sets of 3D electrodes—cylindrical and sidewall electrodes-and features three outlets for separated particles: one for negative dielectrophoresis force and two for positive. The device was tested with red blood cells (RBCs), T-cells, U937-MC cells, and *Clostridium difficile* bacteria. Results showed that sidewall electrodes with a 200 µm gap were optimal for efficient separation, with a maximum separation efficiency of 95.5%. The device effectively separates particles without exceeding cell electroporation thresholds, performing best at specific voltage settings for each separation step.

[63] presented a dielectrophoretic (DEP) method for cell patterning using a dielectrophoretic–hydrodynamic trap. The device uniquely combines conventional electrode-based DEP (eDEP) with insulator-based DEP (iDEP). DEP forces are generated between a top indium tin oxide electrode and a thin CrAu electrode. In contrast, an SU8 photoresist cage around the thin electrode modifies the electric field to create iDEP forces. Cells flowing through the microfluidic channel are trapped in the SU8 cage, enabling cell patterning based on cell size and the SU8 layer thickness. Smaller cells can form 3D structures due to dipole–dipole interactions. This method has potential applications in genetic, biochemical, and physiological cell studies.

5. Challenges and Perspective

Several industrial achievements of dielectrophoretic applications such as bacteria counting (Panasonic), nanoparticle analyser (Shimadzu IG-1000), cell

manipulation (Silicon Biosystems DEPArray), determination of dielectric properties of cells (DEPtech 3DEP), CTCs isolation of tumour cells (ApoStream), isolation of nanoparticular biomarkers (Biological Dynamics) are already marketed. Besides these aplications, translating DEP technology from lab-scale to industry remains challenging due to the complexities of creating large-scale, non-uniform electric fields. Moreover, DEP force magnitudes are relatively low, so DEP is usually employed under quasi-static conditions with slow-moving fluids. The operation of the dielectrophoretic devices at low velocities increases the processing time and requires parallel processing of the samples. For very small (below 50 nm), the Brownian move of the fluid generates forces comparable to or greater than the DEP force, limiting the ability to trap or manipulate particles below a certain size. More complex hydrodynamic effects can influence particle behaviour in flow-focusing systems or systems with higher Reynolds numbers. Another challenge related to the transformation from "chip-in-the-lab" to lab-on-a-chip" is that incorporating DEP-based systems into portable, cost-effective diagnostic devices is a key focus for researchers. Nevertheless, the performance of DEP devices depends on the dielectric properties of materials, which can limit their applicability in complex environments.

Dielectrophoresis' versatility makes it a cornerstone for innovation in diverse scientific fields. Advances in microfabrication, machine learning, and materials science are expected to address current limitations, enabling DEP to achieve its full potential. Developing hybrid systems combining DEP with optical or magnetic forces may unlock new particle manipulation and separation avenues. DEP is a testament to how fundamental physics can drive practical solutions to modern challenges, offering a promising trajectory for future research and application.

6. Conclusions

Recent achievements in DEP underscore its versatility and potential to address challenges across scientific disciplines. Continued innovation in device design, integration with complementary technologies, and exploration of novel applications promise to expand the horizons of DEP in the coming years. Important to note is the fact that DEP represents an isolation method which is label-free, scalable and can be integrated with downstream analysis platforms. For CTCs isolation, DEP presents the unique feature of combining the method's selectivity with the conservation of the cell surface membrane. However, to be widely used and accepted in the clinics, types of DEP buffer, duration of separation and range of voltages have to be optimised in an attempt to preserve

the true state of CTCs. Addressing the major challenges in cell separation using integration will allow a better use of the DEP phenomenon for clinical and biological applications.

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