High throughput leukemia cell(K562) sorting system based on negative dielectrophoretic force

D. Lee*, D. Kim*, Y. Kim* and B. Kim*

*Nano Bio Robotics Lab, Korea Aerospace University
Goyang, Gyeonggi-do 412-791, Korea; Corresponding Author: bkim@kau.ac.kr

ABSTRACT

The negative dielectrophoresis (n-DEP) based cell-sorting system is presented to separate live leukemia cells (K562) from dead leukemia cells (K562). The system comprises meso-size channel and cantilever-type electrode (CE) array for realizing high throughput and high efficiency sorting system. In order to find optimized separation condition, we investigate cell reaction on CE array under various voltage level and frequency, conductivity of medium and flow rate. Conclusively, the target cells are effectively separated under the condition of 7Vpp, 100kHz, 0.2S/m and flow rate of 10uL/min and 20uL/min in the channel 1 and 2 respectively, and we achieve high throughput of 2400cell/sec with high efficiency of 86%.

Keywords: dielectrophoresis, cell separation, leukemia cells, cantilever-type electrode array

1 INTRODUCTION

In the field of micro-particle separation, conventional separation techniques such as fluorescent activated cell sorters (FACS) [1], magnetic-activated cell sorters (MACS) [2] are generally used, because these methods allow the high efficiency and high throughput cell sorting [3-6]. In the conventional methods, however, the immune-labeling process is surely required to separate specific target cells. Therefore, the separation efficiency of these methods is dependent on antibody-antigen reaction based immune-labeling which is invasive method [7]. In order to overcome the limitations of conventional methods, dielectrophoresis (DEP) based cell separator has been suggested since it is non-invasive method and does not require labeling process before separation [8-12].

Thus, in this paper, we suggested the negative dielectrophoresis(n-DEP) based sorting platform that can realize high efficiency and high throughput cell sorting system. Unlike other immune-labeling based cell sorters such as FACS and MACS, the n-DEP based sorter is performed by differentiating intrinsic dielectric property of each cell. For high throughput and high separation efficiency, the proposed sorting platform comprises meso-size vertical channel for transporting many cells and silicon wafer based micro cantilever-type electrodes for generating n-DEP force. In addition, to reduce engineering cost, we adopted the flow regulator to generate gravity based hydrodynamic force instead of micro-syringe pump.

In order to achieve the high efficiency and high-throughput sorting, we investigated the optimal condition for cell separating on CE array. Based on the condition obtained by prior investigation on CE array, we conducted cell separation experiment on the proposed cell sorting system. The experimental study was carried out by employing K562, a leukemia cell lines that widely utilized for the diagnosis or analysis of the immune-mechanisms of various diseases in the field of clinical pathology.

2 WORKING PRINCIPLE AND EXPERIMENTAL SYSTEM SETUP

2.1 Principle of cell separation

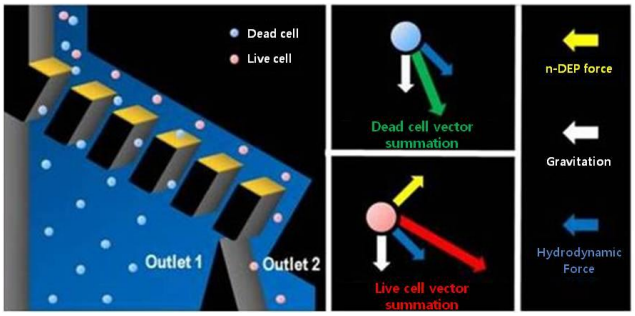


Figure 1: Basic principle of cell deflection in non-uniform electric field.

The dielectrophoresis based cell sorting system separates cells by the differences of dielectric properties. Since live and dead cells have different dielectric properties, the cells will be exposed to different n-DEP forces even if they are exposed to same electric condition (magnitude and frequency of the voltage) and boundary condition (conductivity and permittivity of medium). The basic working principle of cell separating is described in Figure 1. In case of dead cells, they are only influenced by gravitation and hydrodynamic force. On the other hand, live cells are influenced by gravitation, hydrodynamic force and n-DEP force. Thus, dead cells reach outlet 1 through the channels between electrodes, and the deflected live cells flow along the CE array to outlet 2.

2.2 CE array fabrication procedure

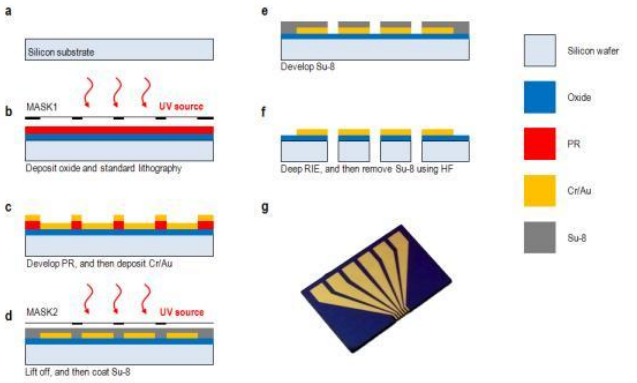


Figure 2: Micro fabrication procedure of the CE array.

The fabrication procedure of the CE array is seen in figure 2: a) Silicon substrate with CMP process(300μm). b) Depositing oxide for insulation and standard lithography using positive PR(AZ 7220). c) Developing PR and deposit evaporation of Cr(300 Å)/Au(2000 Å). d) Lifting off, spin coating and patterning a negative PR(Su-8). e) Developing Su-8. f) Conducting deep RIE and removing Su-8 by HF. g) Finally, CE array is obtained.

2.3 CE array based cell sorting system

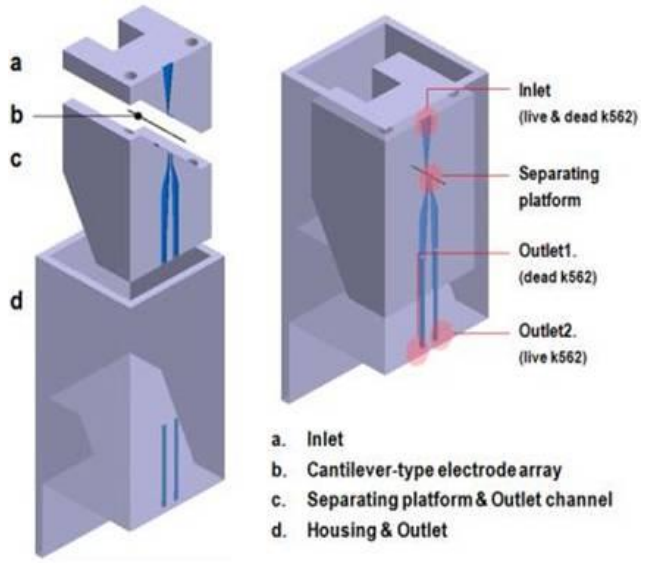
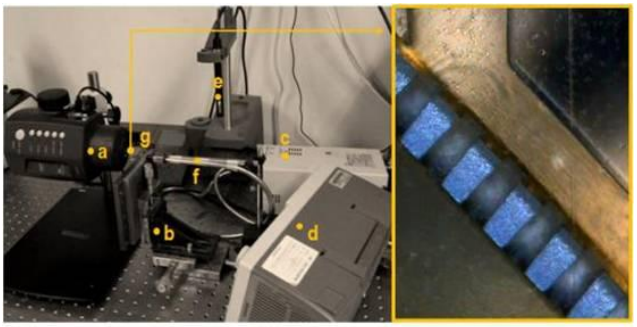


Figure 3: Configuration of the cell sorting system.

The CE array based cell sorting platform comprises four components as shown in figure 3. The part (a) including the inlet channel is the spot where the cells injected. The part (b), CE array, is composed of five electrodes and embedded between the part (a) and (c) with being linked to function generator by electric wires. The part (c) includes the inclined channel with an angle of 45° linked to the channels reaching to the outlet 1 and 2. The part (d) includes the draining passage, outlets, and syringe tube linked to a reservoir.

2.4 Experimental setup



a. Micro cam-scope, b. x-, y- and z micro-stage, c. Light source, d. Function generator
e. Conductivity meter, f. Bifurcated light guide, g. Cell separating system

Figure 4: Experimental system setup for monitoring cell separation.

The experimental system is configured as in Figure 4. It is composed of the micro cam-scope for monitoring cell movement, micro stages for adjusting focal length of the cam-scope properly, function generator for applying some voltage with various frequencies, CE array for applying the voltage and illumination system. For the accurate measurement of medium conductivity, the conductivity meter (InoLab Cond 730, Wissenschaftlich-Technische Werkstatten, GmbH & Co. KG, Germany) is utilized.

2.5 Preparation of cells and buffer solution

K562 cells were cultured in a humidified atmosphere 5% at 37 in 500mL RPMI 1640, supplemented with 50mL heat-inactivated fetal bovine serum and 20μl Penicillin-streptomycin (50x). In the experiment, cells were used after having been grown in an incubator for two days and exposed to 10μg/mL mitomycin-C for 24 hours (to induce apoptosis of K562 cells). Then, the cells were washed and re-suspended with the medium (0.1 × phosphate-buffered saline (PBS) + 1% bovine serum albumin (BSA) + 8.5 % (w/v) sucrose + 0.5 % (w/v) dextrose). The medium used in experiment was the same solution that was used to re-suspend the cells. Final concentration and conductivity of the medium were adjusted to 1.0×10^6cells/mL and 0.201S/m respectively.

After separation, to investigate separation efficiency, we used trypan blue to stain the dead K562 cells.

3 EXPERIMENAL RESULT

3.1 Experimental investigation on CE array

In figure 5, we present n-DEP phenomenon according to various conditions. In case of A (0.1S/m, 5Vpp, 500kHz), due to the n-DEP force, cells at the bottom float in the medium up to 80μm from the electrode array. However, the

cells move to the left side at 10μm/s due to little electro-osmosis. The case B (0.2S/m, 7Vpp, 100kHz) shows the cells at the bottom floating up to 100μm high from electrode array. In addition, electro-osmosis phenomenon is not observed. Finally, in case C (0.2S/m, 8Vpp, 100kHz), the cells float up to 120μm high from electrode array, but the cells move to the left side at 10μm/s due to electro-osmosis like case A. Conclusively, the optimal separation conditions on the CE array is investigated as 7Vpp with 100kHz, and 0.2S/m conductivity of medium. This optimal condition generates prominent n-DEP force without electro-osmosis phenomenon.

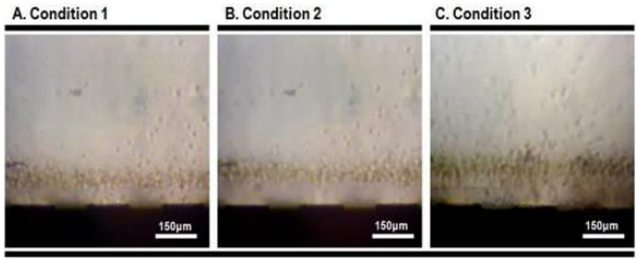


Figure 5: n-DEP phenomenon under different conditions.

3.2 Experimental investigation of the optimal separation condition in cell sorting system

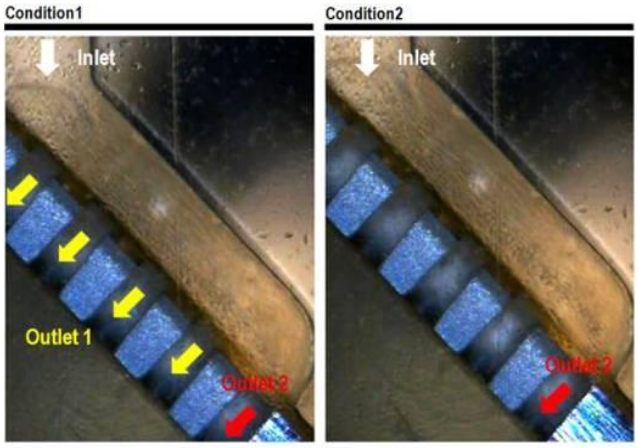


Figure 6: Movement of cells under different conditions.

We have preliminary study to configure optimal separation condition on the CE array based sorting system. The condition is obtained as 7Vpp at 100kHz and 0.2S/m which is similar to result on CE array. To validate this, we conduct cell separating experiment on the proposed platform under the optimized condition.

As shown in the Figure 6, we compare the movement of cells under condition 1 (0.2S/m, 5Vpp, 100kHz) and condition 2 (0.2S/m, 7Vpp, 100kHz). Under the condition 2, most of cells are deflected on the electrodes. According to the investigation result, there is stronger n-DEP force and less electro-osmosis at condition 2 than condition 1. In detail, the velocity driven by electro-osmosis at condition 1 is 10μm/s. Finally, we can separate the target cells successfully and confirm the optimal conditions for K562 separation as 7Vpp at 100kHz and 0.2S/m conductivity.

3.3 Separation efficiency

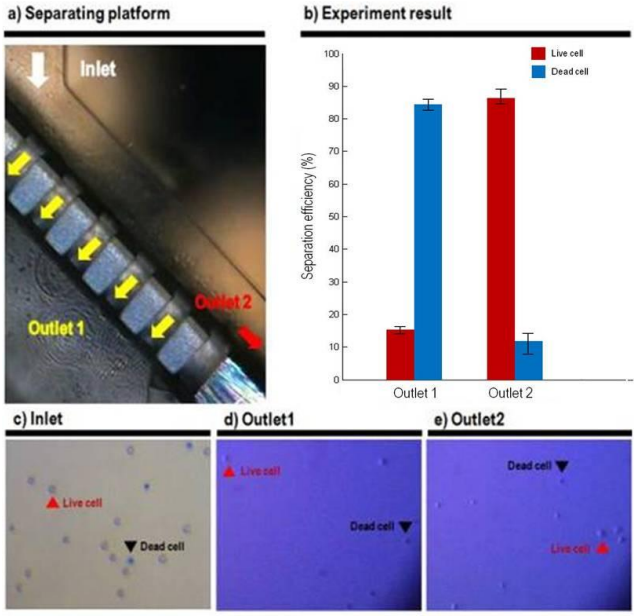


Figure 7: Separation results of the platform; a) Separation system, b) Separation efficiency, c) Injected cells with mixing ratio of (dead:live=1:9) and cell concentration of (1.0×10^6cells/mL), d) Collected cells in the outlet 1, e) Collected cells in the outlet 2.

The separation test was conducted on the proposed cell sorting system. The target cells were effectively separated under the condition of voltage of 7Vpp, frequency of 100kHz, conductivity of 0.2S/m, flow rate of 10uL/min and 20uL/min in the channel 1 and 2 respectively. After separation, to investigate separation efficiency, the dead K562 cells were stained using trypan blue. Experimental results are presented in the figure 7. Injected cells are shown in the figure 7-c. The dead cells were stained by trypan blue, while the live cells were not stained. The collected cells in outlet 1 and outlet 2 are shown in the figure 7-d and 7-e respectively. In conclusion, under the above condition, we achieved high throughput of 2400cell/sec with high separation efficiency (87% and 82% in the channel 1 and 2 respectively).

REFERENCES

[1] P. Malatesta, E. Hartfuss and M. Gotz, "Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage," Development, 127, 5253-5263, 2000.

[2] S. Miltenyi, W. Muller, W. Weiche and A. Radbruch, "High gradient magnetic cell separation with MACS," Cytometry, 11, 231-238, 1990.

[3] A. Radbruch and D. Recktenwald, "Detection and isolation of rare cells," Current Opinion in Immunology, 7, 270-273, 1995.
[4] J. Yang, Y. Huang, X. Wang, F.F. Becker, P.R.C. Gascoyne. "Differential analysis of human leukocytes by dielectrophoretic field-flow-fractionation," Biophysical Journal, 78, 2680-2689, 2000.
[5] N. Pamme and A. Manz, "On-chip free-flow magnetophoresis: continuous flow separation of magnetic particles and agglomerates," Analytical Chemistry, 76, 7250-7256, 2004.
[6] D. Huh, W. Gu, Y. Kamotani, J.B. Grotberg and S. Takayama, "Microfluidics for flow cytometric analysis of cells and particles," Physiological Measurement, 26, 73-98, 2005.
[7] J.D. Adams, U. Kim and H.T. Soh, "Multitarget magnetic activated cell sorter," Proceedings of the National Academy of Sciences of the USA, 105, 18165-18170, 2008.
[8] C.M. Das, F. Becker, S. Vernon, J. Noshari, C. Joyce and P.R.C Gascoyne, "Dielectrophoretic segregation of different human cell types on microscope slides," Analytical Chemistry, 77, 2708-2719, 2005.
[9] C. Iliescu, G. G.L. Xu, P.L. Ong and K.J. Leck, "Dielectrophoretic separation of biological samples in a 3D filtering chip," Journal of Micromechanics and Microengineering, 17, 128-136, 2007.
[10] H. Song, V. Mulukutla, C.D. James and D.J. Bennett, "Continuous-mode dielectrophoretic gating for highly efficient separation of analytes in surface micromachined microfluidic devices," Journal of Micromechanics and Microengineering, 18, 125013-125022, 2008.
[11] N. Demierre, T. Braschler and M.P. Renaud, "Focusing and continuous separation of cells in a microfluidic device using lateral dielectrophoresis," Sensors and Actuators B: Chemical, 132, 388-396, 2008.
[12] J. Vykoukal, D.M. Vykoukal, S. Freyberg, E.U. Alt and P.R.C Gascoyne, "Enrichment of putative stem cells from adipose tissue using dielectrophoretic field-flow fractionation," Lab on a Chip, 8, 1386-1393, 2008.