Dielectrophoretic Selection of Viable Single-cells of Rice and Tobacco

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A carbon fiber microelectrode (CFM) was devised and applied to the dielectrophoretic selection of a viable single-cell of rice and BY-2. When 10 MHz electric field was applied, only a viable single-cell could be pulled and picked up within a few sec. This cell selection system was thought to be useful for the high throughput experiment using single-cells.

Key Words : Single-cell Experiment, Carbon Fiber Microelectrode, Cell Viability, Protoplast

1 Introduction

In the post-genome era, a great deal of genome information is available and accordingly it is frequently required to study not only the function of a particular single gene but also the associative functions of multiple genes in the same cell *in vivo*. From this viewpoint, the single-cell gene experiment has now been recognized as an important method. The single-cell gene experiment implies the selection of a proper single-cell,^{1, 2)} the transportation of the cell to a proper site, the microinjection of genes into the cell,³⁾ the application of a stimulating signal to the cell,^{4, 5)} and the measurement of the gene expression of the cell.⁶⁾ In order to increase the success efficiency as a whole, it is important to check the cell viability and to remove dead or wounded cells at as early step as possible. For this purpose, we have devised a carbon fiber microelectrode (CFM) and applied it to the dielectrophoretic selection of a viable single-cell. The fabrication of the CFM is much easier than more elegant microelectrodes reported formerly.^{1, 2)}

The principle of the dielectrophoresis of viable cells (microbial, plant, and animal cells) was well described elsewhere.⁷⁻⁹⁾ The dielectrophoretic force working between a cell and an electrode (F) depends upon such parameters as the cell diameter (r), the intensity of a non-uniform electric field ($|E|$), the dielectric constants (absolute values of the complex permittivity) of a cell (ϵ_{Cell}) and its suspending solution (ϵ_{Soln}):

$$F = 2\pi r^2 \epsilon_{\text{Soln}} \left(\frac{\epsilon_{\text{Cell}} - \epsilon_{\text{Soln}}}{\epsilon_{\text{Cell}} + 2\epsilon_{\text{Soln}}} \right) \nabla |E|^2$$

In general, the magnitudes of ϵ_{Cell} and ϵ_{Soln} decrease as the frequency increases. However they are not entirely coincident over the frequency range of the applied electric field. At a frequency where ϵ_{Cell} is greater than ϵ_{Soln} , the cell is pulled towards strongest field region (*i.e.* near the carbon fiber microelectrode). We call this the positive force. Where ϵ_{Cell} is smaller than ϵ_{Soln} , the cell moves towards weakest field region (*i.e.* near the counter Pt

plate electrode). We call this the negative force. This study concerns only whether the dielectric force is positive or negative in this sense, and therefore, is aimed at finding such a frequency range where viable and dead cells would move towards opposite directions.

2 Experimental

2.1 Plant cells

Plant cells used in this study are protoplasts of rice (*Oryza sativa* L. japonica cv. Nipponbare) and tobacco (*Nicotiana tabacum* L. cv. line BY-2). The rice protoplasts were prepared as follows. Rice cells were cultured for 4 days with rotation in LQ medium¹⁰⁾ at 25°C. Then the cells (calli) were separated by the centrifugation and rinsed with a washing solution (mannitol 0.5 M, CaCl₂ 0.1 mM). Then the cells were suspended in an enzyme solution (Pectolyase Y-23 (Kikkoman Co. Ltd., Tokyo) 0.05% w/v, Cellulase Onozuka RS (Yakult Honsha Co. Ltd., Tokyo) 2%, CaCl₂·2H₂O 0.01%, K-dextran sulfate 0.1%, mannitol 9%, pH 5.6) and incubated on a shaker (30 rpm) for 1 hr at 30°C in the dark and then stood still for a successive 2 hr without shaking at 30°C. This suspension was passed through a nylon mesh (mesh size 20 μm) to remove callus debris. Then the protoplasts were collected by the centrifugation, washed and finally suspended in a protoplast medium (PP medium)¹¹⁾.

BY-2 cells were cultured in LSD medium¹²⁾ at 28°C for 4 days with rotation. The cells were collected by the centrifugation. Subsequent procedure for the preparation of the protoplast was same as above.

2.2 Fabrication of a carbon fiber microelectrode (CFM)

A carbon fiber with a diameter of 10 μm was inserted in a glass capillary with a diameter of 1 mm. The glass capillary was heated and pulled to form a small tip with a diameter of about 30-50 μm . The tip was finished with a micro forge to seal the possible gap between the carbon fiber and the glass capillary. Carbon powder was put into the capillary from the big end. A lead was inserted from the big end of the capillary to make an electric contact

Fig. 1 Experimental setup for the dielectrophoretic selection of a viable single-cell.

with the carbon powder and then the big end of the capillary was sealed with wax.

2. 3 Measurement of dielectrophoretic behavior

A test cell was set at the center of the microscope view and the tip of a CFM was situated 10 μm apart from the cell (Fig. 1). Alternating sinusoidal electric field was applied between the CFM and a Pt counter electrode. The voltage was 10 V_{pp} (peak to peak) and the frequency was varied from 1 kHz to 10 MHz. When the test cell pulled towards the CFM within a few seconds, the dielectrophoretic force was determined as positive. When the cell did not move, the CFM was brought close to the cell and successively observed if the cell moved away from the CFM. When the cell moved away from the CFM, the dielectrophoretic force was determined as negative. About 20-30 cells were investigated per each frequency and the relative value $100 \times (N_+ - N_-) / N_T$ (%) was plotted versus the frequency, where N_+ , N_- were the numbers of cells that showed positive force and negative force, respectively. N_T was the total number of cells that were assayed.

2. 4 Staining of cells with propidium iodide (PI)

As a comparison, the viability of cells was checked also by a conventional staining method using PI. When cells are treated with saponin, the cell membrane structure is collapsed and consequently the cell will become dead. PI is known to be able to permeate thus collapsed membrane and react with DNA to form a red fluorescent conjugate. Therefore the saponin treated cells and PI-stainable cells may be regarded as dead cells equivalently. Thus we investigated correlation between PI-stainability and dielectrophoretic property of saponin treated cells. Protoplasts were suspended in a PI solution and observed under a fluorescent microscope. Non-fluorescent cells were regarded as viable cells.

3 Results and Discussion

3. 1 Dynamic movement of single-cells in response to the electric field application

When 15 MHz electric field was applied to a rice cell, the cell began to move within 1 sec and reached the CFM around 6 sec (Fig. 2(A)). On the other hand, when

Fig. 2 Dynamic movement of single-cell in response to the electric field application. (A) Single-cell of rice at 15 MHz, positive force. (B) Single-cell of rice at 100 kHz, negative force. (C) Single-cell of BY-2 at 10 MHz, positive force. (D) Single-cell of BY-2 at 100 kHz, negative force.

100 kHz electric field was applied to a rice cell, the cell did not move for 30 sec. Then the CFM was brought close to the cell and 100 kHz electric field was applied to the cell again. Then the cell was repelled immediately (Fig. 2(B)). Similar phenomena were observed with a BY-2 cell (Fig. 2(C),(D)).

3. 2 Frequency dependence of dielectrophoresis

Test cells were treated with 0.3% saponin to obtain the sample of dead cells. The cells used without saponin treatment were regarded as viable control cells. As shown in Fig. 3(A), more than 80% of viable rice cells (27 out of 33 cells) showed negative dielectrophoretic force in the range from 100 kHz to 2 MHz while positive force above 9 MHz. In contrast, most of dead rice cells (18 out of 20 cells) showed no movement at 100 kHz and about 50% of dead cells (10 out of 20 cells) showed negative force in the range from 100 kHz to 10 MHz. Therefore, if the frequency was adjusted above 6 MHz, only viable cells of rice were pulled towards the CFM and could be picked up with the CFM.

BY-2 cells showed a similar property to rice cells. As shown in Fig. 3(B), more than 80% of viable BY-2 cells showed negative force in the range from 100 kHz to 1 MHz while positive force above 6 MHz. The dead cells showed no positive force throughout. Therefore, if the frequency was adjusted above 6 MHz, only viable cells of BY-2 could be picked up.

3. 3 Comparison between the dielectrophoretic property and PI staining property

The protoplasts that could be stained and not be stained with PI were selected, respectively, and each cell was investigated for its dielectrophoretic movement at 15

Fig. 3 Frequency dependence of dielectrophoretic movement. (A) Rice single-cells, (◆)viable cells ($n=33$), (◇)dead cells ($n=20$). (B) BY-2 single-cells, (▲)viable cells ($n=22$), (△)dead cells ($n=20$).

MHz. In the case of rice cells, 19 cells out of 20 stained cells showed negative force while 18 cells out of 20 non-stained cells showed positive force as listed in Table 1(A). Therefore there was a clear correlation between the dielectrophoretic property and the PI staining property. The same correlation was obtained with BY-2 cells (Table 1(B)).

4 Conclusion

The application of a CFM to the dielectrophoretic cell selection was successful in the case of rice and BY-2 protoplasts. The CFM can be prepared easily and its shape is so slim that the CFM is useful for subsequent single-cell experiments on the microscope stage. Especially high throughput microinjection with high success rate is expected.

Table 1 Correlation between the dielectrophoretic movement at 15 MHz and the PI staining property. Positive: movement towards CFM, Negative: movement apart from CFM, N. M.: no movement, (+) stained with PI, (−) not stained with PI. (A) Rice single-cells, (+) $n=20$, (−) $n=20$. (B) BY-2 single-cells, (+) $n=12$, (−) $n=20$.

(A)

		Dielectrophoresis		
		Positive	N.M.	Negative
PI staining	(+)	0	1	19
	(−)	18	2	0

(B)

		Dielectrophoresis		
		Positive	N.M.	Negative
PI staining	(+)	1	2	9
	(−)	20	0	0

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