Technical Paper on Microfluidic Devices — Cell Separation Technology

Joo H. Kang and Je-Kyun Park

1. Introduction

The cell, the basic functional unit of all organisms, has been considered as a key to the solution of human diseases and environmental problems because it contains a lot of information to be disclosed. In many biological and its related experiments, the work of handling a cell is an important initial step to have further experimental results. However, the handling of a cell still requires a skillful hand in spite of modern laboratory techniques[1]. Therefore, the challenge today is to attain higher performance and lower cost of cell manipulation systems, and to find out a novel principle to manipulate cells of interest.

Among cell manipulation techniques, the techniques for cell separation and isolation with high specificity are limiting the rapid growth of cell biology because cell populations are frequently heterogeneous and the cells of interest are suspended in a solution or mixed with different types of chemicals, biomolecules, and cells. For several decades, various technologies have emerged and have been developed for cell separation. Even in current research, novel trials are attempted by the use of not only microfabrication techniques but also conventional tools. Compared with traditional separation methods, microfabricated devices have small working volume and subsequently reduced throughput. Furthermore, crude sample solution containing cells should be diluted to avoid the clogging of the microfluidic channels, which results in greater decrease in separation throughput. Despite these disadvantages, microfabrication-assisted cell separators have considerable potential to overcome current limitations because the microfluidic devices provide unique functions and capability to separate cells in a sensitive manner such as the enhanced dielectrophoresis, magnetophoretic performance, and particular hydrodynamic circumstance.

Microfluidics paved the way for micro total analysis systems (μTAS) and lab-on-a-chip (LOC) which are one of the most promising technologies to accelerate the progress of the present research for biology, chemistry, and bioengineering.[2,3] On the account of its inherent benefit, a microfluidic device such as a μTAS device and a LOC device can provide an automated, reliable and efficient system for cell preparation with respect to current sample preparation procedures. Although the cell manipulation methods have been reported and widely used for several decades, cell treating works are still tedious and time-consuming procedures. In addition, the types of cells that have been separated in microfluidic devices are mainly restricted to a few species such as red blood cell (RBC), white blood cell (WBC) and *Escherichia coli (E. coli)* (Table 1). Therefore, microfluidic cell separation technology is one of the essential research topics in a μTAS or LOC field[4].

Table 1. Separated cell types in microfluidic devices according to each separation principle.

Authors	Cell Type	Separation Principle	Year	Reference No.
A.Y. Fu et. al	*E. coli*	Fluorescence-activated cell sorting	1999	[8]
A.Y. Fu et. al	*E. coli*		2002	[9]
P.C. Li et. al	*Saccharomyces cerevisiae*, *canine erythrocyte*, and *E. coli*		1997	[12]
K. Takahashi et. al	COS cell		2004	[14]
M.A. McClain et. al	*E. coli*		2001	[15]
K.-H. Han et. al	RBC and WBC	Magnetic-activated cell sorting	2004/2005	[35,36]
M. Berger et. al	WBC		2001	[34]
W.C. Chang et. al	HL-60/U-937	Affinity-based cell separation	2005	[40]
J. Yang et. al	T/B-lymphocyte / monocyte / granulocyte	Dielectrophoresis	1999	[7]
Y. Huang et. al	PBMC (peripheral blood mononuclear cell), U-937(monocytic cell) / glioma cell, SH-SY5Y (neuroblastoma cell)		2002	[62]
I. Doh et. al	Live/dead yeast cell		2005	[50]
S.S. Shevkoplyas et. al	RBC and WBC	Hydrodynamic separation	2005	[67]
R.H. Carlson et. al	WBC		1998	[69]
J. Takagi et. al	RBC		2005	[66]
M. Yamada et. al	Plant cell	Aqueous two-phase system	2004	[77]
K.-H. Nam et. al	CHO K1 cell		2005	[78]
F. Petersson et. al	Lipids from blood cells	Ultrasound separation	2004	[80]
J.J. Hawkes et. al	Yeast cell		2004	[90]

In this paper, we will describe the cell separation technology according to the following two categorized principles and what has been realized in the microfluidic cell separation device (Table 2).

Table 2. Recent reports on microfluidic cell separation devices.

Separation principle	Achievements	Reference No.
Fluorescence-activated cell sorting	The μFACS has been used by latex beads and bacterial cells are manipulated with electroosmotic flow (EOF).	[8]
Magnetic-activated cell sorting	The μMACS device is demonstrated using microfabricated Co-Cr-Ta thin film patterns.	[34]
Affinity-based cell sorting	Mimicking the physiological process of leukocyte recruitment to the vessel wall, the authors separate and isolate two types of cells.	[40]
Dielectrophoresis	With DEP/gravitational field-flow-fractionation system, human breast cancer cells, MDA-435 are separated from normal blood cells.	[57]
Hydrodynamic separation	Cell separation is achieved by a microfluidic pinched flow systems according to the cell size.	[65]
Aqueous two-phase system	In PEG and dextran two phase system, plant cells and CHO cells are separated.	[77,78]
Ultrasound separation	Using ultrasonic standing wave, lipid separation from blood and medium exchange is demonstrated in microfluidic channels.	[80~83]

2. Cell separation methods

There are two methods to separate cells of interest: immunological and non-immunological. Conventional cell separation can be carried out by immunoreactions of membrane protein with the capturing antibodies because the type of integrated proteins is specific for their function. Immunological technique is a mainstay of commercialized cell separation methods such as the fluorescence-activated cell sorting and magnetic-activated cell sorting. One of the advantages of this method is high specificity and selectivity because this approach is based on the highly specific immunoreaction between the membrane marker proteins and labeling antibodies. But it also has several disadvantages. The immunologically isolated cells may suffer from damages[5] and overall separation system involves high cost and complicated processes such as immunoreactions and elution of cells from the capturing antibodies.

On the other hand, non-immunological method is a relatively fast and simple technique. This exploits an interactive physical property of cell with the surrounding media. But a disadvantage of this method is its low specificity for cell separation, as cells do not show remarkable differences between each cell type with the exception of immunological properties. In this method, the type of cells is determined and separated according to their cell size, shape and other physical properties. Although these cell characteristics reflect the cell's own function and the type of cell[6], these are not as specific as recognition of cell membrane proteins of an immunological method. In spite of this, non-immunological technique is expected to be a promising separation method. This facilitates researchers to find out a principle to distinguish between subtle differences of cell property[7], which is applicable to the cell therapeutic area.

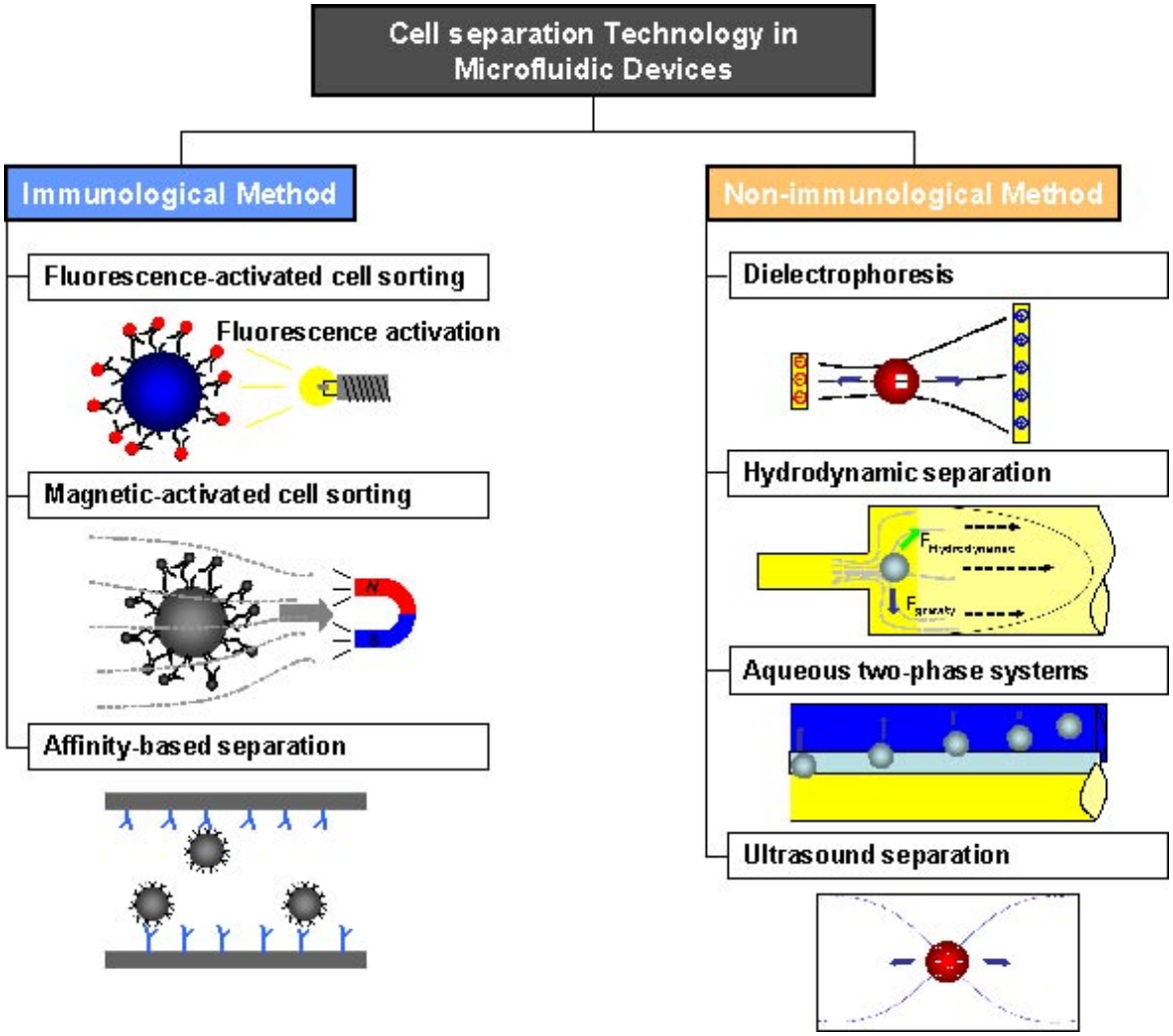

Fig 1. Schematics of microfluidic cell separation techniques.

Figure 1 shows the schematic cell separation principles used in a microfluidic device according to the two methods described. Details are given in the following sections.

3. Immunological techniques used in a microfluidic device

3.1. Fluorescence-activated cell sorting

Fluorescence-activated cell sorter (FACS) is one of the common methods used to evaluate cell population. Despite its effective performance, it is not widely used in every biological laboratories because of its high cost. Since the Quake group has reported a microfabricated FACS device [8], few types of operating principles for μFACS have appeared such as hydrodynamic [9~11], electroosmotic [12,13], electrostatic [14], electrokinetic [15], and dielectrophoretic [16,17] principles. These working principles depend on the fact that the focused cell pathway is deflected after the sorters received the signal of the

determined cell type from the optical detection system. Although the FACS generally requires fluorescence labeling, the μFACS that is without labels can be achieved in certain cases such as autofluorescence property of cells[18].

3.2. Magnetic-activated cell sorting

Magnetic-activated cell sorter (MACS) is an excellent tool for separating cells of interest out of mixed cell populations. The MACS utilizes magnetic micro/nano particles[19,20] conjugated with antibody proteins that are specific to the cell membrane protein of interest, where magnetic particle-bound cells lie in a high magnetic energy gradient and finally the cells change their pathway but non-bound cells have no influence on the magnetic field and keep their pathways.

Since the magnetic separation was one of the useful tools in biotechnology[21], separation technique based on magnetism has been investigated and developed by many research groups. Zborowski and co-workers have reported several impressive cell separation results using magnetic particles[22~33]. Although their works have not conducted in the microfabricated devices, they have reported many physical approaches such as magnetic susceptibility[25,30,33], binding capacity[27~29] and separation using cell samples[30,31]. In spite of these previously reported papers, magnetic cell separation combined with microfabrication technique has achieved few results[34~38]. The results from Han[35,36] and Reich group[37,38] are non-immunological magnetic separation using inherent paramagnetic property of RBC and nanowire-bound cells, respectively.

3.3. Affinity-based cell separation

Affinity-based cell separation uses solid supports where the specific antibody is conjugated. If the heterogeneous cell suspension filters through the antibody-bound solid phase, cells of interest are captured on the solid supports while other contaminants would pass through the way. The bound cells can be released by changing the buffer solution. However, a few microfabricated devices for affinity-based cell separation have been introduced. This is because the microfabricated tools have not a clear good point compared with the conventional affinity cell chromatography technique. Recently, some microfabricated devices based on the affinity-based separation have been reported exploiting the unique function only the microfabrication technology can offer. Revzin and co-workers describe a microfabricated cytometry platform for cell sorting and characterization, providing high-density leukocyte matrix array for isolating, characterizing and releasing the cells[39]. Similarly, Chang demonstrated adhesion-based collection and separation in a microfluidic channel by mimicking the physiological behavior of leukocyte in blood vessel[40]. The separation of sperm and epithelial cells in microfabricated devices has been reported by Horsman using the epithelial cell property of adherence to the glass substrate[41].

4. Non-immunological techniques used in a microfluidic device

4.1. Dielectrophoresis

Dielectrophoresis (DEP) is a valuable method for manipulation of dielectric particles including polymer spheres, cells, proteins, and even DNA. Although it requires the fastidious property of low conductivity of the cell medium, combining inherent advantages of DEP with the recent microfabrication technology, the DEP technique

is considered as one of the most promising tools for cell separation[42~48]. Dielectric particles, including cells, have two different types of behavior; positive and negative DEP, depending on the direction of particle movement under the non-uniform electric field. The DEP cell separators have been developed using positive and negative DEP phenomena. In positive DEP-dependent separator, cells deflected toward the electrodes result in adsorption on electrodes, which may cause reduced cell recovery[49,50]. Therefore, they are not adequate for rare cell recovery after isolation because of the trapped cells on electrodes. Contrarily, the strategy of negative DEP provides the repulsive force acting on the cells, eliminating cell adsorption on the electrodes[51]. Although the negative DEP force is decreased as the cells are moving away from the electrodes, which gives rise to gradually diminished cell deflection velocity, the negative DEP is more adaptable to the continuous-flow cell separation because of its reliable cell recovery ratio.

Research on the DEP cell separation has been carried out using polymer beads as the analogue of cells in order to characterize the devices and principles[51~56]. Gascoyne group has reported various microfabricated DEP devices[56~60] and provided the possibility for cell separation[7]. Live and dead cells were separated by interdigitated array (IDA) electrode[49] and continuous flow system[50]. Likewise, Li and Kaler revealed the continuous cell separation approach, where microfabricated electrodes were individually biased by a variable frequency to improve the efficiency[61]. Huang describes the microfabricated electrode array for isolating six types of cells by modulating DEP frequency[62]. Additionally, other research groups have revealed the DEP devices for the separation of red blood cells[63] and neurons[64].

4.2. Hydrodynamic separation

Hydrodynamic cell separation technique is the most simple and ideal principle to fractionate cells of interest. This method makes the cells separated depending on their cellular properties, such as size, shape, density and stiffness, which are determined by cellular behaviour caused by interaction between the cells and surrounding medium or gravitational force. A pinched flow fractionation method by Yamada[65] and Takagi[66] separates the cells according to their sizes, and Shevkoplyas[67] reported autoseparation of leukocytes in the microfluidic device mimicking blood cell behavior in the vessels. The paper from Carson[68,69] describes separation of white blood cells in the microfabricated lattice structures using a model of activated sticking of cells with the wall. Gravity driven cell sorter (microHYCS) is reported by Huh[70], and Benincasa[71] describes gravitational split-flow thin channel (G-SLPITT) systems even though they do not employ microfabrication methods. Moreover, Blattert presents the simple microfabricated device for blood cell separation from plasma using microchannel bend structures[72] and Crowley reported a device for isolating plasma from whole blood using planar microfilters[73].

4.3. Aqueous two-phase system

The aqueous two-phase systems developed for the separation of macromolecules are useful techniques to fractionate cells of interest from a heterogeneous mixture. When some kinds of water-soluble polymers above critical concentrations are mixed together, an aqueous two-phase system is generated, where the cells are added and distributed between each phase. Dextran and polyethylene glycol (PEG) have been specially used for cell separation, forming a top phase of PEG and a bottom phase of dextrans. Cell separation

in this system depends on cell surface property such as surface charge. Although various conventional cell partitioning methods have appeared[74-76], only a few related results on microfluidic two-phase systems have been reported. Yamada describes a microfluidic continuous cell separation device using the two-phase system.[77] This device is achieved by the increased chance of cells' encounter with the interface of two microfluidic streams, employing two facts of optimized flow rate and width of the phase stream. Recently, Nam reported the microfluidic device for separation of live and dead Chinese hamster ovary (CHO) K1 cells[78].

4.4. Ultrasound separation

Suspended particle separation using ultrasonic standing wave force has been widely used in traditional chemical engineering and material science. In ultrasound standing wave, particles including cells migrate to the pressure node of the standing wave. When the cells are moving, the migration velocity is determined by the diameter of the cell and acoustic contrast factor given by density and compressibility of cells and medium.[79] According to these fundamentals, cells can be separated depending on their size, density, and compressibility. Recently, Laurell group reported four papers. Separation of lipid from blood in a microfluidic channel is described by Petersson.[80] Jönsson suggested the possible clinical implication of the lipid removing microfluidic device.[81] Additionally, Nillsson[82] and Petersson[83] reported suspended particles separation in a microfabricated chip and continuous blood medium exchanging device using ultrasound standing wave. Combining ultrasound force with optical tweezing technique, Takeuchi presented laser ultrasonic micromanipulators.[84] Before applying the ultrasound microfluidic device to cell separation, researchers utilize polystyrene microbeads as a model system. Coakley reported several papers on ultrasonic particle separation[85~90]. Moreover, the conventional ultrasound systems for cells or particle separation have been variously introduced before the appearance of microfluidic ultrasonic devices.[91~98] Concerning the previously reported results on ultrasound cell separation especially in microfluidic devices, they have been usually fundamental and limited to separation of cells from obviously different types of materials such as lipids. Based on this current working principle, it seems that it is difficult to fractionate the cells of interest having subtle differences of size and surface marker.

5. Conclusions

It is obvious that various biology-related researches have been accelerated by up-to-date advanced technologies and facilities. In addition, microfabrication technology can be anticipated as a discipline contributing to the acceleration of the biotechnological progress. In the development of microfluidic cell separators, there are critical issues of what the ultimate objective of the cell separation is and subsequently, how efficient separation can be achieved. For instance, physiological viability of the cells is the most significant condition to be acquired while the cell viability is not a critical issue to the researchers with regards to clinical diagnostics or cell population analysis. Another significant issue in microfluidic cell separation is how to apply the state-of-the-art microfluidic device to actual cell separations achievements. Current research outputs exploit the limited cell types or analogues of cells such as polystyrene microbeads to ascertain the feasibility of the separation principle. Accordingly, the technology of the microfluidic cell separations is required to be developed to meet these needs and to reveal the principles not realized yet.

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Contact Details:
Contact Person: J.K. Park
Address: Department of BioSystems,
Korea Advanced Institute of Science
and Technology (KAIST),
373-1 Guseong-dong,
Yuseong-gu, Daejeon 305-701, Korea
Tel.: +82 42 869 4315
Fax: +82 42 869 4310
Email: jekyun@kaist.ac.kr

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