DEVELOPMENT OF OPTICALLY-INDUCED-DIELECTROPHORESIS (ODEP)-BASED VIRTUAL CELL MICROFILTERS IN A MICROFLUIDIC CHIP FOR THE ISOLATION OF CIRCULATING TUMOR CELL (CTC) CLUSTERS

Tzu-Keng Chiu[1], Wen-Pin Chou[2], Chia-Jung Liao[2], Po-Yu Chu[2], Jia-Long Hong[3], Ping-Hei Chen[3]* and Min-Hsien Wu[2, 4]*

[1]Department of Chemical and Materials Engineering, Chang Gung University, Taoyuan City, Taiwan
[2]Graduate Institute of Biochemical and Biomedical Engineering, Chang Gung University, Taoyuan City, Taiwan
[3]Department of Mechanical Engineering, National Taiwan University, Taipei, Taiwan
[4]Division of Haematology/Oncology, Department of Internal Medicine, Chang Gung Memorial Hospital at Linkou, Taoyuan City, Taiwan
*Email: mhwu@mail.cgu.edu.tw; Tel.: +886-3-2118800 ext. 3599

This study integrated the microfluidic system and ODEP technology for the isolation of CTC clusters from the background leukocytes. The working principle is based on the size difference between the CTC clusters and leukocytes, and thus different magnitude of ODEP force acting on them. ODEP mainly use a controllable light pattern, acting as a virtual electrode, to generate a non-uniform electric field that is in turn utilized to manipulate the electrically-polarized cells. The utilization of ODEP-based mechanism for CTCs isolation has been successfully demonstrated in our previous study [1].

Since 1970, a series of clinical studies have shown that single CTCs may not be the main cause of cancer metastasis, but two or more aggregated CTCs [2, 3]. In order to isolate CTC clusters for back-end analysis. For the biological-based methods (e.g. ^{HB}CTC-Chip [4], or CTC-iChip [5]), although CTC clusters can be specifically separated by antibody-based schemes, but the surface-area-to-volume ratio of CTC clusters is relatively low which might affect the binding efficiency of CTC clusters and antibody. Alternatively, some studies proposed physical-based methods to separate CTC clusters (e.g. ISET [6], FMSA [7], Cluster-chip [8]). Although these methods have been demonstrated to effectively isolate the CTC clusters from the background cells mainly based on their size difference, the influence of shear stress on physical size of CTC cluster, or the viability of the cells isolated is still a problem.

To address this issue, The key advantages of ODEP mechanism for cell isolation including: (1) no need of complex microfabrication process for constructing microfilter structures, and (2) the reduction of shear stress acting on the cells manipulated.

However, the feasibility of using ODEP-based mechanism for the isolation of CTC cluster (i.e. CTC cell aggregates) has not yet been explored. To test its feasibility, a T-shaped microfluidic chip was designed **(Fig.1)**. A virtual microfilter consisting of multiple light patterns was designed at the CTC clusters isolation zone **(Fig.1)**. By continuously moving and rotating the light patterns in the microfilter, the larger CTC clusters can be separated from the background leukocytes, and also transported to the side microchannel **(Fig.2)**. In this work, the optimum ODEP operating conditions (e.g. moving velocity of light pattern) was explored. Results revealed that moving velocity of light pattern that can manipulate the CTC clusters (containing 2-13 cells) was significantly higher than the background of leukocytes **(Fig.3)**. Based on this, the moving velocity of light pattern was set at 100 μm/sec **(Fig.3)**. At a given sample flow rate of 0.5 μl/min, moreover, we found that the rotation speed of light patterns at 14 RPM could significantly increase the purity of CTC clusters isolation **(Fig.4)**. Based on the set operating conditions, the recovery and purity of the isolated CTC clusters were experimentally evaluated to be 70.1 ± 7.1% and 60.8 ± 2.7%, respectively **(Fig.4)**. As a whole, we have established a high purity CTC clusters isolation method that is easy to operate, and is possible to avoid the problem caused by the shear stress acting on the cells or particles.

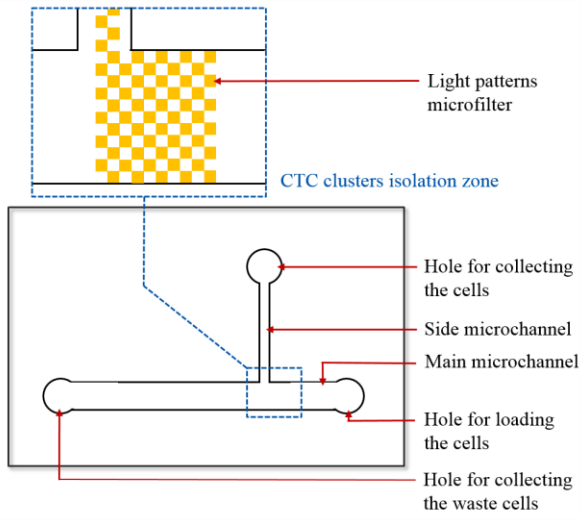

Fig.1 Schematic illustration of top-view layout of the T-shaped microfluidic chip.

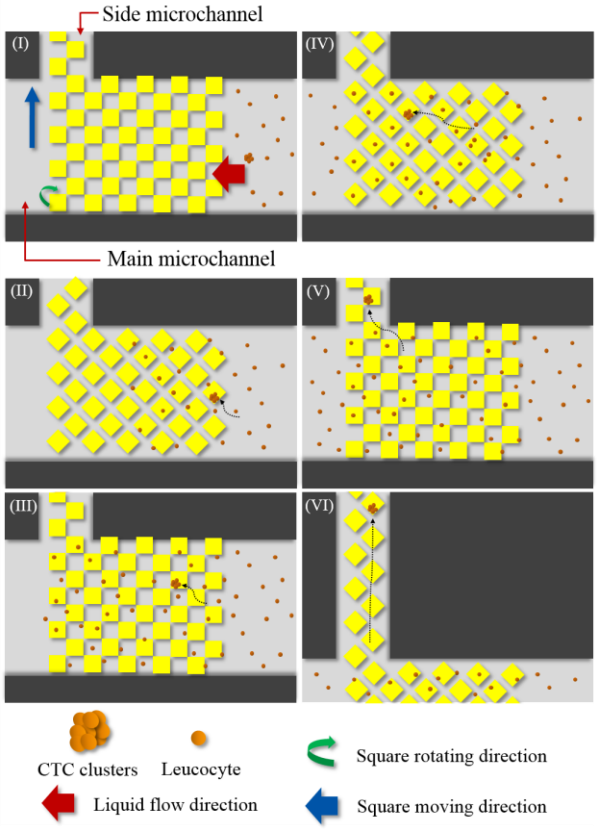

Fig.2 Schematic illustration of (I) the layout of ODEP microfluidic system for CTC clusters isolation, in which light patterns were designed at the cell isolation zones as virtual cell microfilters. The cells suspension flow from right to left in the main microchannel, and in the cell isolation zone, the light pattern moves upward continuously, leading the larger ODEP force of CTC clusters to the side microchannel. At the same time by rotating the light patterns, to avoid the white blood cells in the separation process stuck on the CTC clusters. (II)-(VI) The overall CTC clusters isolation processes.

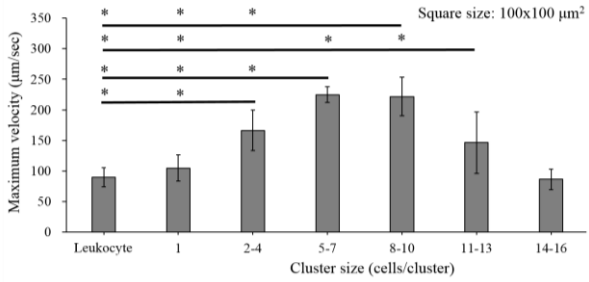

*Fig.3 The quantitative relationship between the cells size (single leukocyte and 1-16 cells size of CTC clusters) and the maximum velocity (μm/sec) of the moving square light patterns that can manipulate H209 cell line clusters and leukocytes (*p<0.05, ANOVA).*

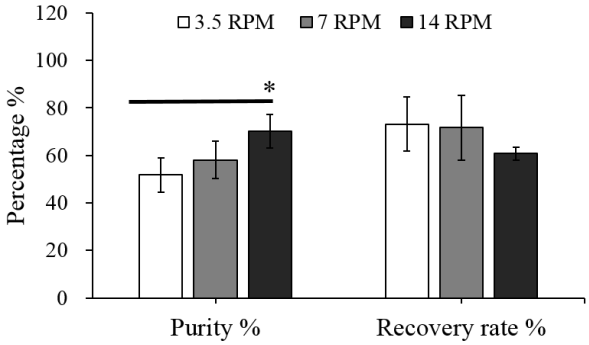

*Fig.4 Effect of square light patterns rotating rate (3.5, 7, and 14 RPM) on the CTC clusters isolation purity (%) and recovery rate (%) at the side microchannel (*p<0.05, ANOVA).*

ACKNOWLEDGMENTS

This work was sponsored by Ministry of Science and Technology (MOST 104-2628-E-182-002-MY3, MOST 105-2221-E-182-028-MY3 and MOST 105-2221-E-002-107-MY3).

REFERENCES:

[1] S.B. Huang, M.H. Wu, Y.H. Lin, C.H. Hsieh, C.L. Yang, H.C. Lin, et al., "High-purity and label-free isolation of circulating tumor cells (CTCs) in a microfluidic platform by using optically-induced-dielectrophoretic (ODEP) force," Lab Chip, **2013**,13, 1371-83.

[2] L.A. Liotta, M.G. Saidel, J. Kleinerman, "The significance of hematogenous tumor cell clumps in the metastatic process," Cancer Res, **1976**, 36, 889-94.

[3] L.A. Liotta, J. Kleinerman, G.M. Saidel, "Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation," Cancer Res, **1974**, 34, 997-1004.

[4] S.L. Stott, C.H. Hsu, D.I. Tsukrov, M. Yu, D.T. Miyamoto, B.A. Waltman, et al., "Isolation of circulating tumor cells using a microvortex-generating herringbone-chip," Proc Natl Acad Sci U S A, **2010**, 107, 18392-7.

[5] E. Ozkumur, A.M. Shah, J.C. Ciciliano, B.L. Emmink, D.T. Miyamoto, E. Brachtel, et al., "Inertial focusing for tumor antigen-dependent and -independent sorting of rare circulating tumor cells," Sci Transl Med, **2013**, 5, 179ra47.

[6] G. Vona, L. Estepa, C. Beroud, D. Damotte, F. Capron, B. Nalpas, et al., "Impact of cytomorphological detection of circulating tumor cells in patients with liver cancer," Hepatology, **2004**, 39, 792-7.

[7] R.A. Harouaka, M.D. Zhou, Y.T. Yeh, W.J. Khan, A. Das, X. Liu, et al., "Flexible micro spring array device for high-throughput enrichment of viable circulating tumor cells," Clin Chem, **2014**, 60, 323-33.

[8] A.F. Sarioglu, N. Aceto, N. Kojic, M.C. Donaldson, M. Zeinali, B. Hamza, et al., "A microfluidic device for label-free, physical capture of circulating tumor cell clusters," Nat Methods, **2015**, 12, 685-91.