Biosensors and Bioelectronics 142 (2019) 111526

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

MEMS impedance flow cytometry designs for effective manipulation of micro entities in health care applications

Mahesh Kumar[a], Supriya Yadav[b], Ashish Kumar[c], Niti Nipun Sharma[d], Jamil Akhtar[a,e], Kulwant Singh[a,*]

[a] Department of Electronics & Communication Engineering, Manipal University Jaipur, 303007, Rajasthan, India
[b] Department of Bioscience, Manipal University Jaipur, 303007, Rajasthan, India
[c] Nano Bio Sensors Group, CSIR-Central Electronics Engineering Research Institute, Pilani, 333031, Rajasthan, India
[d] Department of Mechanical Engineering, Manipal University Jaipur, 303007, Rajasthan, India
[e] Flexible and Non-Silicon Electronics Group, CSIR-Central Electronics Engineering Research Institute, Pilani, 333031, Rajasthan, India

ARTICLE INFO

Keywords:
Cell manipulation
MEMS
Flow cytometry
Microfluidics
Dielectrophoresis
Lab-on-chip

ABSTRACT

Efficient manipulation of micro biological cells has always been a very important task in healthcare sector for which a Micro Electro Mechanical System (MEMS) based impedance flow cytometry has been proven to be a promising technique. This technique utilise the advantage of dielectrophoresis (DEP) force which is generated by non-uniform electric field in a microfluidic channel using an appropriate external AC supply at certain frequency range. The DEP forces generated in micro-channel depend upon various biological and physical parameters of cell and suspending medium. Apart from that design parameters of microfluidic channel and dimension of electrodes used for generating DEP action also plays major role in micro cell/bead manipulation. This article give remarks on the operating parameters which affects the cell manipulation and interrogates the currently accepted various electrode orientations in microfluidic MEMS flow cytometer technologies for effective manipulation of micro entities like healthy human cells (T-lymphocytes, B- lymphocytes, Monocytes, Leukocytes erythrocytes and human kidney cells HEK293), animal cells (neuroblastoma N115 and sheep red blood cells), cancer cells (MCF-7, MDA-435 and $CD34^+$), yeast cells (saccharomyces cerevisiae, listeria innocua and *E. coli*) and micro particles (polystyrene beads) based on their dielectric properties using DEP action. Article focuses on the key electrode orientations for generation of non-uniform electric field in microfluidic flow cytometer like tapered electrodes, trapezoidal electrode arrays, Interdigitated electrodes, curved microelectrode and 3D electrode orientations and give remarks on their advantages and limitations. The cell manipulation with current MEMS impedance flow cytometry orientations targeting possibilities of implementation of the lab-on-chip devices has been discussed.

1. Introduction

Cell is the unit building block of human body which may get damaged by many viruses, bacteria and pathogens (Johnson, 1983). This makes the study of cell behaviour an uttermost important task which has to be monitored over time. Many diseases occur on temporary basis like diabetes, dengue, malaria but some of the diseases like cancer hampers the human body with a tragic situation and propagates its impact with increasing time (Heim and Mitelman, 2015). An early information about the disease like cancer is much crucial for its diagnosis (Hingorani et al., 2003; Wulfkuhle et al., 2003; Xing et al., 2010). The impact study at cell level using different methods like impedance cytometry (Holmes et al. Morgan. 2009; Holmes and Morgan, 2010; Bernabini et al., 2011) and optical cytometry (Krivacic et al., 2004; Lincoln et al., 2004; Durack and Robinson, 2004) using microfluidic approaches is possible by cell analysis. The manipulation and separation of micro cells is possible using many techniques which are available like fluorescence activated cell sorter (FACS) (Villas, 1998; Fu et al., 1999; Yang et al., 2006; Cho et al., 2009), magnetically activated cell sorter (MACS) (Miltenyi et al., 1990; Handgretinger et al., 1998; Smistrup et al., 2005; Furlani and Sahoo, 2006; Han et al., 2006; Liu et al., 2007; Estes et al., 2009; Saliba et al., 2010; Zborowski and Chalmers, 2011), dielectrophoresis (DEP) (Das et al., 2014; Burgarella et al., 2010; Moon et al., 2011; Choi and Park, 2005; Wang et al., 2009; Li and Bashir, 2002; Muller et al., 1999; etin et al., 2009; Kang et al., 2009; Khoshmanesh et al., 2010), acoustic cell sorters (Hawkes and

* Corresponding author.
E-mail addresses: kulwant.singh@jaipur.manipal.edu, eckulwant@gmail.com (K. Singh).

https://doi.org/10.1016/j.bios.2019.111526
Received 6 May 2019; Received in revised form 2 July 2019; Accepted 18 July 2019
Available online 23 July 2019
0956-5663/

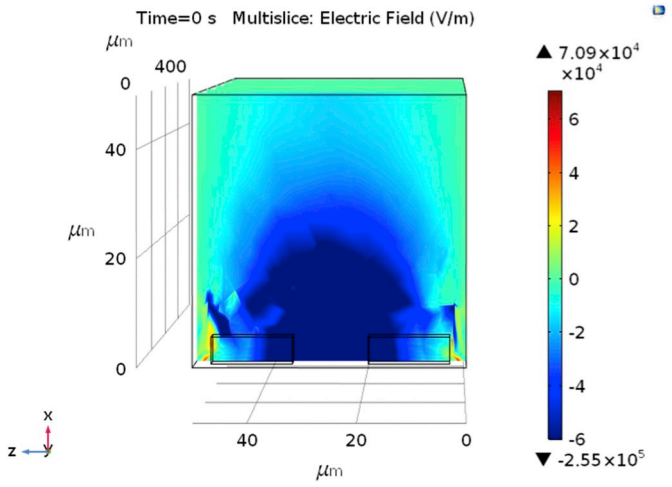

Fig. 1. Electric field distribution in micro-channel with coplanar orientation.

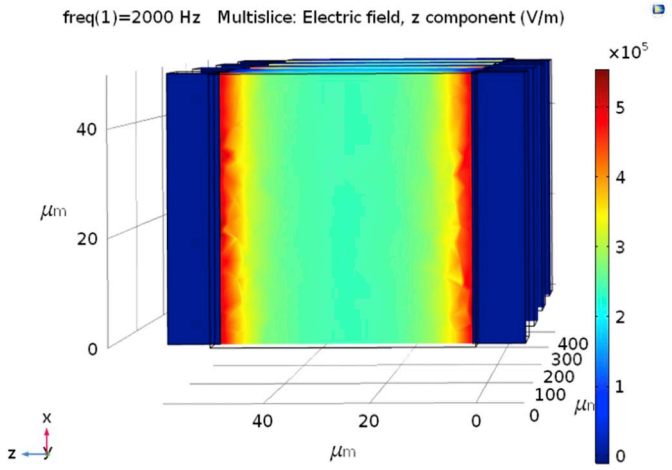

Fig. 2. Electric field distribution in micro-channel with side wall electrodes avoiding dead zone.

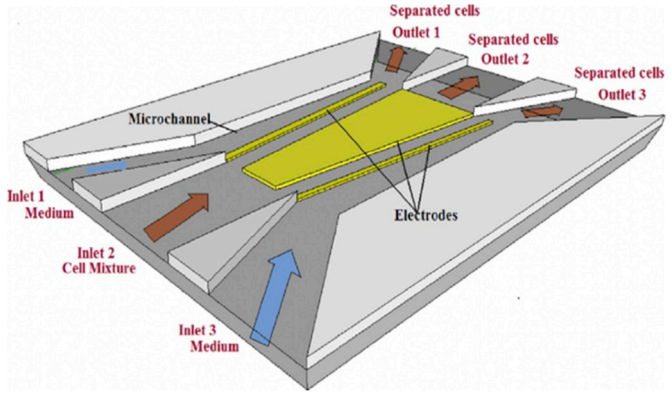

Fig. 3. Tapered electrode oriented microfluidic device (Das et al., 2014).

Coakley, 2001) and filtering methods (Kim et al., 2014; Huang et al., 2014; Liu et al., 2017). Dielectrophoresis which has been globally accepted for cell manipulation techniques due to its advantages like no switching or moving parts, cell specific control, utilises the benefit of dielectric properties of a micro cell (Nagel, 2000). Any cell is made of its cell inner resistance, inner capacitance (internal) and membrane capacitance (external) (Nagel, 2000). When a cell is suspended in a medium in between potentially excited electrodes it experiences

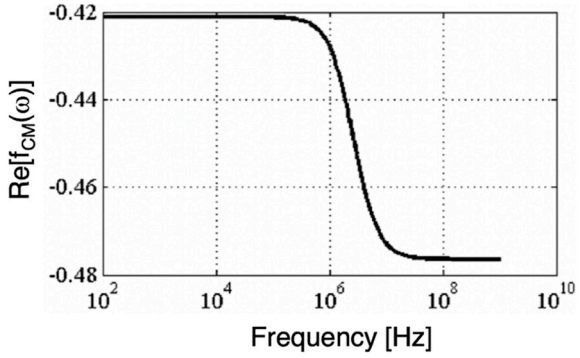

Fig. 4. Frequency cross-over where R_e [f_{cm}] changes its sign (Das et al., 2014).

different forces at different source frequencies. These forces include electro-rotational force and dielectrophoretic force which depend upon strength of electric field, supply frequency, conductivity of cell, conductivity of medium, complex relative permittivity of cell and complex relative permittivity of medium (Pohl, 1958). Out of these six parameters strength of electric field and frequency can be varied to get desired force during the experiments. Stronger electric field generates stronger dielectrophoretic force, but heat generated due to strong electric field may destroy the cell properties (Kotnik and Miklavčič, 2000) so strong electric field at a desired location using less applied potential at electrodes is essential. For this different electrode orientation are implemented by integrating the microfluidic channels along with required electrodes with the help of MEMS technology make complete miniaturized and reliable impedance flow cytometry configurations. Various research groups have demonstrated the integration of MEMS with complex system for biological application which are called as Bio-MEMS technology (Luo et al., 2019; Tsutsui and Ho, 2009; Alam et al., 2018; Chiu et al., 2018; Béné, 2017). There are many articles which provide substantial understanding of MEMS technology for cell manipulation, tissue engineering and etc. (Béné, 2017; Ni et al., 2009; Lee et al., 2015; Huh et al., 2005).

This paper focusses on the different separation techniques using microfluidic impedance flow cytometers based on electrode orientations and materials & makes comparative remarks on these technologies. First working principal of the dielectrophoresis force for cell manipulation technique and its relevance to electro-physical design and operating parameters has been discussed. Author also represents some observation based on simulation of fluid dynamics & electric field distribution in a microchannel using COMSOL Multiphysics 5.3. Then capabilities and performance of DEP manipulation technique with various currently accepted electrode orientations with suitable parameters have been reviewed. Further future alternatives for cell separation in microfluidic techniques targeting lab-on-chip device is discussed.

2. Theory of dielectrophoretic force

The term dielectrophoresis was first time coined in 1958 by H. A. Pohl et al.. (Pohl, 1958). Force induced by the interaction of cell (having certain dielectric properties) in a dielectric medium with an externally applied electric field which is distributed non-uniformly in the microfluidic environment is known as dielectrophoretic force (Jubery et al., 2014). There are basically two different forces which are the part of dielectrophoretic force termed as dielectrophoretic dragging (DEP drag) force and electro-rotational (ER) force. The DEP drag force moves a cell towards the electrodes having either stronger electric field or weaker electric field based on cell & medium properties if the cell is comparatively more polarizable than the medium then the cell is attracted toward the strong electric field (repelled by the weak electric field) but if the cell is less polarizable than the medium then cell is

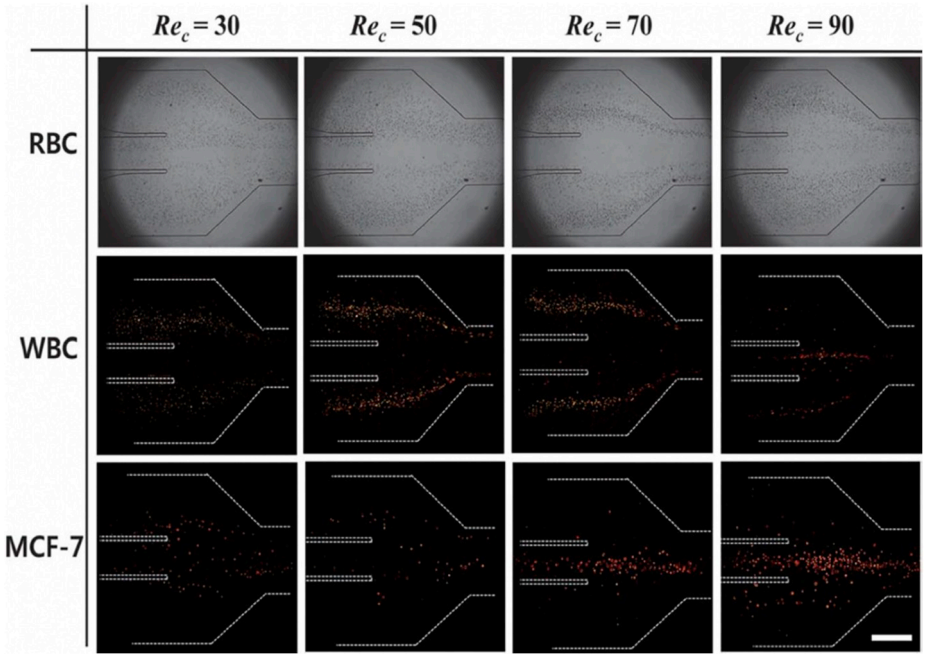

Fig. 5. Cell trajectories through MOFF channel according to channels Reynolds number R_{ec} (Moon et al., 2011).

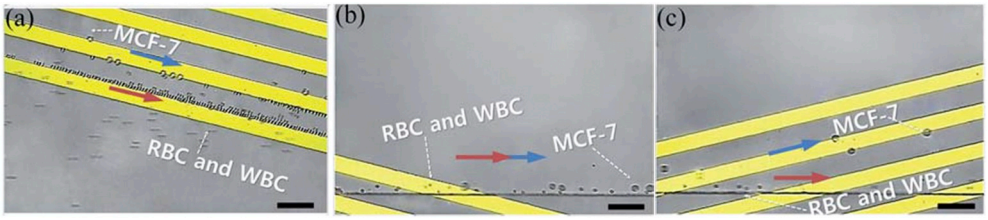

Fig. 6. (a) Generating p-DEP force for effective focusing at 10 V_{pp} and 2 MHz (Moon et al., 2011); (b) Cells focused near the side wall (Moon et al., 2011); (c) Separation of MCF-7 cancer cells using p-DEP at 10 V_{pp} and 900 kHz (Moon et al., 2011).

repelled by the strong electric field (or attracted towards the weak electric field). ER force rolls the cell either in clockwise direction (in direction of flow) or in anti-clockwise direction (in opposite to the direction of flow). Both the forces strongly depend on electric field and source frequency. The mathematical expression dielectrophoretic force is given in equation (1). Here ε_m, a, E are permittivity of medium, radius of the cell/particle and rms value of electric field respectively.

$$<\vec{F}_{DEP}> = 2\pi\varepsilon_m a^3 \,\mathrm{Re}[f_{cm}]\nabla|E|^2 \tag{1}$$

The DEP drag force which plays major role in cell separation, cell trapping and cell dragging depends upon the real part of Clausius Mossotti-factor R_e [f_{cm}]. For positive values of R_e [f_{cm}] the cell experiences the force which acts towards stronger electric field and this force is known as positive dielectrophoresis (p-DEP). When R_e [f_{cm}] is negative, cell experiences the force which drags it towards weaker electric field and this force is known as negative dielectrophoresis (n-DEP). Clausius Mossotti factor depends on complex dielectric permittivity of medium and cell of interest, the expression for Clausius Mossotti factor is given in equation (2).

$$f_{cm} = \frac{\varepsilon_c^* - \varepsilon_m^*}{\varepsilon_c^* + 2\varepsilon_m^*} \tag{2}$$

ε_m^* depends upon the electric field frequency and conductivity of the medium and known as complex dielectric permittivity of the medium. ε_c^* also depends on electric field frequency and cell conductivity and it is known as complex dielectric permittivity of cell. The mathematical expression for both the complex dielectric permittivity ε_m^* and ε_c^* is given below in equations (3) and (4). Here σ_c and σ_m are the electrical conductivities of cell and medium respectively.

$$\varepsilon_c^* = \varepsilon_c - \frac{i}{\omega}\sigma_c \tag{3}$$

$$\varepsilon_m^* = \varepsilon_m - \frac{i}{\omega}\sigma_m \tag{4}$$

The Clausius Mossotti factor can be further represented as equations (5) and (6) with in real and imaginary part using equations (3) and (4)

$$\mathrm{Re}[f_{cm}] = \frac{\left[(\varepsilon_c - \varepsilon_m)(\varepsilon_c + 2\varepsilon_m) + \frac{1}{\omega^2}(\sigma_c - \sigma_m)(\sigma_c + 2\sigma_m)\right]}{\left[(\varepsilon_c + 2\varepsilon_m)^2 + \frac{1}{\omega^2}(\sigma_c + 2\sigma_m)^2\right]} \tag{5}$$

$$\mathrm{Im}[f_{cm}] = \frac{\left[\frac{1}{\omega}\{(\sigma_c - \sigma_m)(\varepsilon_c + 2\varepsilon_m) - (\varepsilon_c - \varepsilon_m)(\sigma_c + 2\sigma_m)\}\right]}{\left[(\varepsilon_c + 2\varepsilon_m)^2 + \frac{1}{\omega^2}(\sigma_c + 2\sigma_m)^2\right]} \tag{6}$$

Here equation (5) is responsible for dielectrophoretic dragging force i.e. DEP and equation (6) is responsible for electro-rotational force. The frequency where Re [f_{cm}] changes its sign from negative to positive or positive to negative is known as dielectrophoretic cross over frequency in hertz which is given by equation (7) & can be calculated by equating R_e [f_{cm}] = 0.

$$f_{crossover} = \frac{1}{2\pi}\sqrt{\frac{(\sigma_m - \sigma_c)(\sigma_c + \sigma_m)}{(\varepsilon_c - \varepsilon_m)(\varepsilon_c + 2\varepsilon_m)}} \tag{7}$$

DEP dragging forces can be utilised for single cell caging for example quadrupole electrodes with all electrodes performing p-DEP/n-DEP action simultaneously on micro cell. By generating equal either p-DEP force or n-DEP force an impedance flow cytometry design can be utilised for efficient trapping. If multiple cells need to be separated then cytometry designs with relevant electrode orientation and channel

Table 1
Design parameters of microfluidic impedance flow cytometers with tapered electrodes.

Electrode materials	Electrode dimension	Cell of Interest	Medium	Techniques Involved	Channel Gap/Gap between working electrodes	Channel Height	Cite
Au/Cr	50 μm × 50 μm with 9°tapering angle	Polystyrene particle of 10 μm and human carcinoma (Hela) cells	phosphate buffered saline (PBS) with an electrical conductivity of 0.156 mS m^{-1} with 5% BSA	DEP Separation	Electrodes separated by 50 μm	50 μm	Das et al. (2014)
Au (250 nm) & Cr (6 nm)	45° tapering angle for Four Caging electrodes, same angle for focusing & separation	*Saccharomyces cerevisiae* (SC) cells of 8 μm and sheep red blood (SRB) cells of 5.2 μm	Aqueous solution with certified electrical conductivity of 435 μS cm^{-1}	Hydrodynamic Focusing, DEP Caging and DEP Separation	Gap between electrodes: 50 μm	400 μm	Burgarella et al. (2010)
Au/Cr	3000Å gold electrode tapered at −15° to 15° angle	Human breast cancer cells (MCF-7) having diameter16-24 μm, blood cells (RBC with 6–9 μm diameter and WBC with 6–10 μm diameter)	Isotonic solution with PBS with 570 μS m^{-1} conductivity and 1% bovine serum albumin (BSA)	Multi-orifice flow fractionation, DEP Focusing, DEP Separation, Hydrodynamic Separation	Electrodes separated by 40 μm	Channel for DEP separation: width 6000 μm, Separation length of 30 mm and of Height 50 μm	Moon et al. (2011)
Not Specified	Multiple electrode sets with glancing angle of 10°and 8°	Polystyrene particles with 10 μm, green and red fluorescent polystyrene particles of 5 μm and non- targeted polystyrene particles of 2 μm diameter	0.1xPhosphate buffer Saline, 1% bovine serum albumin & 20% Glycerol	DEP Separation	Not Specified	40 μm	Kim et al. (2008)

dimension can be used subjected to use of appropriate DEP drag force.

3. Dependancy of design parameters & micro electrode orientations on cell manipulation

3.1. Dead zone

In coplanar electrode orientation designs of impedance flow cytometers the electric field generated by the electrodes at an external applied voltage and frequency there exist some micro-channel area where the electric field is nearly zero or negligible. A typical microchannel with coplanar electrodes shown in Fig. 1. The electric field dead zone can be observed at the top of the microchannel having cross sectional dimensions 50 μm × 50 μm.

These electric field dead zones do not contribute in cell handling process due to which separation efficiency deteriorates significantly as the forces induced by the electrodes do not reach to the cells which are present in dead zone of electric field. Simulation shows that the coplanar electrode orientation (in which electrodes are placed on the bottom surface of the micro-channel) has the strong positive and negative field impact near the electrodes but with increase in channel height the electric field neutralises so the cells which are flowing much far height cannot be manipulated efficiently.

To avoid these dead zones of electric field, electrodes should be formed as the channel walls (Iliescu et al., 2006) so that maximum area should be utilised for the cell separation. Simulation result for electrodes formed as side walls is shown in Fig. 2 where dead zone is significantly minimised. Dead zones can also be avoided with the help of increasing the operating voltage but as the cells membrane are prone to its dielectric breakdown which occurs due to increase in heat at higher voltages.

3.2. Joule heating & bio compatibility

Joule heating in microfluidic device may get generated internally due to applied electrical field (Feng et al., 2007; Kunti et al., 2018) or externally (Green et al., 2000; Gonza´lez et al., 2006) due to illuminance. The thermal effects generated due to heat gradient in a microfluidic device transports the cells in a targeted area if the heating effect in permissible limit of micro bio entity this dragging action due to thermal gradient is known as electrothermal flow. A biological cell consist of cell membrane behaves like a capacitance when cell is suspended in electric field. Higher voltage at electrodes leads to heating effect in micro channel of the flow cytometry device which may damage the cell membrane (Zimmermann et al., 1975) and the cell may lose its semipermeable properties (Sale and Hamilton, 1968) which will lead to loss of capacitive or dielectric effect of cell. As the phenomenon of dielectrophoresis deals with interaction between dielectric properties of cell and the medium, if the cell loses its capacitive effect it will no longer exhibit dielectrophoresis and the separation with DEP will not be possible. Joule heating may vary with different electrode designs of impedance flow cytometer so optimised design with minimum heating effect with maximum DEP force is always desirable.

3.3. Impact of flow rate

The manipulation activities of micro cells in a continuous flow of suspending medium majorly depends on the flow rate. At higher flow rates the hydro-dynamic forces are much higher and may override the DEP action resulting in poor manipulation efficiency (Choi and Park, 2005). For effective separation with high efficiency manipulation process has to be carried out at lower flow rate but due to this MEMS impedance flow cytometry devices have to trade off with manipulation and separation time and this makes device incapable of handling large samples. High separation efficiency with lower flow rates can be achieved in very less time by implementing the parallel focusing and

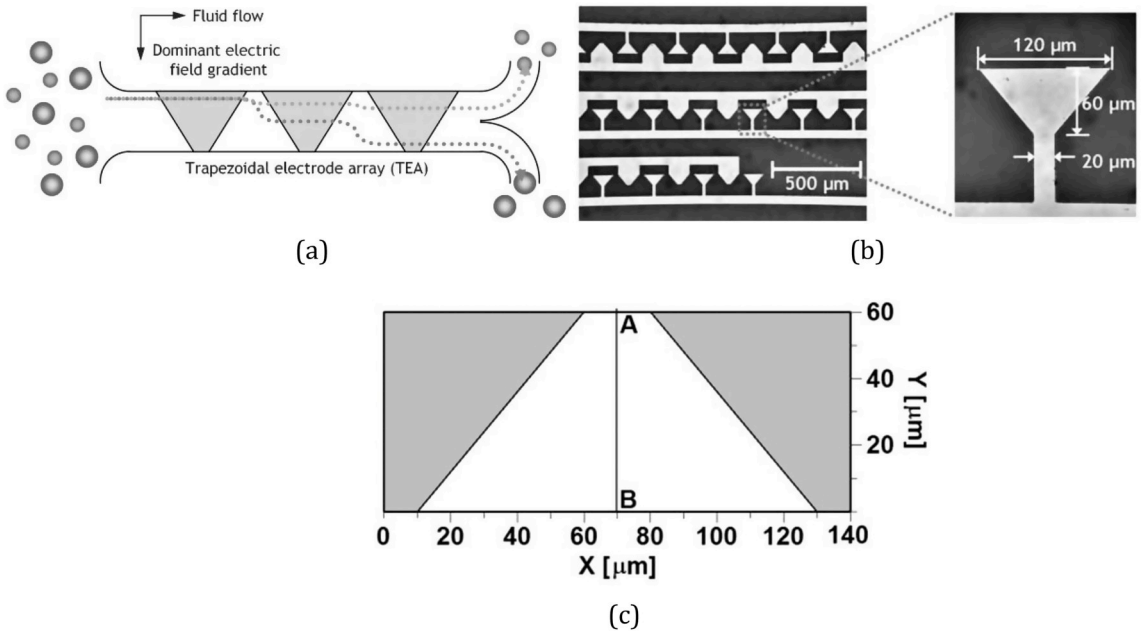

Fig. 7. (a) Trapezoidal electrode microfluidic device scheme (Choi and Park, 2005); (b) Dimensions of single trapezoidal electrodes (Choi and Park, 2005); (c) Gap size of between two electrodes (Choi and Park, 2005).

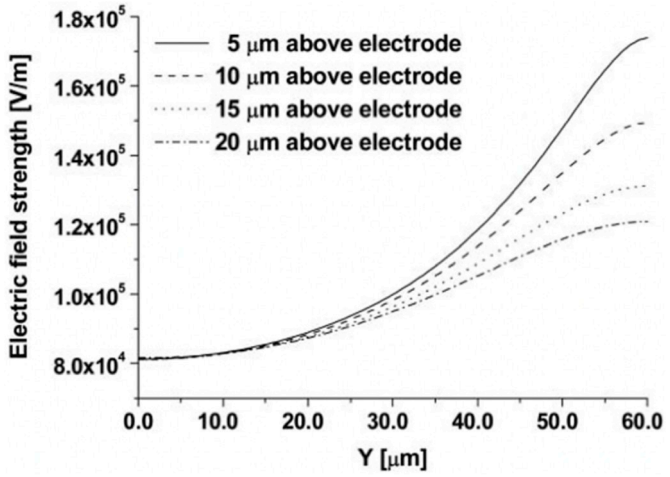

Fig. 8. electric strength at channel height away from electrode (Choi and Park, 2005).

separation in a single microfluidic flow cytometer device. One similar device utilising the parallel flow and separation of suspending medium has been implemented by J. H. Nieuwenhuis et al. (Nieuwenhuis et al., 2005). Parallel cell manipulation requires multiple channels resulting in cell separation in more number of outlets than usual so the collection of separated cells in multiple outlets makes this approach hap-ha-zardous. Collection of multiple cells in a in a single device is more challenging as it is only way possible by implementation of outlet channel crossovers.

3.4. Electrolysis effect

Reliability of any cell handling microfluidic MEMS impedance flow cytometer device depends upon the operating voltage and the time of operation. Electrolysis effect in a microfluidic impedance flow cytometer occurs over the time due to the electrical interaction of electrodes with the suspending medium. At higher operating voltage the electric current passes from one electrode to another via suspending medium due to this continuous current flow electrodes tends to chemically decompose and due to this separation efficiency decreases over time. The electrolysis effect can be avoided for increase in life span of the flow cytometer by optimised selection of medium and material of electrodes. From past many years Ti (Titanium)/Pt (Platinum) electrodes, Au (Gold)/Cr (chromium) electrodes, Whole Si (silicon) electrodes and Carbon electrodes have been used for cell handling.

3.5. Magnetic nanoparticles and magnetic field

Surface functionalization of magnetic nanoparticle (MNP) become an emerging technique in biomedical applications such as, biological cell labelling, magnetic separation, magnetic hyperthermia, drug delivery and contrast enhancement in magnetic resonance imaging (Reisbeck et al., 2016; Gertz and Khitun, 2016; Reddy et al., 2012). Integration of MNPs in microfluidic devices allow the non-optical probing and monitoring of biological sample such as whole blood without any specific sample preparation as the group of MNPs bounded over the target cells displays a higher magnetic moment than the pure biological environment (Reisbeck et al., 2016).

MNP possess physical properties such that it can get magnetically manipulated with the help of external magnetic forces. This type of manipulation activity comes under the magnetophoresis cell/bead manipulation. The prominence of magnetophoretic force is that it can be applied in a microsystem for cell mobilization with control using magnetically engineered micro capsules for guided drug delivery towards an objective location specifically (Pavlov et al., 2013; Kurlyandskaya et al., 2017). Study shows that there exists a magnetoelastic force which deforms the structure of ferrogels containing the magneto-micro entity in a non-uniform magnetic field (Li et al., 2013; Gollwitzer et al., 2008). Yuhui li et al. represented the applications of magnetic field in manipulation and handling of magnetic ferrogels for tissue engineering, drug delivery, enzyme immobilization and cancer therapy (Reddy et al., 2012). These magnetic hydrogels and ferrogels encapsulates the cells and are basically synthesized using blending method, in situ precipitation method and the grafting-onto method (Zr ınyi et al., 1996; Blyakhman et al., 2019; Badawy et al., 2017). Though various MNPs have developed and demonstrated their application in the cell manipulation flow cytometry by various research groups (Reddy et al., 2012), (Li et al., 2013; Gollwitzer et al., 2008; Zr´ınyi

Table 2
Design parameters of microfluidic impedance flow cytometers with tapered electrodes.

Electrode materials	Electrode dimension	Cell of Interest	Medium	Techniques Involved	Channel Gap/Gap between working electrodes	Channel Height	Cite
Au	120 μm (longer base) 20 μm (shorter base) with 60 μm width	Polystyrene particle of 15 μm and green fluorescent particles of 6 μm	Phosphate buffered saline (PBS) with an electrical conductivity of 2.2 mS m^{-1} with 5% BSA	DEP Focusing & DEP Separation	Inverse of electrode dimension	30 μm	Choi and Park (2005)

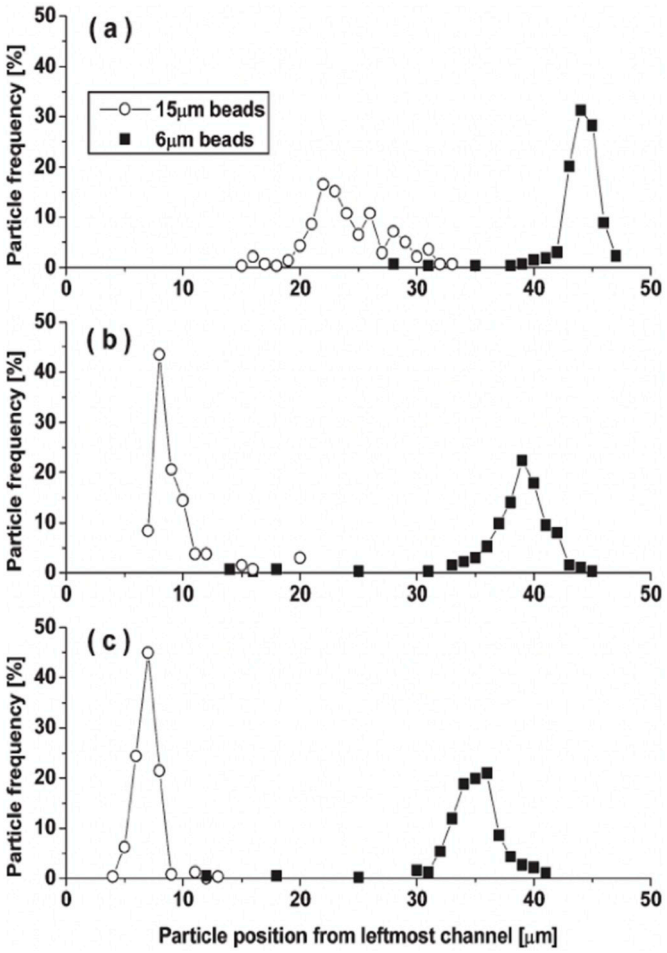

Fig. 9. (a) Separation of beads with 10 electrodes (Choi and Park, 2005) (b) Separation of beads with 20 electrodes (Choi and Park, 2005) (c) Separation of beads with 30 electrodes (Choi and Park, 2005).

et al., 1996; Blyakhman et al., 2019; Badawy et al., 2017), magnetic nanoparticle i. e. ferroso ferric oxide (Fe_3O_4) and maghemite (γ- Fe_2O_3) are widely used due to their biocompatibility and their biodegradability (Reddy et al., 2012). Synthesis technique for MNPs can be decided by the user specification as magnetic moment of MNPs strongly depends on physics stability, size and shape. To harness the advantage of MNPs, several design approach and configuration are used such as inject MNPs in localized area, produce magnetic field gradient and contours formation in device structures. Although much progress has been made towards cell manipulation by MNPs and detection of magnetic finger print of cells in flow cytometry, there are various challenges such as destruction of biological sample in micro heating; yet to address for validate the application of MNPs in pharmacy and clinical medicine.

4. MEMS impedance flow cytometry based cell manipulation designs

In recent years many impedance-based cell manipulation techniques have been developed and came in highlight as these techniques are cheap and less complex in comparison with FACS, MACS and acoustic cell sorting techniques. The principle of cell manipulation based on impedance or dielectric properties require a non-uniform electric field to be generated by the cell surrounding as previously discussed, which can be generated using different electrode dimensions (Das et al., 2014; Burgarella et al., 2010; Moon et al., 2011; Choi and Park, 2005; Wang et al., 2009; Li and Bashir, 2002; Muller et al., 1999; etin et al., 2009; Kang et al., 2009; Khoshmanesh et al., 2010), (Nieuwenhuis et al.,

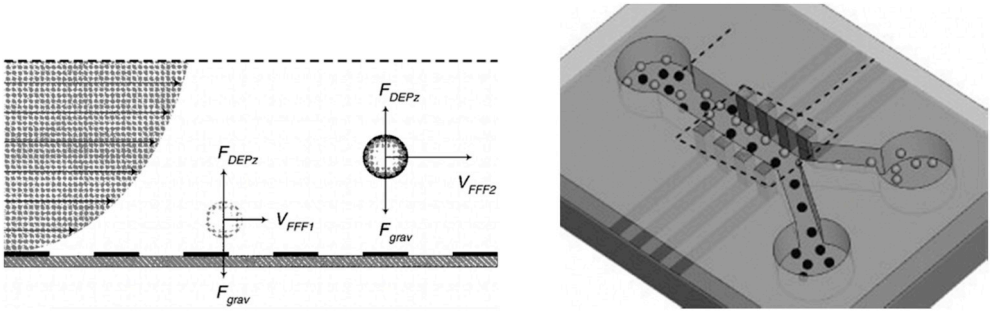

Fig. 10. (a) IDE with coplanar placement of electrodes (Yang et al., 1999); (b) IDE with parallel placement of electrodes (Wang et al., 2009).

2005), (Kim et al., 2008; Iliescu et al., 2004, 2008; Tay et al., 2007), self-aligned electrodes (Hara et al., 2002), multiple frequencies (Braschler et al., 2008) and by some novel wiring scheme for electrodes (Caselli et al., 2018). In past decades researchers have developed many impedimetric sensing and manipulation devices using different dimensions. The impedimetric sensing and cell manipulation devices with different design orientations and dimensions are discussed as follows.

4.1. Tapered electrodes based designs

In this electrode orientation the micro-electrodes are tapered at certain angles to generated non-uniform electric field. Compared to the conventional planner and parallel electrode orientation, tapered electrodes direct the cell movement towards the reservoir at desired angle which makes this an effective separation technique. A tapered electrode microfluidic device is shown in Fig. 3 which has been developed by debanjan das & soumen das (2013) (Das et al., 2014) fabricated using three planner micro-electrodes out of which two electrodes (placed on the sides) used for stronger electric field intensity gradient and one electrode at the centre is used for weaker electric field intensity gradient for cell separation.

The electrodes were tapered at an angle of 9°with maintaining 50 μm gap between the side and central electrode for cell movement. The cross-sectional width of the side electrodes and central are selected as 50 μm and 100 μm (inlet)/1.058 mm (outlet). Result shows that device has potential to separate human carcinoma (HeLa) cells and polystyrene particles in medium of phosphate buffered saline (PBS) having pH value 7.4 and conductivity of 0.156 S m^{-1} at an operating voltage of 10 V_{pp}. The HeLa cells & polystyrene particles were separated above a crossover frequency of 500 kHz as shown in Fig. 4.

In similar tapered electrode orientational design Moon et al. (Moon et al., 2011) has shown that a tapered electrode configuration can be utilised to deviate as well as to align cells in a row. The authors have experimented the microfluidic device with RBC, WBC & MCF-7 cancer cells. The cells were first separated using multi-orifice flow fractionation (MOFF) technique for bulk separation and further separation is performed using dielectrophoresis technique. Fig. 5 shows that separation of RBC, WBC and MCF-7 in bulk quantity is performed with channel's Reynold number varying from 30 to 90 with resolution of 20. The most effective separation has been found at channel's Reynold number 70 where RBC & WBCs were flowing on the sides and MCF-7 cells were focused in the middle of the channel.

After MOFF separation cells were subjected to DEP forces where first all the cells were focused using p-DEP force at applied voltage of 10 V_{pp}, 2 MHz as shown in Fig. 6(a) Focused flow of all the three type of cells can be seen in Fig. 6(b) where cells are flowing near the side wall. In the last stage all the cells were subjected in separation region where DEP electrodes produced high electric field gradient using 10 V_{pp}, 900 kHz. In this MCF-7 cells experienced p-DEP and moved in upward direction as shown in Fig. 6(c) and RBC & WBC were followed their movement trajectory towards central streamline.

As both the MOFF & DEP processes had complemented each other where MOFF separation performed bulk separation and fast filtration, and DEP separation made the separation more precise. Both bulk and precise separation makes this device more suitable for high throughput efficient cell manipulation device. The device overcome the problem of adhesion between cell & electrode which creates a barrier for cell movement using excitation and resting of AC voltage in every 0.5 ms but did not clearly investigate its impact on cells in accountable manner. Similar electrode design oriented cell manipulators are summarised in Table 1.

4.2. Trapezoidal electrode arrays

Trapezoidal electrodes in coplanar orientation for the dielectric deflection using n-DEP has been first proposed by Choi and Park (2005) (2005) as shown in Fig. 7(a) before that researchers had explored the ability of DEP for cell focusing and separation using positive dielectrophoresis and succeeded as well however there was a problem associated with adhesive forces between electrodes and cells which resulted in cells/particles stocking due to forces generated by high electric field gradient with higher electric potential. Same problem of micro particle stocking has been also observed in DC microfluidic MEMS devices (Kumar and Das, 2018). This stocking of cells creates a barrier for other cells to move at high flow rates. In this trapezoidal electrode array based microfluidic sorter the micro beads of polystyrene particles having diameter of 15 μm and another green fluorescent particle of 6 μm diameters were used for interrogating the efficacy of the device. The structure of trapezoidal electrode array with electrode size is shown in Fig. 7(b).

In this configuration the dimension of gap between the two electrodes has been kept same as the dimension of trapezoidal electrode dimension. Gap dimensions are shown in Fig. 7(c) and the electric field distribution over the channel height from the electrodes which is shown in Fig. 8. Detailed design parameters are represented in Table 2.

Results in Fig. 8 shows that electric field gradient is high in region near the electrodes and electric field strength reduces with increase in channel height. To ensure the particle movement towards desired direction an 8 V_{pp} at 50 kHz has been applied to the electrodes. The movement of particles has been observed towards the shorter base of the electrode (or the larger base of the gap).

The trapezoidal electrode array devices can also be utilised with p-DEP force as the design is inspired by interdigitated electrodes configuration. Effect of flow rate for focusing is also observed with trapezoidal electrode array and it is found that increase in flow rate, reduces the effective cell isolation or manipulation (Choi and Park, 2005). It has been observed that at higher flow rates beads with different size are less isolated as comparison to lower flow rates. In that case, high throughput separation and focusing is not possible using this device without utilising any other focusing method or increasing the number

Table 3
Design parameters of microfluidic impedance flow cytometers with interdigitated electrodes.

Electrode materials	Electrode dimension	Cell of Interest	Medium	Techniques Involved	Channel Gap/Gap between working electrodes	Channel Height	Cite
Au/Ti	30 μm–40 μm height	Polystyrene particles of 6–15 μm, human kidney cells (HEK293) and mouse neuroblastoma cells (N115)	Isotonic medium (8.5% w/v sucrose, 0.3% w//v dextrose dissolved in DI water) with conductivity 0.1 mS cm^{-1}	Dual frequency coupled DEP separation	100 μm (Horizontal cell movement area)	40 μm	Wang et al. (2009)
Au/Pt/Cr	15 μm width and 1000Å thick Au over a 100Å Cr seed layer	Live & heat treated rod shaped Listeria innocua cells with 3 μm length & 1 μm diameter	10^5 cells/ml with DI water having conductivity of 2 μS cm^{-1}	Manipulation & DEP Separation	Gap between electrodes: 15 μm	Not revealed	Li and Bashir (2002)
Not mentioned	Electrode width of 50 μm	MDA-435 Breast cancer cells, T- lymphocytes cells, B-lymphocytes cells, $CD34^+$ cells, Monocytes cells, Leukocytes cells and Erythrocytes cells	Isotonic sucrose/dextrose buffer with electrical conductivity of 10 mS m^{-1}	DEP Separation by cell elevation	Height x Width 0.42 × 25 (in mm)	0.42 mm	Wang et al. (2000)

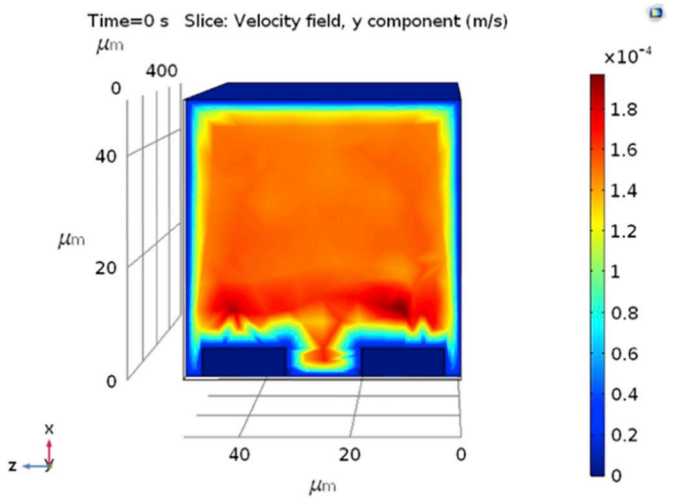

Fig. 11. Medium flow velocity in a coplanar orientation micro channel with 5 μm electrode height.

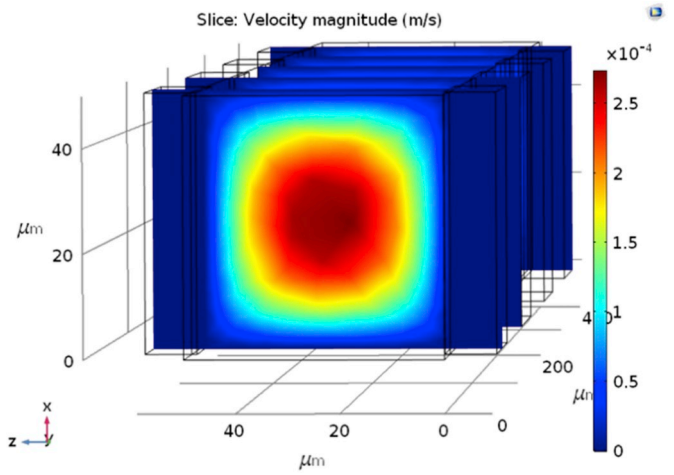

Fig. 12. Medium flow velocity in a micro channel having electrodes as side walls.

of electrodes. Fig. 9 shows the separation at 10 μl h^{-1} with 10, 20 and 30 number of electrodes, it can be observed that beads get good isolation with higher number of electrodes.

4.3. Interdigitated electrodes orientation

Interdigitated electrodes orientation is very less complex to design and comprises two type of design structure as shown in Fig. 10. First one is interdigitated electrode (IDE) orientation with coplanar electrode placement in which electrodes are placed on the bottom floor of the microchannel and the DEP force act vertically (Wang et al., 2009), (Wang et al., 2007). Other one is IDE orientation with parallel electrode placement in which electrodes are formed as a side wall of microchannel where the movement of cells/particles and medium flows (Li and Bashir, 2002), (Wang et al., 2000), here DEP force acts on horizontal plane. Both the structures have their own merits and demerits. Some interdigitated electrode designs are summarised in Table 3.

Coplanar placement of electrodes with significant metal deposition act as barrier for the flow of medium and cells as shown in Fig. 11 So electrodes has to be formed with minimum height in coplanar orientation. To avoid the hap-hazardous fluid dynamics, electrodes can be formed in walls as shown in Fig. 12.

An IDE with coplanar electrodes faces problems with height of the

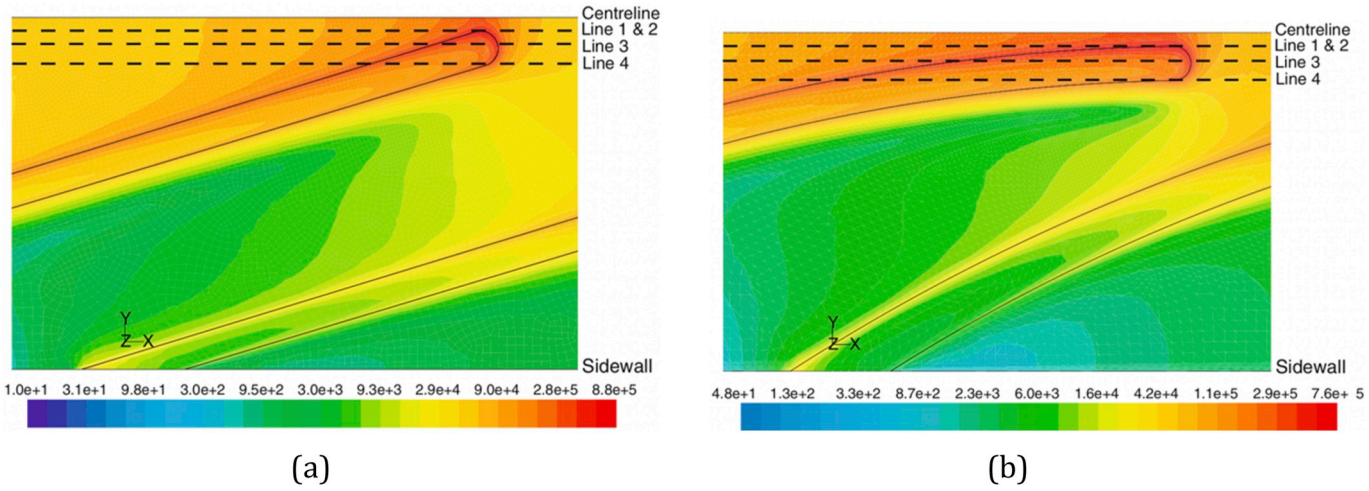

Fig. 13. (a) Electric field distribution in flat electrodes (Khoshmanesh et al., 2010); (b) Electric field distribution in curved electrodes (Khoshmanesh et al., 2010).

channel as there exist a dead zone on the top area of the micro-channel where effects of electrodes are negligible in terms of electric field. Problem associated with electric field dead zone over micro-channel can be overcome using similar electrode structure on the ceiling of the micro-channel (Fiedler et al., 1998) but still there exist cell collection problem for designing reservoir after separation in vertical positions. An IDE with parallel electrode placement overcomes the problem of dead zone of electric field as the electrodes are placed on both side wall and these electrodes generates electric field over entire micro-channel and also it becomes very easy to form cell collection reservoirs as the reservoir has to be formed on horizontal plane and cells after separation can be made to flow in multiple channels along the width of the micro-channel.

4.4. Curved electrode orientation

Curved electrodes orientation have been rarely utilised by the researchers due to the complex design structures although curved electrodes have the advantage in distribution of electric field over more area of the channel as comparison to flat electrodes as shown in Fig. 13. Electrodes with curved orientation are usually moulded in an arc with some specified radius or in elliptical manner (Khoshmanesh et al., 2010).

Overall curved electrodes design can be utilised in such a manner that its sharp electrode corners provide better cell focusing and curved shapes provide better cell isolation. A typical curved electrode-oriented design has been utilised by K. Khoshmanesh et al. (Khoshmanesh et al., 2010) where curve has been provided at a radius range of 2300 μm–3200 μm. The electrode curve in reducing manner making an arrow can be utilised for cell alignment/focusing and curve in opening manner for better isolation of cells. The design parameters of devices introduced by K. Khoshmanesh et al. (Khoshmanesh et al., 2010) and J. H. Nieuwenhuis et al. (Nieuwenhuis et al., 2005) are shown in Table 4.

4.5. 3D electrode orientation

3D electrodes orientation is one of the most efficient designs for performing cell trapping (Iliescu et al., 2004), (Iliescu et al., 2008), caging (Muller et al., 1999), separation (Wang et al., 2009), (Muller et al., 1999) but lack in simplicity of design structure. Devices with this sort of complex electrode designs involve much rigorous process of fabrication with complicated mask designs. Although a 3D electrodes-oriented design can solve all the purpose of cell handling and can be implemented for Lab-on-chip. Cell focusing with multiple electrodes can perform cell alignment at the centre of the flow. 3D electrodes also provide the DEP effect over entire channel height so dead zone of electric field can be avoided. A 3D electrodes oriented device structure is presented in Fig. 14 where cells have been handled by funnel (preliminary focusing), aligner (aligning), cage (trapping for single cell analysis), funnel (focusing) and switch (sorting) in a systematic approach.

T. Müller et al. in 1999 presented that with the use of 3D electrode oriented microfluidic MEMS flow cytometer device with funnel, aligner and switch element type structure, it is possible to handle and cage single cell from a large population of cells where every cell can be controlled individually. The device has been experimented by generating electric field with multiple voltage and frequency combination for different manipulation stage. Cell focusing was achieved in voltage range of 5–11 V_{pp} (rectangular AC) at 5–15 MHz operating frequency and alignment of cells has been achieved at 5–15 MHz Single cell manipulation and caging in a star shaped electrode orientation are achieved at electrodes excited at 5–10 V_{pp} with 5–15 MHz frequency range same as focusing as shown in Fig. 15.

This Device performed focusing action at 300 μm s^{-1} and Caging against laminar flow of 40–200 μm s^{-1}. Overall cell manipulation has been achieved at 3500 μm s^{-1}. In a similar 3D electrode cytometry design by the C. Iliescu et al. multiple cells trapping is achieved using n-DEP in 10–13 s time of operation as shown in Fig. 16. Design parameters of devices with various 3D electrode orientations are presented in Table 5.

5. Summary and conclusion

The impact of electrode dimensions and design orientations on dielectrophoresis force which can be utilised for micro cell manipulation including dragging, caging, focusing and sorting have studied. Basic phenomenon of cell handling process depends on dielectric properties of cells and the medium in which cells are suspended. Appropriate dragging force by DEP can be generated with the various electrode designs and the overall manipulation is greatly impacted by the parameters which are discussed in section III. Tapered electrode orientation can be utilised for cell manipulation which includes cell focusing and cell dragging in a particular direction using electrodes slanted at an appropriate angle. High throughput can be achieved by utilising the complementary combination of hydrodynamic focusing with specific Reynold's number and dielectrophoresis effect. This combination of fluid hydrodynamics and dielectrophoresis resulted in efficient cell sorting performance at even higher flow rates.

It should be noted that it is very important to align all the cells at centre of the channel as cells flowing in aligned manner will experience

Table 4
Design parameters of microfluidic impedance flow cytometers with Curved electrodes.

Electrode materials	Electrode dimension	Cell of Interest	Medium	Techniques Involved	Channel Gap/Gap between working electrodes	Cite
Au/cu	Microelectrodes width: 200 μm at the base & decreased up to 50 μm near the tip for cell alignment; boomerang shaped microelectrodes with 40 μm minimum gap and 820 μm gap between opposite electrodes for separation	*Saccharomyces cerevisiae* yeast cells (in live and dead status with 50-50% mixture)	DI water/DI water- Methanol (50:50) with medium conductivities 0.001–0.14 S m^{-1}	DEP Cell Alignment & DEP Separation	250 μm–40 μm decreasing with the radius range of 2300 μm–3200 μm; minimum electrode gap was 40 μm; (Channel height not specified)	Khoshmanesh et al. (2010)
Au (300 nm)/Cr	Triangular and tetrahedral shape of electrodes of various dimensions	Polystyrene particles with a radius of 12 μm	Watery liquid with a conductivity of 10 mS m^{-1}	Cell Alignment & DEP Separation	1 mm Channel width with 70 μm channel height	Nieuwenhuis et al. (2005)

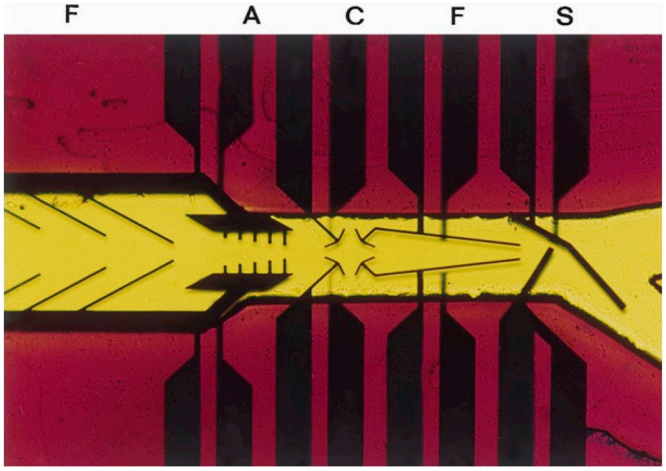

Fig. 14. Schematic of a 3D Micro-electrode oriented DEP sorting device (Muller et al., 1999).

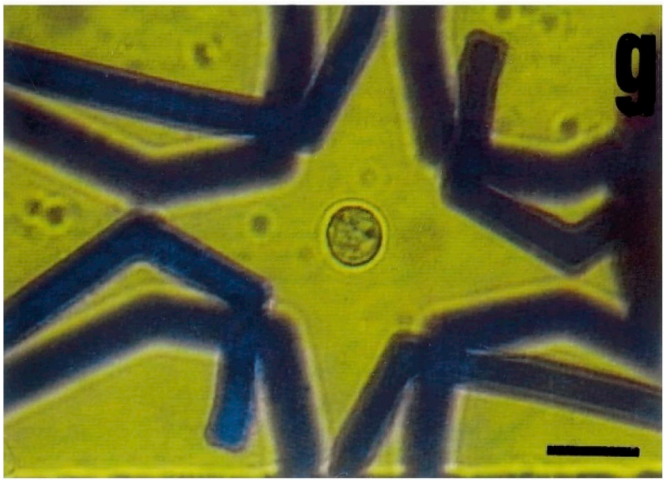

Fig. 15. Single Cell Caged by dielectrophoretic forces (Muller et al., 1999).

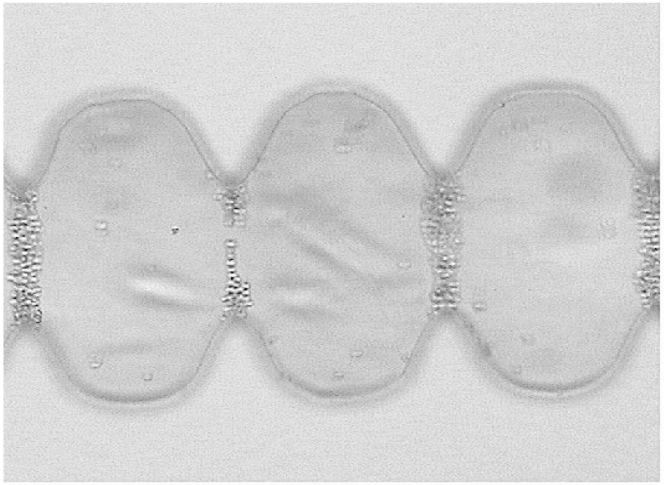

Fig. 16. Multiple cell trapping achieved by n-DEP (Iliescu et al., 2004).

uniform force. If cell which is to be experience the p-DEP is flowing in channel near to the weak electric field region would require high DEP forces as comparison to the same cell flowing at centre of the channel. Similarly cell which is to experience the n-DEP is flowing in channel near to the strong electric field region would require relatively high

Table 5
Design parameters of microfluidic impedance flow cytometers with Curved electrodes.

Electrode materials	Electrode dimension	Cell of Interest	Medium	Techniques Involved	Channel Gap/Gap between working electrodes	Cite
Ti/Pt thin film micro-electrodes	Funnel electrode structure with 20° – 40° tilt for concentrating particles & cells	Eukaryotic cells and latex particles with 14.9 μm diameter, permittivity 3.5 & conductivity of 0.7 mS m^{-1}	Phosphate buffered saline and DI water buffer solution	Focusing, Caging, & DEP Separation	electrode structures separated by a 40 μm thick polymer spacer forming a flow channel with height of 20 μm	Muller et al. (1999)
Si Electrodes	Square Shaped electrodes	Viable and non-viable yeast cells	Phosphate buffered saline & DI water buffer solution mixture with conductivity of 20 μS cm^{-1}	DEP Trapping, Separation & Hydrodynamic dragging	Not specified	Iliescu et al. (2004)
Si Electrodes	Triangular shaped electrodes for strong DEP action	Viable and non-viable yeast cells	Phosphate buffered saline solution with conductivity of 50 μS cm^{-1}	DEP Separation	Not revealed	Iliescu et al. (2008)
Si Electrodes acting as channel walls	100 μm between and bottom layer of glass	*Saccharomyces cerevisiae* yeast cells with 5 μm	Buffer solution with conductivity of 20 μS cm^{-1}	DEP Separation by trapping	Channel gap not revealed; Channel height with 100 μm	Tay et al. (2007)

DEP forces as comparison to the same cell flowing in centre stream line of channel. Moon et al. have shown that a tapered oriented electrodes can be utilised for similar purpose in order to achieve higher separation efficiency.

Higher separation efficiency can also be achieved with the help of electrodes in interdigitated orientation working as a channel walls as electrodes working as walls overcome the problem of electric field dead zone also if electrodes as walls utilised for cell focusing more number of cells will be able to align themselves at central plane (vertically) which will improve the throughput as well as separation efficiency. But here cost of the electrode material will increase as the deposition of metal will be considerably high as comparison to the electrodes for coplanar orientation, where electrodes are formed on bottom surface of the channel but in that case device will have to trade of with electric field dead zone problem. Electrodes formed on bottom floor of the channel with higher amount of metal deposition may avoid the dead zone problem but there exist the flow issue as shown in section 4.3.

6. Future prespective

It is always been a research problem to achieve higher throughput with higher separation efficiency and efficient cell manipulation at considerable low cost, if the electric field distribution can be increased over channel area where cell trajectories are expected separation efficiency can be improved. Curved electrode with sharp tip generates strong DEP effect on large area so that cells can experience DEP force for more channel distance this increases the separation efficiency considerably. Multiple curved electrodes with multiple outlets using triangular and tetrahedral shapes (J. H. Nieuwenhuis et al. (Nieuwenhuis et al., 2005)) can serve high throughput without hampering the cell separation efficiency which further leads to prevention of cells from thermal effect or joule heating. Although most of the electrode orientation serve the cell manipulation with good impact but to solve the problems in compatibility with healthcare sector in market the lab-on-chip device for overall cell handling processes like trapping, aligning, sorting, single cell caging and disease detection have to be implemented with complex three dimension electrode with biocompatible environment. A good attempt has been made by T. Müller et al. in 1999 (Muller et al., 1999) to achieve all the manipulation problems but technology still strives for a complete Lab-on-chip device which can be implemented to diagnose the diseases occurring due to infectious cells & blood cell destruction. Good attempts are proceeding in the healthcare sector by magnetophoresis using ferrogels but synthesis of these ferrogels and magnetically engineered micro-capsules for guided drug delivery requires specialized resources and complex study of parameters.

CRediT authorship contribution statement

Mahesh Kumar: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing - original draft, Writing - review & editing. **Supriya Yadav:** Conceptualization, Visualization, Software, Writing - original draft, Writing - review & editing. **Ashish Kumar:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Validation, Writing - review & editing. **Niti Nipun Sharma:** Conceptualization, Funding acquisition, Resources, Writing - review & editing. **Jamil Akhtar:** Conceptualization, Formal analysis, Resources, Writing - review & editing. **Kulwant Singh:** Conceptualization, Funding acquisition, Formal analysis, Methodology, Project administration, Supervision, Validation, Writing - review & editing.

Acknowledgment

The authors would like to thank Multiscale Simulation Research Centre (MSRC), Manipal University Jaipur for providing simulation facilities of COMSOL Multiphysics 5.3. Mahesh Kumar and Supriya

Yadav are also thankful to administration of Manipal University Jaipur for providing Dr. Ramdas pai Fellowship.

References

Alam, M.K., Emmanuel, K., Heng, Z., Changqing, Y., Cheuk-Wing, L., Tao, X., Mengsu, Y., 2018. Recent advances in microfluidic technology for manipulation and analysis of biological cells (2007–2017). Anal. Chim. Acta 1044, 29–65.

Badawy, M.E., Taktak, N.E., Awad, O.M., Elfiki, S.A., El-Ela, N.E.A., 2017. Preparation and characterization of biopolymers chitosan/alginate/gelatin gel spheres cross-linked by glutaraldehyde. J. Macromol. Sci., Part B 56 (6), 359–372.

Béné, M.C., 2017. Microfluidics in flow cytometry and related techniques. Int. J. Lit. Humanit. 39, 93–97.

Bernabini, C., Holmes, D., Morgan, H., 2011. Micro-impedance cytometry for detection and analysis of micron-sized particles and bacteria. Lab Chip 11 (3), 407–412.

Blyakhman, F.A., Makarova, E.B., Fadeyev, F.A., Lugovets, D.V., Safronov, A.P., Shabadrov, P.A., Shklyar, T.F., Melnikov, G.Y., Orue, I., Kurlyandskaya, G.V., 2019. The contribution of magnetic nanoparticles to ferrogel biophysical properties. Nanomaterials 9 (2), 232.

Braschler, T., Demierre, N., Nascimento, E., Silva, T., Oliva, A.G., Renaud, P., 2008. Continuous separation of cells by balanced dielectrophoretic forces at multiple frequencies. Lab Chip 8 (2), 280–286.

Burgarella, S., Merlo, S., Dell'Anna, B., Zarola, G., Bianchessi, M., 2010. A modular microfluidic platform for cells handling by dielectrophoresis. Microelectron. Eng. 87 (11), 2124–2133.

Caselli, F., DeNinno, A., Reale, R., Businaro, L., Bisegna, P., 2018. A novel wiring scheme for standard chips enabling high-accuracy impedance cytometry. Sens. Actuators B Chem. 256, 580–589.

Chiu, T.K., Chao, A.C., Chou, W.P., Liao, C.J., Wang, H.M., Chang, J.H., Chen, P.H., Wu, M.H., 2018. Optically-induced-dielectrophoresis (ODEP)-based cell manipulation in a microfluidic system for high-purity isolation of integral circulating tumor cell (CTC) clusters based on their size characteristics. Sens. Actuators B Chem. 258, 1161–1173.

Cho, S.H., Chen, C.H., Tsai, F.S., Lo, Y.-H., 2009. Micro-fabricated fluorescence-activated cell sorter. In: 2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE, pp. 1075–1078.

Choi, S., Park, J.-K., 2005. Microfluidic system for dielectrophoretic separation based on a trapezoidal electrode array. Lab Chip 5 (10), 1161–1167.

Das, D., Biswas, K., Das, S., 2014. A microfluidic device for continuous manipulation of biological cells using dielectrophoresis. Med. Eng. Phys. 36 (6), 726–731.

Durack, G., Robinson, J.P., 2004. Emerging Tools for Single-Cell Analysis: Advances in Optical Measurement Technologies, vol. 8 John Wiley & Sons.

Estes, M.D., Ouyang, B., Ho, S.-m., Ahn, C.H., 2009. Isolation of prostate cancer cell subpopulations of functional interest by use of an on-chip magnetic bead-based cell separator. J. Micromech. Microeng. 19 (9) p. 095015.

etin, B.C,, Kang, Y., Wu, Z., Li, D., 2009. Continuous particle separation by size via ac-dielectrophoresis using a lab-on-a-chip device with 3-d electrodes. Electrophoresis 30 (5), 766–772.

Feng, J., Krishnamoorthy, S., Sundaram, S., 2007. Numerical analysis of mixing by electrothermal induced flow in microfluidic systems. Biomicrofluidics 1 (2) p. 024102.

Fiedler, S., Shirley, S.G., Schnelle, T., Fuhr, G., 1998. Dielectrophoretic sorting of particles and cells in a microsystem. Anal. Chem. 70 (9), 1909–1915.

Fu, A.Y., Spence, C., Scherer, A., Arnold, F.H., Quake, S.R., 1999. A microfabricated fluorescence-activated cell sorter. Nat. Biotechnol. 17 (11), 1109.

Furlani, E., Sahoo, Y., 2006. Analytical model for the magnetic field and force in a magnetophoretic microsystem. J. Phys. D Appl. Phys. 39 (9), 1724.

Gertz, F., Khitun, A., 2016. Biological cell manipulation by magnetic nanoparticles. AIP Adv. 6 (2), 025308.

Gollwitzer, C., Turanov, A., Krekhova, M., Lattermann, G., Rehberg, I., Richter, R., 2008. Measuring the deformation of a ferrogel sphere in a homogeneous magnetic field. J. Chem. Phys. 128 (16), 164709.

Gonza´lez, A., Ramos, A., Morgan, H., Green, N.G., Castellanos, A., 2006. Electrothermal flows generated by alternating and rotating electric fields in microsystems. J. Fluid Mech. 564, 415–433.

Green, N.G., Ramos, A., Gonza´lez, A., Castellanos, A., Morgan, H., 2000. Electric field induced fluid flow on microelectrodes: the effect of illumination. J. Phys. D Appl. Phys. 33 (2), L13.

Han, K.-H., Han, A., Frazier, A.B., 2006. Microsystems for isolation and electrophysiological analysis of breast cancer cells from blood. Biosens. Bioelectron. 21 (10), 1907–1914.

Handgretinger, R., Lang, P., Schumm, M., Taylor, G., Neu, S., Koscielnak, E., Niethammer, D., Klingebiel, T., 1998. Isolation and transplantation of autologous peripheral cd34+ progenitor cells highly purified by magnetic-activated cell sorting. Bone Marrow Transplant. 21 (10), 987.

Hara, T., Ichiki, T., Horiike, Y., Yasuda, K., 2002. Fabrication of on- chip sorter devices with submicrometer scale channels and self-aligned microelectrodes. In: Micro Total Analysis Systems 2002. Springer, pp. 124–126.

Hawkes, J.J., Coakley, W.T., 2001. Force field particle filter, combining ultrasound standing waves and laminar flow. Sens. Actuators B Chem. 75 (3), 213–222.

Heim, S., Mitelman, F., 2015. Cancer Cytogenetics: Chromosomal and Molecular Genetic Aberrations of Tumor Cells. John Wiley & Sons.

Hingorani, S.R., Petricoin, E.F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M.A., Ross, S., Conrads, T.P., Veenstra, T.D., Hitt, B.A., et al., 2003. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 4 (6), 437–450.

Holmes, D., Morgan, H., 2010. Single cell impedance cytometry for identification and counting of cd4 t-cells in human blood using impedance labels. Anal. Chem. 82 (4), 1455–1461.

Holmes, D., Pettigrew, D., Reccius, C.H., Gwyer, J.D., van Berkel, C., Holloway, J., Davies, D.E., Morgan, H., 2009. Leukocyte analysis and differentiation using high speed microfluidic single cell impedance cytometry. Lab Chip 9 (20), 2.

Huang, T., Jia, C.-P., Sun, W.-J., Wang, W.-T., Zhang, H.-L., Cong, H., Jing, F.-X., Mao, H.-J., Jin, Q.-H., Zhang, Z., et al., 2014. Highly sensitive enumeration of circulating tumor cells in lung cancer patients using a size-based filtration microfluidic chip. Biosens. Bioelectron. 51, 213–218.

Huh, D., Gu, W., Kamotani, Y., Grotberg, J.B., Takayama, S., 2005 Feb 1. Microfluidics for flow cytometric analysis of cells and particles. Physiol. Meas. 26 (3), R73.

Iliescu, C., Xu, G.L., Samper, V., Tay, F.E., 2004. Fabrication of a dielectrophoretic chip with 3d silicon electrodes. J. Micromech. Microeng. 15 (3), 494.

Iliescu, C., Tay, F.E., Xu, G., Yu, L.M., Samper, V., 2006. A dielectrophoretic chip packaged at wafer level. Microsyst. Technol. 12 (10–11), 987–992.

Iliescu, C., Yu, L., Tay, F.E., Chen, B., 2008. Bidirectional field-flow particle separation method in a dielectrophoretic chip with 3d electrodes. Sens. Actuators B Chem. 129 (1), 491–496.

Johnson, P.T., 1983. Diseases caused by viruses, rickettsiae, bacteria, and fungi. Bio Crustac Pathobiol 6, 1–78.

Jubery, T.Z., Srivastava, S.K., Dutta, P., 2014. Dielectrophoretic separation of bioparticles in microdevices: a review. Electrophoresis 35 (5), 691–713.

Kang, Y., Cetin, B., Wu, Z., Li, D., 2009. Continuous particle separation with localized ac-dielectrophoresis using embedded electrodes and an insulating hurdle. Electrochim. Acta 54 (6), 1715–1720.

Khoshmanesh, K., Zhang, C., Tovar-Lopez, F.J., Nahavandi, S., Baratchi, S., Mitchell, A., Kalantar-Zadeh, K., 2010. Dielectrophoretic- activated cell sorter based on curved microelectrodes. Microfluid. Nanofluidics 9 (2–3), 411–426.

Kim, U., Qian, J., Kenrick, S.A., Daugherty, P.S., Soh, H.T., 2008. Multitarget dielectrophoresis activated cell sorter. Anal. Chem. 80 (22), 8656–8661.

Kim, J., Erath, J., Rodriguez, A., Yang, C., 2014. A high-efficiency microfluidic device for size-selective trapping and sorting. Lab Chip 14 (14), 2480–2490.

Kotnik, T., Miklavcic, D., 2000. Theoretical evaluation of the distributed power dissipation in biological cells exposed to electric fields. In: Bioelectromagnetics: Journal of the Bioelectromagnetics Society, the Society for Physical Regulation in Biology and Medicine, vol. 21. The European Bio- electromagnetics Association, pp. 385–394 no. 5.

Krivacic, R.T., Ladanyi, A., Curry, D.N., Hsieh, H., Kuhn, P., Bergsrud, D.E., Kepros, J.F., Barbera, T., Ho, M.Y., Chen, L.B., et al., 2004. A rare-cell detector for cancer. Proc. Natl. Acad. Sci. 101 (29), 10 501–510 504.

Kumar, M., Das, D., 2018. Mems based flow cytometer with instrumentation system for detection of micro particles for health care applications. In: 2018 Recent Advances on Engineering, Technology and Computational Sciences (RAETCS). IEEE, pp. 1–5.

Kunti, G., Dhar, J., Bandyopadhyay, S., Bhattacharya, A., Chakraborty, S., 2018. Energy-efficient generation of controlled vortices on low-voltage digital microfluidic platform. Appl. Phys. Lett. 113 (12), 124103.

Kurlyandskaya, G., Litvinova, L., Safronov, A., Schupletsova, V., Tyukova, I., Khaziakhmatova, O., Slepchenko, G., Yurova, K., Cherem- pey, E., Kulesh, N., et al., 2017. Water-based suspensions of iron oxide nanopar- ticles with electrostatic or steric stabilization by chitosan: fabrication, characterization and biocompatibility. Sensors 17 (11), 2605.

Lee, G.H., Kim, S.H., Ahn, K., Lee, S.H., Park, J.Y., 2015. Separation and sorting of cells in microsystems using physical principles. J. Micromech. Microeng. 26 (1) p.013003.

Li, H., Bashir, R., 2002. Dielectrophoretic separation and manipulation of live and heat-treated cells of listeria on microfabricated devices with interdigitated electrodes. Sens. Actuators B Chem. 86 (2–3), 215–221.

Li, Y., Huang, G., Zhang, X., Li, B., Chen, Y., Lu, T., Lu, T.J., Xu, F., 2013. Magnetic hydrogels and their potential biomedical applications. Adv. Funct. Mater. 23 (6), 660–672.

Lincoln, B., Erickson, H.M., Schinkinger, S., Wottawah, F., Mitchell, D., Ulvick, S., Bilby, C., Guck, J., 2004. Deformability-based flow cytometry. Cytometry Part A: the journal of the International Society for Analytical Cytology 59 (2), 203.

Liu, Y.-J., Guo, S.-S., Zhang, Z.-L., Huang, W.-H., Baigl, D., Xie, M., Chen, Y., Pang, D.-W., 2007. A micropillar-integrated smart microfluidic device for specific capture and sorting of cells. Electrophoresis 28 (24), 4713–4722.

Liu, C., Guo, J., Tian, F., Yang, N., Yan, F., Ding, Y., Wei, J., Hu, G., Nie, G., Sun, J., 2017. Field-free isolation of exosomes from extracellular vesicles by microfluidic viscoelastic flows. ACS Nano 11 (7), 6968–6976.

Luo, T., Fan, L., Zhu, R., Sun, D., 2019. Microfluidic single-cell manipulation and analysis: methods and applications. Micromachines 10 (2), 104.

Miltenyi, S., Müller, W., Weichel, W., Radbruch, A., 1990. High gradient magnetic cell separation with macs. Cytometry: The Journal of the International Society for Analytical Cytology 11 (2), 231–238.

Moon, H.-S., Kwon, K., Kim, S.-I., Han, H., Sohn, J., Lee, S., Jung, H.-I., 2011. Continuous separation of breast cancer cells from blood samples using multi-orifice flow fractionation (moff) and dielectrophoresis (dep). Lab Chip 11 (6), 1118–1125.

Müller, T., Gradl, G., Howitz, S., Shirley, S., Schnelle, T., Fuhr, G., 1999. A 3-d microelectrode system for handling and caging single cells and particles. Biosens. Bioelectron. 14 (3), 247–256.

Nagel, J.H., 2000. Biopotential amplifiers. In: The Biomedical Engineering Handbook, vol. 2. pp. 1300.

Ni, M., Tong, W.H., Choudhury, D., Rahim, N.A.A., Iliescu, C., Yu, H., 2009. Cell culture on MEMS platforms: a review. Int. J. Mol. Sci. 10 (12), 5411–5441.

Nieuwenhuis, J.H., Jachimowicz, A., Svasek, P., Vellekoop, M.J., 2005. Optimization of

microfluidic particle sorters based on dielectrophoresis. IEEE Sens. J. 5 (5), 810–816.
Pavlov, A.M., De Geest, B.G., Louage, B., Lybaert, L., De Koker, S., Koudelka, Z., Sapelkin, A., Sukhorukov, G.B., 2013. Magnetically engi- neered microcapsules as intracellular anchors for remote control over cellular mobility. Adv. Mater. 25 (48), 6945–6950.
Pohl, H.A., 1958. Some effects of nonuniform fields on dielectrics. J. Appl. Phys. 29 (8), 1182–1188.
Reddy, L.H., Arias, J.L., Nicolas, J., Couvreur, P., 2012. Magnetic nanoparticles: design and characterization, toxicity and biocompatibility, pharmaceutical and biomedical applications. Chem. Rev. 112 (11), 5818–5878.
Reisbeck, M., Helou, M.J., Richter, L., Kappes, B., Friedrich, O., Hayden, O., 2016. Magnetic fingerprints of rolling cells for quantitative flow cytometry in whole blood. Sci. Rep. 6, 32838.
Sale, A., Hamilton, W., 1968. Effects of high electric fields on micro- organisms: iii. lysis of erythrocytes and protoplasts. Biochim. Biophys. Acta Biomembr. 163 (1), 37–43.
Saliba, A.-E., Saias, L., Psychari, E., Minc, N., Simon, D., Bidard, F.-C., Mathiot, C., Pierga, J.-Y., Fraisier, V., Salamero, J., et al., 2010. Microfluidic sorting and multimodal typing of cancer cells in self-assembled magnetic arrays. Proc. Natl. Acad. Sci. 107 (33), 14524–14529.
Smistrup, K., Kjeldsen, B., Reimers, J., Dufva, M., Petersen, J., Hansen, M.F., 2005. On-chip magnetic bead microarray using hydrodynamic focusing in a passive magnetic separator. Lab Chip 5 (11), 1315–1319.
Tay, F.E., Yu, L., Pang, A.J., Iliescu, C., 2007. Electrical and thermal characterization of a dielectrophoretic chip with 3d electrodes for cells manipulation. Electrochim. Acta 52 (8), 2862–2868.
Tsutsui, H., Ho, C.M., 2009. Cell separation by non-inertial force fields in microfluidic systems. Mech. Res. Commun. 36 (1), 92–103.
Villas, B., 1998. Flow cytometry: an overview. Cell Vis. J. Anal. Morphol. 5 (1), 56–61.
Wang, X.-B., Yang, J., Huang, Y., Vykoukal, J., Becker, F.F., Gascoyne, P.R., 2000. Cell separation by dielectrophoretic field-flow-fractionation. Anal. Chem. 72 (4), 832–839.
Wang, L., Flanagan, L., Lee, A.P., 2007. Side-wall vertical electrodes for lateral field microfluidic applications. Journal of microelectromechanical systems 16 (2), 454–461.
Wang, L., Lu, J., Marchenko, S.A., Monuki, E.S., Flanagan, L.A., Lee, A.P., 2009. Dual frequency dielectrophoresis with interdigitated sidewall electrodes for microfluidic flow-through separation of beads and cells. Electrophoresis 30 (5), 782–791.
Wulfkuhle, J.D., Liotta, L.A., Petricoin, E.F., 2003. Early detection: proteomic applications for the early detection of cancer. Nat. Rev. Cancer 3 (4), 267.
Xing, L., Todd, N.W., Yu, L., Fang, H., Jiang, F., 2010. Early detection of squamous cell lung cancer in sputum by a panel of microrna markers. Mod. Pathol. 23 (8), 1157.
Yang, J., Huang, Y., Wang, X.-B., Becker, F.F., Gascoyne, P.R., 1999. Cell separation on microfabricated electrodes using dielectrophoretic/gravitational field-flow fractionation. Anal. Chem. 71 (5), 911–918.
Yang, S.-Y., Hsiung, S.-K., Hung, Y.-C., Chang, C.-M., Liao, T.-L., Lee, G.-B., 2006. A cell counting/sorting system incorporated with a micro- fabricated flow cytometer chip. Meas. Sci. Technol. 17 (7), 2001.
Zborowski, M., Chalmers, J.J., 2011. Rare Cell Separation and Analysis by Magnetic Sorting.
Zimmermann, U., Pilwat, G., Riemann, F., 1975. Preparation of erythrocyte ghosts by dielectric breakdown of the cell membrane. Biochim. Biophys. Acta Biomembr. 375 (2), 209–219.
Zrínyi, M., Barsi, L., Büki, A., 1996. Deformation of ferrogels induced by nonuniform magnetic fields. J. Chem. Phys. 104 (21), 8750–8756.