Analysis of Dielectrophoretic Movement of Floating Myoblast near Surface Electrodes in Flow Channel

Shigehiro HASHIMOTO, Ryota MATSUZAWA, Yuji ENDO

Biomedical Engineering, Department of Mechanical Engineering,
Kogakuin University, Tokyo, 163-8677, Japan
shashimoto@cc.kogakuin.ac.jp http://www.mech.kogakuin.ac.jp/labs/bio/

ABSTRACT

The dielectrophoretic movement of the floating biological cell near the surface electrodes has been analyzed in the micro flow channel *in vitro*. A pair of asymmetric surface electrodes of titanium (thickness of 200 nm) were incorporated in the flow channel by the photolithography technique: a triangular electrode with the tip angle of 0.35 rad, and a rectangular electrode of the flat edge as reference. The cyclic alternating electric current of the square wave (between 0.25 μs and 0.3 μs of periods) was introduced between the surface electrodes. The suspension of C2C12 (mouse myoblast cell line) was injected into the flow channel, and the flow rate was controlled by the pressure head between the inlet and the outlet. The experimental results show that the absolute value of the amplitude of the acceleration by the electric field, which is perpendicular to the flow direction, increases with the diameter of the cell.

Keywords: Biomedical Engineering, Dielectrophoresis, Surface Electrode and C2C12.

1. INTRODUCTION

The movement of a biological cell suspended in the medium is governed by several factors: movement of the medium, gravity, electric force, Van der Waals force, and affinity of surface. Various methods have been applied to control the movement of cells *in vitro*: the flow [1], the shear field [2, 3], the filter, the slit [4–6], the magnetic field [7], the gravitational field [8–10], and the electric field [11]. These methods might contribute to several applications of manipulation of cells [12, 13]: detection of targeted cells [14–16], sorting of cells [17–21], arrangement of cells to make a tissue, and measurement of the character of cells [22]. Movement of a charged particle depends on the electric field. The effect is applied to the electrophoresis device [23]. When a particle is subjected to a non-uniform electric field, a force acts even on a non-charged particle, because the polarization generates in the particle. The phenomenon is called dielectrophoresis [8], which depends on the several parameters: the electrical property of the particle, shape and size of the particle [24], the electrical property of the medium, and frequency [25] of the electric field. Electrophoresis is a phenomenon, in which a particle is moved by the Coulomb force between the charge of the particle and the electric field. Dielectrophoresis [26], on the other hand, is a phenomenon, in which a particle moves due to the interaction between the electric field and the charge induced in a neutral particle, when the particle is placed in a non-uniform electric field.

With the aid of the micromachining technique [27], many kinds of micro systems were designed. In some systems, dielectrophoresis was applied to sorting of biological cells floating in the medium. In the present study, a micro flow channel with the surface electrodes has been designed to analyze the dielectrophoretic movement of a flowing biological cell *in vitro*.

2. METHODS

Surface Electrode

The titanium film was used for the surface electrode. Titanium was coated on the glass plate (36 mm × 28 mm × 1 mm, Matsunami Glass Ind., Ltd., Japan). The thickness of coating is 200 nm. The tip angle of the triangle shape of the one of the surface electrodes is 0.35 rad. The other reference electrode has a flat edge. The distance between electrodes is 0.1 mm (Fig. 1). The shortest connecting line between electrodes is vertical to the flow direction. The micro flow channel with surface electrodes was manufactured by the photolithography technique.

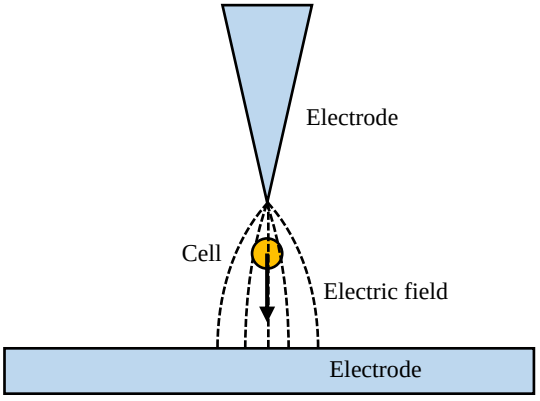

Fig. 1: Dielectrophoretic movement of cell in non-uniform electric field.

The pattern of the electrode with the flow channel was drawn on the mask with a laser drawing system (DDB-201K-KH, Neoark Corporation, Hachioji, Japan). The thickness of the layer (SU-8 100) on the surface electrode was measured by the stylus (with 3×10^{-5} N) of the contact profilometer (Dektak XT-E, Bruker Corporation).

Flow Channel

The upper plate of polydimethylsiloxane has holes with the diameter of 5 mm for the inlet and the outlet of the suspension. The upper plate was exposed to the oxygen gas (0.1 Pa, 30 cm^3/min) in the reactive ion etching system (FA-1) (50 W, 20 Pa, for two hours). Aminopropyltriethoxysilane (APTES) was coated on the upper plate. After ten minutes, the upper plate was adhered on the lower plate under the pressure of 0.5 N/m^2 by sandwiching between plates of poly-methyl-methacrylate. A rectangular parallelepiped channel of 18 mm length × 0.5 mm width × 0.035 mm height is formed between upper and lower plates. The flow channel is placed on the stage of the inverted phase-contrast microscope (IX71, Olympus Co., Ltd., Tokyo).

Electric Stimulation

The electric stimulation (E) of the alternating rectangular cyclic wave (0.25 μs < period (T) < 0.3 μs; −15 V < Ea < +15 V) was generated with an electric stimulator (Fig. 2a). The stimulator was connected to the titanium film electrode, and the electric stimulation was applied to the medium of cells. An electric resistance (R) of 2 kΩ is serially inserted between the electrode and the stimulator (Fig. 2b) to measure the electric current (I < ±7.5 mA). The electric voltage (V) between the terminals of the resistance (R) is monitored by an oscilloscope during electric stimulation applied between the titanium surface electrodes (Fig. 2b).

Cell

C2C12 (mouse myoblast cell line originated with cross-striated muscle of C3H mouse) was used in the test. D-MEM (Dulbecco's Modified Eagle Medium) containing 10% FBS (Fetal Bovine Serum) and 1% penicillin/ streptomycin was used for the medium.

Before the flow test, the inner surface of the flow channel was hydrophilized by the oxygen plasma ashing. The bovine serum albumin solution was prefilled in the flow channel, and incubated for thirty minutes at 310 K in the incubator.

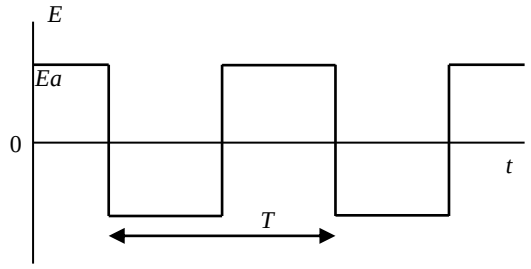

Fig. 2a: Voltage (E) tracings of rectangular wave: time (t), period (T), amplitude (Ea).

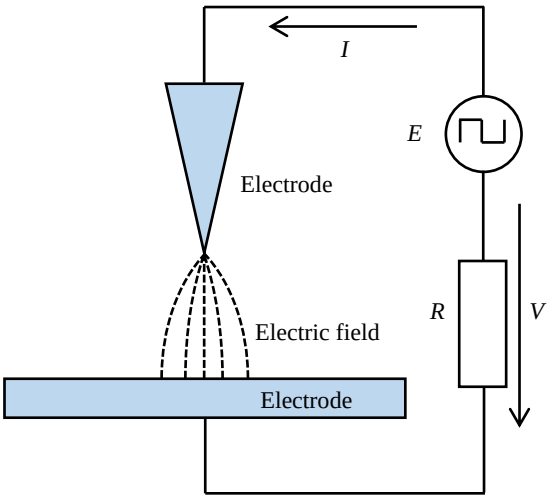

Fig. 2b: Electric circuit: alternating electric rectangular wave (E), resistance (R), voltage (V), and electric current (I).

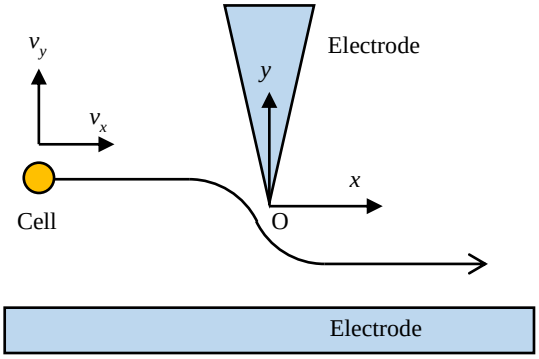

Fig. 3: Two-dimensional movement of cell flowing near tip of electrode: position (x, y), velocity (v_x, v_y) of cell.

Before the flow test, the cells were exfoliated from the plate of the culture dish with trypsin including EDTA (ethylenediaminetetraacetic acid), and suspended in the medium. The suspension of cells was poured at the inlet of the flow channel. The flow occurs by the pressure difference between the inlet and the outlet, which was kept by the gravitational level of the medium (< 5 mm).

Each cell passing between the electrodes was observed by the inverted phase-contrast microscope (IX71), and recorded by the camera (DSC-RX100M4, Sony Corporation, Japan), which is set at the eyepiece of the microscope. The movement of the cell was analyzed by "Kinovea (Ver. 8.23, Commons Attribution)" at the video images: 30 frames per second. To trace the movement of the cell, the coordinates are defined as that in Fig. 3. The main flow direction of the medium is defined as x. The perpendicular direction from the reference electrode to the tip of the triangular electrode is defined as y. The origin is adjusted at the tip of the triangular electrode.

3. RESULTS

Fig. 4 exemplifies the microscopic image, which shows cells

flowing near the tip of the triangular electrode.

Fig. 5 shows the tracings of movement of twenty cells. Fig. 5a shows movement of cells without the electric field. Every cell moves at the constant velocity according to the flow to the x direction. The period of rectangular cyclic wave is 0.3 μs in Fig. 5b, and 0.25 μs in Fig. 5c. Every cell shows the movement perpendicular to the flow direction near the triangular electrode. The movement at 0.25 μs (period) is larger than that at 0.3 μs in the alternating electric field.

Fig. 6 shows the tracings of velocity of twenty cells: v_x (Fig. 6a), and v_y (Fig. 6b). The period of rectangular cyclic wave is 0.3 μs. The velocity v_x is almost constant (0.2 mm/s < v_x < 0.7 mm/s). The velocity v_y is almost zero, except near the triangular electrode.

Fig. 7 shows the tracings of velocity of twenty cells: v_x (Fig. 7a), and v_y (Fig. 7b). The period of rectangular cyclic wave is 0.25 μs. The velocity v_x is almost constant (0.2 mm/s < v_x < 0.6 mm/s). The velocity v_y is almost zero, except near the triangular electrode. The maximum absolute value of v_y is larger at 0.25 μs of the period than at 0.3 μs of the period, although v_x of the both periods are in the similar range.

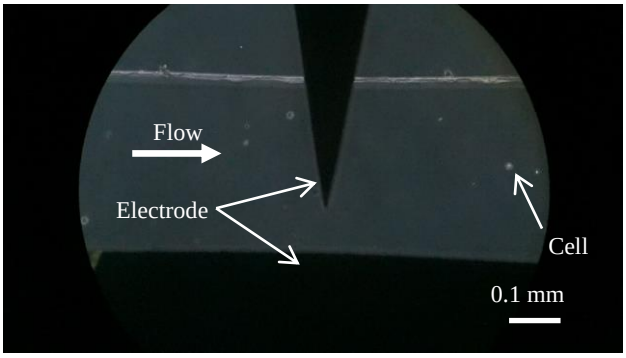

Fig. 4: Cells in flow channel around triangle electrode.

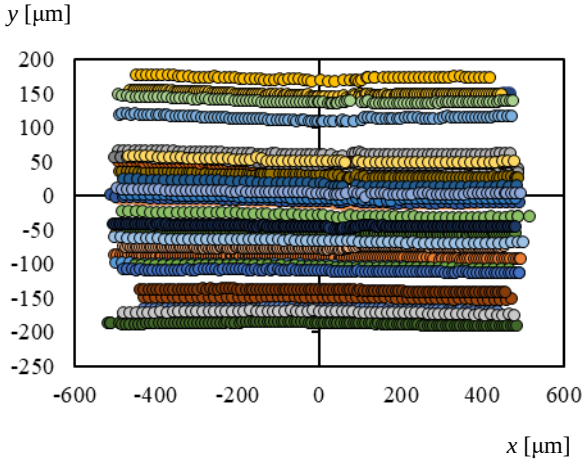

Fig. 5a: Tracings of movement of 20 cells [μm]: without electric field.

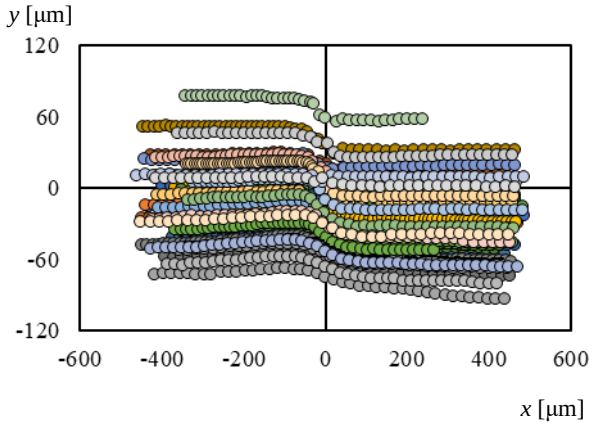

Fig. 5b: Tracings of movement of 20 cells [μm]: period of 0.3 μs, amplitude of ±15 V.

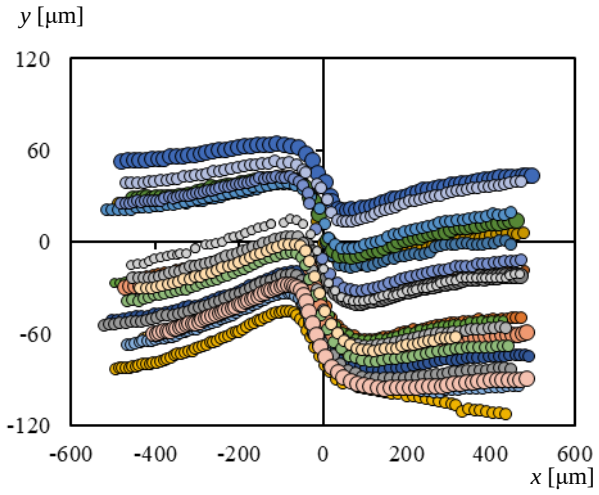

Fig. 5c: Tracings of movement of 20 cells [μm]: period of 0.25 μs, amplitude of ±15 V.

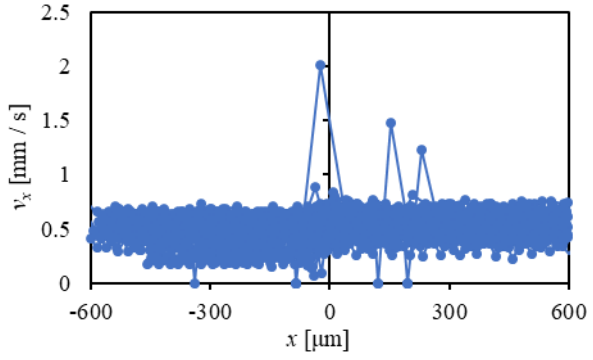

Fig. 6a: Velocity (v_x) tracings of 20 cells: period of 0.3 μs, amplitude of ±15 V.

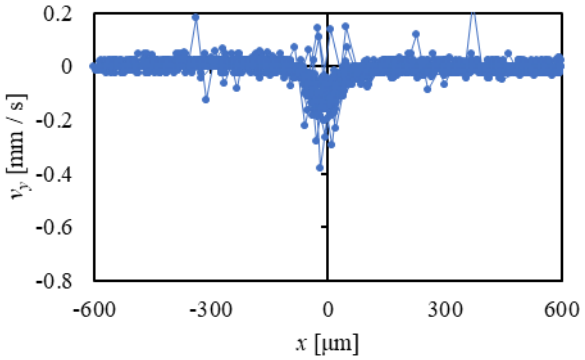

Fig. 6b: Velocity (v_y) tracings of 20 cells: period of 0.3 μs, amplitude of ±15 V.

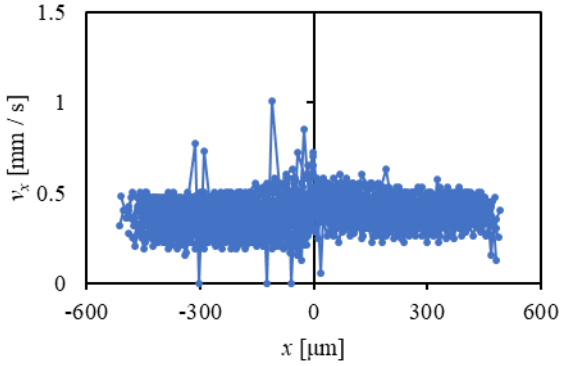

Fig. 7a: Velocity (v_x) tracings of 20 cells: period of 0.25 μs, amplitude of ±15 V.

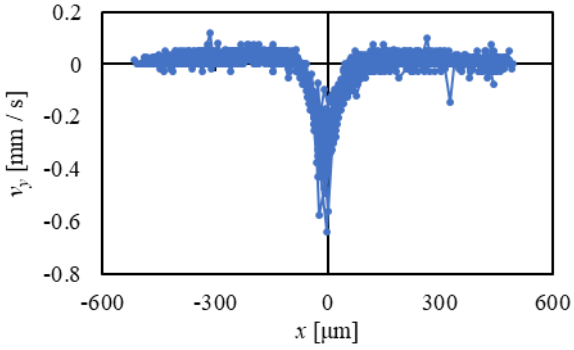

Fig. 7b: Velocity (v_y) tracings of 20 cells: period of 0.25 μs, amplitude of ±15 V.

Fig. 8 exemplifies the tracings of velocity (v_y) of three cells, which have variations on the radius of the cells. The period of rectangular cyclic wave is 0.25 μs. The absolute value of the peak velocity near the tip of the triangular electrode is higher at the bigger cell than at the smaller cell.

Fig. 9 shows the tracings of the acceleration (α_y) of 20 cells. The period of rectangular cyclic wave is 0.25 μs. The cells are accelerated to the apart direction (the direction perpendicular to the flow) from the tip of the triangular electrode before passing through the tip of the electrode, and decelerated after passing through the tip of the electrode.

Fig. 10 shows the relationship between the acceleration (α_y) and the radius (r) of the cell. The period of rectangular cyclic wave is 0.25 μs. The absolute value of the peak velocity near the tip of the triangular electrode increases with the radius of the cell.

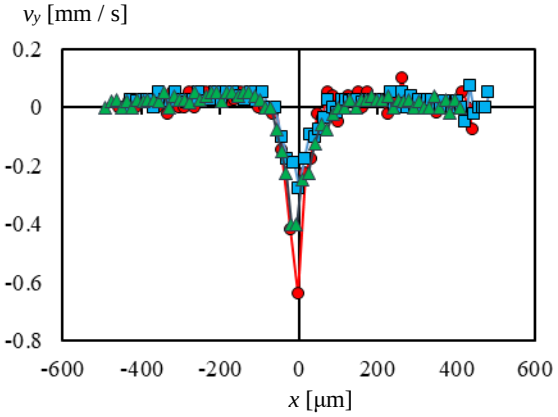

Fig. 8: Velocity (v_y) tracings: : 9.1 μm, y_0 = 22 μm, (circle), 6.6 μm, y_0 = 24 μm, (triangle), 5.9 μm, y_0 = 22 μm, (square): period of 0.25 μs, amplitude of ±15 V.

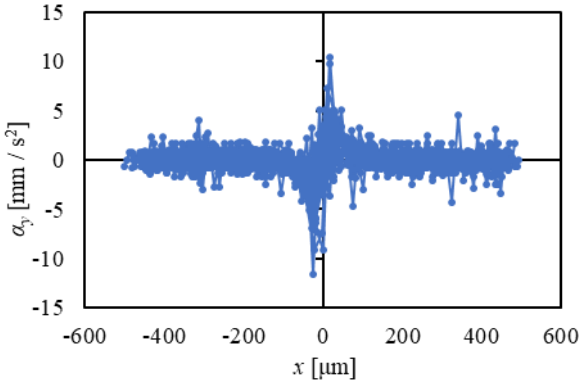

Fig. 9: Acceleration (α_y) tracings of 20 cells: 9.1 μm (circle), 6.6 μm (triangle), 5.9 μm (square): period of 0.25 μs, amplitude of ±15 V.

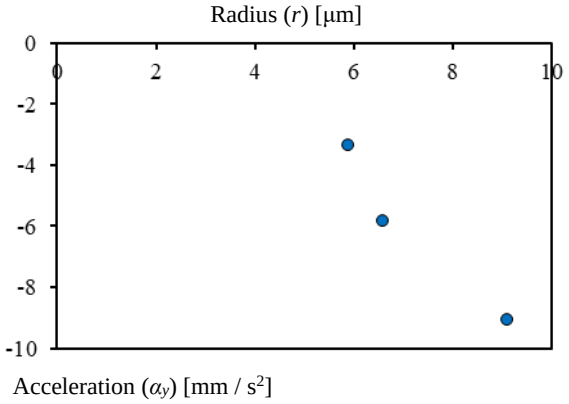

Fig. 10: Relationship between acceleration (α_y) and radius (r): period of 0.25 μs, amplitude of ±15 V.

4. DISCUSSION

The dielectrophoresis was applied to the cell sorting system as minimally invasive method in the previous studies [12, 28]. The shift distance [15, 26] has been very small (< 0.1 mm) in the present experiment. If the shift movement of the cell adjacent to the tip of the electrode is enlarged at the downstream by the inertial movement, the method can be applied to the cell sorting. The relationship between the streamline of the cell and the position of the side wall of the flow channel should be considered for the continuous movement of the cell at the downstream. To apply the present system for cell sorting, both the initial position and the velocity [29] should be controlled before the non-uniform electric field in the flow channel. In another way, the accumulated shift by the multiple electrodes might enlarged the shift movement of the cell [30].

In the present study, the dielectrophoretic movement of the cell has been tried to be observed by the optical microscope. The couple of electrodes arrangement with the shorter distance each other has not been designed, because the electrodes in the present study are not optically transparent [31]. The shift might depend on the passing route of the cell. The dielectrophoretic effect might be highest adjacent to the tip of the electrode [32–34].

The movement of cells between surface electrodes depends on the morphology of surface electrodes (the angle of the tip) [32, 33], which relates to non-uniformity of electric field. The higher slope of electric field with non-uniformity is necessary to enlarge the movement of cells around the electrode.

In principle, dielectrophoretic effect increases with the diameter of the particle [25]. The present experiment shows that the acceleration of the cell along the electric field (perpendicular to the flow direction) increases with the radius of the cell (Fig. 10).

The voltage between electrodes [13, 29, 35] higher than ±15 V might enlarge the shift. The amplitude of the electric stimulation has been limited within the threshold value to prevent electrolysis [23] in the present study.

Figs. 5–10 show the movement of each cell in the *x*-*y* plane. The movement of the direction (*z*) perpendicular to the flow is very small, because the height of the channel is 0.035 mm in the present experiment.

In the previous studies, dielectrophoresis was tried to be applied to the biological cell manipulation technology [12]. The micro grooves, on the other hand, were used for trapping of flowing cells in the previous study [9].

5. CONCLUSION

The dielectrophoretic movement of the floating C2C12 (mouse myoblast cell line) near the surface electrodes has been analyzed in the micro flow channel *in vitro*. A pair of asymmetric surface electrodes of titanium (thickness of 200 nm) were incorporated in the flow channel by the photolithography technique: a triangular electrode with the tip angle of 0.35 rad, and a rectangular electrode of the flat edge as reference. The rectangular cyclic alternating electric current (0.25 μs of period) was introduced between the surface electrodes. The experimental results show that the amplitude of the acceleration by the electric field, which is perpendicular to the flow direction, increases with the diameter of the cell.

ACKNOWLEDGMENT

The authors thank to Mr. Daisuke Hasegawa for his assistance of the experiment.

REFERENCES

[1] A. Tabll and H. Ismail, “Chapter 7: The Use of Flow Cytometric DNA Ploidy Analysis of Liver Biopsies in Liver Cirrhosis and Hepatocellular Carcinoma”, **Liver Biopsy**, Edited by H. Takahashi, IntechOpen Limted, 2011, pp. 88–107.

[2] S. Hashimoto, H. Sugimoto and H. Hino, “Effect of Couette Type of Shear Stress Field with Axial Shear Slope on Deformation and Migration of Cell: Comparison Between C2C12 and HUVEC”, **Journal of Systemics, Cybernetics and Informatics**, Vol. 17, No. 2, 2019, pp. 4–10.

[3] S. Hashimoto, Y. Endo, H. Sugimoto, Y. Kachi, S. Toma and R. Matsuzawa, “Cell Migration in Shear Field: Comparative Study between MC3T3-E1 and 3T3-L1”, **Proc. 23rd World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 4, 2019, pp. 88–93.

[4] S.M. McFaul, B.K. Lin and H. Ma, “Cell Separation Based on Size and Deformability Using Microfluidic Funnel Ratchets”, **Lab on a Chip**, Vol. 12, 2012, pp. 2369–2376.

[5] Y. Takahashi, S. Hashimoto, H. Hino and T. Azuma, “Design of Slit between Micro Cylindrical Pillars for Cell Sorting”, **Journal of Systemics, Cybernetics and Informatics**, Vol. 14, No. 6, 2016, pp. 8–14.

[6] S. Hashimoto, K. Kakishima and Y. Takahashi, “Cell Deformation Passing through Slit between Micro-Machined Surfaces in Vitro: Comparison among Cell Types”, **Proc. 23rd World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 4, 2019, pp. 100–105.

[7] M. Heijazian, W. Li and N.T. Nguyen, “Lab on a Chip for Continuous-flow Magnetic Cell Separation”, **Lab on a Chip**, Vol. 15, No. 4, 2015, pp. 959–970.

[8] H. Imasato and T. Yamakawa, “Measurement of Dielectrophoretic Force by Employing Controllable Gravitational Force”, **Journal of Electrophoresis**, Vol. 52, No. 1, 2008, pp. 1–8.

[9] Y. Takahashi, S. Hashimoto, H. Hino, A. Mizoi and N. Noguchi, “Micro Groove for Trapping of Flowing Cell”, **Journal of Systemics, Cybernetics and Informatics**, Vol. 13, No. 3, 2015, pp. 1–8.

[10] S. Hashimoto, A. Hayasaka and Y. Endo, “Sorting of Cells with Flow Channel: Movement of Flowing Myoblast Cell at Oblique Micro Grooves”, **Proc. 23rd World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 4, pp. 82-87, 2019.

[11] D.R. Gossett, W.M. Weaver, A.J. Mach, S.C. Hur, H.T.K. Tse, W. Lee, H. Amini and D. DiCarlo, “Label-free Cell Separation and Sorting in Microfluidic Systems”, **Analytical and Bioanalytical Chemistry**, Vol. 397, 2010, pp. 3249–3267.

[12] H. Shafiee, J.L. Caldwell, M.B. Sano and R.V. Davalos, “Contactless Dielectrophoresis: a New Technique for Cell Manipulation”, **Biomedical Microdevices**, Vol. 11, No. 5,

2009, pp. 997–1006.

[13] B. Yafouz, N.A. Kadri and F. Ibrahim, "Dielectrophoretic Manipulation and Separation of Microparticles Using Microarray Dot Electrodes", **Sensors**, Vol. 14, No. 4, 2014, pp. 6356–6369.

[14] P.R.C. Gascoyne, X.B. Wang, Y. Huang and F.F. Becker, "Dielectrophoretic Separation of Cancer Cells from Blood", **IEEE Transactions on Industry Applications**, Vol. 33, No. 3, 1997, pp. 670–678.

[15] N. Demierre, T. Braschler, R. Muller and P. Renaud, "Focusing and Continuous Separation of Cells in a Microfluidic Device Using Lateral Dielectrophoresis", **Sensors and Actuators B: Chemical**, Vol. 132, 2008, pp. 388–396.

[16] S. van den Driesche, V. Rao, D. Puchberger-Enengl, W. Witarski and M.J. Vellekoop, "Continuous Cell from Cell Separation by Traveling Wave Dielectrophoresis", **Sensors and Actuators B: Chemical**, Vol. 170, 2012, pp. 207–214.

[17] S. Fiedler, S.G. Shirley, T. Schnelle, and G. Fuhr, "Dielectrophoretic Sorting of Particles and Cells in a Microsystem", **Analytical Chemistry**, Vol. 70, No. 9, 1998 p. 1909–1915.

[18] B.H. Lapizco-Encinas, B.A. Simmons, E.B. Cummings and Y. Fintschenko, "Dielectrophoretic Concentration and Separation of Live and Dead Bacteria in an Array of Insulators", **Analytical Chemistry**, Vol. 76, No. 6, 2004, pp. 1571–1579.

[19] M.D. Vahey and J. Voldman, "An Equilibrium Method for Continuous-Flow Cell Sorting Using Dielectrophoresis", **Analytical Chemistry**, Vol. 80, No. 9, 2008, pp. 3135–3143.

[20] J. An, J. Lee, S.H. Lee, J. Park and B. Kim, "Separation of Malignant Human Breast Cancer Epithelial Cells from Healthy Epithelial Cells Using an Advanced Dielectrophoresis-activated Cell Sorter (DACS)", **Analytical and Bioanalytical Chemistry**, Vol. 394, No. 3, 2009, pp. 801–809.

[21] X. Chen, Y. Ren, W. Liu, X. Feng, Y. Jia, Y. Tao and H. Jiang, "A Simplified Microfluidic Device for Particle Separation with Two Consecutive Steps: Induced Charge Electro-osmotic Prefocusing and Dielectrophoretic Separation", **Analytical Chemistry**, Vol. 89, 2017, pp. 9583−9592.

[22] T. Müller, A. Pfennig, P. Klein, G. Gradl, M. Jäger and T. Schnelle, "The Potential of Dielectrophoresis for Single-Cell Experiments", **IEEE Engineering in Medicine and Biology Magazine**, No. 11/12, 2003, pp. 51–61.

[23] G. Mernier, N. Piacentini, T. Braschler, N. Demierrea and P. Renaud, "Continuous-flow Electrical Lysis Device with Integrated Control by Dielectrophoretic Cell Sorting", **Lab on a Chip**, Vol. 10, No. 16, 2010, pp. 2077–2082.

[24] Y. Kang, D-D. Li, S.A. Kalams and J.E. Eid, "DC-Dielectrophoretic Separation of Biological Cells by Size", **Biomedical Microdevices**, Vol. 10, No. 2, 2008, pp. 243–249.

[25] S. Hashimoto, D. Hasegawa, Y. Takahashi and R. Matsuzawa, "Design of Flow Channel with Surface Electrodes to Detect Dielectrophoretic Movement of Floating Myoblast", **Proc. 23rd World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 4, 2019, pp. 94–99.

[26] H.C. Shim, Y.K. Kwak, C.S. Han and S. Kim, "Effect of a Square Wave on an Assembly of Multi-walled Carbon Nanotubes Using AC Dielectrophoresis", **Physica E**, Vol. 41, 2009, pp. 1137–1142.

[27] B.H. Jo, L.M. Van Lerberghe, K.M. Motsegood and D.J. Beebe, "Three-Dimensional Micro-Channel Fabrication in Polydimethylsiloxane (PDMS) Elastomer", **Journal of Microelectromechanical Systems**, Vol. 9, No. 1, ,2000, pp.76–81.

[28] J. Yoshioka, Y. Ohsugi, T. Yoshitomi, T. Yasukawa, N. Sasaki and K. Yoshimoto, "Label-Free Rapid Separation and Enrichment of Bone Marrow-Derived Mesenchymal Stem Cells from a Heterogeneous Cell Mixture Using a Dielectrophoresis Device", **Sensors**, Vol. 18. No. 9, 3007, 2018, pp.1–8.

[29] J.G. Gao, R. Riahi, M.L.Y. Sina, S. Zhang and P.K. Wonga, "Electrokinetic Focusing and Separation of Mammalian Cells in Conductive Biological Fluids", **Analyst**, Vol. 137, No. 22, 2012, pp. 5215–5221.

[30] X. Xuan, B. Xu and D. Li, "Accelerated Particle Electrophoretic Motion and Separation in Converging-diverging Microchannels", **Analytical Chemistry**, Vol. 77, No. 14, 2005, pp. 4323–4328.

[31] Y. Takahashi, S. Hashimoto, R. Yamauchi, H. Hino and T. Yasuda, "Myoblast Behavior around Surface Electrodes in Flow Channel", **Proc. 21st World Multi-Conference on Systemics Cybernetics and Informatics**, Vol. 2, 2017, pp. 251–256.

[32] Y. Wakizaka, M. Hakoda and N. Shiragami, "Effect of Electrode Geometry on Dielectrophoretic Separation of Cells", **Biochemical Engineering Journal**, Vol. 20, No. 1, 2004, pp. 13–19.

[33] N. Demierre, T. Braschler, P. Linderholm, U. Seger, H. van Lintel and P. Renaud, "Characterization and Optimization of Liquid Electrodes for Lateral Dielectrophoresis", **Lab on a Chip**, Vol. 7, No. 3, 2007, pp. 355–365.

[34] Y. Takahashi, S. Hashimoto, M. Watanabe and D. Hasegawa, "Dielectrophoretic Movement of Cell around Surface Electrodes in Flow Channel", **Journal of Systemics Cybernetics and Informatics**, Vol. 16, No. 3, 2018, pp. 81–87.

[35] H. Chu, I. Doh and Y.H. Cho, "A Three-dimensional (3D) Particle Focusing Channel Using the Positive Dielectrophoresis (pDEP) Guided by a Dielectric Structure between Two Planar Electrodes", **Lab on a Chip**, Vol. 9, No. 5, 2009, pp. 686–691.