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Electric Pulse–Induced Fusion of 3T3 Cells in Monolayer Culture

Abstract. *Swiss mouse 3T3-C2 fibroblasts, grown to confluence in monolayer culture, are shown to fuse when exposed to electric fields. Exposure to five repetitive electric pulses of about 1 kilovolt per centimeter with a duration of 50 microseconds caused approximately 20 percent of the cells to become fused (multinucleate) when 1 millimolar magnesium was present in the medium. The effects of minimum thresholds of field strength, pulse duration, and number of pulses were determined. Cell disruption was observed when the electric field exceeded 2.0 kilovolts per centimeter or the pulse was of longer duration than 120 microseconds.*

The major nonthermal effect of an electric field on a closed bilayer vesicle is the induction of a membrane potential (*1*). When cells are clustered, as during the development of the mold *Dictyostelium* or in Pronase-treated red cell ghosts that have been stacked by dielectrophoresis, strong electric pulses induce fusion of cell membranes (*2*). In this report we present evidence that this phenomenon is a general property of cells in close contact. No chemical or physical treatments are required. The technique may be extended to different types of cells, and fusions have also been observed with human myeloma and *Dictyostelium* cells in our laboratory (*3*). The efficiency of cell fusion induced by voltage pulses is generally high (10 to 20 percent), and experiments with 3T3 cells suggest that the fused cells are viable. Thus the technique may find application in the study of genetic transfer between cells.

The electric pulses were generated by a Cober 605 pulse generator connected to two electrodes. The signal was monitored as previously described (*4*). The electrodes were either two platinum wires or two stainless steel razor blades, parallel to each other at a distance of 3 mm. The electrodes were immersed in the cell-bathing buffer and seated firmly against the bottom of the plastic cell-culture dish; the polarity of the electrodes could be reversed by a switch. The temperature increase due to Joule heating was estimated to be less than 2°C. The initial temperature was 22° ± 2°C. Square wave pulses of controlled voltage and duration could be applied to the cell monolayers and the impulses repeated at intervals ranging from 1 to 15 seconds.

Swiss mouse 3T3-C2 fibroblasts were grown on plastic dishes as described (*5*) and were used in experiments 2 to 8 days after they reached confluence. For the electric pulse experiments, the culture medium was replaced with 3.5 ml of 272 m*M* sucrose in 7 m*M* phosphate buffer (*p*H 7.2), with or without 1 m*M* $MgSO_4$. This change of medium reduced ionic strength and, thus, the Joule heating of the cultured cells. After the cells had been treated with the electric pulses the sucrose solution was removed and replaced with the culture medium. The pulsed cells were then returned to the incubator (37°C; 95 percent air and 5 percent CO_2) for at least 2 hours. The monolayers were subsequently washed with phosphate-buffered saline (PBS), fixed with methanol, and stained with Giemsa blue. Cells were scored as fused if a continuous membrane surrounded at least two Giemsa blue–stained nuclei as viewed by phase-contrast microscopy. The viability of the pulsed and control cells was routinely monitored by trypan blue exclusion and by the incorporation of tritiated leucine into pulsed cells. The monolayers were incubated with 200 μCi of L-[4,5-^{3}H(N)]leucine (New England Nuclear) for 2 hours at 37°C. The cells were then extensively washed with PBS and autoradiographed with Ilford L4 emulsion for 1 week at −70°C.

When an electric pulse of 1.6 kV/cm

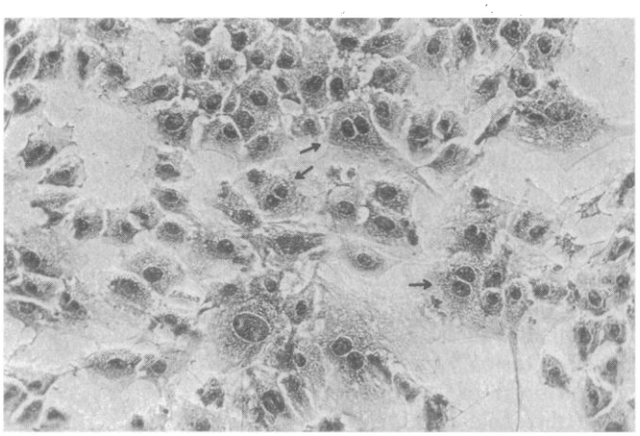

Fig. 1. Electric field–induced fusion of 3T3-C2 fibroblasts in monolayer culture. The cells were treated with five electric pulses of 1.6 kV/cm field strength and 100-μsec duration. A significant fraction of cells fused. Several binucleate cells are identified (see arrows for examples of fused cells). Fused cells were judged viable by the criteria discussed in the text.

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Table 1. Voltage dependence of the fusion of 3T3-C2 cells. Cell monolayers were subjected to electric field pulses as described in the text and then incubated for at least 2 hours. Pulse duration and the number of successive pulses (delay of 1 second between pulses) in experiments 1 and 2 were, respectively, 100 μsec and five pulses, and 30 μsec and four pulses. Fusion was observed under a phase-contrast microscope after staining (−, no fusion; +/−, less than 5 percent fusion or uncertain because of cell disruption at high voltage; +, 5 to 10 percent fusion; ++, 10 to 20 percent fusion). Integrity of the membrane was checked by the penetration of trypan blue (+, no rapid penetration of trypan blue; +/−, partial penetration; −, severe penetration). Cell disruption refers to the observation that after voltage treatment of monolayer cells, cells became detached from the culture medium (+) or were unaffected (−). See text for details. N.D., means not determined.

E (kV/cm)	Fusion	Membrane integrity	Cell disruption
Experiment 1. No Mg^{2+} present			
0.66	−	+	−
1.00	−	+	−
1.10	+	+	−
1.30	+	+	−
1.60	+	+	−
2.00	+/−	+/−	+
2.10	+/−	−	+
Experiment 2. Mg^{2+} (1 mM) present			
0.42	+/−	N.D.	−
0.66	+/−	N.D.	−
1.00	+	N.D.	−
1.30	++	N.D.	−
1.66	++	N.D.	−
2.00	+/−	N.D.	+
2.50	+/−	N.D.	+

and 100 μsec in duration was applied five times to the 3T3 cells, a significant percentage of the cells became fused (Fig. 1). This percentage was increased by the addition of Mg^{2+}; with 1 m*M* $MgSO_4$ 10 percent of the cells became binucleate (that is, 20 percent fusion). Trypan blue exclusion showed that the cell membranes were intact after exposure to the pulse or underwent repair during the subsequent incubation period. Autoradiography indicated comparable rates of incorporation of leucine into the treated and control cells.

The degree of fusion depended on (i) the magnitude of the applied field, (ii) the duration of the pulse, (iii) the number of pulses, and (iv) the presence of magnesium. The major limitation observed with pulses that were too strong or of too long a duration was detachment of the cells from the monolayer. The mechanical force due to the electric pulse either disrupted the cells per se or their binding to the plate. As shown in Table 1, for a given pulse duration, the electric field induced fusion of cells only within a certain range of field strength. A minimum threshold (1 kV/cm) was clearly demonstrated. At too high a field, that is, 2.0 kV/cm, cells were detached from the plate. Furthermore, trypan blue exclusion showed that above this value of field strength, the cell membranes became leaky in a manner not repaired during the 2-hour incubation period after exposure to the electric field. Similarly, Table 2 shows that for a given field strength, fusion depended on the pulse duration. Here again, if the pulse conditions were too drastic, that is, of greater duration than 250 μsec, the cells were irreversibly damaged.

Several pulses applied to the cells on one plate increased the rate of fusion (Table 3). If more than ten pulses were applied, most of the cells were damaged. Applying pulses of alternating polarity or increasing the delay between pulses (1, 5, or 15 seconds) did not remedy this situation. Data in Tables 1 and 2 also indicate that the presence of 1 m*M* Mg^{2+} increased the rate of fusion by lowering the thresholds of both the field (0.6 kV/cm) and the pulse duration (30 μsec). A recovery period after exposure to the electric pulses appeared to be required, since cells that were fixed immediately after exposure became detached from the plate.

Strong electric fields have been shown to cause perforation of cell membranes. The channels opened are either transient (*1*) or permanent (*6*), and the size of the pores depends on the strength and duration of the electric pulse (*7*). Electric fields also energize bilayer membranes. In the case of chloroplast and mitochondrial membranes, this membrane energization can trigger synthesis of adenosine triphosphate (ATP) through the action of ATP synthetases (*8*). We propose that this transient pore formation and energization of cell membranes brought about by the electric field are responsible for the fusion of cells in close contact. Electric field pulses applied to cells in suspension did not induce cell fusion.

Table 2. Dependence of cell fusion on pulse duration. The experimental conditions and notations used are given in Table 1.

Duration (μsec)	Fusion	Membrane integrity	Cell disruption
No Mg^{2+} present, five pulses at 1.6 kV/cm			
11	−	+	−
20	−	+	−
34	−	+	−
45	+	+	−
60	+	+	+/−
120	+/−	+/−	+
180	+/−	+/−	+
No Mg^{2+} present, two pulses at 1.6 kV/cm			
100	+/−	+	−
120	+	+	−
200	+	+/−	−
250	+/−	−	+
400	+/−	−	+
1 mM Mg^{2+} present, four pulses at 1.6 kV/cm			
10	+/−	N.D.	−
30	++	N.D.	−
120	++	N.D.	−
300	+/−	N.D.	+

Table 3. Dependence of 3T3-C2 cell fusion on number of repetitive pulses (no Mg^{2+} present). Voltage pulses at 1.6 kV/cm and of 50-μsec duration were applied with 1-second delay between pulses. The other experimental conditions and notations used are as given in Table 1.

Number of pulses	Fusion	Cell disruption
1	−	−
2	−	−
5	+	−
10	+/−	+
20	+/−	+

The advantages of cell fusion induced by an electric field are numerous. First, no additives to the medium, such as virus, polyethylene glycol (*9*), or liposomes (*10*), are required. Thus, no chemical modification of the cells is involved. Second, the technique is easily performed and quite rapid. Third, an excellent yield of fused cells is obtained. Fourth, no specific receptor is required on the surface of the cells, so this method may be generally applicable to many cell types.

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