Cellular Thermal Measurement and Characteristic Analysis of Yeast Cells by Dielectrophoresis

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***Abstract*—In this paper we measured the cellular thermal changes by dielectrophoresis when we used yeast cell for the target cell. First, we conformed about heat damage evaluation of the yeast cells in the suspension. This is because to check the effectiveness of our proposed experimental systems and our electrodes. As a result, it was found that cells activity could be checked from shunt voltage changes. Second, we conformed about cellular thermal measurement using yeast cell. As the results, yeast cell was trapped between the electrodes by the DEP force and shunt voltage changes were measured as the impedance changes between the electrodes. Shunt voltage increased with the temperature rise, and decreased with the temperature drop. In addition, this phenomenon was confirmed only when the cells have been trapped. Also, when the number of trapped cell increased, voltage changes became larger.**

***Index Terms*—dielectrophoresis, impedance measurement, yeast cell, protoplast, cellular thermal measurement**

I. Introduction

In the field of food and beverage, it is important to measure the intracellular temperature. Yeast cells are used commonly for manufacture of food and beverage such as beer, bread, and so on. Various studies have been made about the method on how to measure the intracellular temperature [1]-[3]. This is because to control temperature of the cell is more productive and warranty of quality control in the food and beverage industry. Furthermore, measuring the intracellular temperature is demanded in the area of biology and medicine because cellular functions are concerned with intracellular temperature.

On the other hand, dielectrophoresis is developed recently, and using for various application and methods in the world as well. For example, it can sort cell which is viable and non-viable cell by DEP force [4]-[8]. This is because viable and non-viable cell has different frequency dependence for different permittivity and conductivity. Also, DEPIM (Dielectrophoresis impedance measurement) has recently attracted and used for cellular activity measurement using impedance changes by short-circuiting of pearl chain [9], [10].

In prior studies we focused on DEPIM as a thermal measurement technique. In the case of carrot protoplasts, it has been identified that the cellular impedance changes due to the temperature change [11], [12].

In this paper, we inspected impedance measurement of the cell which has not only cell membrane but also wall. As the target of the cell, we prepared yeast cells. In addition, we inspected extra experiment that is to measure cellular activity in the suspension by yeast cell.

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 cells are spherical structures, the time-averaged DEP force applied on them is calculated as below [13]:

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

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

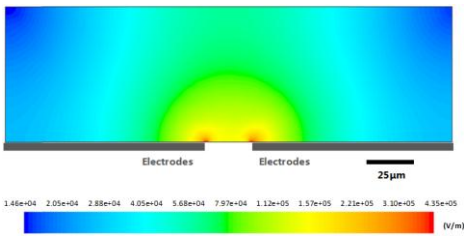

Figure 1. The electric field distribution in the electrodes.

Fig. 1 is the contours of E produced by the plane double electrodes at 10Vp-p, obtained numerical simulation. The blue color shows the weakest electric field and red one is the strongest electric field. The gap width is about 25μm and the thickness of the electrodes is about 100nm; it is same scale used in the experiment. The electric field distribution in the electrodes is depicted in Fig. 1. High electric field is generated in the gap between adjusting electrodes, especially near the electrode edge.

B. Cellular Thermal Measurement by DEPIM

In general, cell is trapped on the electrodes when applied AC voltage to the electrodes. Then cells stand in

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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. 2 shows the schematic of the biological cell model placed inside a DEP chip. 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 [14]. 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 [15].

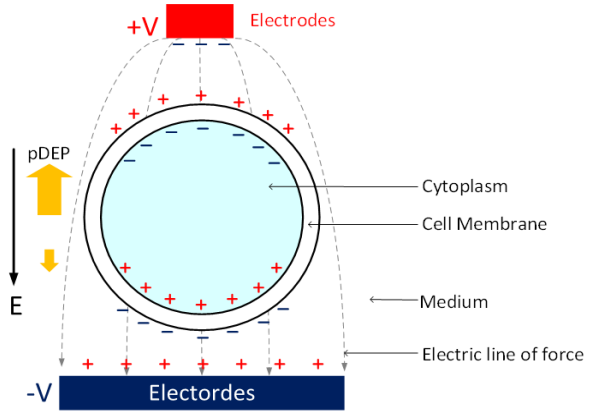


Figure 2. Schematic of the cell model placed inside a DEP chip.

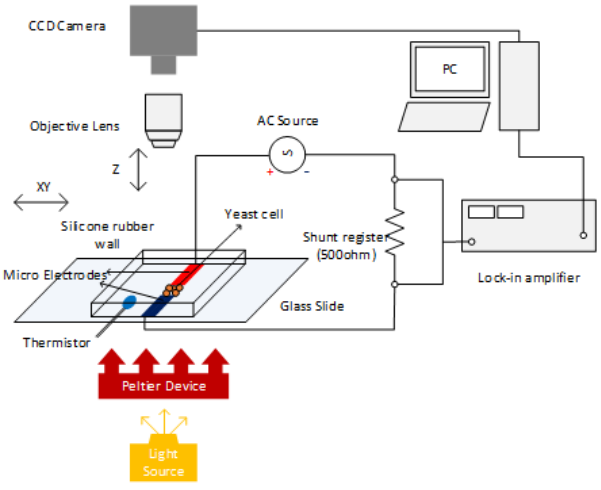


Figure 3. The micro measurement chip using DEP and the measurement circuit.

III. Experimental Set up

Fig. 3 depicts an outline of DEPIM (Dielectrophoresis impedance measurement) system. The circuit is built with the electrodes, an AC source, and a shunt resistor (500ohm) connected in series. Using a lock-in amplifier, the value of the voltage changes across the shunt resistor is taken continuously with a high degree of accuracy. The electrode gap impedance can be calculated from the values taken. As the target cell, yeast cell is prepared in this research.

First, we conformed about heat damage evaluation of the yeast cells in the suspension. This is because to check the effectiveness of experimental systems and electrodes.

In prior studies we prepared three types of micro tubes (30, 50, 80℃).

In this paper, we investigated more deeply in the five types of tubes. As the experimental method, we prepared yeast cells because these were die about 80℃. We prepared 5 micro tubes with yeast cells which were applied differences heat damage (30, 40 50, 60, 70, 80℃ for 15min). It is into the constant temperature bath at each temperature for 15min to process heat damage. After heat damage, cells were put onto the micro measurement chip. At this time, the temperature in the suspension is room temperature. Afterward, cells were trapped by the pDEP when we applied 200 kHz, 5Vpp by the AC source. After about 15 min the cells were trapped well, we checked the shunt voltage each other. Afterward, we drew the graphs which were performed normalization like initial value of the shunt voltage is set to 1. In this experiment, peltier device and thermistor is not used.

The next step, we tried to measure the cellular temperature measurement using DEPIM. We applied heat to the cells by the peltier device and measure the temperature through the thermistor's resistance. We applied heat to the cells by the peltier device and measured the temperature through the thermistor's resistance. As the experimental method, yeast cell was put onto the chip and applied AC source for 10 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 four times similar experiments at different density of the cell in the suspension such as with "Milli-Q", "MilliQ+YPD 40μl", "MilliQ+yeast 40μl", "MilliQ+yeast 200μl". "MilliQ" is the ultra pure water and "YPD" is the culture solution of yeast cell. Also, "yeast" includes yeast cell and YPD solution. We drew each result to the graphs as well.

IV. Result and Discussion

A. Heat Damage Evaluation of Yeast Cell

Fig. 4 shows the microscope images of electrodes and cells which is processed heat damage or not. Each figure was taken after 15min from applied 200 kHz, 5Vpp by the AC source. Fig. 4(a) is applied heat damage at 30℃ for 15min. In this situation, cells aren't applied heat damage and they are fresh and healthy, so almost cells are trapped on the electrodes by pDEP. In Fig. 4(b), almost cells died and cells were not trapped on the electrodes. These results show only viable cells were trapped on the electrodes and it can sort the viable and non-viable cell by the differences of dielectric constant.

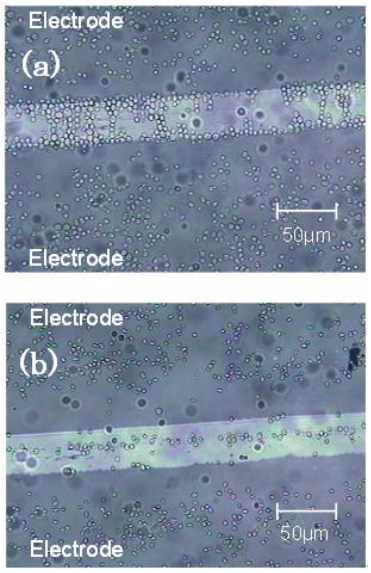


Figure 4. Microscope images of electrodes and cells, (a) at 30 ℃ for 15min. (b) at 80 ℃ for 15min.

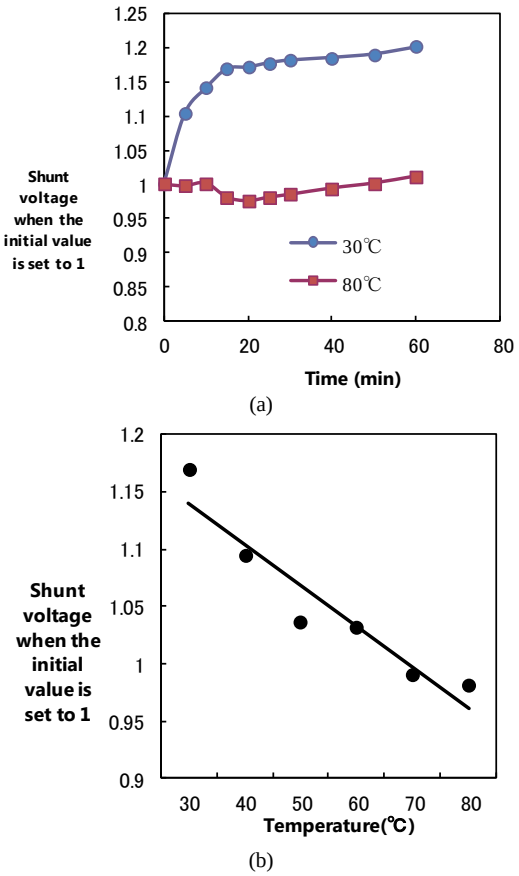


Figure 5. The relationship of shunt voltage when the initial value is set to 1 of each situation, (a) time and shunt voltage, (b) temperature and shunt voltage.

Fig. 5(a) shows the relationship of shunt voltage when the initial value is set to 1 of each situation for 60min. This graph shows the fresh cell rose shunt voltage with time elapsed, and the cell which is applied heat damage didn't change the shunt voltage from the initial value.

In addition, we inspected the cell activity changes with each situation. We prepared micro tube which is into the yeast cell and damaged 30, 40, 50, 60, 70, 80℃ each other. We applied 200 kHz, 5Vpp and trapped cell between the electrodes for 15min. After about 15 min the cells were trapped well, we checked the shunt voltage in each situation and drew the graph which is performed normalization like initial value of the shunt voltage is set to 1. Fig. 5(b) shows the relationship of temperature and shunt voltage when the initial value is set to 1 of each situation. It was measured after trapped 15min by pDEP in each situation.

As the results, the applied heat damage is increased and shunt voltage changes was decreased. From these experimental results, it was found that cells activity could be checked from shunt voltage changes.

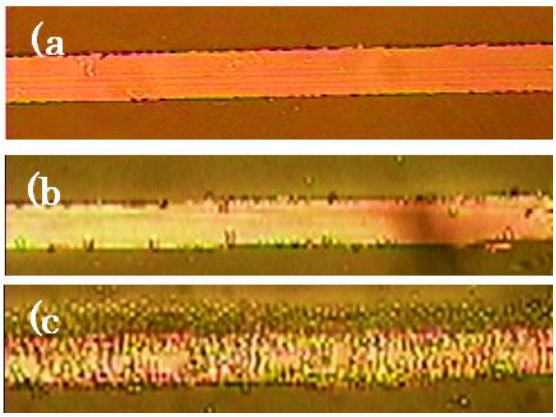


Figure 6. Note Microscope images of the electrodes (a) with MilliQ, (b) with MilliQ+Yeast 40μl, (c) with MilliQ+Yeast 200μl.

Fig. 6(a) is the microscope image of the plane electrodes. The gap width is about 25μm and electrodes width is about 15mm. The electrodes trapped nothing like this picture when we used MilliQ and MilliQ+YPD 40μl as the solution. Fig. 6(b) and Fig. 6(c) are the microscope images after 10 minutes from turn on the peltier device each other. They are added each yeast cell 40μl and 200μl. In addition, YPD solution has been mixed as the culture as well. As the result, Fig. 6(b) trapped some yeast cells between the electrodes by pDEP force. However it isn't short-circuiting as well. Fig. 6(c) trapped a lot of cells between the electrodes, and it is short-circuiting completely. At each situation, we conformed the number of trapped cell was different by density of the cell suspension.

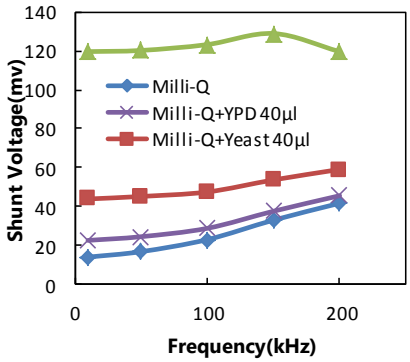


Figure 7. The characteristics in capturing the number of yeast by frequency and shunt voltage.

B. Cellular Thermal Measurement Using Yeast Cell

Fig. 7 shows the characteristics in capturing the number of yeast by frequency and shunt voltage. This graph shows the change in shunt voltage with respect to the frequency. In each of the frequency band, shunt voltage showed a high value when cell are trapped much.

Fig. 8 shows the thermal characteristic of the thermistor. It shows that the thermistor's resistance decreases when the temperature rises.

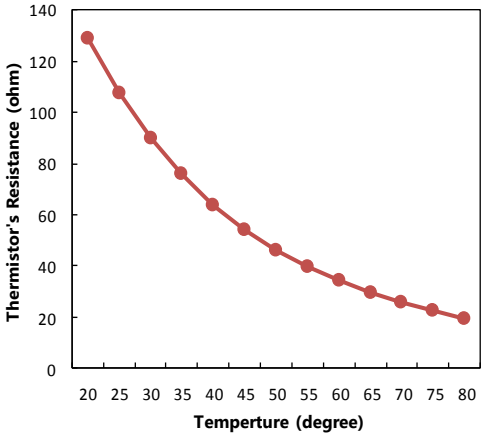
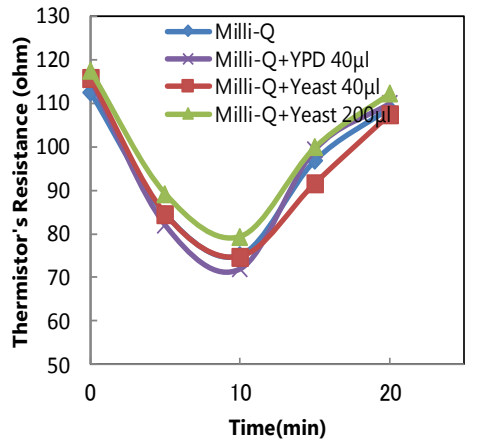
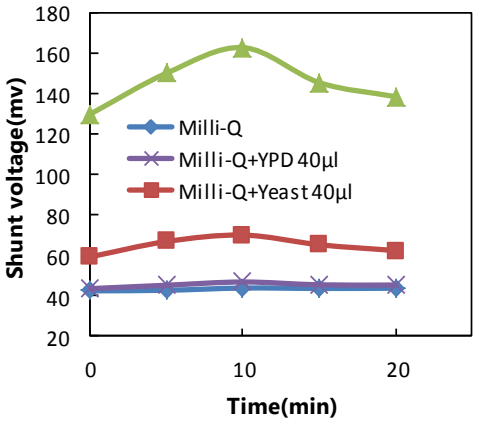


Figure 8. The thermal property of the thermistor.



(a)



(b)

Figure 9. The changes of the (a) temperature and (b) shunt voltage when applying heat by peltier device.

Fig. 9(a) 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 about 20℃ at the beginning time as the room temperature. As the results, the temperature was about 35℃ after 10 mitutes 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.

Fig. 9(b) shows the changes of shunt voltage at each situation when applying heat by peltier device. When the situation of "MilliQ", and "MilliQ+YPD 40µl", cells were not trapped between the electrodes, the shunt voltage didn't change. And when the situation of "MilliQ+yeast 40µl", and "MilliQ+yeast 200µl", cells were trapped, the shunt voltage changed with temperature changes. In addition, it shows that voltage change increases when the number of trapped cell is increased. This shows the shunt voltage depend on the number of trapped cells and the temperature changes.

V. Conclusion

In this paper we inspected two experiments. First, we demonstrated carefully about cellular activity evaluation using heat damage. Next, we demonstrated about the cellular thermal measurement using peltier device which has cell wall such as the yeast cell.

From these experimental results, it was found that cells activity could be checked from shunt voltage change.

As the next step, we demonstrated cellular thermal measurement using yeast cells. Shunt voltage increased with the temperature rise, and decreased with the temperature drop. We will try to develop the micro-thermometer in cellular level.

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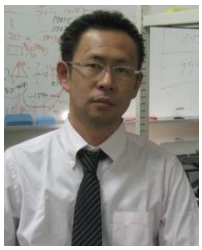

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