Cell Fusion and Distinction between Viable Cell and Non-Viable Cell Using Dielectrophoresis and Optical Tweezers

Yoshihiro Mizuta, Hayashi Takuya, Trang D. Nguyen, Kozo Taguchi
Ritsumeikan University
Kusatsu, Shiga, Japan

***Abstract*—A lot of researchers have studied cell fusion and manipulation during the past decades, and this field was pursued low cost and small setup. There is a lot of ways that can manipulate cell such as optical tweezers and dielectrophoresis (DEP). Particularly, optical tweezers can manipulate cells with non-contact and non-invasive so this technique was noticed in biotechnology. In this paper we introduce three techniques based on DEP and optical tweezers: Distinction between viable cells and non-viable cells using DEP, Manipulating cells captured by positive DEP (pDEP) using optical tweezers and PEG fusion combining optical tweezers and DEP. About cell fusion, we would suggest efficient PEG fusion that can fuse cell in selective. We are considering a variety of system combined DEP and optical tweezers now. The systems will be useful to perform cell operation and other applications.**

Keywords-optical tweezers; dielectrophoresis; yeast;protoplast; fusion lab-on-chip system

I. Introduction

Cell technology has been studied during the past decades, this field is raising expectations for new technology. Specifically, evaluation of cell using electricity or optics and gene manipulation were noticed by all over the world institution. Furthermore, researchers are pursuing more efficient, low cost and small setup now. In the background, cell manipulation using electric, magnetic and optics was researched. In 1970th, Ashkin discovered force that can capture microparticles when light irradiates it in focus point, and then he noticed gradient force and proposed basics of optical tweezers [1]. This technique realizes manipulating micro particles in three-dimensional space, and has been utilized biological systems since this technique discovered.

Dielectric particles were known to move in AC field. In 1950th, Pohl called this phenomena dielectrophoresis (DEP) [2]. From that time, theory of DEP has been investigated by several experiments and is applied applications in biological systems. DEP can manipulate particles using DEP force that are occurred when particles is placed in a non-uniform electric field, so that are used in biological applications such as separation, trapping and sorting [3].

In this paper we introduce three techniques based on DEP and optical tweezers: Distinction between viable cells and non-viable cells using DEP, Manipulating cells captured by positive DEP (pDEP) using optical tweezers and PEG fusion combining optical tweezers and DEP. DEP was performed by Au-thin film that made by easily ways. About manipulating cells captured by DEP using optical tweezers, we also examined relationship between voltage applied on the two electrodes of the DEP and the laser power output of the optical tweezers. About third technique, we suggest PEG fusion with combining optical tweezers and DEP in order to realize efficient cell fusion method. And then we would suggest a system combined both DEP and optical tweezers that would be a useful tool in biotechnology.

II. Principle

A. *Optical Tweezers*

In the past decades, the methods which can control and operate micro object with non-contact were studied. Optical tweezers is one of the important methods, and it is applies to manipulate microparticle and single cell with non-invasive and non-contact so it is important for biological objects [1]. Optical tweezers uses principle of momentum preservation, there are mainly two types in optical tweezers. The first is method using microscope objective lens, and second is using optical fiber.

B. *Dielectrophoresis*

As shown in Figure. 1, dielectric particle moves and is trapped between two electrodes with non-uniform AC field. This phenomenon is known to DEP, this technique is used to manipulate cell in biomechanics. Dielectric particles are affected a translational force as a consequence of the interaction of the polarization of the particle that are caused by non-uniform AC field. There are two types of movement by changing frequency of the applied electric field. Dielectric particles move toward the regions of strong electric field and attach to electrode in pDEP as shown in Figure.1 (a), while move toward the regions of low electric field in negative DEP (nDEP) as shown in Figure.1(b). Using pDEP, a particle is trapped to electrodes, while a particle repel from electrode using nDEP.

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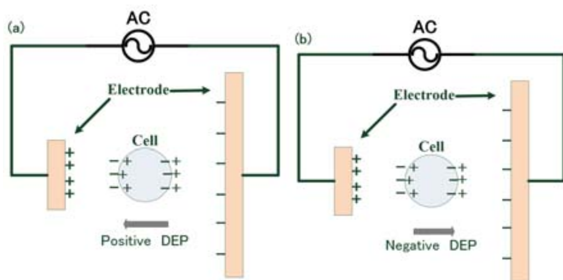

FIGURE I. (A) DIELECTRIC PARTICLE MOVES TO THE STRONG FIELD IN PDEP. (B) DIELECTRIC PARTICLE REPELS FROM THE STRONG FIELD IN NDEP.

C. *Fusion by Polyethylene Glycol (PEG)*

In the natural world, cell fusion occurred with various methods. Polyethylene glycol is mainly used by cell fusion. Cell fusion using PEG has two process, the first is that PEG make cell adhered with each other, and then the second is trigger of fusion cell. When we add PEG to cells, membranes are adhered by a positive and negative charge and cell fuse in some minutes. But probability of cell adhering is not good, that is disadvantage and mainly reason of being not fusion.

III. MATERIALS AND METHODS

A. *Yeast Cells and Protoplast of Plant*

In this experiment, yeast cells (BY4741 S.cerevisiae) were used in DEP. Cells are almost spherical and has diameter in the range of 5 to 10 µm. Non-viable cells of yeasts are prepared for separation system. Non-viable cells were obtained by a heat treatment of 80 degrees Celsius in 10 minutes. And they are stained with methylene blue. Methylene blue works only for non-viable cells, while viable cells maintain non-stained.

Protoplasts were prepared as reported the former research. The pieces of red cabbage were penetrated by enzyme containing Onozuka R-10, Macerozime R-10, Pectolyase Y-23, KCl, CaCl2 and 0.5 M mannitol in one hour. After removing surplus of red cabbage, that were centrifuged for 10 min and replace enzyme with 0.5M mannitol. So protoplasts of red cabbage were collected in the bottom. Viable protoplasts are stained with Fluorescein diacetate (FDA), that are observed by fluorescence microscope.

B. *Optical Tweezers Operation*

For microparticle manipulation, we assembled the optical tweezers setup. We used laser diode at 980nm (SNO534289, Lumics, Germany). It was focused using microscope immersion objective lens (100×/1.25, Edmund, USA). We prepared microsphere (10µm) and yeast (Saccharomyces cerevisiae) as target microparticle and confirmed optical tweezers operation. Target yeast and microsphere was trapped using focused laser beam with 30mW average laser power.

IV. EXPERIMENT

A. *Distinction between Viable Cells and Non-Viable Cells Using DEP*

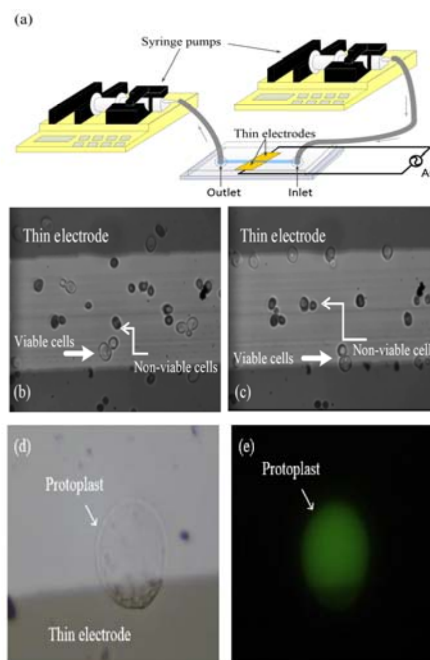

FIGURE II. (A) LAB-ON-CHIP SYSTEM EQUIPPED THIN ELECTRODE AND FLOW SEPARATION.(B) INCLUDE VIABLE CELLS(WHITE CELLS) AND NON-VIABLE CELLS STAINED WITH METHYLENE BLUE(BLACK CELLS). (C) ONLY VIABLE CELLS ATTACHED TO THE ELECTRODES. (D) PROTOPLAST WAS ATTACHED TO ELECTRODE BY PDEP. (E)PROTOPLAST ATTACHED WAS STAINED BY FDA.

In this experiment, we performed distinction between viable cell and non-viable cell using a lab-on-chip system. We made lab-on-chip equipped thin electrodes and flow separation as shown in Figure.2 (a) and concerned distinction with yeast cells and protoplasts of red cabbage. The lab-on-chip can perform pDEP with viable yeast cells in frequency range 300 kHz to 15 MHz, and they form pearl chain. While non-viable didn't show behaviour in frequency range 300 kHz to 15 MHz.So we can perform distinction between viable yeast cells and non-viable yeast cells using DEP that cause different behaviour.

First, yeast cells including both viable cells and non-viable cells stained with methylene blue were given pDEP. And then only viable cells attached to the electrodes. Second, we washed non-viable yeast cells away with syringe pumps applied 100 µl/m. The results are shown in Figure.2 (b) and (c). Viable cells keep attaching to the electrodes, while non-viable cells were flowed into syringe pump.

In case of protoplasts, viable cells were affected by pDEP and were attached to electrode in frequency range 20 kHz to 15 MHz as shown in Figure.2 (d).From Figure. 2 (e), protoplast attached was viable cell and we are considering possibility of distinction with protoplast now.

B. Manipulating Cells Captured By PDEP Using Optical Tweezers

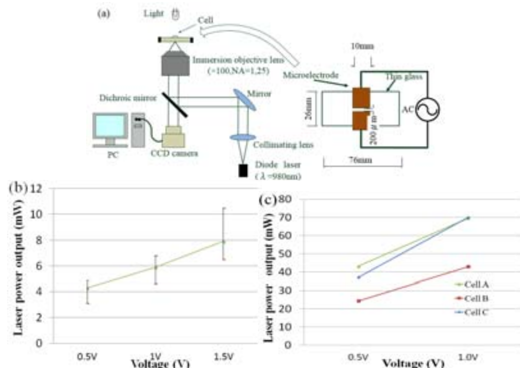

FIGURE III. (A) COMBINING OPTICAL TWEEZERS AND DEP EXPERIMENTAL SETUP. (B) RELATIONSHIP BETWEEN VOLTAGES THAT FUNCTION GENERATOR SUPPLIES TO DEP AND LASER POWER OUTPUT WITH YEAST CELLS. (C) RELATIONSHIP BETWEEN VOLTAGES THAT FUNCTION GENERATOR SUPPLIES TO DEP AND LASER POWER OUTPUT WITH PROTOPLASTS OF RED CABBAGE.

In the second section, we moved cells captured by pDEP using the optical tweezers. To manipulate cells attached electrode by pDEP would take advantage of applications such as cell isolation and fusion. And we examined relationship between voltage applied on the two electrodes of the DEP and the laser power output of the optical tweezers. According to the theory of DEP [4,5], DEP force is known to be in proportional to the applied voltage of the DEP. Trapping power of optical tweezers also turns strong when laser power output is increase. In optical tweezers, we used laser diode at 980nm and laser was focused by a micro-scope immersion objective lens as shown in Figure.3 (a) and then we choice yeast cells and protoplasts of red cabbage as target cells. In DEP, we made lab-on-chip equipped microelectrodes. First we carry out pDEP to target cells, the applied voltage was 0.5, 1.0 and 1.5 V peak to peak, and frequency of 500kHz. Second we repel viable cell from the electrode of the DEP using the optical tweezers. The result of our experiment clearly shows that the laser power of the optical tweezers was increased gradually when more and more voltage was applied, as shown in Figure.3 (b) and (c). These observations appear to match the theoretical formula of DEP force.

C. PEG Fusion Combining Optical Tweezers and DEP

In this experiment, we considered method of efficient cell fusion combining optical tweezers and DEP. First we checked whether PEG fusion was affected by laser in order to apply optical tweezers to cell fusion. Second we constructed a lab-on-chip system equipped flow chamber as shown in Figure.4 (a), suggest PEG fusion system of combing optical tweezers and DEP that can fuse cell in selectively.

As shown in Figure.5 (a) and (b), we add PEG to cell-cell adhered by DEP and cell-cell was irradiated by laser in 30 s. Fusion processes were demonstrated as shown in Figure.5 (c) and (d). According to performing cell fusion, cell fusion was unaffected by laser so that optical tweezers could be applied to cell fusion. Thus we considered PEG fusion system of combing optical tweezers and DEP that can fuse cell in selectively. First protoplasts of red cabbage were sprinkled on channel flowing mannitol with syringe pumps applied 20 µl/m as shown in Figure.4 (a).That setting lead to prevent backflow of cells into isolation room as shown in Figure.4 (b). Second two protoplasts were manipulated into isolation room equipped electrodes by optical tweezers with against the flow. When we were manipulating the second cell to isolation room using optical tweezers then, the first cell was being fixed electrode in isolation room by DEP as shown in Figure.6 (a). The second cell was moved near the first cell and they were adhered by DEP as shown in Figure.6 (b). Finally we added PEG to the solution and observed fusion process. In fact, the fusion progressed a little and the pair of protoplast wasn't fused in perfectly because efficiency of PEG was low. We need to consider other trigger of fusion without PEG. Now we are considering femtosecond laser as new trigger as shown in Figure.7. This method utilizes focused laser beam of ultrashort pulses to create pores on the cell membrane and subsequently induces cell fusion, such processes are called laser-induced cell fusion. Femtosecond laser was used for surgery and isolation as cell applications without inducing thermal pressure [6].

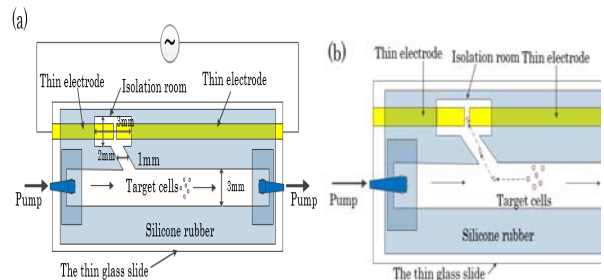

FIGURE IV. (A)LAB-ON-CHIP SYSTEM EQUIPPED FLOW CHAMBER.(B) ILLUSTRATION OF CELL MOVEMENT WITH OPTICAL TWEEZERS.

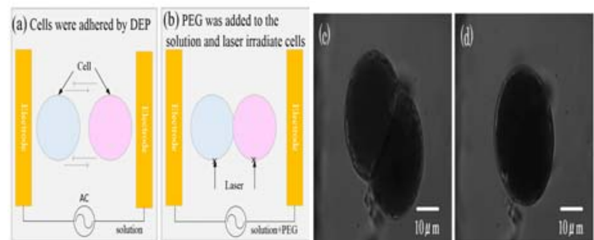

FIGURE V. CELL FUSION PROCEDURE; (A), (B), (C) AND (D). ACCORDING THIS EXPERIMENT,CELL FUSION WAS UNAFFECTED BY LASER.

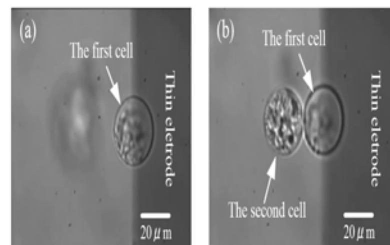

FIGURE VI. (A)THE FIRST CELL MANIPULATED WAS FIXED BY DEP. (B) THE SECOND CELL WAS MANIPULATED NEAR THE FIRST CELL BY OPTICAL TWEEZERS AND THEY WARE ADHERED BY DEP.

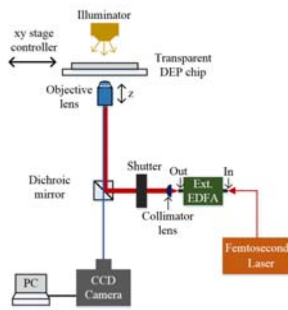

FIGURE VII. SETUP OF THE EFFICIENT CELL FUSION SYSTEM USING DEP AND FEMTOSECOND LASER.

V. CONCLUSION

Using DEP and optical tweezers, we can perform distinction between viable cells and non-viable cells with yeast cells and can repel cells captured by DEP as mentioned above. DEP can distinct between viable cells and non-viable cells easily, moreover we are considering distinction not yeast cells but also protoplast of red cabbage. About cell fusion combing DEP and optical tweezers, the fusion progressed a little but the cell wasn't fused in perfectly. This mainly reason is PEG, so we must think other trigger of fusion without PEG. From cell fusion unaffected by laser, optical tweezers could be applied to cell fusion and could manipulate only viable cells at will. From this study, we showed probabilities of experiments combined DEP and optical tweezers, furthermore we are deeply engaged in the lab-on-chip combing DEP and optical tweezers. Then we make lab-on-chip system that is applied to other cells without yeast cells and protoplast. For example, firstly we carried out distinction of using pDEP, we could get only activity cells. And then we can remove non-viable cells with microfluidic system equipped syringe pump. Then that activity cells are manipulated by optical tweezers of being low power, after that we could perform cell operation, cell transfection and fusion changing high power. As the above account thoughts, lab-on-chip system equipped both DEP and optical tweezers would have a lot of expectations in cell applications.

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REFERENCES

[1] Ashkin, Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime, Biophysical Jounal, 61,pp.569-582,1992.

[2] Herbert A. Pohl&Joe S. Crane, Dielectrophoresis of Cell, 11, pp.711–727, 1971.

[3] H. Morgan, M. P. Hughes, &N. G. Green, Separation of Submicron Bioparticles by Dielectrophoresis, 77, Issue 1, pp.516–525, 1991.

[4] [W.J. Liu, J. Zhang, L.J.Wan, K.W. Jiang, B.R. Tao, H.L. Li,W.L. Gong,&X.D. Tang, Dielectrophoretic manipulation of nano-materials and its application to micro/nano-sensors, 133, Issue 2, pp. 664–670, 2008.

[5] Zhang, K. Khoshmanesh, A. Mitchell,&K. Kalantar-zadeh, Dielectrophoresis for manipulation of micro/nano particles in microfluidic systems, 396, pp 401-420, 2010.

[6] Uchugonova, M. Lessel, S. Nietzsche, C. Zeitz, K.Jacobs, et al, Nanosurgery of cells and chromosomes using near-infrared twelve-femtosecond laser pulses, J. Biomed. Opt. 17, pp. 101502, 2012.