Cellular Thermal Measurement by Dielectrophoresis and the Impedance Changing between Electrodes

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***Abstract*—Measuring the cellular thermal measurement is demanded in the area of biology and medicine because cellular functions are concerned with intracellular temperature. Also, dielectrophoresis impedance measurement method has recently attracted attention because the method is simple and immediately. In prior studies, it has been considered to change the impedance between electrodes due to the short-circuiting by pearl chain between electrodes [1], [2]. In this paper, we inspect impedance measurement by thermal changes under various situations and demonstrated that the impedance changes by heating even without short-circuiting by pearl chain.**

***Index Terms*—dielectrophresis, impedance measurement, plant protoplast**

I. Introduction

Dielectrophoresis (DEP) is developed recently, and there are a lot of applications and methods. For example, it can sort yeast cell which is viable and non-viable cell by DEP force [3]. This is because viable and non-viable cell has different frequency dependence for different permittivity and conductivity. Also, contactless dielectrophoresis (cDEP) is a recently developed method of cell manipulation in which the electrodes are physically isolated from the sample. In this method, an electric field is crated in the sample micro channel using electrodes inserted into two conductive micro chambers, which are separated from the sample channel by thin insulating barriers [4].

The concept of this paper is to measure cellular temperature. This technique can be used many applications in the various field such as biology, medicine, food and beverage industries. In biology field, it is expected to analysis of the cellular metabolic mechanism because cellular functions are concerned with cellular temperature. In medicine field, it is known that cancer cell is weak against heat compared with normal cell. Cellular temperature measurement shows promise for the new way to diagnose cancer and effective hyperthermia therapy. Also, we have already demonstrated to measure cellular thermal changes by dielectrophoresis [5]. It can be precisely measured through measuring the impedance changes between the electrodes. This is because the cells are trapped electrodes and are short-circuiting by pearl chain. Cell acts as the capacitor and can be measured impedance changes when the temperature changes. This paper is considered more deeply about the phenomenon of thermal changing by DEPIM. In this research, we demonstrated that impedance changes are happen without short-circuiting pearl chain by the wide gap electrodes.

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II. Theory

A. Dielectrophoresis

Dielectrophoresis (DEP) is the force which moves micro particles such as a biological cell toward high electric field strengths region under non-uniform electric field. It was described by Pohl in 1952, and was the movement of particles such as a biological cell which was electrically neutral. If it is assumed that plant protoplasts are spherical structures, the time-averaged DEP force applied on them is calculated as below [6]:

$$F_{DEP} = 2\pi a^3 e_m \mathrm{Re}[K(\omega)]\nabla(E^2) \quad (1)$$

where a is the radius of the particles and E is the electric filed; $\nabla(E^2)$ is the gradient of the acquired electric field; $K(\omega)$ is the real part of Clausius-Mosotti (CM) factor.

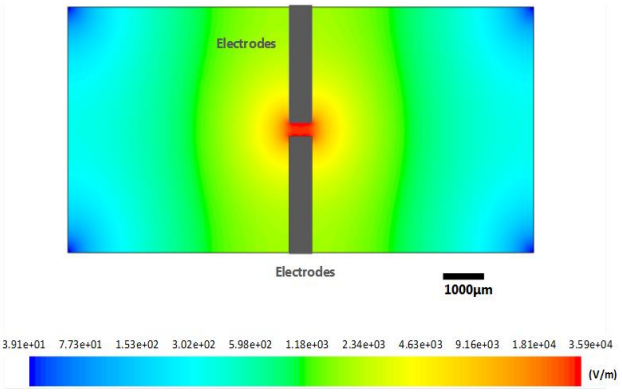

Figure 1. The electric field distribution in the rod-shaped wide gap electrodes.

Fig. 1 is the contours of E produced by the rod-shaped wide gap electrodes at 10Vp-p, obtained numerical simulation. The blue colour shows the weakest electric field and red one is the strongest electric field. The gap

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width is about 300μm and electrode width is about 500μm; it is same scale used in the experiment. The electric field distribution in the rod-shaped wide gap electrodes is depicted in Fig. 1. High electric field is generated in the gap between adjusting electrodes, especially near the electrode edge.

B. Fabrication of the Measurement Chip

In this paper, we created a micro measurement chip using DEP which was previously reported about how to fabricate. Fig. 2 shows schematic drawing of the micro measurement chip. It consists of two micro-electrodes, a peltier device, and a thermistor. Thermistor is a type of resistor whose resistance varies sensitively with temperature changes. We also used a peltier device to heat the cells by controlling the current with a DC power supply. The thermistor's resistance decreases when the temperature rises.

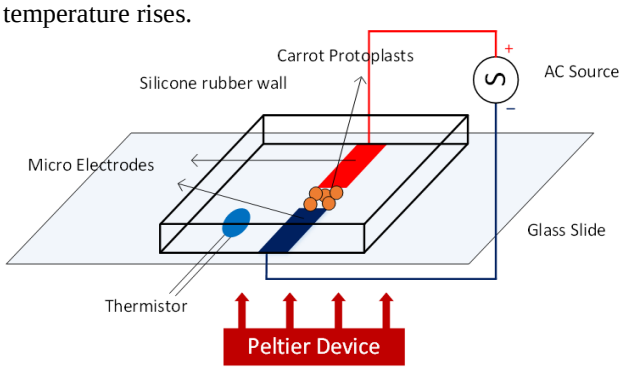


Figure 2. The micro measurement chip using the DEP.

C. Cellular Thermal Measurement by DEPIM

In general, plant protoplasts are trapped on the electrodes when applied AC voltage to the electrodes. Then cells stand in lines along the electric field and they form pearl chains. The impedance between electrodes changes by the pearl chain's condition and it is already confirmed to depend on the density of the cell in the suspension. Fig. 3 shows the schematic of the biological cell model placed inside a DEP chip.

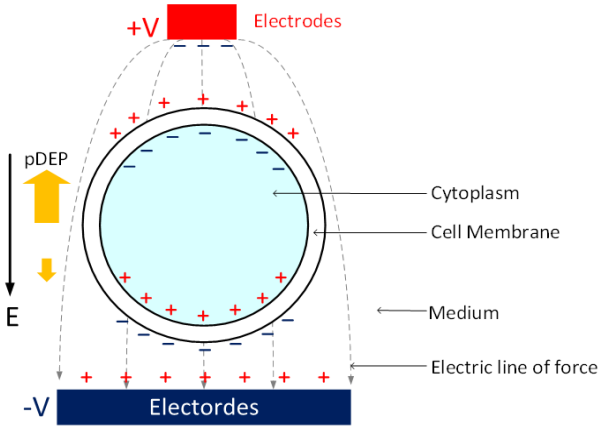


Figure 3. Schematic of the biological cell model placed inside a DEP chip.

Fig. 4 shows the change of shunt voltage when applying heat by peltier device and the microscope image of the electrodes with cells. When applying non-uniform high frequency alternating electric field to the biological cell, it moves toward high electric field strength regions. This phenomenon is called positive DEP (pDEP), while negative DEP repels the cell from high electric field strength regions. Then, the medium, which has lower conductivity, induces charges on the other side of the cell membrane. This phenomenon causes the cell membrane to act as a capacitor when the applied potential difference appears across the cell membrane [7]. Because the capacitor has thermal property, the cell membrane has thermal property as well. Therefore, as cells are trapped by pDEP, we can measure precisely cellular temperature through measuring the impedance changes between the two electrodes of the dielectrophoresis (DEP) chip. For the method of to measure the impedance between the electrodes, we used a lock-in amplifier. It measured current passing through the pDEP microelectrode via shunt resistance (500Ω), and the data were transferred to the PC to calculate the impedance change [8].

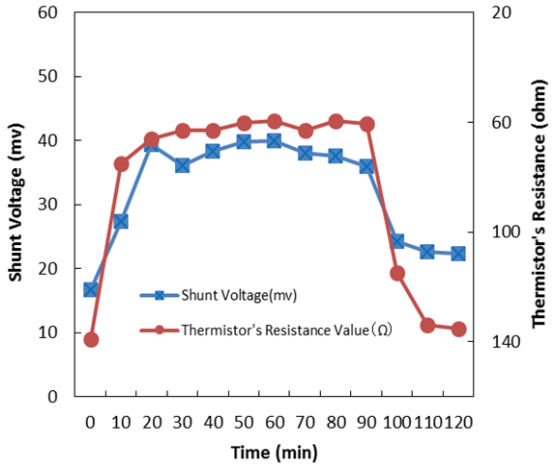


Figure 4(a). The changes of shunt voltage when applying heat by peltier device.

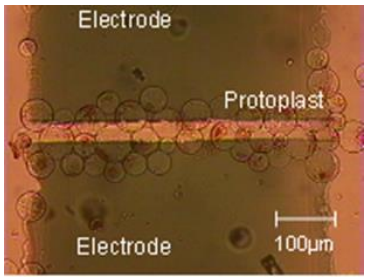


Figure 4(b). Microscope image of the electrodes with cell.

Fig. 4 shows changes of shunt voltage when we applied heat to the chip with 0.8A current through the peltier device. The blue line shows shunt voltage changes versus time and red line is thermistor's resistance value as the temperature changes in the suspension. The blue line shows shunt voltage changes versus time and red line is thermistor's resistance value as the temperature changes in the suspension. The thermistor's resistance was stable after about 20 minutes. The temperature at that time was about 40 ℃. As a result, they show the shunt voltage rose with the increase in temperature and it fell with the decrease in temperature without time-delay even if the

number of trapped cell doesn't change. This shows that cell has the thermal property. Meanwhile, we were confirmed no change in the shunt voltage when cells were not trapped between the electrodes. Plant protoplast is a cell which doesn't have cell wall. In this paper, we prepared carrot protoplast as the target cell because it is used for basic experiment field of biology and medicine such as cell fusion. It is previously reported about how to make carrot protoplast. Also, we are used mannitol as the cell suspension which was adjusted to 0.7M as well.

III. Result and Discussion

A. Cellular Thermal Measurement by the Heating of Two Steps

We measured shunt voltage as the impedance change between the electrodes. It is the same way as previously method about the observing method on electrodes, electric circuits, and analyser [5]. However, we measured by changing the pattern of heating and the distance of the electrodes gap. We measured by changing pattern of heating which is the heating of two steps.

As the experimental method, carrot protoplasts were put onto the micro measurement chip. Afterward, cells were trapped when we applied 100 kHz, 5Vpp by the AC source. After about 20min the cells were trapped well, we begin heating the cells by turning on the peltier device for 30 minutes which was applied 0.6A. Afterward, we applied 0.9A to the peltier device 30 minutes as well. We drew the graphs that show the changing of shunt voltage and temperature in total 60 minutes, measured since the moment of turning on the peltier device.

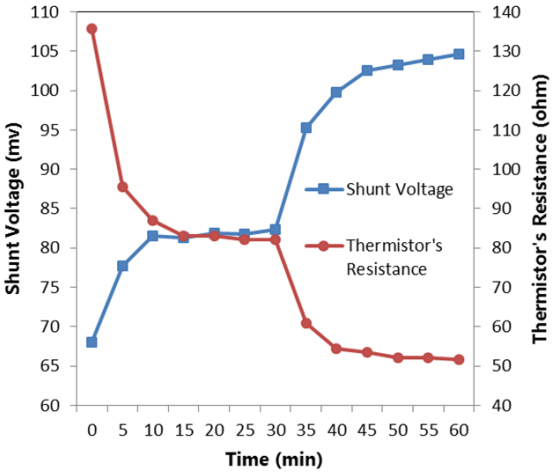


Figure 5. The changes of shunt voltage when applying heat by peltier device.

As a result, when the Fig. 5 shows the shunt voltage rose with the increase in temperature without time-delay by heating of two steps. This graph shows the 20 ℃ at the beginning time as the room temperature. After 30 minutes from turn on the peltier, temperature is about 33 ℃. After 60 minutes from turn on the peltier, temperature is about 45 ℃. This shows that impedance changes is happen when the temperature changes at the different temperature zone. Also, we confirmed that the impedance changes don't happen without cell.

B. Cellular Thermal Measurement by the Wide Gap Electrodes

We measured shunt voltage as the impedance change between the electrodes. We create new electrode which have wide distance between the electrodes. The gap width is about 300μm and electrode width is about 500μm. This is because to check the impedance changes can be measured without short-circuiting by pearl chain of the protoplast.

As the experimental method, carrot protoplasts were put onto the chip and applied AC source for 20 minutes as well. Afterward, we applied 0.7A to the peltier device for 10 minutes. We drew the graphs that show the change of shunt voltage and temperature in total 20 minutes, measured since the moment of turning on the peltier device as well. We repeated three times similar experiments at different density of the cell in the suspension. Finally, we drew the result of each to the graphs.

Fig. 6(a) shows the microscope images of the wide gap electrodes. The gap width is about 300μm and electrodes width is about 500μm. Fig. 6(b) and Fig. 6(c) are after 10minutes from turn on the peltier device. They are added each protoplasts 20, 40μl and we use 0.7M mannitol as the suspension. As the results of Fig. 6(b), some protoplasts are trapped between the electrodes by pDEP force. It forms line by pearl chain. However it isn't short-circuiting as well. As shown in Fig. 6(c), a lot of cells are trapped between the electrodes, and it has some pearl chains and short-circuiting completely. At each situation, we confirmed the number of trapped cell was different by density of the cell suspension.

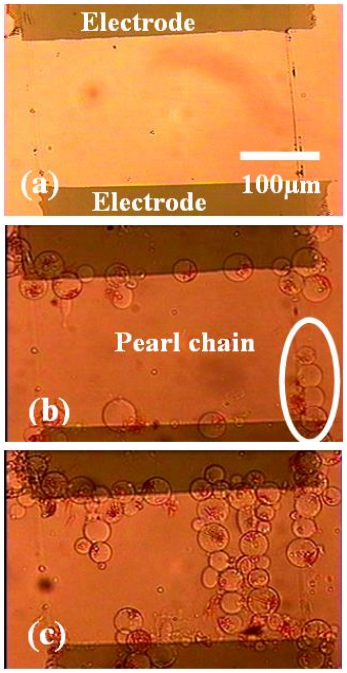
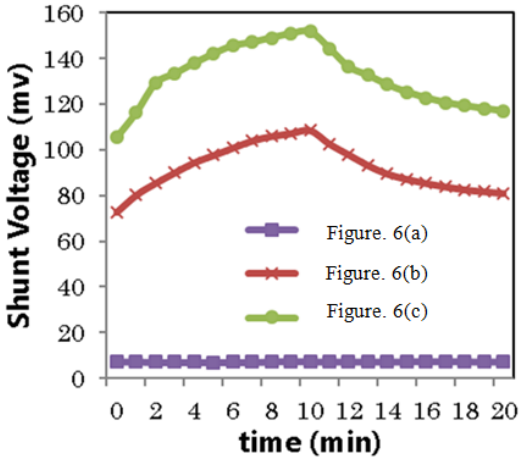


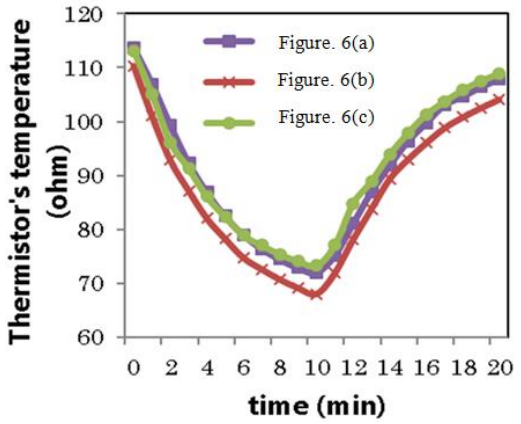
Figure 6. Microscope images of the electrodes (a) without cell, (b) with cells 40μl, (c) with cells 80μl.

Fig. 7(i) shows the changes of shunt voltage at each situation when applying heat by peltier device. When the Fig. 6(a)'s situation, cells were not trapped between the

electrodes, so the shunt voltage didn't change. And when the Fig. 6(b, c)'s situation, cells were trapped, and the shunt voltage changed with temperature changes. Fig. 7(ii) shows the changes of thermistor's resistance which is depend on the temperature changes. At each situation, we applied 0.7A to the peltier device. The graph shows 20℃ at the beginning time as the room temperature. As the results, the temperature was about 35℃ after 10 minutes from turned on the petier device at each situation. After that we turned off the peltier device and the temperature decreased and got back to room temperature.



(i)



(ii)

Figure 7. The changes of the (i) shunt v oltage and (ii) temperature when applying heat by peltier device.

IV. Conclusions

In precisely paper, we demonstrated that the cellular thermal changes could be measured by observing the change in shunt voltage. In this paper, we examined carefully about cellular thermal measurement by DEP. We demonstrated that the amount of the voltage changes depended on the number of trapped cells between the electrodes. Also, we could verify that the impedance changed without short-circuiting by the pearl chain. It is expected to measure single cellular measurement by DEP. In the future, we want to measure single cellular thermal measurement with isolation by optical tweezers.

Acknowledgmnt

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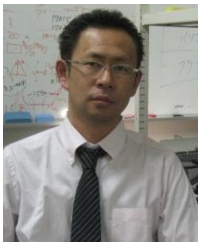

Kozo Taguchi was born in Kyoto, Japan, on December 18, 1968. He received the B.E., M.E., and Dr. Eng. Degrees in electrical engineering from Ritsumeikan University, Kyoto, Japan, in 1991, 1993, and 1996, respectively. In 1996, he joined Fukuyama University, Hiroshima, Japan, where he had been engaged in research and development on the optical fiber trapping system, semiconductor ring lasers and their application for optoelectronics devices, and polymeric optical waveguides for optical interconnection. In 1996-2003, he worked as an assistant and lecturer in Fukuyama University. In 2003, he moved to Ritsumeikan University, Shiga, Japan, and currently he is a professor of department of electric and electronic engineering. From 2006 to 2007, he was a visiting professor at University of St Andrews (Scotland, United Kingdom). From 2014 to 2015, he was a visiting professor at Nanyang Technological University (Singapore). His current research interests include cells trap, microfluidic cell based devices, dye sensitized solar cell, biofuel cells. Dr. Taguchi is a member of the SPIE.