An improved electrofusion technique for production of mouse hybridoma cells

J. Vienken and U. Zimmermann

Lehrstuhl für Biotechnologie der Universität Würzburg, Röntgenring 11, D-8700 Würzburg, FRG

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An experimental procedure is described for the reproducible production of hybridoma cells using the electrofusion technique. High yields can be obtained when fusion is performed in isotonic inositol solutions containing Ca^{2+} and Mg^{2+} in a ratio of 1:5 in the millimolar range. The hybridoma cells are transferred 10 min after the field pulse application into a balanced salt solution for 30 min at 37 °C.

Hybridoma *Electrofusion* *Fusion medium*

1. INTRODUCTION

In the last few years it has been possible to demonstrate that a great diversity of plant and animal cells can be fused by means of electrical fields [1–8]. In the meantime, this technique, which was developed in our laboratory [9–12], has become established in many laboratories, and a number of reports on electrofusion have already been published [13–24]. In the light of these reports, the viability of electrofused hybrids should be beyond any doubt.

In many cases, the recently published methods are the same with respect to the application of one or more DC pulses of short duration, but differ in the various methods of inducing the membrane contact necessary for fusion. For example, in the magneto-electrofusion technique [25], membrane contact is achieved by the action of inhomogeneous magnetic fields on magnetically labelled cells. In some published studies fusion is induced by the application of electric field pulses to a monolayer of cells in which cell contact has been achieved by the cells growing together [18,19] or by means of a specific chemical reaction of pretreated cells [26].

The electrofusion of lymphocytes and myeloma cells for the production of hybridoma cells and, in turn, the manufacture of monoclonal antibodies is of particular interest. It is possible to manufacture mouse and human hybridoma cells by electrofusion [7,21,27], but so far the yields have been low. In this communication we report on the production of high yields of mouse hybridoma cells by electrofusion and on the cloning of hybrids obtained in this manner.

2. MATERIALS AND METHODS

2.1. *Cells*

HAT-sensitive SP2/0 myeloma cells were cultured in RPMI 1640 medium supplemented with HT as well as 10% fetal calf serum (FCS). The myeloma cells were used for fusion when they reached the logarithmic growth phase. Lymphocytes were isolated from the spleen of a mouse (BALB/c), and any undesirable erythrocytes were lysed with the aid of NH_4Cl (0.85%). Lymphocytes isolated in this manner were stored in RPMI 1640 at 4°C until required for fusion.

2.2. *Fusion pretreatment*

Myeloma cells and lymphocytes were mixed in a ratio of 1:10 or 1:5, finally producing a total number of cells of 1.1×10^7 per ml. Before being introduced into the fusion chamber, the cells were twice washed in a medium of the following com-

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position: 280 mM inositol, 1 mM phosphate buffer (K_2HPO_4/KH_2PO_4), 0.5 mM magnesium acetate and 0.1 mM calcium acetate.

2.3. *Fusion chamber*

140 μl of cell suspension prepared in this way were transferred into the so-called helical chamber [8,28]. The helical chamber consists of two perspex cylinders, one inside the other and each sealed at one end. Two platinum wires (diameter 0.2 mm) are wound round the inner cylinder in such a way that the distance between them is 0.2 mm throughout. The result is a helical arrangement of the wires [8,28]. The fusion device marketed by GCA Corp. Chicago, Ill, USA, was used for the fusion of the cells.

3. RESULTS AND DISCUSSION

Once in the helical chamber, the cells were made to align themselves in pearl chains by applying an alternating electric field. The field strength of the applied alternating electric field was 250 $V \cdot cm^{-1}$, the frequency 1.5 MHz. The alternating electric field was applied for 30 s. After 30 s three square pulses of 3.5 $kV \cdot cm^{-1}$ and 20 μs duration were applied at intervals of 1 s, which induced an electrical breakdown of the cell membranes in the membrane contact zones. The alternating electric field which had been switched off during the application of the square pulses was then re-applied for a further 30 s so that the cells were maintained in their relative positions to one another. The helical chamber was subsequently transferred to an incubation chamber (or water bath) for 10 min at 37°C. After incubation the inner core with the wire helix was carefully removed, and warm (37°C) post-fusion medium of the following composition was carefully added to the cell suspension: 132 mM NaCl, 8 mM KCl, 10 mM phosphate buffer, 0.5 mM magnesium acetate and 0.1 mM calcium acetate. The cells were incubated in this medium for a further 30 min in an incubation chamber at 37°C. After 30 min the cell suspension was centrifuged at $200 \times g$, and the supernatant decanted. The cells were resuspended in RPMI 1640/10% FCS/HAT and transferred in units of 3×10^6 cells to a previously prepared feeder layer of peritoneal macrophages (24-well Costar microtitre plate). The density of the macrophages was about 10^4 cells per well.

The described procedure resulted in the recovery of a large number of live mouse hybridoma cells from the microtitre wells 4 days after fusion. On average up to 100 clones were recovered from each well containing RPMI 1640/HAT medium. Division of the hybridoma cells was normally observed after 3 to 4 days.

In contrast to the studies of Teissié and coworkers [18,19] and Lo et al. [26], cell contact in our experiments was always established with the aid of dielectrophoresis. This means that the process is not restricted to certain cell types nor does it rely on complicated chemical reactions such as the adhesion of antigen/antibody structures to cell surfaces. The method thus retains its universal applicability.

The main prerequisites for a high yield of viable hybridoma cells are the fusion medium, the resealing medium and the intervals between the addition of the various media. Contrary to our earlier published studies of hybridoma cells [21,27], the addition of pronase is no longer necessary if cells in the logarithmic growth phase are fused in the medium described above. Since the formation of a new spherical hybrid – after the breakdown pulse has been applied – needs some time, it is necessary to allow a certain amount of time between the application of the electric field pulse and the addition of the resealing medium. A period of about 10 min has been found to be appropriate, although incompletely rounded-off fusion products can still be observed after 30 min.

Since the membrane is only incompletely resealed 10 min after pulse application at 37°C, the addition of substances that might be toxic to the permeabilized cells must be avoided under all circumstances. This includes the addition of nutrient media containing dyes such as neutral red or phenol red.

The use of these solutions and field conditions leads to a high yield of hybridoma cells in a very reproducible manner. A further increase in the yield of hybridoma cells can be expected in the light of the results obtained for the fusion of yeast cells [28,29]. With yeast cells the yield of hybrids could be increased from 60 hybrids per run [28] to more than 4000 [29], by using an adequate fusion medium and appropriate field and incubation conditions which have a beneficial effect on the growth (regenerative capacity) of these cells. We

therefore believe that further efforts in this field should be directed towards further improvement of the composition of the fusion and post-fusion media and further optimisation of the field conditions. Alternative ways of establishing membrane contact, as mentioned in the introduction, may be very useful for certain applications, but in our opinion they place too great a restriction on the diverse and universal applications of this method. The potential of this method for the manufacture of hybridoma cells should be further augmented by the fact that it is possible, by means of electrical fields, to separate out antibody-secreting lymphocytes for alignment with myeloma cells in the alternating electric field [6]. The resulting hybridoma cells secrete monoclonal antibodies per se under these conditions.

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