ELECTRIC FIELD-INDUCED FUSION: ELECTRO-HYDRAULIC PROCEDURE FOR PRODUCTION OF HETEROKARYON CELLS IN HIGH YIELD

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1. Introduction

Electric-field-mediated fusion is described for plant protoplasts [1–3], mammalian cells [1,4,5], sea urchin eggs [6], vesicles and artificial liposomes [1]. The cells, eggs or vesicles are exposed to an alternating, non-uniform electric field of low intensity which brings about the formation of pearl chains of varying length [7,8]. Fusion is induced by electrical breakdown in the membrane contact zone both between the cells, eggs or vesicles within a given pearl chain and between cells of adjacent pearl chains. Reversible electrical breakdown is induced by the application of a field pulse of high intensity but very short duration [9].

With the appropriate suspension density and electrode arrangement the number of cells to be fused can be preselected. Viable giant cells which can be impaled by microelectrodes can be produced by fusing hundreds of cells [1,4,5]. However, when the electric-field-mediated fusion technique is employed for the production of heterokaryon hybrids consisting of 2 cells of different provenience, there are problems regarding yield. Low suspension densities and weak non-uniform alternating fields favour the production of 2-cell combinations. For statistical reasons however only ~30% of these aggregates exhibit the desired cell combination.

Here, we introduce an electro-hydraulic procedure which produces high yields of the desired combination of 2 heterokaryon cells. This new technique was tested for the production of murine hybridoma cells [10,11]. Murine hybridoma cells, the products of fusion between mouse lymphocytes and mouse myeloma cells, are capable of manufacturing monoclonal antibodies of predetermined antigenic specificity and thus have great potential in medicine and biotechnology.

2. Material and methods

Myeloma cells were obtained from Organon-Trust (Oss). The cell line was grown in suspension in supplemented Dulbecco's modified Eagle's medium (DME) to which 15% fetal calf serum was added. To obtain lymphocytes the spleen of a NMRI mouse was dissected into 2 halves, and lymphocytes as well as erythrocytes were squeezed out into a Petri dish containing DME. The erythrocytes were subsequently lysed with the aid of 150 mM NH_4Cl.

For fusion, murine myeloma cells and lymphocytes were washed twice in 280 mM mannitol and 20 mM histidine, suspended in the same solution containing 1 mg pronase P/ml (Serva GmbH, Heidelberg) just before field application. After the field pulse the fused aggregates were suspended in a solution of 150 mM NaCl and 1 mM $CaCl_2$, so that they could regain their spherical shape.

3. Results and discussion

Fig.1 shows a schematic representation of the electro-hydraulic system used for the production of hybridoma cells. Two small microslides (25 mm long and 5 mm wide) are each mounted on plexiglass spacers (100 μm apart) onto a slide (10 cm long and 2 cm wide). The gap between the 2 small microslides is 70 μm. The chamber thus formed between the 2 small slides and the large slide is fed or emptied by a syringe containing a cell suspension. The left-hand

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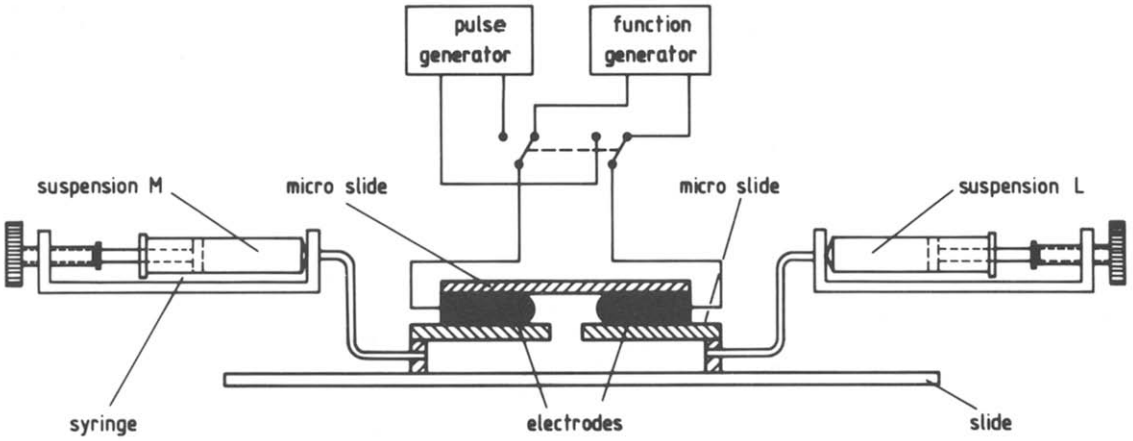

Fig.1. Set-up for the electro-hydraulic production of heterokaryon cell combinations. For details see text.

syringe is connected to a reservoir containing a very dilute suspension of murine myeloma cells (M), while the right-hand syringe is connected to a reservoir containing a very dilute suspension of murine lymphocytes (L). The cells are suspended in an isotonic mannitol solution containing 20 mM histidine. The electrical conductivity of the solution was $<10^{-4}$ S/cm with pH 7.

As desired, one or the other cell suspension was pressed into or withdrawn from the chamber with the aid of a plunger whose position could be altered by means of a micrometer screw. This procedure is very gentle and avoids the occurrence of turbulences. Two platinum plates (25 mm long, 10 mm wide and 100 μm thick) are glued onto the 2 smaller (upper) microslides (fig.1). The 2 electrodes are each positioned at ~10 μm from the edge of the gap between the 2 upper microslides. A high yield of lymphocyte/myeloma cell aggregates is achieved in the following manner: An alternating voltage of 100 V/cm and a frequency of 1 MHz is applied. By displacing the left-hand plunger, myeloma cells are then forced into the gap between the 2 electrodes. Because of the slight uniformity of the field, the myeloma cells migrate towards the electrodes and form a monolayer of cells on the electrodes (fig.2a). This process is controlled under the microscope. In the second stage of the procedure, the remaining myeloma cells are withdrawn from the chamber, and instead lymphocytes are introduced into the gap between the electrodes by operating the right-hand plunger. Mutual dielectrophoresis [7,8] causes the lymphocytes to align themselves on

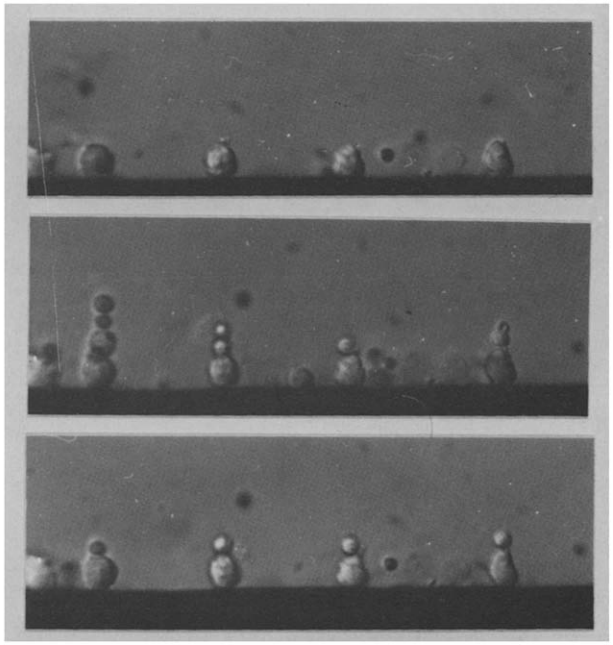

Fig.2. Formation of 2-cell combinations by the electro-hydraulic procedure. A dilute suspension of myeloma cells is forced into the gap between the 2 electrodes. Due to dielectrophoresis the cells form a monolayer on the electrode surface (a). Now lymphocytes are introduced into the electrode gap. Mutual dielectrophoresis causes the cells to align themselves on top of the myeloma cells (b). Superfluous lymphocytes are removed with the aid of a small hydrostatic pressure exerted by one of the plungers. Since the electrodes are slightly displaced from the gap between the microslides, 2-cell aggregates are not exposed to the hydrodynamically created stream. In this manner M/L combinations can be achieved.

top of the monolayer of myeloma cells covering the electrodes. Since the myeloma cells are larger than the lymphocytes, the field surrounding them is distorted and therefore less uniform. As a result, the forces exerted between 2 cells approaching each other are much higher than one would expect from the inhomogeneity of the electric field in the bulk solution between the 2 electrodes. The main prerequisite for the success of this procedure is that the 2 electrodes are exactly parallel. Otherwise the cells may migrate in parallel with the wires towards the smallest gap between the electrodes. This in turn would prevent a uniform production of myeloma cell/lymphocyte (M/L) aggregates. Since the lymphocytes are smaller than the mouse myeloma cells, mutual dielectrophoretic forces may lead to undesirable combinations such as M/L/L, in addition to the desired combination M/L (fig.2b). The superfluous lymphocytes can be removed with the aid of a small hydrostatic pressure exerted by the displacement of the right-hand plunger. Since the electrodes are slightly displaced from the gap between the 2 upper microslides, the 2-cell (M/L) aggregates on the electrodes are not exposed to the hydrodynamically created stream, so that they are not detached. In this manner, a high yield (60–80%) of M/L combinations can be achieved within a few minutes as a matter of routine. Fig.2c shows a section of the electrode covered with lymphocyte/myeloma cell combinations. Fusion of the cells is achieved by injecting a field pulse of 4 kV/cm over 20 μs, provided that 1 mg pronase/ml or 10 μg dispase/ml was added to the mannitol–histidine solution prior to the experiment [5]. These enzymes protect cell membranes against high field intensities [4,5], probably by creating lipid domains within the membrane [12,13]. Fusion is initiated immediately on application of the breakdown pulse. Fig.3 shows the fusion of a lymphocyte/myeloma cell aggregate at higher magnification. The cells regain their spherical shape after 5 min when isotonic electrolyte solution is added. The hybridoma cells can be selectively removed from the electrodes and transferred to nutrient solutions by means of the pressure probe or 2-electrode micropincers.

This procedure rules out the necessity to select hybridoma cells by culturing them in HAT medium. Preliminary experiments have shown that the hybridoma cells are viable and that they will grow in nutrient medium (in preparation). The electro-hydraulic technique produces high yields of heterokaryon cells, particularly if very long, parallel electrodes are used.

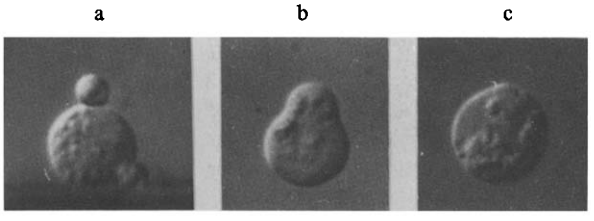

Fig.3. Electrically-induced cell fusion between a murine myeloma cell and a murine lymphocyte resulting in the formation of a viable hybridoma cell. Micrographs show the time course of fusion 15 min (b) and 20 min (c) after the application of the electric field pulse (field strength 4 kV/cm; 20 μs duration). The cell regains a spherical shape when isotonic electrolyte solution is added.

Acknowledgements

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