Microfluidic System for Cell Sorting

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Abstract. The need for efficient cell sorting, which is a useful technique in medical and biological research, has boosted the development of microfluidic cell sorter. This review paper concludes the basic principle of microfluidic sorter which covers active and passive sorting techniques. The work process, sorting efficiency, advantages, disadvantages, and avenue for improvement are explored for each type by introducing several typical sorters. The industrial implementation and potential development are also conveyed. In conclusion, developing efficient microfluidic cell sorter offers a greater control over cell distribution and is fundamental in realizing efficacious cell sorting systems.

1. Introduction

Cell sorting, a crucial technology that separating cells from complex fluid environment, is widely used in a variety of medical and biological studies [1]. For instance, capturing the heterogeneity of cell types within their tissue environment is fundamental to understand the physiology and pathology of disease and is of great importance to assure the efficacy and safety of cell-based therapy [1,2]. With the quantities of molecular species that need to be tracked continuing to increase in the cell biology studies, individual cells are more and more valued as the basic in our biological understanding [3]. In order to reduce time, improve cost-efficiency, and overcome difficulties in some procedures that are impossible to be conducted by macroscopic devices during the sorting process, microfluidic cell sorter is developed. Since 1990s, lots of studies have been conducted to improve the mechanism, broaden the applications, and promote the efficiency of the sorter. By using fluorescent-activated cell sorting, which was an important instrument for measuring the fluorescence and light scattering of microscopic particles and biological cells and separating them based on their size and fluorescence color, Sun et al. developed a microfluidic cell sorting chip with micromachining technology, where electroosmotic flow (EOF) was exploited to drive and switch cells [4]. Chen et al. demonstrated a low-power and low-voltage on-chip piezoelectrically actuated micro-sorter that could deflect single particle or cell at high-speed [5]. Weigl et al. integrated passively driven pumping systems, a kind of self-contained power source, and microfluidic channels onto a simple, disposable chip to make the microfluidic system more integrated as well as more practical to manufacture and implement [6]. Based on this, Cho et al. developed a micro-scale integrated system using related concept using related concept to classify motile sperm from non-
motile cells powered by gravity [6,7]. Chen et al. described the design, simulation, manufacturing, and experimental verification of an off-chip-controlled microfluidic switch for cell and embryo sorting, which greatly broadened the applications [8]. Wu et al. developed a new method to separate bacteria from human blood cells based on soft inertial force. Inside this microfluidic device, migration with flow fixed curved and focused sample flow are induced [9]. Pritchard et al. reported ‘vortex-actuated cell sorting’ (VACS), a new technique that deflected cells individually via the generation of transient microfluidic vortex by thermal vapor bubble. This was a novel mechanism, which could sort cells based on fluorescently labelled molecular markers [10].

In this review, the basic principles of the microfluidic sorter are explored by categorizing their basic technology and principles into two types including active sorting techniques and passive sorting techniques. In each type of techniques, some representative examples are chosen to help review how these techniques facilitate the microfluidic sorter to improve its efficiency and sorting accuracy. In addition, some of the opportunities and challenges for both fundamental research and implementation of microfluidic sorter are put forward. For instance, microfluidic devices show great potential for applications in the food industry because it ensures the reduction of analysis costs and the amount of waste, enhances mass and heat transfer, and improves analytical performances etc. [11]. Finally, our thoughts on the development of microfluidic sorter in the near future are conveyed.

2. Active sorting techniques

Generally speaking, active sorting techniques can be classified into the following sorting ways [3], which are fluorescence-activated cell sorting (FACS) [12-14], electrophoretic sorting [15,16], magnetic sorting [17,18], optical sorting [19], acoustic sorting [20] etc. Here we choose two specific kinds of sorters to explain the mechanism and development of active sorting techniques.

2.1. Piezoelectric sorter

The sorter, called piezoelectric zirconate titanate (PZT), is an actuator controlled by voltage shown in Fig. 1. The bending direction and degree of the piezoelectric actuator are controlled by the polarity and magnitude of the input voltage. The piezoelectric film of the device is made of lead zirconate titanate.

Under the condition of providing a certain fluid volume displacement, bending rate, and the target particles entering the separation junction, the bending actuator of piezoelectric ceramics will temporarily interfere with the flow of the fluid to the left or right, causing the particles to deviate to different channels. Then, the sorter will distinguish the harmful particles from the target particles and finally, discharge the fluid. The diameter of the piezoelectric actuator is 20mm, which is easy to carry [5].

Fig. 1 Structure of PZT sorter [5]
The device is used to suspend the prepared E. coli cells in the solution for low-speed separation, apply a certain voltage and frequency (20 Hz, 3 Vp-p activations), and record the separation speed. The performance of the cell deflection device was characterized by frame-by-frame images. The flow rate is ranged from 2 μL/min to 18 μL/min, which can improve the sorting throughput. At the highest total flow rate (18 μL/min), the flow with cell propagation speed being 6 cm/s in the corresponding central flow makes the E. coli cells passing through the sorting junction.

Compared to hybrid systems, the integrated approach offers several advantages. First, the PZT-actuated sorting module is low-voltage and consumes minimum power. Second, precise control of the magnitude of transverse cell and particle deflection to enable single particle/cell sorting. Third, the PZT sorter has an intrinsically much faster response than conventional check or membrane valves and low timing jitter.

It is found that the sorting efficiency of the device is less than 100%, and the classification error caused by the flow interference is not ideal. To improve the result more effectively, we can improve the limitation of traffic and timing jitter.

2.2. Electroosmotic microfluidic cell sorting chip with switching structures

The electroosmotic microfluidic cell sorting chip is shown in Fig. 2. The chip consists of two optical systems: cell operation and cell detection. The working principle of cell sorting chip: For the blood cells prepared outside the chip, the target cells are labeled with fluorescent markers. These labeled and unlabeled cell mixtures are transported through micro-channels via electroosmotic flow (EOF), which is controlled by platinum electrodes in the input and output containers [4].

The chip has a branch channel system for focusing the sample solution and narrowing the cells so that they can be arranged and moved forward gradually. In the detection area, the light source comes from a laser diode and is integrated into the chip to excite fluorescence. Identify and count targeted cells by fluorescence reaction. In the subsequent switching, electroosmotic conversion can be used to divide the cell into two different output channels.

In the application of cell classification, in order to facilitate the classification of two different types of cells, the switch structure is designed. The switch structure guides the sample to the appropriate outlet after focusing and optical detection. Two parameters, switching speed and switching accuracy, are introduced to evaluate the performance of the structure. Switching speed refers to the speed of fluid switching from one direction to another. The switching accuracy indicates that the targeted cells can be successfully manipulated into a predetermined memory [4].

For the switch structure design, Y-junction switch structures with different angles (and switch angles) are simulated and tested, and all dimensions are the same as those shown in Fig. 3. The inlet potential is kept constant at 100 V and the voltage at the collection and waste storage tanks is switched between 0 V and floating state. H₂O is used as a buffer. The PIV system measures the flow rate and leakage distance.
As shown in Table 1, the flow rates with switching angles of $\theta = 90^\circ$ and $\theta = 180^\circ$ are presented. According to the data, the velocity is greater than that of $\theta = 90^\circ$ in the waste liquid channel. It means that the leakage distance is larger. When the switch angle decreases, the switch precision is lower and the switch time is shorter. The design of the switch structure depends on the application to a great extent. When accuracy is the main factor, the large switch angle is more appropriate. If throughput is more important, it is good to switch from a smaller angle. According to the accuracy and throughput parameters, it is appropriate to use a Y-junction switch structure with switch angle.

The direction of electroosmotic flow can be adjusted by an electric switch. When the fluorescence threshold of cells exceeds the preset value, active sorting occurs. Since the EOF will respond to the direction of the applied electric field immediately, the switching time can be significantly shortened, and the EOF has high efficiency. Theoretically, when the cell is moved to the collection container, the electroosmotic flow is completely blocked. But in fact, due to the wrong switching, it will lead to the leakage of fluid, resulting in a waste of cost.

### Table 1. The flow velocities of the Y-junction structures

| Y-junction switching structure | Inlet speed ($\mu m/s$) | Collection channel speed ($\mu m/s$) | Waste channel speed ($\mu m/s$) |
|-------------------------------|-------------------------|-------------------------------------|-------------------------------|
| $\theta = 90^\circ$           | 257                     | 229                                 | 21.8                          |
| $\theta = 180^\circ$          | 265.1                   | 254.7                               | 9.6                           |

Passive sorting techniques can be categorized into the following types [3], pillar and weir structures [21], pinched flow fractionation [22], hydrodynamic filtration [23], inertial forces [24-27], affinity-based separation [28], biomimetic separation [29] etc. Three specific kinds of sorters are presented here to explain the mechanism and development of passive sorting techniques.

#### 3.1. A pressure-driven microfluidic sorter designed for drosophila embryos

A pressure-driven microfluidic sorter was introduced in paper [8]. The sorter structure is shown in Fig.4. Because of the different pressure of two control inlets and two outlets, the main flow is generated and cells are manipulated following the flow due to the drag forces. In microchannels, the laminar flow is needed to achieve higher accuracy, while unwanted turbulence will weaken the ability to control the flow.
Fig. 4 Illustration of the mechanism of the pressure-driven sorter [8]

The switching time is measured from the instant that the pressure changes in control inlets to the instant that the main flow switches totally from one outlet to the other [8]. In the paper [8], the authors simulated and analysed the effects of the control pressure, chamber length, and entrance length on the switching time of fluid between different micro-channels. Parameters are listed in Table 2.

Table 2. The switching time ($T_{SW}$) study

| Case              | $P$(Pa) | $L_c$(μm) | $L_e$(μm) | $T_{SW}$(μs) |
|-------------------|---------|-----------|-----------|--------------|
| Control pressure  | 9000    | 1000      | 1500      | 796          |
|                   | 10000   |           |           | 741          |
|                   | 20000   |           |           | 513          |
|                   | 40000   |           |           | 360          |
| Chamber length    | 20000   | 250       | 1500      | 374          |
|                   |         | 500       |           | 357          |
|                   |         | 1000      |           | 506          |
|                   |         | 1500      |           | 650          |
| Entrance length   | 20000   | 1000      | 1500      | 506          |
|                   |         |           | 2000      | 562          |
|                   |         |           | 3000      | 673          |
|                   |         |           | 4500      | 840          |

From Table 2, we can easily find the switching time drops from 796 to 360 us non-linearly when the control pressure is increased from 9000 to 40,000 Pa and increases linearly from 360 to 650 us with increasing chamber length from 500 to 1500 um. However, the switching time increases slightly when chamber length is reduced to 250 um because the switch fork is too close to the convergence area of the three inlets. Besides, for entrance lengths from 1500 to 4500 um, the delta of switching time $\Delta T$ increases linearly with increasing entrance length $L_e$ and the relationship can be represented by $\Delta T = 0.1114\Delta L_e$.

The type of device has a promising prospect for a microfluidic switch for embryo and cell sorting, which provides higher accurate automated operations in micro-manipulating of biological cells. However, the switching time of the microfluidic sorter is limited by the response time of the electromagnetic three-way valve used to control the pressure.
3.2. Vortex-actuated cell sorting

A novel mechanism called ‘vortex-actuated cell sorting’ (VACS) is developed by Pritchard, R. H. et al., which is applied to sort particles individually through a microfluidic vortex generated by the interaction of a thermal ink-jet (TIJ)-style actuator with the channel geometry [10]. In the prior arts, the inertial vortex has been applied in microfluidic sorters to separate cells in different paths based on their physical properties. Compared to the prior art, instead of persisting at certain positions, in VACS, the vortex is transient and will drop and flow downstream. Therefore, the VACS is able to sorter particles based on the fluorescently-labelled molecular markers.

The mechanism of vortex-actuated cell sorting is shown in Fig. 5(a). This device incorporates a TIJ-style actuator to generate the micro-bubble. By designing a sharp edge feature in the micro-channels of ~100μm dimension, the bubble collapse and transient flows generate, leading to particles deflection caused by partial inertial vortex in the flow.

Fig. 5(b) shows the VACS device. The microheater is a sputtered layer of titanium on a borosilicate glass substrate, while the conductor tracks are gold on a titanium adhesion layer. The micro-channels are cast in a layer of PDMS, bonded to the glass substrate by plasma surface treatment [10].

‘Sort envelope rate’, the maximum rate to sort the individual cell, of the device is 43 kHZ. And two other rates are defined by authors, the ‘peak sort rate’, representing the inverse of the minimum repeat time of actuation, and the ‘sustained sort rate’, showing the average rate to continuously manipulate the device. The ‘peak sort rate’ is up to 10 kHZ without significant degradation or recovery and ‘sustained sort rate’ is lower than ‘peak sort rate’ due to the limit of the micro-resistor. In the paper [10], ‘sustained sort rate’ is limited to 1-2 kHZ.

The innovation of creating the transient inertial vertex contributes to high-purity and high-recovery with an input rate of up to $10^4$ cells per second. Also, the device has a promising prospect to achieve practical processing of millions or billions of cells in a batch by a high density of parallelization on-chip. In addition, with the advantage of the comparatively simple sorting mechanism and the usage of bio-compatible materials on device construction, VACS is suited to design low-cost disposable devices in a variety of research and applications.

However, the sustained actuation rate and a lifetime of the micro-resistor limit the electrical current devices. High sustained actuation rates tend to cause thermal or mechanical damage on the micro-resistor, probably due to the high stress or temperature in the electron device. The analogy with the TIJ printing, to solve these problems, adding thin-film passivation and anti-cavitation layers on the current devices to prevent these failures matters. These layers can increase the lifetime under high sustained actuation rates. Also, errors in particle focusing may have a negative influence on the output purity from the current devices.
3.3. Soft inertial microfluidics sorter

When people send fluid with particles into a channel. For some geometric configurations like sudden turn and expansion in the channel, the particles and fluid will lose momentum and get inertial force due to the wall restriction. The inertial effects between particles and fluid are different. If the inertial effect for particles is big enough, the particle will be deflected from the fluid streamline and leave the original fluid finally. Based on this observation, a microfluidics sorter was developed in [9].

The structure of the microfluidic device is shown in Fig.6. The sample fluid will go into a small channel under the external pressure from the stronger acting flow. Because the velocity of the sample fluid at the beginning doesn’t match with the velocity of sample flow that is under the effect of acting flow, sample fluid will lose momentum and accelerate near the entrance of a smaller channel. As shown in figure 6, the particle in the sample fluid experience two main phases of momentum loss. From point 1 to 2, the change in momentum is toward the wall. From point 2 to 3, the direction of change in momentum is far away from the original flow axis. Some particles will leave the original fluid due to the acceleration caused by the change of momentum [9]. After that, the different flows will flow parallel to each other in the mid-channel and go into the container because they are laminar flow.

As shown in Table 3, different flow rates are tested for bacterial cells and blood cells. According to the data, we can see the purity of bacterial cells is still high underflow rate of 18μL/min. The whole system is effective.

| Flow rate(μL/min) | 2  | 5  | 10 | 15 | 18 |
|------------------|----|----|----|----|----|
| Purity (%)       | 99.88 | 99.78 | 99.86 | 99.87 | 99.71 |
| Throughput (cells/s) | 7653 | 19133 | 38267 | 57400 | 68880 |

The soft inertial microfluidics device has several advantages. Firstly, the whole device can be fabricated in polymer, which makes it easy to get at a low cost. Secondly, the device can effectively separate bacteria samples from blood cells. These samples are sufficient for the study on bacteria. Thirdly, the soft inertial makes it possible to further study other microorganisms in blood at the molecular level [9].
This device still has some issues. For some bacterial cells that are close to the size of red blood cell, this method will not work. And this device cannot get high purity of bacterial, large number of bacteria collected and a high bacterial cell recovery at the same time [9].

4. Conclusion

In this paper, several advanced microfluidic cell separation technologies are reviewed and categorized as active or passive ones according to their separation principles. The development and application of microfluidic cell sorter technology have significant impacts in biology, medicine, and clinical science. The use of microfluidic sorters greatly reduces the requirements for samples and reagents, resulting in less pollution due to the retrieved material. Moreover, the decrease of processing time in the sorters contributes to less waiting time before receiving the result due to the micro geometry. Additionally, a self-sufficient and automatic system can effectively prevent pollution and decrease the influence of human factors to achieve higher accuracy. And the most important point is that the portability facilitates the use of the devices in areas with serious shortage of technicians.

In conclusion, with the development of nanotechnology and other advanced technologies, we expect that the microfluidic sorters will become more integrated, portable, and functional to meet the demand of high accuracy and low cost simultaneously. Meanwhile, the application and implementation of the microfluidic sorters keep strong connections with projects and studies in other disciplines. For instance, these devices represent great potential in water pollution treatment to detect the heavy metal pollutant by continuous detection, due to their fast reaction speed, efficient working performance, and economy. However, the lifetime of the sorters is still limited by the materials, structure, electrical current devices or other practical factors and there are still a lot of applications to be explored.

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