



Recent advances in microfluidic technologies for separation of biological cells

Lujing Sun¹ · Wenguang Yang¹ · Shuxiang Cai¹ · Yibao Chen¹ · Honghui Chu¹ · Haibo Yu^{2,3} · Yuechao Wang^{2,3} · Lianqing Liu^{2,3}

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Abstract

Cell separation has always been a key topic in academic research, especially in the fields of medicine and biology, due to its significance in diagnosis and treatment. Accurate, high-throughput and non-invasive separation of individual cells is key to driving the development of biomedicine and cellular biology. In recent years, a series of researches on the use of microfluidic technologies for cell separation have been conducted to solve bio-related problems. Hence, we present here a comprehensive review on the recent developments of microfluidic technologies for cell separation. In this review, we discuss several cell separation methods, mainly including: physical and biochemical method, their working principles as well as their practical applications. We also analyze the advantages and disadvantages of each method in detail. In addition, the current challenges and future prospects of microfluidic-based cell separation were discussed.

Keywords Microfluidic technology · Cell separation

1 Introduction

Cell separation has always been a problem facing researchers in the fields of medicine and biology (Xiang et al. 2019; Bacon et al. 2020; Nagase et al. 2020). It is of great importance in biomedical research, including diagnosis and cellular biology. For instance, some cells are found at extremely low numbers in the blood, such as circulating tumor cells (CTCs), fetal nucleated red blood cells (NRBCs), circulating endothelial cells, immune cells subgroup, and stem cells (Myung et al. 2010; Li et al. 2015; Jones and Watt 1993; Tayoun et al. 2019; Safarpour et al. 2020). Separation and investigation of these cells can help with the diagnosis of certain diseases. For example, CTCs are tumor cells that spread into peripheral

blood and circulate with the blood due to primary focus or metastasis of solid tumors shed into blood. Studies have shown that CTCs can lead to the occurrence of tumor metastasis (Tanaka et al. 2009; Martin et al. 2017; Kim et al. 2019; Gupta and Massagué 2006; Rengan et al. 2015). The effective capture of CTCs and the detection of their genes and secretions offer important guidance for the early diagnosis, postoperative evaluation, personalized treatment and evaluation of tumor resistance. NRBCs play an important role in prenatal diagnosis (Kumo et al. 2017; Wei et al. 2019; Antfolk and Laurell 2017). NRBCs contain complete genetic information of the fetus. Moreover, they are present in maternal peripheral blood in early pregnancy at higher contents than other fetal cells, and disappear from the mother a few days after delivery, which ensures that they are not confused with maternal cells. All these make NRBCs the preferred target cells for minimally invasive prenatal diagnosis. In terms of treatment, certain components of the blood have therapeutic effects. For instance, platelets are often injected during surgery. Islet transplantation is a method to treat diabetes. The key to successful islet transplantation is to isolate islet cells with high levels of purity and vitality from the donor.

Therefore, the separation of biological cells is essential for bio-related researches. Conventional cell separation methods fall into two categories: labeled and unlabeled (Henighan et al. 2010; Bhagat et al. 2010; Wyatt Shields Iv et al. 2015).

✉ Wenguang Yang
ytu_yangwg@163.com

✉ Lianqing Liu
lqliu@sia.cn

¹ School of Electromechanical and Automotive Engineering, Yantai University, Yantai 264005, China

² State Key Laboratory of Robotics, Shenyang Institute of Automation, Chinese Academy of Sciences, Shenyang 110016, China

³ Institutes for Robotics and Intelligent Manufacturing, Chinese Academy of Sciences, Shenyang 110016, China

Labeled methods separate cells by labeling cell antibodies and using the combination of antibodies and specific antigens. At present, fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) are the two most common marker screening methods. Unlabeled methods separate cells by using the physical properties of cells, such as cell size, deformation, and movement. However, the development of cell separation methods has been bottlenecked by throughput, sensitivity and costs.

The microfluidic technology is one of the most advanced technologies in the world. As a highly interdisciplinary technology that integrates physics, life science, microelectronics, materials and computer science, it is mainly applied in the fields of chemistry and life science (Brouzes et al. 2009; Andersson and Van Den Berg 2003; Delamarche et al. 2005). As the microfluidic technology keeps developing to match the scale of cells, the cell separation technology has become increasingly sophisticated and efficient. With the microfluidic technology, the entire process spanning from sample pretreatment reaction separation and enrichment to detection can be completed on a microfluidic chip, which has the advantages of low costs, fast analysis and compact size. These advantages make it a better method for the separation and purification of molecules and cells in time and space. The combination of the microfluidic technology and traditional cell separation methods has taken the research on cell separation to the next level.

Although these mentioned methods have been summarized by other researchers (Chen et al. 2012; Ding et al. 2013; Yan et al. 2017; Dalili et al. 2018), it is urgent need a comprehensive and systematic review on engineering-facilitated microfluidic-based cell separation techniques. As a consequence, this review focuses on recent advances in microfluidic technologies for cell separation. First, we present a series of cell separation methods, followed by a discussion about how they work and how they are used in real-world situations. Then, we analyze the advantages and disadvantages of each method. Finally, we summarize the current challenges and future directions of microfluidic-based cell separation.

2 Microfluidic-based separating techniques

Based on the working principle, cell separation methods can be divided into two categories: physical and biochemical. Depending on the physical field used, physical cell separation methods can be divided into the single hydrodynamic physical field separation method and the multi-physical field-assisted separation method. There are two primary categories of biological cell separation methods: immunoaffinity-based and cell adhesion-based. Table 1 is a summary of cell separation methods by category.

2.1 Hydrodynamic-based cell separation

The hydrodynamic-based cell separation method depends heavily on how cells behave in the laminar flow, which is determined by their physical properties including size, shape, and deformability. Cells are moved under the action of a hydrodynamic lift force and the magnitude of this force relies on the physical properties of the cells. Therefore, different cells will be dragged in different paths.

2.1.1 Pinched flow fractionation

Pinched flow fractionation (PFF) is a typical cell separation method based on fluid dynamics. It relies on the difference in cell size in the laminar flow for continuous cell separation (Oakey et al. 2002). The working principle of PFF is shown in Fig. 1 (Yamada et al. 2004). The device contains two inlets, a pinched segment, and a widened channel outlet. A sample solution containing cells with different sizes and a buffer pass through the two inlets, respectively. By controlling the flow rate of the two solutions, the cells in the sample can be moved along one side of the pinched channel. In the laminar flow, the streamline of the larger particle is close to the center of the channel, while the streamline of the smaller particle is close to the channel wall. Therefore, when the solution enters the widened channel from the pinched segment, the streamline of the fluid diffuses, thus aggravating the distance between cells with different sizes and achieving cell separation (Vig and Kristensen 2008). Obviously, the pinched flow fractionation can achieve rapid cell separation without labeling. However, this method can easily cause cell blockage in the pinched segment and thus damages to the cells, which may affect the experimental results.

2.1.2 Hydrodynamic filtration

Hydrodynamic filtration is another cell separation method based on hydrodynamics. Similar to pinched flow fractionation (PFF), this method also depends on the different physical properties of cells that make them behave differently in the laminar flow. The different physical properties lead the cells to experience different lift forces in the laminar flow, which makes the cells move in different streamlines. As shown in Fig. 2, the difference in cell size causes the cells to flow out from different side channels to achieve cell separation. In this microchannel, the buffer causes the cells to move along one side of the channel (Yamada et al. 2007). Since smaller cells are nearer to the channel wall than larger cells, they filter out earlier than the larger ones. This method has the advantages of simple structure of microfluidic device, rapid separation and high throughput, and is most commonly used to separate blood cells or tumor cells. Yamada et al. first designed a

Table 1 Microfluidic-based techniques for cell separation

Separation techniques		Mechanism	Advantages	Disadvantages	
Physical	Hydrodynamic-based	Pinched flow fractionation (PFF)	Hydrodynamic force	Label-free and rapid separation	Clogging and damages to cells
		Hydrodynamic filtration	Hydrodynamic force	Label-free	Clogging and damages to cells
		Inertia-based microfluidic methods	Shear-induced and wall-induced lift	Label-free	Shear-induced damage to cells
	Physical field-assisted	Magnetic-based	wall-induced lift magnetic field	High specificity	Low throughput and requiring magnetic particles
		Acoustic-based	Ultrasonic standing waves	Robust and contact-free	Requiring special materials for device fabrication
		Dielectrophoresis-based	Inhomogeneous electric field	Label-free	Requiring preparation of different mediums
Biochemical	Immunoaffinity-based	Specific binding to cell surface markers	High specificity and purity	Requiring cell-specific markers	
	Cell adhesion-based	Cell interaction with surface	Label-free and high specificity	High requirement for liquid flow and shear stress	

hydrodynamic filter and used it to concentrate white blood cells and liver cells (Yamada and Seki 2005, 2006).

This hydrodynamic-based method separates cells primarily relying on the different physical properties of cells that determine the cells' behaviors in the laminar flow. In other words, the device structure must be designed based on the cells'

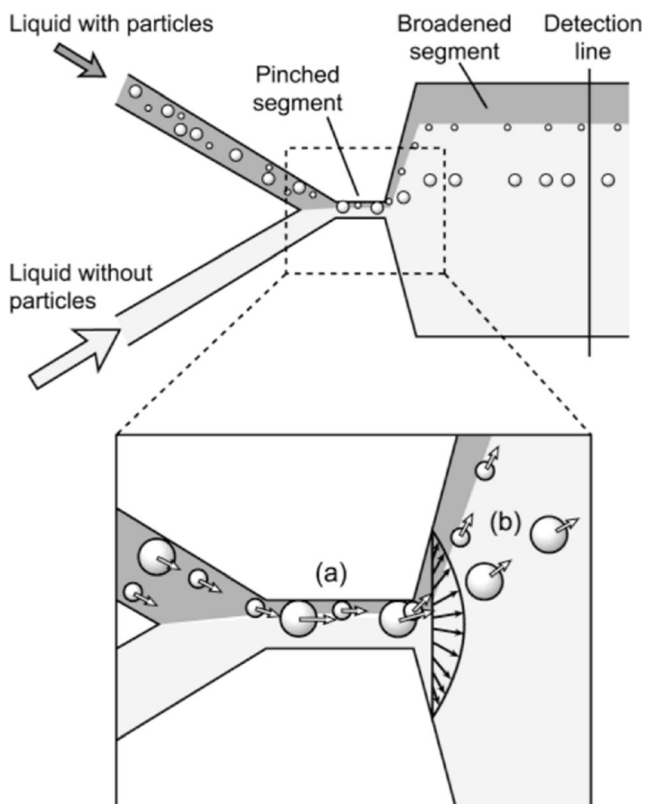
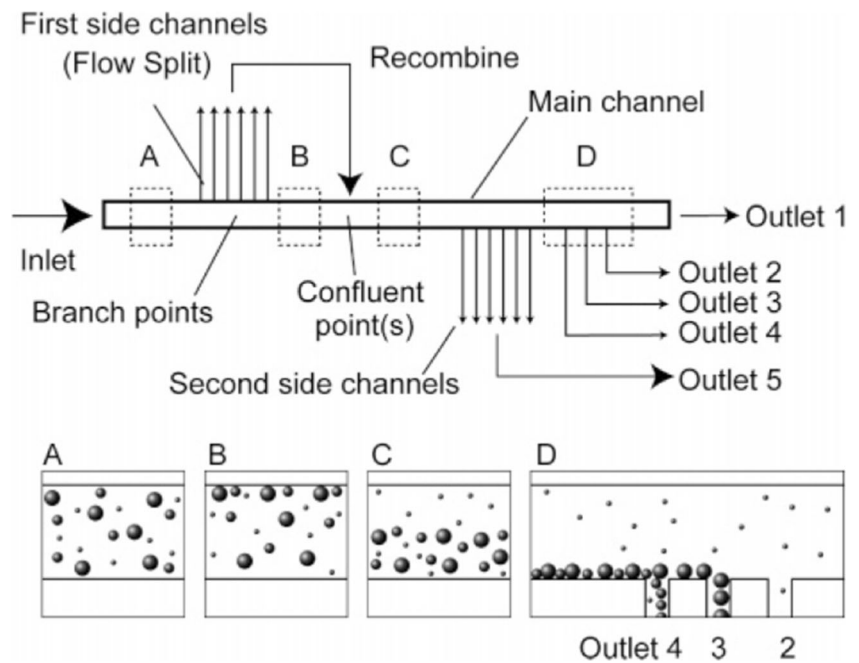


Fig. 1 The working principle of pinched flow fractionation. Reproduced with permission from Analytical chemistry 76.18 (2004). Copyright © 2004 American Chemical Society

behaviors to achieve cell separation. Of course, different streamlines can be designed based on the cells' behaviors in the laminar flow, so that the cells move in a certain path and the cell separation method is more flexible. Deterministic lateral displacement (DLD) technology utilizes this principle to achieve a specific trajectory of cell separation. DLD is one of the methods of cell separation by hydrodynamic filtration (Huang et al. 2004; McGrath et al. 2014). It realizes continuous deterministic lateral displacement according to the size of the particles or cells by designing a micro-pillars array with a specific structure. As shown in Fig. 3, the regularly arranged array designed by DLD divides the fluid into different streamlines. It allows cells smaller than the critical size to move along the streamline without migration, and cells larger than the critical size to collide with the micropillar and migrate to the adjacent streamline to separate cells of different sizes. The shape of the micropillar can not only be designed as a square, but also a circle or a triangle. Huang et al. designed a micropillar array with a circular structure based on this principle, and isolated white blood cells and red blood cells based on the size difference between nucleated cells and non-nucleated cells with an efficiency as high as 99.99% (Huang et al. 2008). The DLD method is sensitive to cell size and deformability and can be used for high-throughput cell separation, but it can also cause blockage problems.

Another type of hydrodynamic filtration method is the microstructural filtration method. This method also uses hydrodynamic principles to separate cells of different physical properties. However, it is different from the two types of methods mentioned above in that it relies more heavily on the structures of the device. The method is to capture larger cells by designing membranes, holes and other structures in the device. This method can be divided into three types according to the structure of the separation device: membranes (Zheng et al. 2007;

Fig. 2 Principle of cell separation according to size based on Hydrodynamic filtration. Reproduced with permission from *Analytical chemistry* 78.4 (2006). Copyright © 2006 American Chemical Society



Li et al. 2014; Kang et al. 2015; Hernández-Castro et al. 2017), pillars (Alvankarian et al. 2013; Wang et al. 2014; Kang et al. 2017), and weirs (Stott et al. 2010). They can also be classified by flow direction: dead-end filters or cross-flow filters (Cheng et al. 2016), as shown in Fig. 4. In the case of dead-end filters, the flow is perpendicular to the filter plane, while in the cross-flow case, the flow follows the filter structure. This method is commonly used to separate blood plasma from blood cells by placing membranes or holes in the device to block blood cells on the filter surface and having the plasma filtered out through the membranes or holes. This method can also achieve high-throughput cell separation, but at a low efficiency due to the severe clogging.

In general, the hydrodynamic filtration device is simple in structure, easy to operate, and requires no labeling. It has the potential to achieve high-throughput and efficient cell separation, but the biggest obstacle is the problem of blockage. In addition, further research is needed on non-destructive cell separation.

2.1.3 Inertia-based microfluidic methods

In addition to PFF and hydrodynamic filtration methods, inertia forces can also be used as a hydrodynamic-based approach to cell separation. When the fluid presents a laminar flow state in the microfluidic channel, the boundary effect of the fluid flow near the wall of the microfluidic channel will generate lift forces, i.e., shear lift and wall lift, respectively (Park et al. 2009). These two forces balance the cells at different positions on the cross section of the microchannel according to the size of the cell relative to the microchannel, thus separating cells of different sizes, as shown in Fig. 5. The curved channel allows cells to concentrate in the same place using the principle of inertia. The researchers used asymmetric serpentine microchannels to enrich platelets in the blood by a factor of 100 using the inertial migration of cells (Di Carlo et al. 2008). Like serpentine channels, cells can be sorted in helical microchannels because of

Fig. 3 The regularly arranged array designed by DLD divides the fluid into different streamlines. Reproduced with permission from *Chemical engineering science* 73 (2012). Copyright © 2012 Elsevier Ltd.

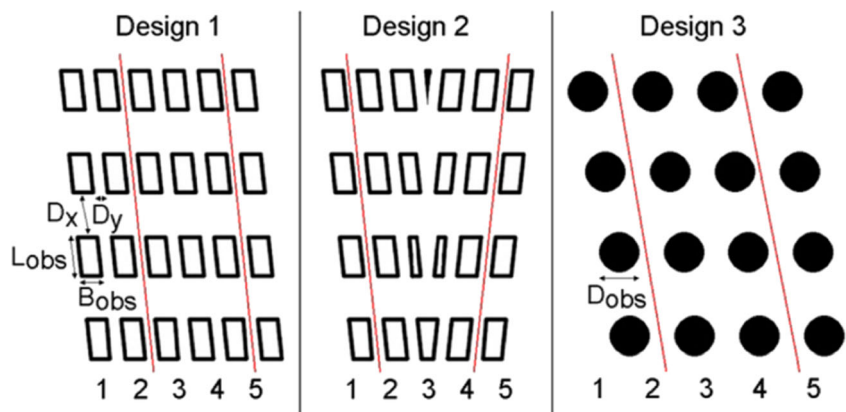
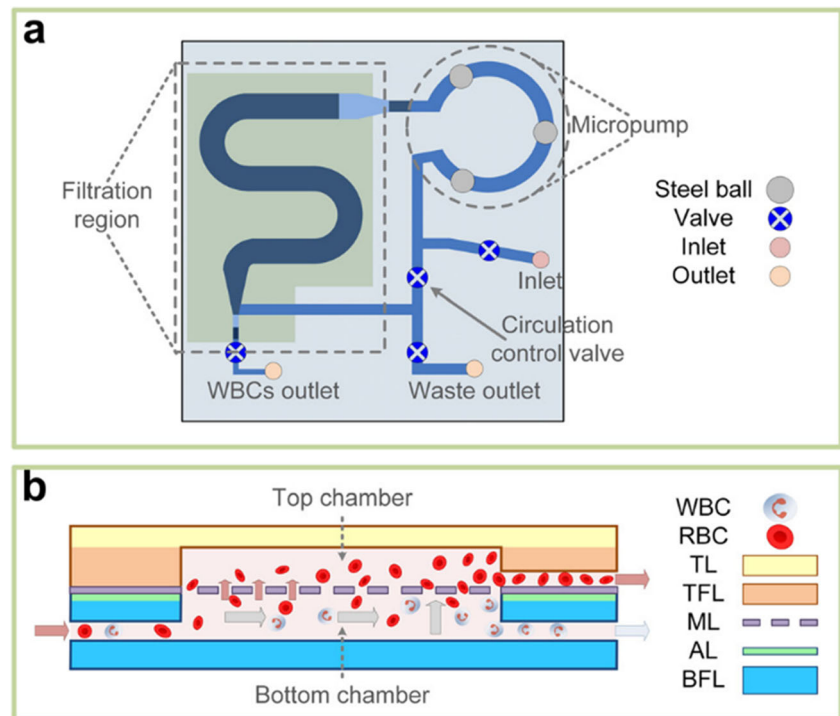


Fig. 4 Microfluidic filtration platform for whole blood cell separation. **a** the schematic of designed microfiltration chip. **b** the cross-section of the chip in the filtration region. Reproduced with permission from *Biomicrofluidics* 10.1 (2016). Copyright © 2016 AIP Publishing LLC



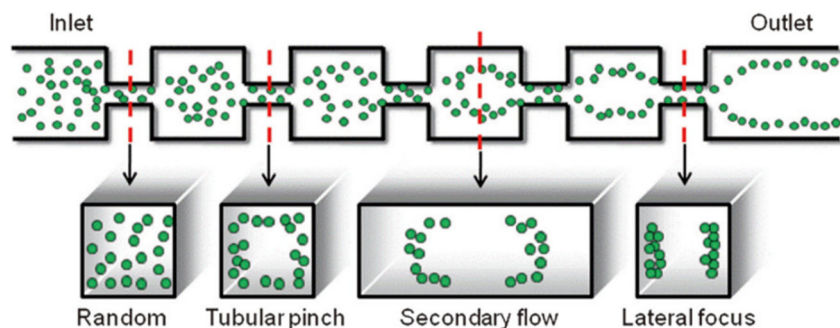
their different focal points, allowing different cells to branch rapidly into multiple channels (Kuntaegowdanahalli et al. 2009). Helical microchannels can also be used to separate neuroblastoma cells from glioma cells with an efficiency of up to 80% and a cell survival rate of up to 90% (Wu et al. 2009).

The principle of inertia can be used to achieve high-throughput cell separation in much shorter times. Compared with the PPF and hydrodynamic filtration methods, this method can eliminate the influence of blockage, but its shear-induced damage to cells is greater.

2.2 Physical field-assisted cell separation

Applying external physical fields such as sound field, magnetic field, electric field, and light field to the fluid field can improve the efficiency of cell separation. In this section, we will cover in detail the principles and applications of cell separation methods assisted with other physical fields.

Fig. 5 Rigid spheres were moving in the poiseuille flow under inertial lift forces. Reproduced with permission from *Lab on a Chip* 9.7 (2009). Copyright © 2009, Royal Society of Chemistry (Great Britain)



2.2.1 Magnetic-based cell separation

Cell separations based on a magnetic field have attracted wide attention due to their advantages of simplicity, cleanliness and less damage to samples (Shen et al. 2014). With a permanent magnet or electromagnetic coil installed in the separation device, these methods separate cells by applying a magnetic force to magnetic cells or magnetic marked cells. The magnetic cell separation system (MACS) produced by combining magnetic techniques with fluorescence activated cell sorting (FACS) greatly improves the efficiency of cell separation. One of the advantages of using MACS to separate cells is that the magnetic field does no harm to cells or biological tissues, and it does not affect the immune chemistry required for magnetic markers (Berger et al. 2001). In addition, the magnetic cell separation system (MACS) can also separate a variety of cells or particles at the same time. There are two ways of magnetic separation of cells: batch separation (Kwak et al. 2018; Grodzinski et al. 2003; Furduliu and Harrison 2004; Lee

et al. 2004) and continuous separation (Ngamsom et al. 2016; Vojtisek et al. 2012; Pamme and Wilhelm 2006; Tarn and Pamme 2017; Hoshino et al. 2011). Batch separation utilizes a magnetic field to bind magnetically labeled cells in a separation chamber, so other unlabeled cells or particles can be removed. Then the target particles can be released when the magnetic field is turned off. Hoshino (Hoshino et al. 2011) designed a batch separation device and used antibodies to mark CTCs coupling nano magnetic beads. Labeled cells by magnetic beads under a magnetic field adsorbed on the surface of silicon wafer and CTCs can be isolated from blood cells (Fig. 6).

Continuous separation refers to a process in which different magnetic field forces are exerted on cells with different magnetism or susceptibility to result in different cell migration distances that causes different cells to flow out of different outlets. In the device designed by Pamme and Wilhelm (Pamme and Manz 2004) (Fig. 7), the migration distance of particles is affected by the size and magnetic susceptibility of particles. The larger particles have a larger migration distance due to the larger magnetic field force, which separates them from the smaller particles. The same group of researchers used the technique to successfully separate mouse macrophages from human ovarian cancer cells (Pamme and Wilhelm 2006).

In another continuous separation device, the separation of cells depends not on the labelling of the magnetic material but on the nature of the cell itself. The device is used to separate red blood cells from white blood cells in the blood. As shown in Fig. 8, the ferromagnetic nickel wire is placed in a separation chamber (Han et al. 2006). Since the white blood cells in the blood are diamagnetic and the red blood cells are paramagnetic, when the magnetic field is perpendicular to the ferromagnetic nickel wire, the red blood cells deviate from the ferromagnetic nickel wire and flow out from the exits on both sides. When the magnetic field is parallel to the ferromagnetic nickel line, the white blood cells deviate from the line and flow out of the outlet on either side of the device to separate the cells. The system is also fabricated on silicon and

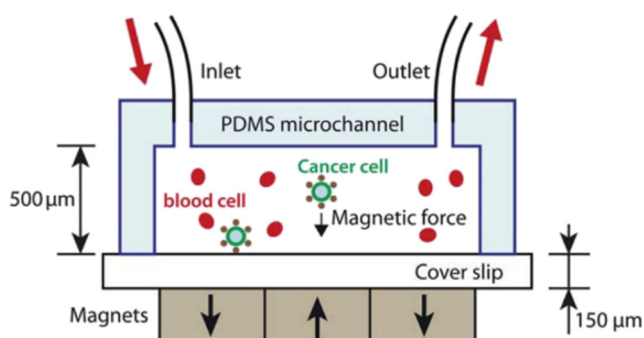


Fig. 6 Magnetic isolation of CTCs labelled with EpCAM functionalized Fe₃O₄ magnetic nanoparticles in microfluidic devices. Reproduced with permission from Lab on a Chip 11.20 (2011). Copyright © 2011, Royal Society of Chemistry (Great Britain)

glass substrates using microfabrication and stereoscopic lithography.

In summary, cell separation methods based on a magnetic field greatly improve the efficiency of cell separation, and cause little damage to cells. At present, the development of these methods is restricted by the selection of specific ligands. Their practical application has been hindered by such factors as poor preservation of antibodies, harsh reaction conditions, and high costs. Therefore, the key to moving these methods forward is to find a specific ligand with strong specificity and low costs.

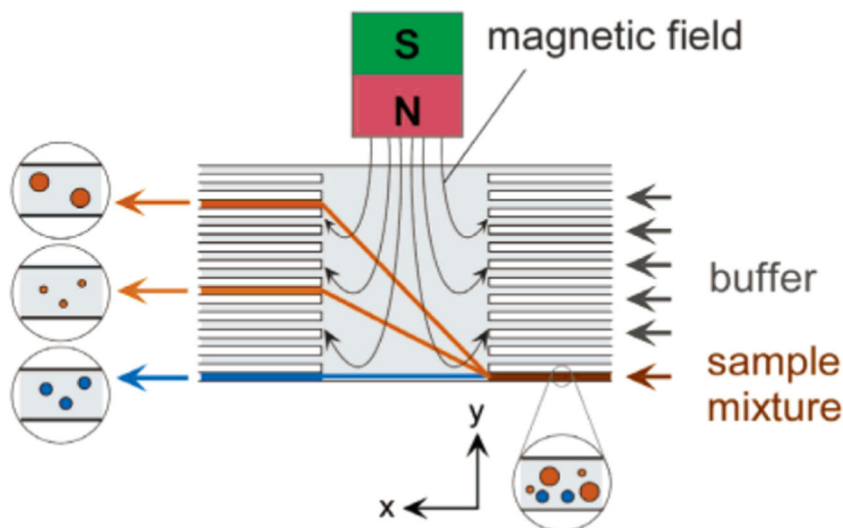
2.2.2 Acoustic-based cell separation

Acoustic-based cell separation is to apply a sound field around a microfluidic chip, so that particles suspended in it are under the action of an acoustic radiation force and tend to move to the position of the pressure wave abdomen or pressure wave nodes. Moreover, ultrasonic wave are well suited for cell manipulation because they provide rapid and accurate spatial control within a microfluidic chip without affecting cellular activity, thereby enabling cell separation through sound fields (Burguillos et al. 2013; Laurell et al. 2007; Lenshof et al. 2012). Acoustic-based cell separation methods can be divided into three categories: bulk standing wave separation, surface standing wave separation, and traveling wave separation (Johansson et al. 2009).

The magnitude of the acoustic radiation force produced by the interaction between the acoustic field and the cell is determined by the volume, density and compressibility of the cell and the density of the fluid. John Wiley and Sons demonstrated a device that combines a standing wave with split-flow fractionation to separate particles (Kumar et al. 2005). Under the action of an acoustic field, the particles all move towards the wave point of the standing wave, but the larger particles move faster because of the larger acoustic radiation force. Therefore, the relationship between the current and the channel can be adjusted to separate large particles from small ones. Kumar et al. (2005) used this principle to establish a model for predicting particle trajectories and used it to separate mixed tumor cells from *Lactobacillus rhamnosus* cells (Fig. 9).

Using this principle, Dykes et al. (Dykes et al. 2011) isolated platelets from peripheral blood endothelial cells, and Yang et al. (Yang and Soh 2012) isolated live cells from dead cells. By an optimized device, Chen et al. (Dolatmoradi and El-Zahabs 2016) identified over 85% of WBCs and RBCs and recovered over 80% of platelets. This device has an upper inlet and a lower outlet, and the node of the standing wave is located on the wall of the upper channel, which makes it easier for the particles to move. The buffer flows in from the upper inlet, causing the cells to move along the channel wall (Fig. 10). Under the action of a sound field, the red blood cells and white blood cells rapidly shift upward due to their larger volume

Fig. 7 On-chip free flow magnetophoresis. Reproduced with permission from Analytical chemistry 76.24 (2004). Copyright © 2004 American Chemical Society



than that of the platelets, and eventually flow out from the upper outlet, while the platelets flow out from the lower outlet.

In contrast to bulk standing waves, surface standing waves are formed by placing an interdigital transducer (IDT) around a microfluidic channel, providing the necessary mechanical disturbance to move the cell along a clear streamline at the top of the fluid (Shi et al. 2009). This technique provides a wide range of frequencies that allow for flexible control of single cells as well as multiple channels for cell separation (He et al. 2012; Wang and Zhe 2011). Nam et al. used the standing wave surface acoustics (SSAWs) technology to separate cells of different sizes (Nam et al. 2011). The mixed cell sample flows in from the middle air inlet, and a buffer is set on both sides of the air inlet to concentrate the cells in the channel on the central streamline (Fig. 11). The pressure nodes are positioned on the wall and the resistance points are at the center of the channel. Due to acoustic radiation, the cells begin to move towards the channel wall on either side, during which the larger cells move

faster and in turn separate from the smaller cells. Later, they used this method to separate platelets from whole blood and *Escherichia coli* from peripheral monocytes in the same way (Ai et al. 2013).

The difference between the traveling wave (Destgeer et al. 2014, 2016; Ma et al. 2016; Ung et al. 2017) and standing wave (Park et al. 2017) is the node. The node travels with the wave, while that of the standing wave does not travel with the wave. Therefore, the traveling wave is a wave that transmits energy, while the standing wave can only vibrate *in situ*. It has been found that when the wavelength is close to the particle size, the acoustic radiation force applied to the particle increases nonlinearly. That is, smaller wavelengths can more precisely control and separate particles or cells, which traveling waves can do (Collins et al. 2016). It can be seen from the device that IDT does not need to be parallel to the channel (Fig. 12) (Destgeer et al. 2013). The device has two inlets and three outlets, and the sample cells enter from the left channel,

Fig. 8 Ferromagnetic wire operated in diamagnetic mode for continuous magnetophoretic separation of suspended cells (WBC and deoxyhemoglobin RBC) using their native magnetic properties. Reproduced with permission from Proc. Nanotech 1 (2005). Copyright © 2004 American Chemical Society

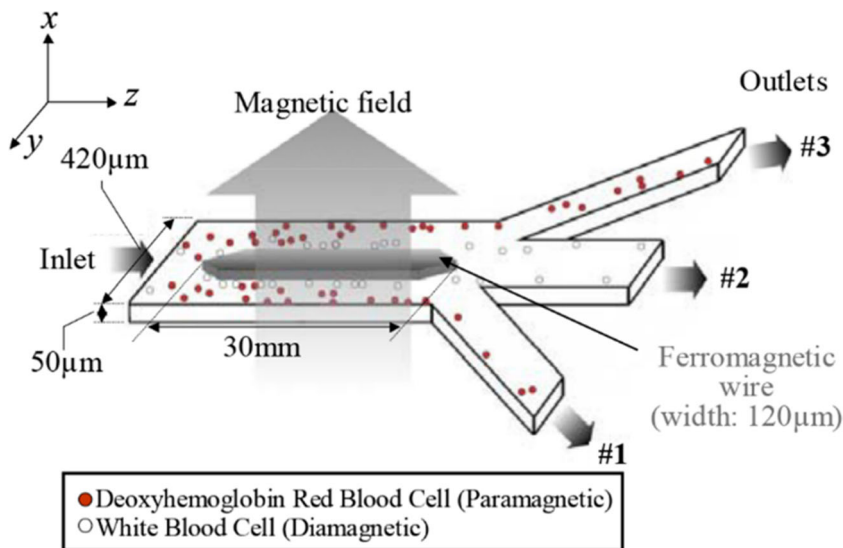
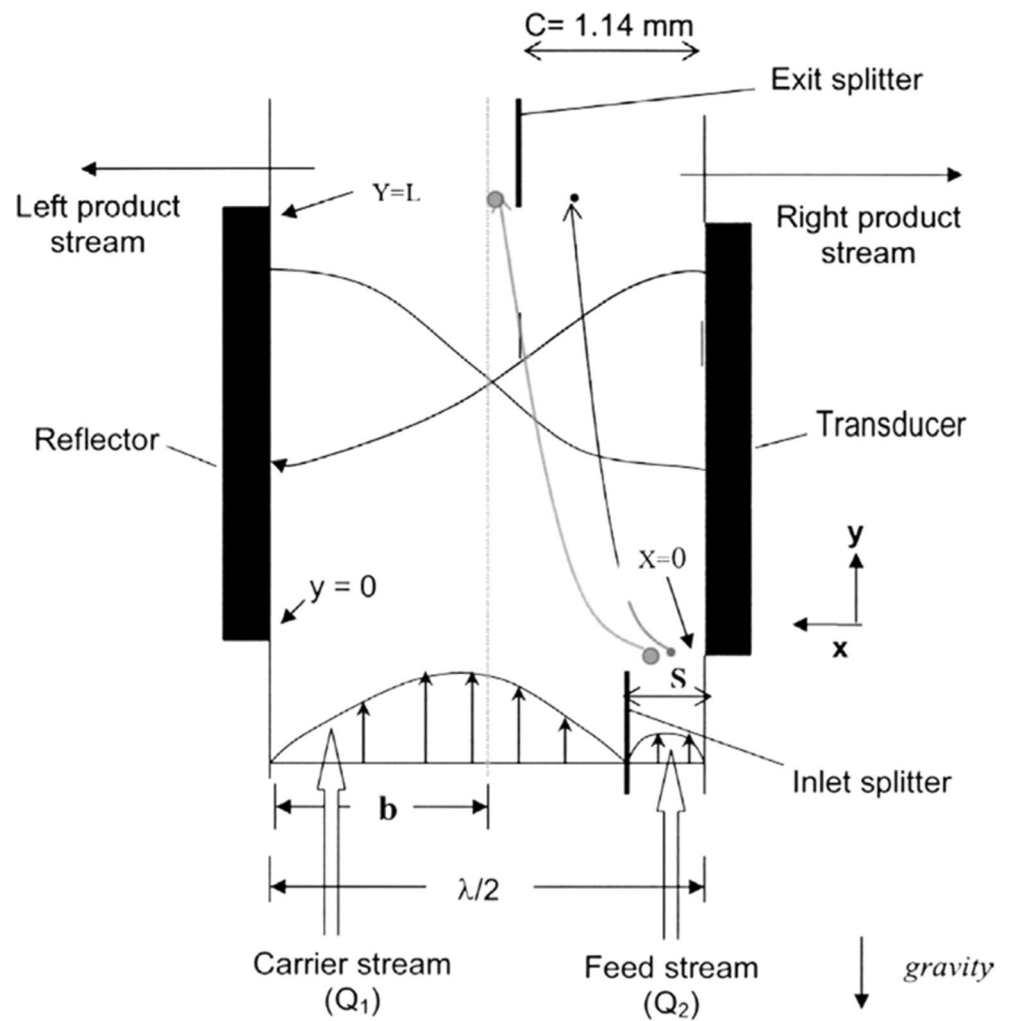


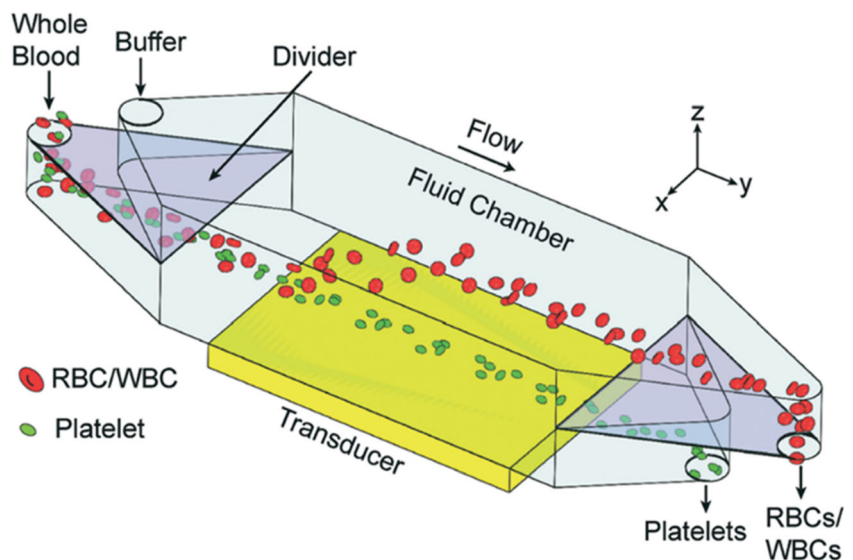
Fig. 9 The schematic of the acoustic fractionation concept. Reproduced with permission from *Biotechnology and bioengineering* 89.2 (2005). Copyright © 2004 Wiley Periodicals



flowing along the channel wall on one side because of the buffer. After that, the cells are gradually offset by the gradually increasing acoustic radiation force, making the cells of

different sizes move along the streamline of different positions. As a result, the cells or particles of different sizes can be separated with an efficiency of up to 100%. Using this

Fig. 10 Schematic of acoustic separation device for separating platelets from whole blood. Reproduced with permission from *Lab on a Chip* 16.18 (2016). Copyright © 2016, Royal Society of Chemistry (Great Britain)



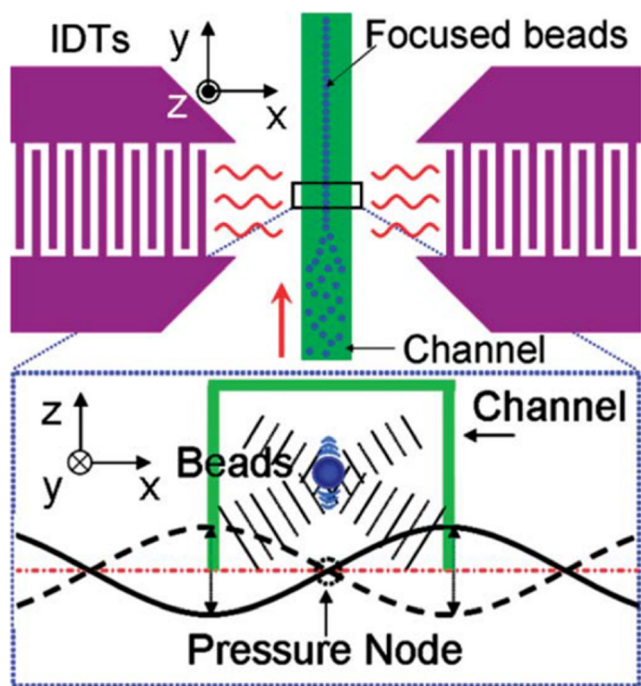


Fig. 11 Schematic of a standing surface acoustic wave device. Reproduced with permission from Lab on a Chip 8.2 (2008). Copyright © 2008, Royal Society of Chemistry (Great Britain)

technique, Wang et al.(Wang et al. 2018)combined SSAW and TSAW to separate CTCs from blood samples.

In summary, cell separation based on acoustic radiation is relatively mild, robust and contactless, and therefore holds

great potential for further research and application. However, due to the small physical difference between cells, the acoustic radiation force affected by this difference is extremely small, which leads to low separation efficiency. In addition, cell separation devices based on an acoustic field are complex and require a high degree of fabrication. Therefore, more efforts are called for to study acoustic field based devices with higher levels of sensitivity and separation efficiency.

2.2.3 Dielectrophoresis-based cell separation

Dielectrophoresis works for cell separation in the following way: In a non-uniform electric field (Huang et al. 2002; Dürr et al. 2003), due to the different dielectric properties of different cells, the dielectrophoresis force produced by the cells is different in size and direction, which will make the cells drift in different directions. In terms of cell isolation, Becker et al. 1 first used a dielectric affinity column to isolate cancer cells. As shown in Fig. 13, the device is equipped with an array of microelectrodes(Hughes 2002). Due to the different permittivity between cells, the voltage and frequency applied to the electrodes are adjusted to generate a dielectrophoretic force for the absorption of cancer cells on the electrodes, while other blood cells flow out of the device along with the fluid. Finally, the voltage is turned off and the cancer cells on the electrode are collected. In this way, cancer cells can be separated with an efficiency of more than 95%.

Fig. 12 Schematic of a travelling surface acoustic wave-based separation device. Reproduced with permission from Lab on a Chip 13.21 (2013). Copyright © 2013, Royal Society of Chemistry (Great Britain)

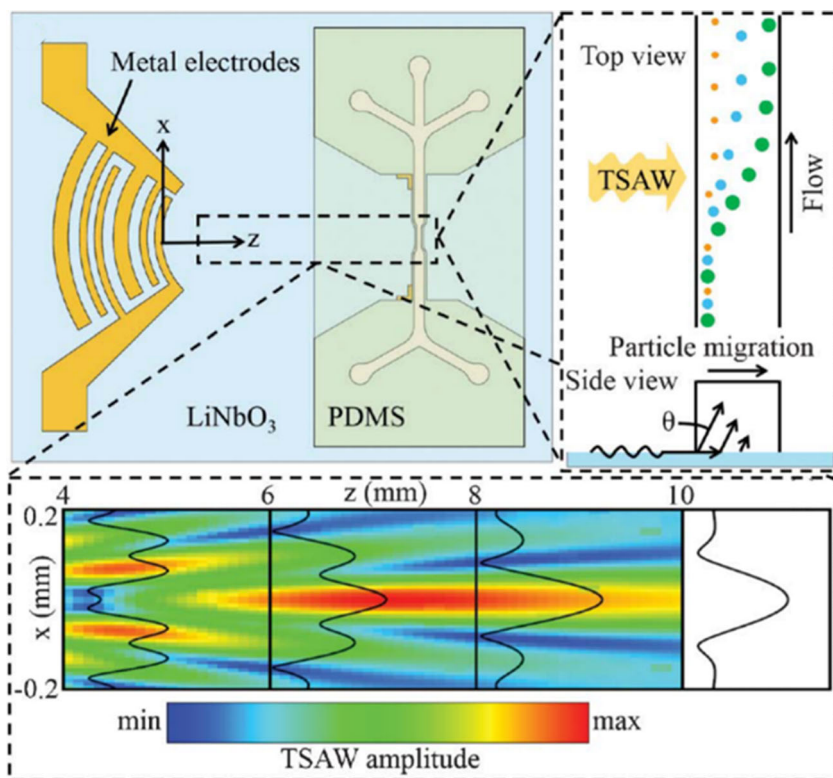
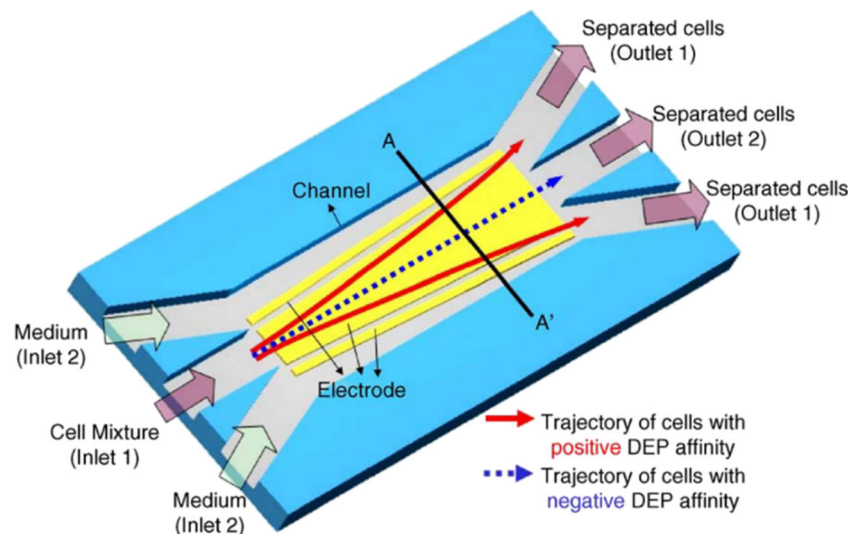


Fig. 13 Conceptual view of hydrodynamic dielectrophoresis (DEP) process. Reproduced with permission from Sensors and Actuators A: Physical 121.1 (2005). Copyright © 2005 Elsevier B.V



In addition to setting a microelectrode array, electrodes can also be set at the bottom of the device, which enables continuous high-throughput separation of cells. Positive dielectrophoresis and negative dielectrophoresis can be used to separate active and inactive yeast cells. Due to the different dielectric properties between active yeast cells and inactive yeast cells, dielectrophoresis forces in different directions are produced. The inactive yeast cells experience a negative dielectrophoresis force and flow from the middle outlet along the central flow line of the channel, while the active yeast cells flow from the channel on both sides under the action of a positive dielectrophoresis force (Fig. 13).

The electrodes in the above devices are all set on a two-dimensional (2D) plane, while the application of three-dimensional (3D) electrodes can make cell separation more efficient. Due to the limitations of 2D electrodes, the height of the fluid in the channel is very small, which limits the throughput of the device. To address this problem, some researchers have designed and built 3D electrode devices that increase both throughput and separation speed. Recently, 3D carbon electrodes have been developed due to their low cost and low electrolysis rate (Cheng et al. 2015; Hughes 2016). Using a 3D carbon electrode, Yildizhan et al. successfully isolated live and dead U937 monocytes without dissolving the live cells (Yagmur et al. 2017). In another 3D electrode device (Ling et al. 2012), a triangular periodic array is arranged at the bottom of the device and continuous electrodes at the top, so this is a device with asymmetric electrodes. The device can be used to obtain pure live cells from NIH-3 T3 live/dead hybrid cells, and to separate MG-63 cells from red blood cells, with an efficiency of 80%.

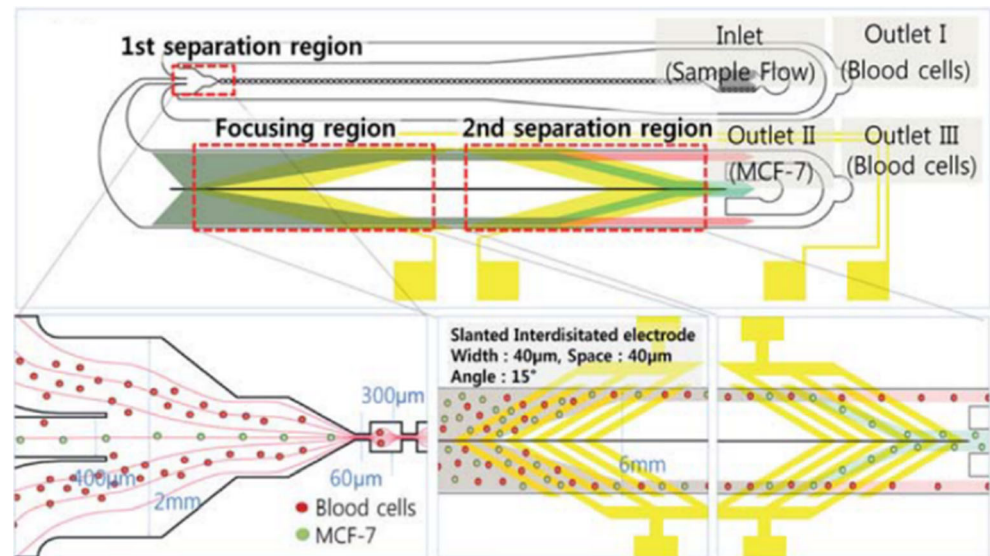
DEP is often combined with other separation methods to improve cell separation efficiency. For example, multi-orifice flow fractionation (MOFF) separation is based on cell size only and cannot separate cells of similar sizes, such as CTCs

and WBCs. This problem can be solved by the combination of MOFF separation and DEP (Moon et al. 2011). The corresponding device is shown in Fig. 14. In the separation chamber, the MOFF in the first part separates cells with large size differences. In the second part, the DEP generated by the electrode can separate cells of similar sizes. This method can not only separate CTCs from a mixture of cells of similar sizes, but also deliver an efficiency 94.23% and 99.24% for the separation of WBCs and RBCs, respectively.

There has also been another method for cell separation based on DEP named optically-induced dielectrophoresis (ODEP) (Chiou et al. 2005). The principle of ODEP is similar to DEP and the only difference is that non-uniform electric field was no longer generated by metal-based microelectrodes. Instead, the optical pattern as virtual electrode projected onto a-SiH can produce this electric field. Since its emergence, this technique has attracted researchers' attention. Liao et al. fabricated an ODEP-based microfluidic device and CTC cells can be isolated with a high purity (Liao et al. 2018). Huang et al. combined ODEP with microfluidic technology and generated six moving light-bar to separate oral cancer cells (OEC-M1) and prostate cancer cells (PC-3) from the leukocytes (Huang et al. 2013). The high purity and high recovery rate can be achieved based on this label-free process. Moreover, after the separating operation, the survival rate of cells is more than 90%. Furthermore, the separation of tumor cells with different viabilities can be achieved using ODEP-based microfluidic technology. Chu et al. succeeded in separating tumor cells based on the difference of ODEP force generated by cells with different levels of viability (Chu et al. 2019). Similar to metal-based DEP, ODEP for cell separation is label-free. But due to optical pattern replaced metal electrode, ODEP is more flexible and cost-effective.

Cell separation methods based on the DEP technology have been widely used in recent years. The DEP technology

Fig. 14 A combination of MOFF and DEP, which is used for separation of breast cancer cells (MCF-7) from blood samples. Reproduced with permission from Lab on a Chip 11.6 (2011). Copyright © 2011, Royal Society of Chemistry (Great Britain)



can be easily integrated with other technologies, and researchers have combined it with the field flow fractionation technology or optical technology to achieve label-free cell separation. However, this technology offers extremely low separation efficiency when there is no difference in the size and dielectric properties of the cells. In addition, the complex structure of DEP-based cell separation devices and the variety of samples used limit the practical application of this technology. Therefore, further research is required to improve this technology.

2.3 Biochemical cell separation

There are two kinds of biochemical cell separation methods: immunoaffinity-based and cell adhesion-based. The immunoaffinity-based method refers to the use of specific antibodies or aptamers to modify specific cells to achieve cell capture. The principle of cell adhesion method is that the adhesive ability of the target cell can be enhanced by changing the structure of the channel surface, for example, by setting a micro-nano structure on the channel surface. The cell adhesion-based method is mainly used to separate tumor cells, blood cells and plasma in the blood based on the leukocyte margination and Zweifach–Fung effects.

2.3.1 Immunoaffinity-based cell separation

Immunoaffinity is a common biological cell isolation method in which antibodies or artificial aptamers are used as affinity ligands, i.e., some cell types are identified specifically and attached to the device and the others pass through the device. When it comes to the separation of tumor cells, the specificity of such cells is also key to their separation from other cells. The EpCAM antibody is modified on the microchannel

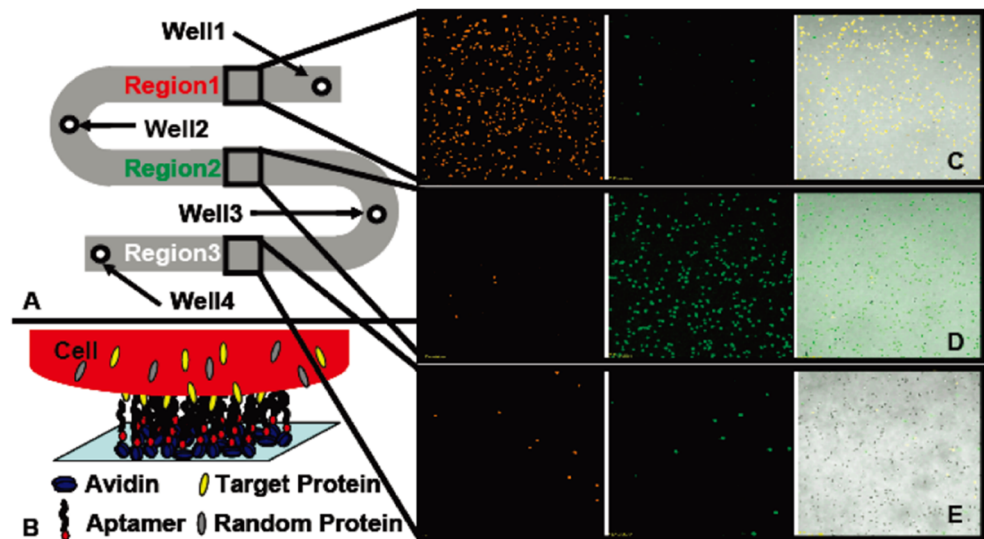
micropores on the chip to specifically identify cancer cells and separate them from the blood (Nagrath et al. 2007). In order to improve the separation efficiency, the research group went a step further by developing a microturbo herringbone chip. The well-designed herringbone chip can increase the interaction between the target cancer cells and the surface of the antibody modified microchannel by generating microvortices, so as to achieve higher cell capture ability in microfluidics.

In addition to traditional antibodies, artificial chemical antibodies with high molecular recognition affinity have been fabricated and named aptamers (Fang and Tan 2010). Aptamers, like antibodies, are effective in capturing targeted tumor cells specifically, and they can bind to microfluidics to efficiently isolate and identify cancer cells (Shen et al. 2013; Wan et al. 2011). The device shown in Fig. 15 is a cell-specific aptamer immobilized in the channel, which can recognize the specific cancer cells corresponding to the fluid and immobilized in the channel to separate cancer cells (Xu et al. 2009). To collect and capture many different tumor cells in the same microfluidic channel, different specific aptamers can be used to modify the channel to identify and collect many different types of tumor cells in a complex sample. In addition, cells can be captured and isolated using a platform approach that synthesizes biologically-inspired 3D networks of multivalent DNA adaptors in microfluidic channels (Zhao et al. 2012). This method leads to more efficient cell capture and higher cell purity. At present, aptamers have shown a strong potential for application, but they still suffer from the limited aptamer library and low cell specific aptamer sorting efficiency, which need to be solved urgently.

2.3.2 Cell adhesion-based cell separation

Cell adhesion-based cell separation is one of the few methods that do not require labeling and do not depend on cell size

Fig. 15 Aptamer affinity-based cell isolation in the microfluidic device. Reproduced with permission from Analytical chemistry 81.17 (2009). Copyright © 2009 American Chemical Society



(Didar and Tabrizian 2010). The separation is achieved by the interaction of the target cell's specific adhesion ability with the surface of the microchannel. Cell adhesion varies with the cell type, shear stress, and fluid flow rate. The adhesive ability of the target cell can be enhanced by changing the structure of the channel surface, for example, by setting a micro-nano structure on the channel surface. The device shown in Fig. 16 separates human breast cancer cells from epithelial cells by placing a micro-nano structure in the microfluidic channel. Various nanostructures (columnar, vertical and parallel) are prepared on the PDMS surface by UV-assisted capillary molding (Kwon et al. 2007). The experiments show that the adhesion strength of MCF10A cells is higher than that of MCF7 cells, which is not affected by the surface structure and culture time of the microchannel, so the cancer cells can be isolated.

Biological cell isolation is mainly used to separate tumor cells. There are a variety of specific receptors on tumor cells, and the combination of specific antibodies or aptamers with the receptors can achieve highly specific cell separation, which greatly improves the purity of separation. However, this approach is limited by antibody and aptamer search. Cell adhesion-based separation methods do not require labeling, but they require high fluid flow and shear stress and are difficult to control, which impedes their development and application.

3 Conclusion and prospective

Separating cells of interest from a heterogeneous mixture which can minimize both the presence and effect of unwanted, background cells, is critical in a variety of biomedical applications including therapeutics, diagnostics and cell biology (Rengan et al. 2015; Huang et al. 2019; Wang et al.

2015; Zhang et al. 2017). Blood, for example, is a highly complex bio-fluid consist of red blood cell, blood platelet, white blood cell, and circulating tumor cells (CTCs) (Bole and Manesiotis 2016; Fachin et al. 2017). Isolation of CTCs from these extremely rich component for accurate analysis are challenging and time-consuming. However, the previous researches have shown that microfluidic technologies with advantages of integration, flow control, ease of fabrication and reduction in the samples needed, provide immeasurable assistance for cellular separation. In this review, the current state-of-the-art microfluidic technologies for cells separation was presented. Based on the working principle, cell separation methods fall into two categories: physical and biological. Both methods were demonstrated through working principles, results and the functions for cells separation. However, it is difficult to rely on a single method for achieving perfect cell separation. And there is an urgent demand for new device for new application and new requirements. Therefore, some new challenges need to be solved in future before the microfluidic-based cell separation can be used outside the lab.

Continuous improvement and optimization of microfluidic chip design are required. For both physical and biochemical approaches, microfluidic chip designs are the basis for realizing the function and application. For example, hydrodynamic-based cell separation depends on the different physical properties of cells that make them behave differently in the laminar flow (Liu et al. 2019; Yin et al. 2019). When fluid velocity and number of cells increases, cell blockage occurs most frequently during cells separation which damages to the cells and affect the experimental results. Thus, exploring and optimizing structure of hydrodynamic-based chip is of significant importance to separate cells. In contrast of physical methods, the throughput of biochemical is lower and limited by the interactions between surface coated with antibody and cells. Therefore, researchers in this field should try best to improve

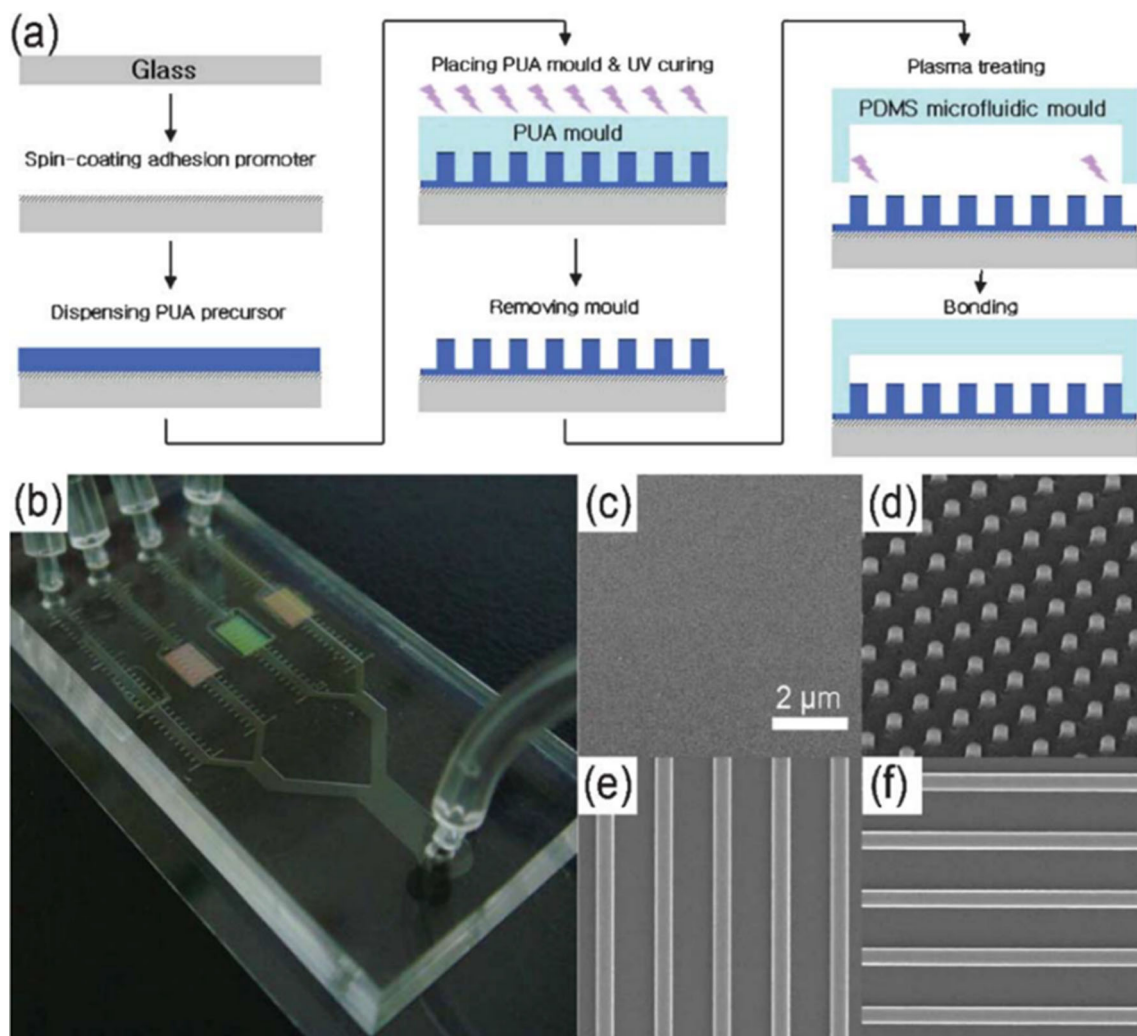


Fig. 16 A scheme of fabrication of microfluidic channels integrated with a nanopatterned substrate. **a** The schematic diagram fabrication steps of microfluidic chip. **b** The image of fabricated device with four branch channels. The SEM image of **c** flat surface, **d** pillars array, **e**

perpendicular lines, **f** parallel lines. Reproduced with permission from Lab on a Chip 7.11 (2007). Copyright © 2007, Royal Society of Chemistry (Great Britain)

the efficiency of interactions between cells and immune-coated surface.

Combination of different technologies is the trend of microfluidic-based cells separation. As we mentioned above, the hydrodynamic-based techniques is label-free, while it is easy to clog and make damage to cells. Magnetic-based and acoustic-based techniques show high specificity and robust. Although DEP-based technique is label-free, it requires preparation of different mediums. And it is generally known that it is difficult to separate cells with similar physical properties for physical label-free techniques. Meanwhile, immunoaffinity-based method could achieve cell capture by using specific antibodies or aptamers to modify specific cells. Thus, combination of physical and biochemical techniques which integrating the respective advantages of different technologies, is an ideal solution to separate cells with high purity and efficiency (Luo et al. 2018). At the same time, the

combination of different techniques with different work mechanisms both increases the complexity of device design and bring together inherent drawbacks. Furthermore, various processes and functions, such as cell mixer, labeling, counting, separation and analysis, may be integrated in a portable and inexpensive microfluidic device for improving efficiency of diagnosis.

In a word, high efficiency, high purity, high throughput and easy to use are required for future microfluidic-based separation of cells. And the microfluidic device should be designed with lower cost, small size and integrated all the necessary complex processes and functions. Furthermore, these developed technologies should solve the biomedical challenges and promote the clinical application. Ultimately, both techniques and device should accelerate the industrialization and commercialization process of microfluidic-base cell separation which will bring mankind plenty of profits.

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References

- Y. Ai, C.K. Sanders, B.L. Marrone, Separation of *Escherichia coli* bacteria from peripheral blood mononuclear cells using standing surface acoustic waves. *Anal. Chem.* **85**, 9126–9134 (2013)
- J. Alvankarian, A. Bahadorimehr, Yeop Majlis, B., A pillar-based microfilter for isolation of white blood cells on elastomeric substrate. *Biomicrofluidics* **7**, 14102 (2013)
- H. Andersson, A. Van Den Berg, Microfluidic devices for cellomics: A review. *Sensors Actuators B Chem.* **92**, 315–325 (2003)
- M. Antfolk, T. Laurell, Continuous flow microfluidic separation and processing of rare cells and bioparticles found in blood – A review. *Anal. Chim. Acta* **965**, 9–35 (2017)
- K. Bacon, A. Lavoie, B.M. Rao, M. Daniele, S. Menegatti, Past, present, and future of affinity-based cell separation technologies. *Acta Biomater.* **112**, 29–51 (2020)
- M. Berger, J. Castelino, R. Huang, M. Shah, R.H. Austin, Design of a microfabricated magnetic cell separator. *Electrophoresis* **22**, 3883–3892 (2001)
- A.A.S. Bhagat, H. Bow, H.W. Hou, S.J. Tan, J. Han, C.T. LIM, Microfluidics for cell separation. *Med. Biol. Eng. Comput.* **48**, 999–1014 (2010)
- A.L. Bole, P. Manesiotis, Advanced materials for the recognition and capture of whole cells and microorganisms. *Adv. Mater.* **28**, 5349–5366 (2016)
- E. Brouzes, M. Medkova, N. Savenelli, D. Marran, M. Twardowski, J.B. Hutchison, J.M. Rothberg, D.R. Link, N. Perrimon, M.L. Samuels, Droplet microfluidic technology for single-cell high-throughput screening. *Proc. Natl. Acad. Sci.* **106**, 14195–14200 (2009)
- Burguillos, M. A., Magnusson, C., Nordin, M., Leshof, A., Augustsson, P., Hansson, M. J., Elm RE, , Lilja, H., Brundin, P., Laurell, T. & Deierborg, T. 2013. Microchannel acoustophoresis does not impact survival or function of microglia, leukocytes or tumor cells. *PLoS One*, **8**, e64233
- J. Chen, J. Li, Y. Sun, Microfluidic approaches for cancer cell detection, characterization, and separation. *Lab Chip* **12**, 1753–1767 (2012)
- I.F. Cheng, W.L. Huang, T.Y. Chen, C.W. Liu, Y.D. Lin, Su, W. C., Antibody-free isolation of rare cancer cells from blood based on 3D lateral dielectrophoresis. *Lab Chip* **15**, 2950–2959 (2015)
- Y. Cheng, X. Ye, Z. Ma, S. Xie, W. Wang, High-throughput and clogging-free microfluidic filtration platform for on-chip cell separation from undiluted whole blood. *Biomicrofluidics* **10**, 014118 (2016)
- P. Chiou, A.T. Ohta, Wu, M. C., Massively parallel manipulation of single cells and microparticles using optical images. *Nature* **436**, 370–372 (2005)
- P.-Y. Chu, C.-J. Liao, C.-H. Hsieh, H.-M. Wang, W.-P. Chou, P.-H. Chen, Wu, M.-H., Utilization of optically induced dielectrophoresis in a microfluidic system for sorting and isolation of cells with varied degree of viability: Demonstration of the sorting and isolation of drug-treated cancer cells with various degrees of anti-cancer drug resistance gene expression. *Sensors Actuators B Chem.* **283**, 621–631 (2019)
- D.J. Collins, Z. Ma, Y. Ai, Highly localized acoustic streaming and size-selective submicrometer particle concentration using high frequency microscale focused acoustic fields. *Anal. Chem.* **88**, 5513–5522 (2016)
- A. Dalili, E. Samiei, M. Hoorfar, A review of sorting, separation and isolation of cells and microbeads for biomedical applications: Microfluidic approaches. **144**, 87–113 (2018)
- E. Delamarque, D. Juncker, H. Schmid, Microfluidics for processing surfaces and miniaturizing biological assays. *Adv. Mater.* **17**, 2911–2933 (2005)
- G. Destgeer, K.H. Lee, J.H. Jung, A. Alazzam, H.J. Sung, Continuous separation of particles in a PDMS microfluidic channel via travelling surface acoustic waves (TSAW). *Lab Chip* **13**, 4210–4216 (2013)
- G. Destgeer, B.H. Ha, J.H. Jung, H.J. Sung, Submicron separation of microspheres via travelling surface acoustic waves. *Lab Chip* **14**, 4665–4672 (2014)
- G. Destgeer, A. Alazzam, H.J. Sung, High frequency travelling surface acoustic waves for microparticle separation. *J. Mech. Sci. Technol.* **30**, 3945–3952 (2016)
- D. Di Carlo, J.F. Edd, D. Irimia, R.G. Tompkins, M. Toner, Equilibrium separation and filtration of particles using differential inertial focusing. *Anal. Chem.* **80**, 2204–2211 (2008)
- T.F. Didar, M. Tabrizian, Adhesion based detection, sorting and enrichment of cells in microfluidic lab-on-Chip devices. *Lab Chip* **10**, 3043–3053 (2010)
- X. Ding, P. Li, S.C. Lin, Z.S. Stratton, N. Nama, F. Guo, D. Slotcavage, X. Mao, J. Shi, F. Costanzo, T.J. Huang, Surface acoustic wave microfluidics. *Lab Chip* **13**, 3626–3649 (2013)
- A. Dolatmoradi, El-Zahab, B., Thermally-assisted ultrasonic separation of giant vesicles. *Lab Chip* **16**, 3449–3453 (2016)
- M. Dürr, J. Kentsch, T. Müller, T. Schnelle, M. Stelzle, Microdevices for manipulation and accumulation of micro- and nanoparticles by dielectrophoresis. *Electrophoresis* **24**, 722–731 (2003)
- j. Dykes, a. Leshof, M.I.B. Åstrand-Grundstr, T. Laurell, S. Scheduling, Efficient removal of platelets from peripheral blood progenitor cell products using a novel micro-chip based acoustophoretic platform. *PLoS One* **6**, e23074 (2011)
- F. Fachin, P. Spuhler, J.M. Martel-Foley, J.F. Edd, T.A. Barber, J. Walsh, M. Karabacak, V. Pai, M. Yu, K. Smith, H. Hwang, J. Yang, S. Shah, R. Yarmush, L.V. Sequist, S.L. Stott, S. Maheswaran, D.A. Haber, R. Kapur, M. Toner, Monolithic chip for high-throughput blood cell depletion to sort rare circulating tumor cells. *Sci. Rep.* **7**, 10936 (2017)
- X. Fang, W. Tan, Aptamers generated from cell-SELEX for molecular medicine: A chemical biology approach. *Acc. Chem. Res.* **43**, 48–57 (2010)
- V.I. Furdul, D.J. Harrison, Immunomagnetic T cell capture from blood for PCR analysis using microfluidic systems. *Lab Chip* **4**, 614–618 (2004)
- P. Grodzinski, J. Yang, R.H. Liu, M.D. Ward, A modular microfluidic system for cell pre-concentration and genetic sample preparation. *Biomed. Microdevices* **5**, 303–310 (2003)
- G.P. Gupta, J. Massagué, Cancer metastasis: Building a framework. *Cell* **127**, 679–695 (2006)
- K.H. Han, A. Han, A.B. Frazier, Microsystems for isolation and electrophysiological analysis of breast cancer cells from blood. *Biosens. Bioelectron.* **21**, 1907–1914 (2006)
- Y. He, B.L. Huang, D.X. Lu, J. Zhao, B.B. Xu, R. Zhang, X.F. Lin, Q.D. Chen, J. Wang, Y.L. Zhang, H.B. Sun, “Overpass” at the junction of a crossed microchannel: An enabler for 3D microfluidic chips. *Lab Chip* **12**, 3866–3869 (2012)
- T. Henighan, A. Chen, G. Vieira, A.J. Hauser, F.Y. Yang, J.J. Chalmers, R. Sooryakumar, Manipulation of magnetically labeled and unlabeled cells with Mobile magnetic traps. *Biophys. J.* **98**, 412–417 (2010)
- J.A. Hernández-Castro, K. Li, A. Meunier, D. Juncker, T. Veres, Fabrication of large-area polymer microfilter membranes and their application for particle and cell enrichment. *Lab Chip* **17**, 1960–1969 (2017)

- K. Hoshino, Y.Y. Huang, N. Lane, M. Huebschman, J.W. Uhr, E.P. Frenkel, X. Zhang, Microchip-based immunomagnetic detection of circulating tumor cells. *Lab Chip* **11**, 3449–3457 (2011)
- Y. Huang, S. Joo, M. Duhon, M. Heller, B. Wallace, Xu, X., Dielectrophoretic cell separation and gene expression profiling on microelectronic chip arrays. *Anal. Chem.* **74**, 3362–3371 (2002)
- L.R. Huang, E.C. Cox, R.H. Austin, J.C. Sturm, Continuous particle separation through deterministic lateral displacement. *Science* **304**, 987–990 (2004)
- R. Huang, T.A. Barber, M.A. Schmidt, R.G. Tompkins, M. Toner, D.W. Bianchi, R. Kapur, W.L. Flejter, A microfluidics approach for the isolation of nucleated red blood cells (NRBCs) from the peripheral blood of pregnant women. *Prenat. Diagn.* **28**, 892–899 (2008)
- S. Huang, M. Wu, Y. Lin, C. Hsieh, C. Yang, H. Lin, C. Tseng, G. Lee, High-purity and label-free isolation of circulating tumor cells (CTCs) in a microfluidic platform by using optically-induced-dielectrophoretic (ODEP) force. *Lab Chip* **13**, 1371–1383 (2013)
- X. Huang, J. Tang, L. Hu, R. Bian, M. Liu, W. Cao, H. Zhang, Arrayed microfluidic chip for detection of circulating tumor cells and evaluation of drug potency. *Anal. Biochem.* **564–565**, 64–71 (2019)
- M.P. Hughes, Strategies for dielectrophoretic separation in laboratory-on-a-chip systems. *Electrophoresis* **23**, 2569–2582 (2002)
- M.P. Hughes, Fifty years of dielectrophoretic cell separation technology. *Biomicrofluidics* **10**, 032801 (2016)
- L. Johansson, F. Nikolajeff, S. Johansson, S. Thorslund, On-chip fluorescence-activated cell sorting by an integrated miniaturized ultrasonic transducer. *Anal. Chem.* **81**, 5188–5196 (2009)
- P.H. Jones, F.M. Watt, Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. *Cell* **73**, 713–724 (1993)
- Y.T. Kang, I. Doh, Y.H. Cho, Tapered-slit membrane filters for high-throughput viable circulating tumor cell isolation. *Biomed. Microdevices* **17**, 45 (2015)
- Y.-T. Kang, I. Doh, J. Byun, H.J. Chang, Y.-H. Cho, Label-free rapid viable enrichment of circulating tumor cell by photosensitive polymer-based microfilter device. *Theranostics* **7**, 3179–3191 (2017)
- T.H. Kim, H.J. Yoon, S. Fouladdel, Y. Wang, M. Kozminsky, M.L. Burness, C. Paoletti, L. Zhao, E. Azizi, M.S. Wicha, S. Nagrath, Characterizing circulating tumor cells isolated from metastatic breast cancer patients using graphene oxide based microfluidic assay. *Adv. Biosyst.* **3**, 1800278 (2019)
- M. Kumar, D.L. Feke, J.M. Belovich, Fractionation of cell mixtures using acoustic and laminar flow fields. *Biotechnol. Bioeng.* **89**, 129–137 (2005)
- T. Kumo, Y. Tomizawa, M. Kita, H. Takabayashi, Y. Takamura, Concentration and extraction chip of fetal nucleated red blood cells (NRBCs) by micro gap with diaphragm for fetal DNA diagnosis from maternal blood. *Microsyst. Technol.* **23**, 5351–5355 (2017)
- S.S. Kuntaegowdanahalli, A.A. Bhagat, G. Kumar, I. Papautsky, Inertial microfluidics for continuous particle separation in spiral microchannels. *Lab Chip* **9**, 2973–2980 (2009)
- B. Kwak, J. Lee, J. Lee, H.S. Kim, S. Kang, Y. Lee, Spiral shape microfluidic channel for selective isolating of heterogenic circulating tumor cells. *Biosens. Bioelectron.* **101**, 311–316 (2018)
- K.W. Kwon, S.S. Choi, S.H. Lee, B. Kim, S.N. Lee, M.C. Park, P. Kim, S.Y. Hwang, K.Y. Suh, Label-free, microfluidic separation and enrichment of human breast cancer cells by adhesion difference. *Lab Chip* **7**, 1461–1468 (2007)
- T. Laurell, F. Petersson, A. Nilsson, Chip integrated strategies for acoustic separation and manipulation of cells and particles. *Chem. Soc. Rev.* **36**, 492–506 (2007)
- H. Lee, A.M. Purdon, R.M. Westervelt, Manipulation of biological cells using a microelectromagnet matrix. *Appl. Phys. Lett.* **85**, 1063–1065 (2004)
- A. Lenshof, C. Magnusson, T. Laurell, Acoustofluidics 8: Applications of acoustophoresis in continuous flow microsystems. *Lab Chip* **12**, 1210–1223 (2012)
- X. Li, W. Chen, G. Liu, W. Lu, J. Fu, Continuous-flow microfluidic blood cell sorting for unprocessed whole blood using surface-micromachined microfiltration membranes. *Lab Chip* **14**, 2565–2575 (2014)
- P. Li, Z. Mao, Z. Peng, L. Zhou, Y. Chen, P.-H. Huang, C.I. Truica, J.J. Drabick, W.S. El-Deiry, M. Dao, S. Suresh, T.J. Huang, Acoustic separation of circulating tumor cells. *Proc. Natl. Acad. Sci.* **112**, 4970–4975 (2015)
- C.J. Liao, C.H. Hsieh, T.K. Chiu, Y.X. Zhu, H.M. Wang, F.C. Hung, W.P. Chou, Wu, M. H., An optically induced dielectrophoresis (ODEP)-based microfluidic system for the isolation of high-purity CD45(neg)/EpCAM(neg) cells from the blood samples of Cancer patients-demonstration and initial exploration of the clinical significance of these cells. *Micromachines (Basel)* **9** (2018)
- S.H. Ling, Y.C. Lam, K.S. Chian, Continuous cell separation using dielectrophoresis through asymmetric and periodic microelectrode array. *Anal. Chem.* **84**, 6463–6470 (2012)
- G. Liu, F. He, X. Li, H. Zhao, Y. Zhang, Z. Li, Z. Yang, Multi-level separation of particles using acoustic radiation force and hydraulic force in a microfluidic chip. *Microfluid. Nanofluid.* **23**, 23 (2019)
- T. Luo, L. Fan, Y. Zeng, Y. Liu, S. Chen, Q. Tan, R.H.W. Lam, D. Sun, A simplified sheathless cell separation approach using combined gravitational-sedimentation-based prefocusing and dielectrophoretic separation. *Lab Chip* **18**, 1521–1532 (2018)
- Z. Ma, D.J. Collins, J. Guo, Y. Ai, Mechanical properties based particle separation via traveling surface acoustic wave. *Anal. Chem.* **88**, 11844–11851 (2016)
- O.A. Martin, R.L. Anderson, K. Narayan, M.P. Macmanus, Does the mobilization of circulating tumour cells during cancer therapy cause metastasis? *Nat. Rev. Clin. Oncol.* **14**, 32–44 (2017)
- J. McGrath, M. Jimenez, H. Bridle, Deterministic lateral displacement for particle separation: A review. *Lab Chip* **14**, 4139–4158 (2014)
- H.S. Moon, K. Kwon, S.I. Kim, H. Han, J. Sohn, S. Lee, H.I. Jung, Continuous separation of breast cancer cells from blood samples using multi-orifice flow fractionation (MOFF) and dielectrophoresis (DEP). *Lab Chip* **11**, 1118–1125 (2011)
- J.H. Myung, C.A. Launiere, D.T. Eddington, S. Hong, Enhanced tumor cell isolation by a biomimetic combination of E-selectin and anti-EpCAM: Implications for the effective separation of circulating tumor cells (CTCs). *Langmuir* **26**, 8589–8596 (2010)
- K. Nagase, R. Shukuwa, H. Takahashi, N. Takeda, T. Okano, Enhanced mechanical properties and cell separation with thermal control of PIPAAm-brushed polymer-blend microfibers. *J. Mater. Chem. B* **8**, 6017–6026 (2020)
- S. Nagrath, L.V. Sequist, S. Maheswaran, D.W. Bell, D. Irimia, L. Ulkus, M.R. Smith, E.L. Kwak, S. Digumarthy, A. Muzikansky, P. Ryan, U.J. Balis, R.G. Tompkins, D.A. Haber, M. Toner, Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature* **450**, 1235–1239 (2007)
- J. Nam, Y. LEE, S. Shin, Size-dependent microparticles separation through standing surface acoustic waves. *Microfluid. Nanofluid.* **11**, 317–326 (2011)
- B. Ngamsom, M.M. Esfahani, C. Phurimsak, M.J. Lopez-Martinez, J.C. Raymond, P. Broyer, P. Patel, N. Pamme, Multiplex sorting of foodborne pathogens by on-chip free-flow magnetophoresis. *Anal. Chim. Acta* **918**, 69–76 (2016)
- j. Oakey, j. Allely, D.W.M. Marr, Laminar-flow-based separations at the microscale. *Biotechnol. Prog.* **18**, 1439–1442 (2002)
- N. Pamme, A. Manz, On-chip free-flow magnetophoresis: Continuous flow separation of magnetic particles and agglomerates. *Anal. Chem.* **76**, 7250–7256 (2004)
- N. Pamme, C. Wilhelm, Continuous sorting of magnetic cells via on-chip free-flow magnetophoresis. *Lab Chip* **6**, 974–980 (2006)

- J.S. Park, S.H. Song, H.I. Jung, Continuous focusing of microparticles using inertial lift force and vorticity via multi-orifice microfluidic channels. *Lab Chip* **9**, 939–948 (2009)
- K. Park, J. Park, J.H. Jung, G. Destgeer, H. Ahmed, H.J. Sung, In-droplet microparticle separation using travelling surface acoustic wave. *Biomicrofluidics* **11**, 064112 (2017)
- A.K. Rengan, A.B. Bukhari, A. Pradhan, R. Malhotra, R. Banerjee, R. Srivastava, De, A., *In vivo* analysis of biodegradable liposome gold nanoparticles as efficient agents for photothermal therapy of cancer. *Nano Lett.* **15**, 842–848 (2015)
- H. Safarpour, S. Dehghani, R. Nosrati, N. Zebardast, M. Alibolandi, A. Mokhtarzadeh, M. Ramezani, Optical and electrochemical-based nano-aptasensing approaches for the detection of circulating tumor cells (CTCs). *Biosens. Bioelectron.* **148**, 111833 (2020)
- Q. Shen, L. Xu, L. Zhao, D. Wu, Y. Fan, Y. Zhou, W.H. Ouyang, X. Xu, Z. Zhang, M. Song, T. Lee, M.A. Garcia, B. Xiong, S. Hou, H.R. Tseng, X. Fang, Specific capture and release of circulating tumor cells using aptamer-modified nanosubstrates. *Adv. Mater.* **25**, 2368–2373 (2013)
- S. Shen, C. Ma, L. Zhao, Y. Wang, J.C. Wang, J. Xu, T. Li, L. Pang, J. Wang, High-throughput rare cell separation from blood samples using steric hindrance and inertial microfluidics. *Lab Chip* **14**, 2525–2538 (2014)
- J. Shi, H. Huang, Z. Stratton, Y. Huang, T.J. Huang, Continuous particle separation in a microfluidic channel via standing surface acoustic waves (SSAW). *Lab Chip* **9**, 3354–3359 (2009)
- S.L. Stott, C.H. Hsu, D.I. Tsukrov, M. Yu, D.T. Miyamoto, B.A. Waltman, S.M. Rothenberg, A.M. Shah, M.E. Smas, G.K. Korir, F.P. Floyd Jr., A.J. Gilman, J.B. Lord, D. Winokur, S. Springer, D. Irimia, S. Nagrath, L.V. Sequist, R.J. Lee, K.J. Isselbacher, S. Maheswaran, D.A. Haber, M. Toner, Isolation of circulating tumor cells using a microvortex-generating herringbone-chip. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 18392–18397 (2010)
- F. Tanaka, K. Yoneda, N. Kondo, M. Hashimoto, T. Takuwa, S. Matsumoto, Y. Okumura, S. Rahman, N. Tsubota, T. Tsujimura, K. Kuribayashi, K. Fukuoka, T. Nakano, S. Hasegawa, Circulating tumor cell as a diagnostic marker in primary lung cancer. *Clin. Cancer Res.* **15**, 6980–6986 (2009)
- M.D. Tarn, N. Pamme, On-Chip magnetic particle-based immunoassays using multilaminar flow for clinical diagnostics. *Methods Mol. Biol.* **1547**, 69–83 (2017)
- T. Tayoun, V. Faugeroux, M. Oulhen, A. Aberlenc, P. Pawlikowska, F. Farace, CTC-derived models: A window into the seeding capacity of circulating tumor cells (CTCs). *Cells* **8**, 1145 (2019)
- W.L. Ung, K. Mutaopulos, P. Spink, R.W. Rambach, T. Franke, D.A. Weitz, Enhanced surface acoustic wave cell sorting by 3D microfluidic-chip design. *Lab Chip* **17**, 4059–4069 (2017)
- A.L. Vig, A. Kristensen, Separation enhancement in pinched flow fractionation. *Appl. Phys. Lett.* **93**, 203507 (2008)
- M. Vojtisek, M.D. Tarn, N. Hirota, N. Pamme, Microfluidic devices in superconducting magnets: On-chip free-flow diamagnetophoresis of polymer particles and bubbles. *Microfluid. Nanofluid.* **13**, 625–635 (2012)
- Y. Wan, J. Tan, W. Asghar, Y.T. Kim, Y. Liu, S.M. Iqbal, Velocity effect on aptamer-based circulating tumor cell isolation in microfluidic devices. *J. Phys. Chem. B* **115**, 13891–13896 (2011)
- Z. Wang, J. Zhe, Recent advances in particle and droplet manipulation for lab-on-a-chip devices based on surface acoustic waves. *Lab Chip* **11**, 1280–1285 (2011)
- M. Wang, Y. Zeng, X. Liang, X. Lu, G. Feng, D. Han, G. Yang, Full-field optical micro-angiography. *Appl. Phys. Lett.* **104**, 053704 (2014)
- J. Wang, W. Lu, C. Tang, Y. Liu, J. Sun, X. Mu, L. Zhang, B. Dai, X. Li, H. Zhuo, X. Jiang, Label-free isolation and mRNA detection of circulating tumor cells from patients with metastatic lung Cancer for disease diagnosis and monitoring therapeutic efficacy. *Anal. Chem.* **87**, 11893–11900 (2015)
- K. Wang, W. Zhou, Z. Lin, F. Cai, F. Li, J. Wu, L. Meng, L. Niu, H. Zheng, Sorting of tumour cells in a microfluidic device by multi-stage surface acoustic waves. *Sensors Actuators B Chem.* **258**, 1174–1183 (2018)
- X. Wei, B. Cai, K. Chen, L. Cheng, Y. Zhu, Z. Wang, Y. Sun, W. Liu, S.-S. Guo, Y. Zhang, X.-Z. Zhao, Enhanced isolation and release of fetal nucleated red blood cells using multifunctional nanoparticle-based microfluidic device for non-invasive prenatal diagnostics. *Sensors Actuators B Chem.* **281**, 131–138 (2019)
- Z. Wu, B. Willing, J. Bjerketorp, J.K. Jansson, K. Hjort, Soft inertial microfluidics for high throughput separation of bacteria from human blood cells. *Lab Chip* **9**, 1193–1199 (2009)
- C. Wyatt Shields Iv, C.D. Reyes, G.P. López, Microfluidic cell sorting: A review of the advances in the separation of cells from debulking to rare cell isolation. *Lab Chip* **15**, 1230–1249 (2015)
- N. xiang, J. Wang, Q. Li, Y. Han, D. Huang, Z. Ni, Precise size-based cell separation via the coupling of inertial microfluidics and deterministic lateral displacement. *Anal. Chem.* **91**, 10328–10334 (2019)
- Y. Xu, J.A. Phillips, J. Yan, Q. Li, Z.H. Fan, W. Tan, Aptamer-based microfluidic device for enrichment, sorting, and detection of multiple cancer cells. *Anal. Chem.* **81**, 7436–7442 (2009)
- Y. Yagmur, E. Nurdan, I. Monsur, M.D. Rodrigo, E. Meltem, Dielectrophoretic separation of live and dead monocytes using 3D carbon-electrodes. *Sensors* **17**, 2691 (2017)
- M. Yamada, M. Seki, Hydrodynamic filtration for on-chip particle concentration and classification utilizing microfluidics. *Lab Chip* **5**, 1233–1239 (2005)
- M. Yamada, M. Seki, Microfluidic particle sorter employing flow splitting and recombining. *Anal. Chem.* **78**, 1357–1362 (2006)
- M. Yamada, M. Nakashima, M. Seki, Pinched flow fractionation: Continuous size separation of particles utilizing a laminar flow profile in a pinched microchannel. *Anal. Chem.* **76**, 5465–5471 (2004)
- M. Yamada, K. Kano, Y. Tsuda, J. Kobayashi, M. Yamato, M. Seki, T. Okano, Microfluidic devices for size-dependent separation of liver cells. *Biomed. Microdevices* **9**, 637–645 (2007)
- S. Yan, J. Zhang, D. Yuan, Li, W., Hybrid microfluidics combined with active and passive approaches for continuous cell separation. *Electrophoresis* **38**, 238–249 (2017)
- A.H. Yang, H.T. Soh, Acoustophoretic sorting of viable mammalian cells in a microfluidic device. *Anal. Chem.* **84**, 10756–10762 (2012)
- D. Yin, X. Zhang, X. Han, J. Yang, Hu, N., Multi-Stage Particle Separation based on Microstructure Filtration and Dielectrophoresis., 10 (2019)
- Y. Zhang, W. Li, Y. Zhou, A. Johnson, A. Venable, A. Hassan, J. Griswold, D. Pappas, Detection of sepsis in patient blood samples using CD64 expression in a microfluidic cell separation device. *Analyst* **143**, 241–249 (2017)
- W. Zhao, C.H. Cui, S. Bose, D. Guo, C. Shen, W.P. Wong, K. Halvorsen, O.C. Farokhzad, G.S. Teo, J.A. Phillips, D.M. Dorfman, R. Kamik, J.M. Karp, Bioinspired multivalent DNA network for capture and release of cells. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 19626–19631 (2012)
- S. Zheng, H. Lin, J.Q. Liu, M. Balic, R. Datar, R.J. Cote, Y.C. Tai, Membrane microfilter device for selective capture, electrolysis and genomic analysis of human circulating tumor cells. *J. Chromatogr. A* **1162**, 154–161 (2007)

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