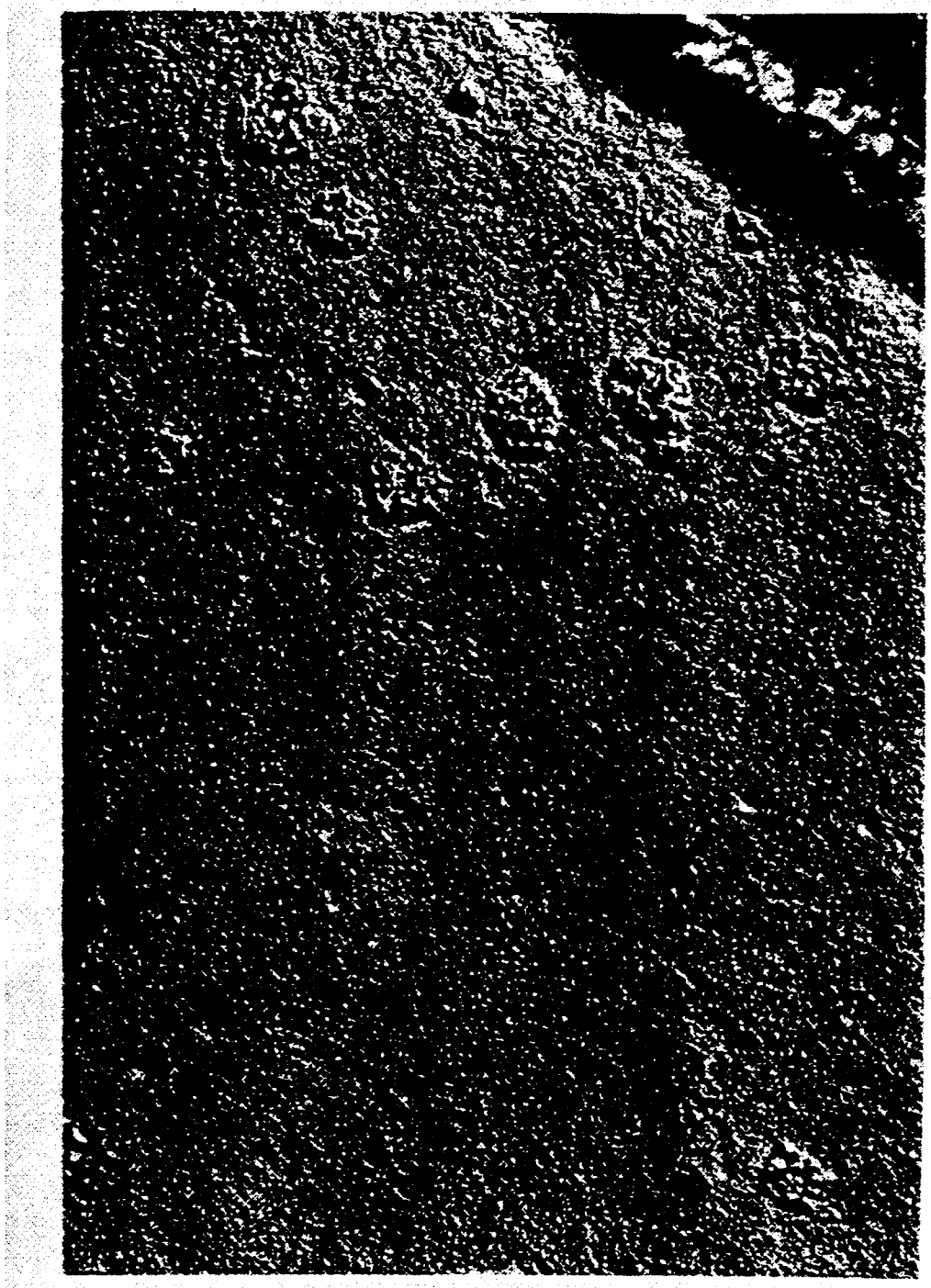

FIG. 15

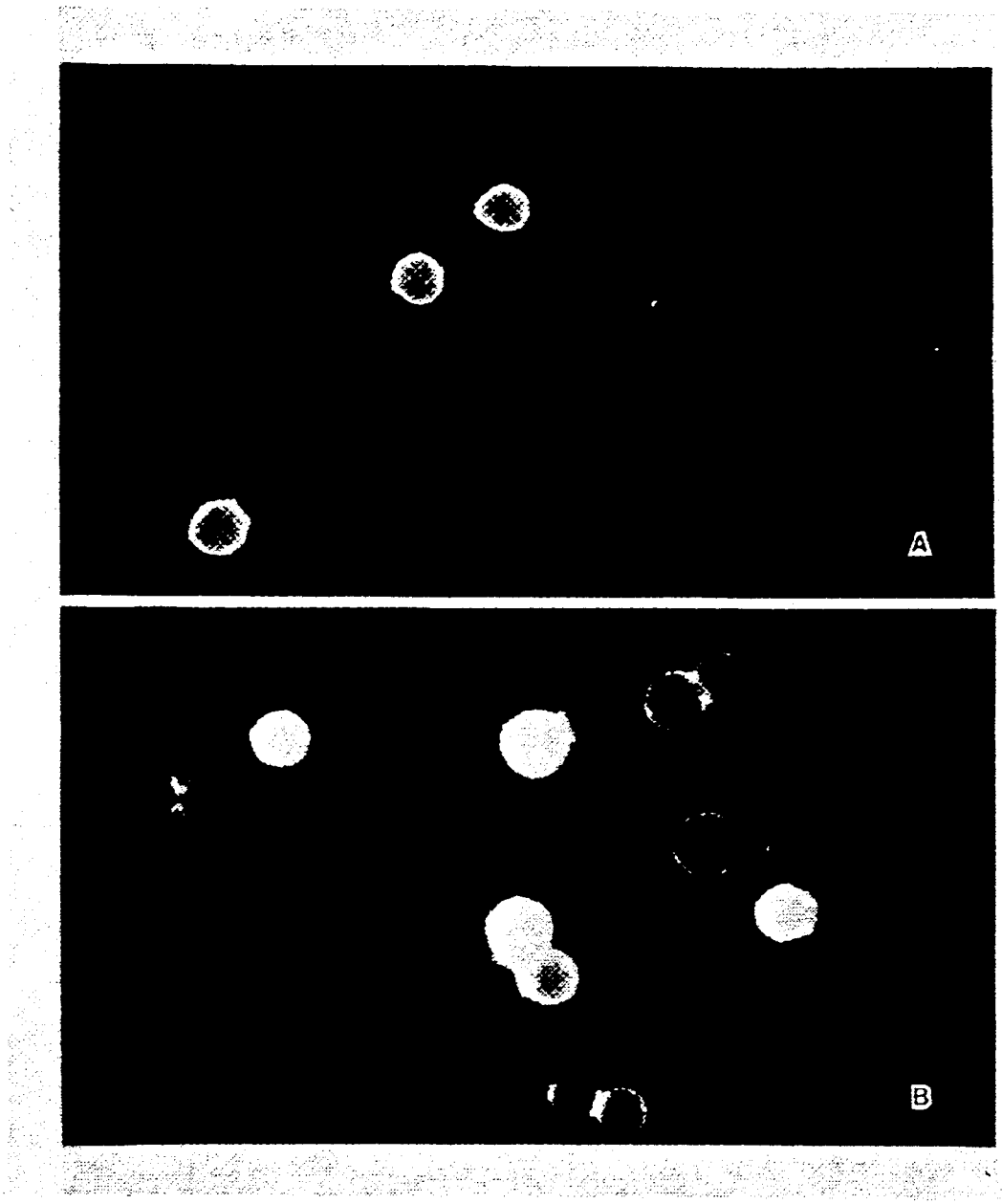

FIG. 16

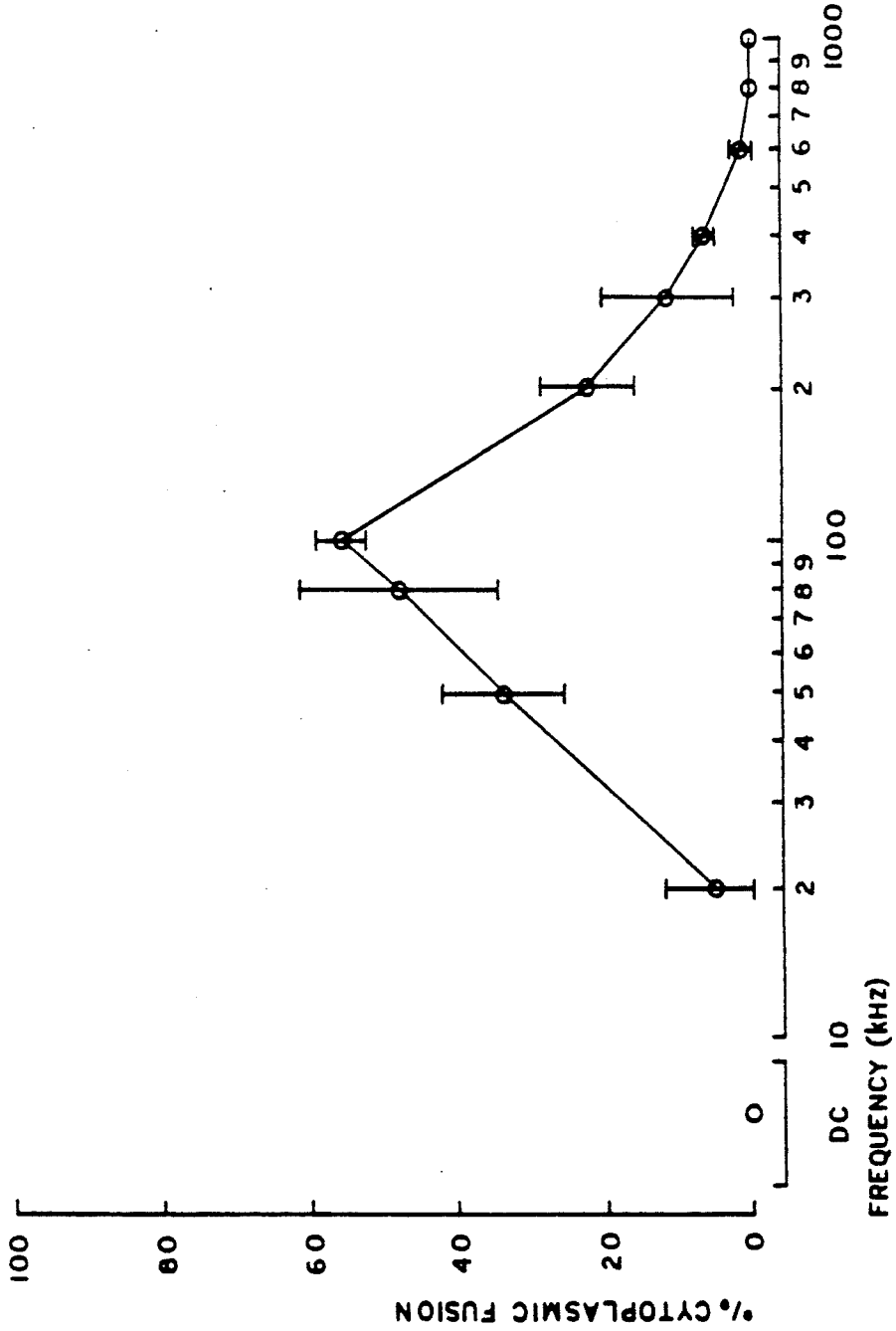
% CYTOPLASMIC FUSION
0
20
40
60
80
100
DC
0
10
2
3
4
5
6
7
8
9
100
2
3
4
5
6
7
8
9
1000
FREQUENCY (kHz)

FIG. 17

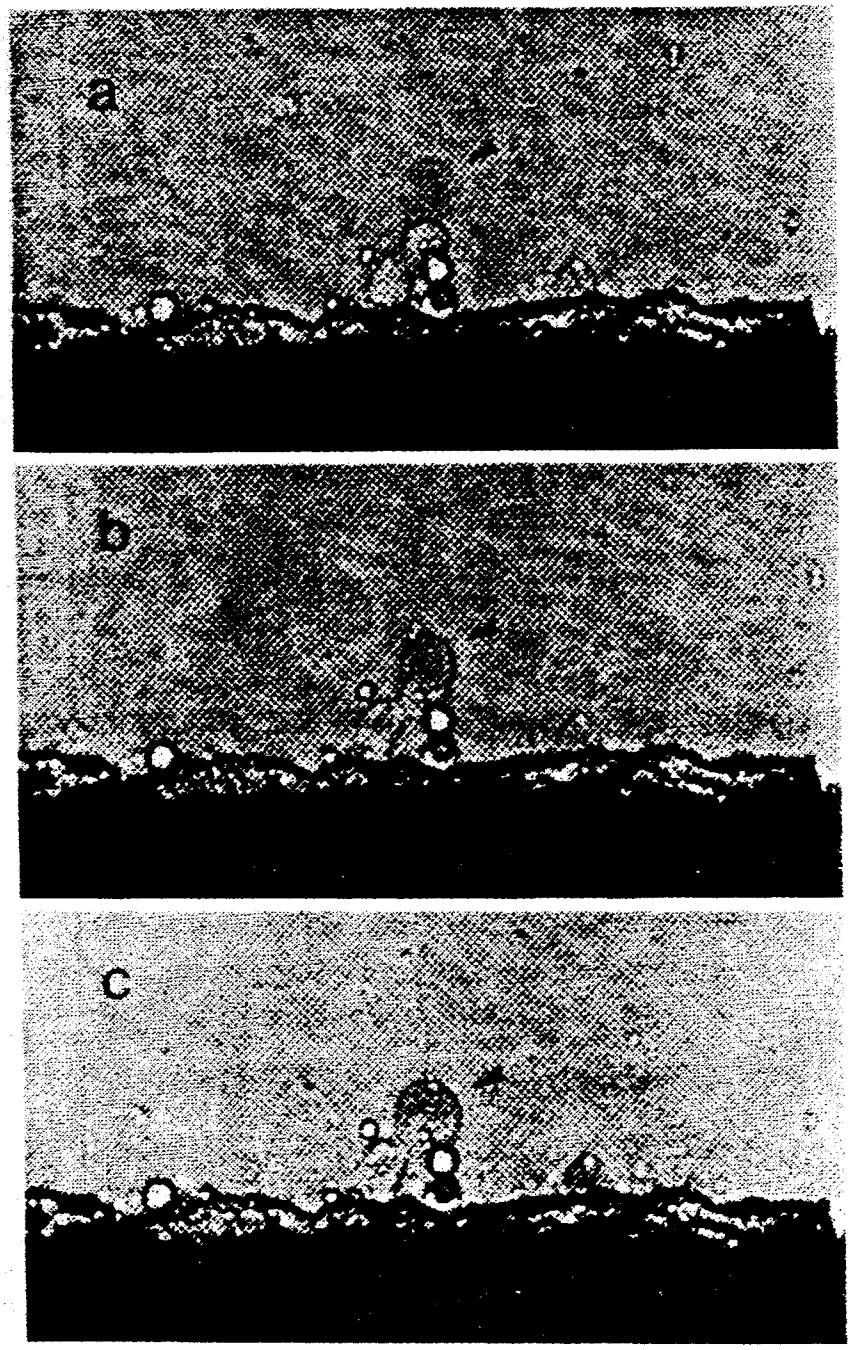

FIG. 18

METHOD OF AND APPARATUS FOR CELL PORTION AND CELL FUSION USING RADIOFREQUENCY ELECTRICAL PULSE

CROSS REFERENCE TO RELATED APPLICATIONS

This Application is a continuation-in-part of Applicants' co-pending application Ser. No. 238,670 filed Aug. 30, 1988, now U.S. Pat. No. 4,970,154 which was a continuation-in-part of application Ser. No. 106,282 filed Oct. 9, 1987, now U.S. Pat. No. 4,822,470.

FIELD OF THE INVENTION

This invention relates to the field of poration and fusion of biological cells by application of a high-power pulsed radiofrequency electric field. More particularly, it relates to permeabilizing and fusing cells in a wide variety of fields including gene transfection, microinjection of cells, production of monoclonal antibodies and making new biological species by hybridization.

BACKGROUND

Cell poration and cell fusion play a very important role in modern biotechnology. For example, one key procedure in genetic engineering is the introduction of exogeneous genetic material into a host cell. This insertion of genes is accomplished by either permeabilizing the cell membrane to allow entry of genetic material (i.e., gene transfection) or fusing the host cell with a cell containing the desired genetic material. Cell fusion is also important in the production of monoclonal antibodies. The process of producing monoclonal antibodies requires the fusion of antibody producing cells with continuously dividing cancer cells. (Galfre, G. et al., Nature 266:550–552 (1977); Lo, M. M. S. et al., Nature 310:794–796 (1984)). Additionally, one highly effective method of delivering drugs which normally cannot enter a cell is to fuse the cell with liposomes or red blood cell ghosts that have been pre-loaded with specific drugs. (Schlegel & Lieber, *Cell Fusion*, ed. by A.E. Sowers, Plenum Press (1987)).

The conventional techniques of cell fusion rely mainly on the actions of viruses (White, J. et al., J. Cell Biol. 89:674–679 (1981)); or chemical agents such as polyethylene glycol (Davidson, R.L. et al., Somatic Cell Genetics 2:271–280 (1976)). Virus-induced and chemical-induced fusion methods have many shortcomings. Not only is the fusion yield often very poor, typically less than 0.01%, but the standard fusion techniques may also cause severe side effects on the fused cells, thus greatly limiting their usefulness for many systems.

Alternative methods which induce cell fusion and cell poration by electric fields have been developed. (Pohl, U.S. Pat. No. 4,476,004; Sowers, U.S. Pat. No. 4,622,302; Schoner, U.S. Pat. No. 4,578,167; Neumann, E. et al. Naturwissenschaften 67:414–415 (1980); Zimmerman, U. and Nienken, J., J. Membrane Biol. 67:165–182 (1982); Bates G. W., et al., *Cell Fusion*, Plenum Press pp. 367–395 (1987)). The basic principle of these methods of electrofusion is to apply a pulsed high strength direct-current (DC) electric field across the cell. This DC field is usually generated by briefly switching on a DC power source or by discharging a capacitor. The applied DC field has a strength of several kilovolts per centimeter. This external electric field induces a large cell membrane potential. When the membrane potential is of sufficient magnitude, a reversible breakdown of a small area of the cell membrane occurs. The breakdown results in the formation of physical pores at the surface of the cell. This process is called electroporation. Intracellular and extracellular material can exchange through the pore while it is open. After the DC field is removed, the pore will normally reseal quickly. When a pore is created between two closely adjacent cells a cytoplasmic bridge is formed via the pore. When the DC field is turned off the pore cannot reseal. Instead, the cytoplasmic bridge usually begins to enlarge, eventually causing the two cells to fuse.

Although the DC electrofusion method has been used successfully for a number of biological cells, including plant protoplasts (Zimmerman, U. et al., Biochem. Biophys. ACTA 641:160–165 (1981); Bates, G.W. et al., *Cell Fusion*, Plenum Press pp. 479–496 (1987)); blood erythrocytes (Sowers, A.E., J. Cell. Biol. 102:1358–1362 (1986); Chang and Hunt, *Proceedings of the International Symposium on Molecular Mechanisms of Membrane Fusion*, Buffalo, N.Y. pp. 26 (1987); Stenger, D.A. and Hui, S.W., J. Membrane Biol. 93:43–53 (1986)); tumor cells (Lo, M.M.S. et al., Nature 310:794–796 (1984); Tessie, J. et al., Science 216:537–538 (1982)); yeast cells (Halfmann, H.J., et al., Archiv. Microbiol. 134:1–4 (1983)); and blastomerers and eggs (Kubiac, J.Z. and Jarkowski, A.K., Exp. Cell Res. 157:561–566 (1985)), there are still many limitations to the use of this method. First, not all cell types can be fused with the same ease. In fact many cell types are extremely difficult to fuse with DC pulses. Second, there are many unknown factors which influence fusion yield. Fusion of certain cell types may be successful in one laboratory but not in others. The DC pulse method is still more of an art than a well understood procedure. Third, it is very difficult to use the DC pulse method to fuse cells of different sizes. This later problem occurs because the membrane potential induced by the external DC field is proportional to the diameter of the cell. Thus, the induced potential is larger for bigger cells. It is nearly impossible to chose a proper field strength of external field in order to fuse cells of two different sizes. When the external field is just sufficient to cause membrane breakdown in the larger cell, it is inadequate to induce a critical membrane potential in the smaller cell. On the other hand, if the external field is elevated to cause a membrane breakdown in the small cell, the large potential induced in the larger cell will cause an irreversible membrane breakdown and destroy the cell.

The present invention provides an improved method of cell Poration and cell fusion which overcomes the above problems. Unlike the conventional electrofusion method which employs DC pulses to induce membrane breakdown, the present invention uses a pulse or pulses of radiofrequency (RF) electric field to reversibly permeabilize cells and induce cell fusion. The high-power RF field produces an oscillating motion of the cell membrane through a process of electro-compression. Permeabilization of the cell membrane is caused by a combination of electrical breakdown and a localized sonication induced from the RF field. Thus, this oscillating electric field is more effective in breaking down the cell membrane than a DC field. Since this new method uses only physical means (i.e., RF electrical energy) to induce cell poration and cell fusion, it is free of biological or chemical contamination. The present invention produces results in seconds, provides much higher yields than conventional methods, and has mini-