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BRIEF COMMUNICATION

ELECTRICALLY STIMULATED FUSION OF HUMAN UC 729–6 CELL LINE POTENTIALLY USEFUL FOR GENERATING ANTIBODY–SECRETING HUMAN–HUMAN HYBRIDOMAS¹

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Antibodies against Hanganutziu and Deicher (HD) antigens, which were detected in sera of patients who had received therapeutic injections of animal antiserum, are often elevated in pathological conditions such as tumors.^{2,5)} We intend to make human monoclonal antibodies to the heterophilic HD antigens. This is because human–human monoclonal antibodies are more convenient than murine–derived monoclonal antibodies for immunodiagnosis and immunotherapy, due to possible interspecies reaction.⁵⁾ This will require fusion of a human myeloma cell line with antibody–producing B lymphocyte from a patient with elevated anti–HD antibodies. However, the number of such B cells from a patient and the availability of the fusion partner cell lines are limited compared to those of mouse. We hope to overcome this limitation in terms of raising the fusion rate by using electrofusion technique. In this paper, we report the determination of the optimum fusion conditions for the UC 729–6 cell line, a human lymphoblastoid B cell line.

UC 729–6 cells which were purchased from University of California, San Diego, were maintained in HB 104 growth medium (Hana Media Inc., Berkeley, California) with 0.1mM 6–thioguanine.

Medium was changed a day before fusion to induce cells to be in logarithmic growth

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phase.³⁾ Fusions were done in a fusion chamber. This is a circular dish with two platinum electrodes, 0.2 mm apart, connected to pulse generator (Shimadzu Somatic Hybridizer SSHI; Shimadzu, Kyoto). The cells in the fusion chamber were made to come close together and align themselves by applying an alternating electric current.¹⁾ This dielectrophoresis was achieved by 40 V/cm at a frequency of 2 MHz for 3 minutes (Fig. 1). Fusions were then induced with square direct current pulses of high intensity and short duration.

Cell fusion is mediated by membrane fusion.⁸⁾ Therefore, a reversible membrane breakdown is a prerequisite to cell fusion. Electric field has to be high enough to induce breakdown in contact zones of apposed membranes of two cells. Moreover, cells have to remain viable for culturing. To allow close cell apposition we treated the cells with a proteolytic enzyme to remove the outer calyx.¹⁾ Pronase E (Kaken Kagaku Co., Tokyo) treatment at a concentration of 0.05mg/ml facilitated fusion with higher viability than 0.5mg/ml, although both had similar fusion rates. Pulse duration of 20 μ s had high deleterious effect on viability. Pulse duration of 15 μ s had higher fusion rate than 10 μ s and was thus preferable. Mannitol and inositol fusion buffers had inverse relationship with respect to fusion rate and viability.^{4,6)} Inositol buffer was selected for its superior fusion rate (Tab. 1).

Field strength between 1 and 2 KV/cm of direct current produced similar viability, but 2.5 KV/cm had much less. Two KV/cm of field strength was considered optimum for fusion induction. The number of direct pulses had inverse effect on fusion rate and viability. Two pulses per sample were considered appropriate (Tab. 2). The above conditions were successful for homokaryon hybridoma formation.

Similar fusion conditions will be employed to make hybridomas of the UC 729-6 lymphoblastoid cell line with HD antibody-producing lymphocytes. Using a larger percentage of lymphocytes to UC 729-6 cells, myeloma-lymphocyte heterokaryon formation is favored.

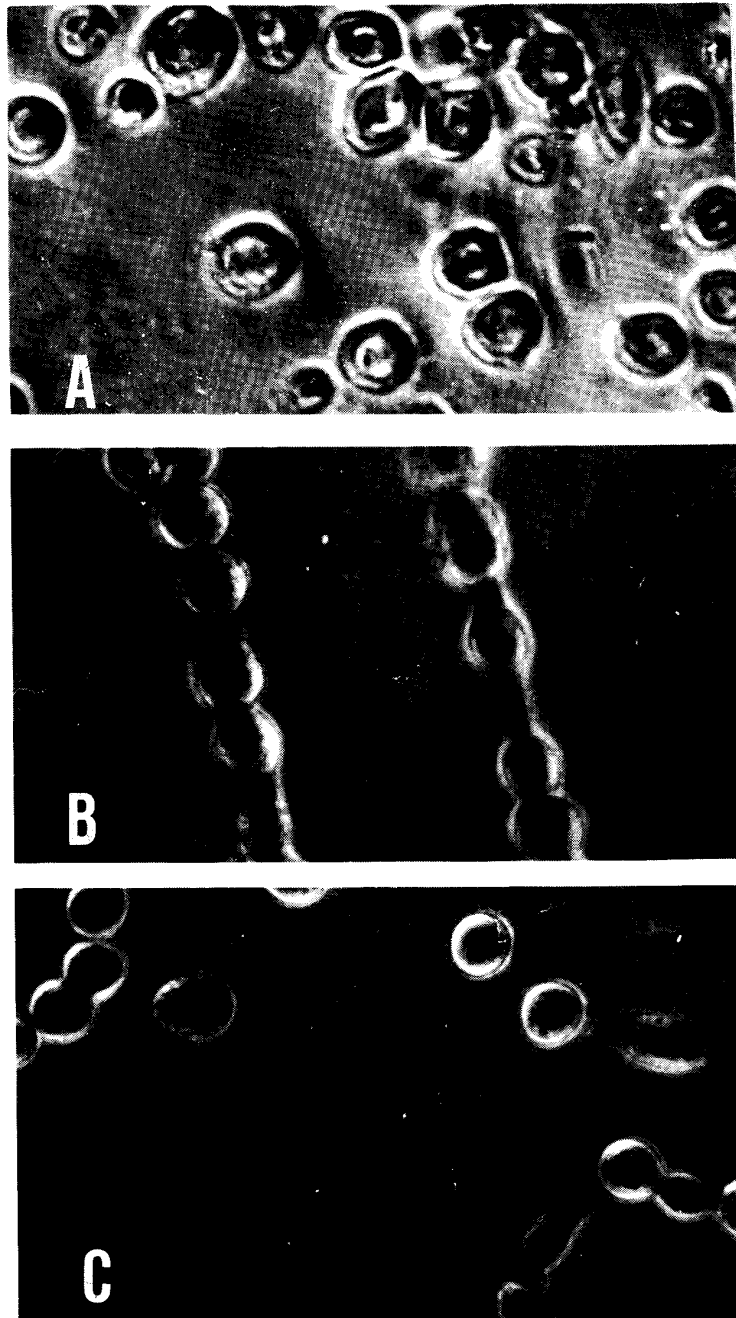


FIGURE. 1 Electric field induced pearl formation and fusion of UC 729-6 cells. The cells were treated with alternating current of 40V/cm at 2 MHz for 3 minutes for pearl formation, then fused with two direct electric pulses of 2.0 KV/cm field strength and 15 μ s duration. A) Before any current, B) Pearl formation after alternating current, C) Fused cells after direct current application. Fused cells can be seen (arrows). Magnification $\times 380$

TABLE 1 Effects of pronase E concentration, pulse duration and buffers on UC 729-6 cell viability and fusion rate.

		Cell viability(%)	Fusion rate(%)
Pronase E ¹ :	0.50 mg/ml	50.000	14.30
	0.05 mg/ml	65.32	14.54
Pulse duration ² :	10 μ s	52.17	8.70
	15 μ s	53.57	14.29
	20 μ s	40.00	14.29
Buffers ³ :	Mannitol	67.19	11.10
	Inositol	52.94	16.00

Cells at logarithmic growth phase were suspended in mannitol buffer at a concentration of $1-2 \times 10^5$ cells /ml.

- 1) The cells were treated with pronase E at 0.5 mg/ml and 0.05 mg/ml in mannitol buffer for 20 minutes at room temperature. The cells were washed twice with inositol fusion buffer. Four hundred microliter cell suspensions were transferred to fusion chamber and allowed to settle for a minute and fused with two pulses of 2 KV/cm direct current for 15 μ s.
- 2) Fusion was induced by two exposures of 2 KV/cm direct current at increasing pulse durations of 10, 15 and 20 μ s.
- 3) Cells were washed after the enzyme treatment and suspended in the respective fusion buffer for fusion; Mannitol buffer: 0.26 M Mannitol, 4 mM Dextrose and 2.5mM Calcium chloride; Inositol buffer: 0.28 M Inositol, 1 mM Phosphate buffer (K_2HPO_4/KH_2PO_4), 0.5mM Magnesium acetate and 0.1 mM Calcium acetate. Cells were fused with two pulses of 2 KV/cm for 15 μ s. Fusion rate was scored by microscopic observation (Figure 1) and cell viability was examined by staining with 0.06% trypan blue solution.

TABLE 2 Effects of electric field strength and number of pulses on UC 729-6 cell viability.

KV/cm ¹	Cell viability(%)	Number of pulses ²	Cell viability(%)
1	80.00	1	88.24
1.5	85.71	2	77.88
2	83.33	3	58.82
2.5	58.30	4	50.00

- 1) After pronase E treatment at a concentration of 0.05 mg/ml 400 μ l cell suspensions (same concentration as described in Table 1), were transferred to fusion chamber and fused with increasing direct current of 1, 1.5, 2 and 2.5 KV/cm applied two times for 10 μ s each with 30 sec intervals or
- 2) different number of pulses applied at 2 KV/cm for 10 μ s each. Viability was scored as described in Table 1.

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