mal biological side effects. Thus, it is a clean, fast, efficient and safe method.

The improved efficiency of cell poration and cell fusion offered by the method of this invention has a particular significance in medical applications. One example is to produce antibodies for therapeutic uses. Since the human body usually rejects animal antibodies, such therapeutic antibodies must be produced by hybridomas of human cells; however, human hybridomas are extremely difficult to form by conventional methods (including electro-fusion by DC field). The method of the present invention will help to improve the efficiency in forming human hybridomas. Another example of medical application of this method is gene therapy. Many genetic diseases can be treated by inserting a therapeutic gene into the patient's cells in vitro and then transplanting the cells back to the patient's body. The conventional methods of cell poration (including the DC field method) usually require a large number of cells (typically 5–10 million cells) to perform a gene transfection and, as a result, are unsuitable for use in human therapy. In contrast, the method of the present invention has been demonstrated to be able to transfect cells in small numbers with high efficiency, and will be highly useful for gene therapy.

SUMMARY OF THE INVENTION

An object of the present invention is a method for the poration of cells.

An additional object of the present invention is a method for the fusion of cells.

A further object of the present invention is a device for the poration and fusion of cells.

Another object of the present invention is a method for inserting genetic materials into biological cells.

A further object of the present invention is the treatment of genetic disease by inserting therapeutic genes into cells that are transplanted into diseased patients.

Another object of the present invention is a method for the formation of hybridoma cells by the fusion of cells with RF electric field.

An additional object of the present invention is a method which greatly enhances the efficiency of producing monoclonal antibodies.

Another object of the present invention is the formation of a new species by the fusion of cells from different species using high-power RF pulses.

An additional object of the present invention is the introduction of chemicals and biological molecules into cells by the procedures of poration and/or fusion.

Thus, in accomplishing the foregoing objects there is provided in accordance with one aspect of the present invention a method for poration of biological particles comprising the steps of placing a plurality of biological particles in solution between two electrodes and applying a high-power pulsed RF oscillating field across the electrodes for porating the particles. The biological particles can either be suspended cells in solution or attached cells in cell culture. An additional embodiment of this method includes fusing the biological particles by placing the suspended biological particles in a container which allows the biological particles to congregate before applying the pulsed RF field.

An alternative method includes fusing the biological particles by applying a low power (e.g., 20 to 900 V/cm) alternating current (AC) electrical field before and/or after the pulsed RF oscillating field. The low-power electric field can cause the particles to move dielectrophoretically to form "pearl chains".

The biological particles can be a variety of materials including biological cells (human, animal or plant cells), liposomes, vesicles, erythrocyte ghosts, protoplasts, bacteria, and yeasts.

The pulsed RF field applied for the poration and fusion of cells can be an oscillating field of a single frequency or a mixed frequency. The RF oscillating field may be in the frequency range of 1.0 KHz to 100 MHz with a pulse width of about 1 μsec to 100 msec and a pulse amplitude of up to about 20 KV/cm. In a preferred embodiment the RF oscillating field is about 0.02 to 10 MHz and the pulse width is about 20 to 5000 μsec and the pulse amplitude is about 1–10 KV/cm. The wave form of the RF field may be sinusoidal, triangular, sawtooth, or square waves.

Another aspect of the present invention is the fusion of cells for the formation of new species, the introducing of chemical agents and natural or man-made genetic material into cells, and the formation of hybridoma cells. By the appropriate selection of cell types and materials new species can be formed either by the combining of genetic material from two different species by the fusion of their cells, or by the isolation or synthesis of the genetic material, and then the introduction of the genetic material into cells by either poration or fusion. Hybridoma cells are made by the fusion of antibody producing cells with continuously dividing cancer cells. Chemicals, drugs, DNA, RNA and other molecules can be introduced into cells by preloading vesicles, liposomes or erythrocyte ghosts before fusion with target cells.

Another aspect of the present invention is a device for the poration or fusion of biological particles comprising a container of non-conducting material capable of holding liquid and biological particles. The device also includes electrodes positioned equidistant from each other and inserted into the container. A high-power function generator is attached to the electrodes and is capable of generating a high intensity electrical field including a pulsed RF electric field and/or an alternating electric field. In one embodiment the container is shaped to allow the biological particles to congregate.

An additional aspect is a device for poration and fusion of biological particles comprising a glass chamber and used with an optical microscope for observation of the poration and fusion of cells.

A further aspect is a cell poration and fusion device which can be hand-held. This device includes a handle and equidistant electrodes. The electrodes can be side-attached or bottom-attached and can be designed in a variety of shapes including rings, circles, double helices, squares, ellipses, concentric rings, concentric squares, interdigitating arrays, spirals and parallel plates.

Other and further objects, features and advantages will be apparent from the following description of the presently preferred embodiment of the invention given for the purpose of disclosure when taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be more readily understood from a reading of the following specification by reference to accompanying drawings, forming a part thereof, where examples of embodiments of the invention are shown and wherein:

FIG. 1 is a schematic of one form of the present invention using a chamber which allows for the congregation of cells by gravity. 1A is a top view of the device and 1B is a cross-sectional view of the device showing the fusion chamber.

FIG. 2 is a graph of examples of the radiofrequency (RF) pulses used in the present invention. 2A is a single-frequency symmetrical RF pulse, 2B is an asymmetrical RF pulse, 2C is a multiple-frequency RF pulse, 2D is consecutive RF pulses of different frequencies and 2E is a low-power AC field followed by a high-power RF pulse followed by a low-power AC field.

FIG. 3 is a schematic of one form of the present invention showing a large volume chamber for cell poration and/or cell fusion. 3A is a top view of the fusion chamber and 3B is a cross-sectional view showing the arrangement of electrodes in the chamber.

FIG. 4 is a schematic of a chamber for cell poration and/or cell fusion for optical microscopic observation. 4A is a elevational view of the chamber and 4B is a cross-sectional view of the chamber.

FIG. 5 is a schematic of a hand-held device for cell poration and/or cell fusion using a side contact configuration. 5A shows an elevational view of the device and 5B shows a cross-sectional view of the electrode inserted inside the cell container.

FIG. 6 is a schematic of a double helical design for the side-contact electrode assembly. 6A shows a elevational view of the helical design for the electrode assembly and 6B shows a side view of the same assembly.

FIG. 7 is a schematic view of a segmented ring design for the side-contact electrode assembly. 7A shows an elevational view of the electrode assembly, 7B shows the connection of the electrode rings in the electrode assembly and 7C is a top view of a single electrode ring.

FIG. 8 is schematic of a rectangular electrode assembly for cell poration and cell fusion. 8A shows an elevational view of the electrode assembly and 8B shows the connection of the electrode squares in the electrode assembly.

FIG. 9 is a schematic of a cell fusion and cell poration device with a bottom-contact configuration of electrodes.

FIG. 10 is a schematic of the double spiral design for the bottom-contact electrode assembly. 10A shows a elevational view and 10B shows top view of the electrode.

FIG. 11 is a schematic view of a concentric ring design for the bottom-contact electrode assembly. 11A shows an elevational view and 11B shows a top view of the electrode.

FIG. 12 is a schematic view of different designs for a bottom-contact electrode assembly. 12A is a top view of a square spiral assembly, 12B is a top view of a concentric square assembly, 12C is a top view of an interdigitating array assembly and 12D is a top view of a parallel plate assembly.

FIG. 13 is a schematic of a probe for cell poration and cell fusion of a small number of cells using the RF method. The exterior of the metal electrode is designed to fit inside the wells of a 96-well cell culture plate 13A is a three-dimensional view of the probe, 13B is a cross-sectional view and 13C is a fragmentary elevational view of the electrode.

FIG. 14 is a block diagram of the apparatus which provides the source of the AC field for dielectrophoresis and the high-power RF pulses for cell poration and/or cell fusion.

FIG. 15 is an electron micrograph showing the surface of a human red blood cell following RF poration treatment. Three RF electric field pulses were applied with a one second interval. The cells were rapidly frozen in liquid freon which was cooled by liquid nitrogen (Temperature 90° K.). The frozen sample is examined by freeze-fracture electron microscopy. Magnification 50,000×.

FIG. 16 is fluorescent micrographs showing the events of fusion between human red blood cells. Red cells were lined up in pearl chains by the process of dielectrophoresis. Roughly 10% of the cells were prelabelled with a fluorescent dye which produced bright images under a fluorescence microscope. The unlabelled cells could not be seen. 16A shows how the cells looked before applying the RF pulses. No transfer of dye between labelled and unlabelled cells was seen. 16B shows how the cells looked 4 minutes after 3 RF pulses (40 μsec wide, 300 KHz, 5 KV/cm) were applied. Some of the labelled cells fused with their unlabelled neighboring cells, allowing the fluorescent dye to transfer between them.

FIG. 17 is a graph showing the measured fusion yield between human red blood cells using three electrical pulses (4 KV/cm, 100 μsec). The fusion yield is shown to vary with the oscillating frequency.

FIG. 18 is a time series of optical micrographs showing the fusion of a xanthophore cell with a fish tumor cell induced by pulsed RF fields. 15A is before fusion but after the xanthophore (marked by the arrow) was brought into close contact with two tumor cells by dielectrophoresis. 15B is two minutes after application of the RF pulses showing that the xanthophore has already begun fusing with one of the tumor cells. 15C is 4 minutes after application of the RF pulses showing that the xanthophore and tumor cell have completely merged into a single round cell.

DETAILED DESCRIPTION

In the description which follows, like parts are marked throughout the specifications and drawings with the same referenced numerals. The drawings are not necessarily to scale and certain features of the invention may be exaggerated in scale or shown in schematic form in the interest of clarity and conciseness. It will be readily apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

One embodiment comprises a method for poration of biological particles comprising the steps of placing the biological particles in solution between two electrodes and applying a pulsed radiofrequency (RF) oscillating electric field across the electrodes, FIG. 1. A variety of biological particles can be used including biological cells, erythroycte ghosts, liposomes, protoplasts, bacteria and yeasts. The biological particles can be suspended cells in solution or can be attached cells in cell culture.

When a cell is placed in an electric field, an electrical potential is induced across the cell membrane. For a spherical cell, the membrane potential induced by an external electric field is

$$V_m = 1.5\ rE \cos\eta \quad (1)$$

where r is the radius of the cell, E is the strength of the external field and θ is the angle between the

direction of the external field and the normal vector of the membrane at the specific site.

The induced electric field within the membrane is

$$E_m = V_m/d = 1.5\ (r/d)\ E \cos\theta \quad (2)$$

where d is the thickness of the membrane. Since d is much smaller than r (d is about 6×10^{-7} cm while r is in the order of several microns), E_m is about 1000 fold larger than the applied field, E. The large electric field within the membrane produces two effects. First, it exerts a strong force on the phosphate head group of the lipid molecules in the membrane and tends to move them in the direction of the field. Secondly, it compresses the membrane. When the external electric field oscillates, the lipid molecules within the membrane also undergo an oscillating motion.

In this arrangement, the cell itself functions as an antenna and the membrane is a transducer which converts the electrical oscillation into a mechanical oscillation. Thus, it is possible to generate an ultrasonic motion in the cell membrane by applying an external RF field. Because the induced potential at a given site of the membrane is a function of the angle between the orientation of the membrane and the electric field vector, the induced potential is not uniform over the entire cell surface. The applied energy is focused at the poles of the cell, that is, at $\theta=0°$ or 180°. The combined action of mechanical oscillation and electrical compression on the membrane can cause a localized breakdown of the cell membrane at the poles. Experiments indicated that this localized membrane breakdown induced by the externally applied pulsed RF field is reversible. That is, the pore(s) induced by the RF field reseal quickly (within minutes) after the field is turned off. Furthermore, most of the cells apparently stay viable.

Such temporary permeabilization of the cell membrane is called cell poration. During this time period when pores are formed, a brief exchange of intracellular and extracellular materials occur. Many molecules, including drugs, antibodies, and gene segments, which normally cannot penetrate the cell membrane, can enter the cell through the temporarily opened pores that were induced by the pulsed RF field.

Another embodiment of this invention comprises a method for fusing cells. In order for biological particles to be fused, they must be in close proximity. When cells are in close proximity they are said to congregate. Two alternative procedures may be used to congregate the cells before fusion. In one, a container with a shape that allows the biological particles to congregate by gravity is used. For example, the bottom of the container can be made in a concave shape (see FIGS. 1 and 3). This allows the cells to congregate. When the cell membranes are permeabilized by the applied RF field, the closely adjacent cells can form cytoplasmic bridges. This process results in the fusion of cells.

Alternatively, a low amplitude continuous alternating current (AC) electrical field can be applied across the two electrodes. The frequency ranges from about 60 Hz to about 50 MHz. Typically a 100–400 V/cm field strength is used. Under the low amplitude AC field the cells act as dipoles and line up parallel to the field, eventually forming a long chain of cells which appear like "pearl chains". This process is called "dielectrophoresis" (Schwan, H.P. and Sher, L.D., J. Electrochem. Soc. 116:22C–26C (1969); Pohl, H.A. et al., J. Biol. Phys. 9:67–86 (1981)). Formation of this pearl chain normally takes about a few seconds to one minute.

The present invention uses a pulsed RF field to porate and/or fuse cells and has a clear advantage over the conventional electro-fusion method that uses a pulsed DC field. First, the RF field is a much more efficient means of transmitting energy to the cell membrane than the direct current field. The present invention utilizes a combination of localized sonication and electrical compression to break down the cell membrane. This method is much more effective than the DC pulse method which relies solely on the electrical breakdown. The cell membrane is composed of macromolecules which have characteristic frequencies of thermal motion. When the frequency of the applied oscillating field matches one of these natural frequencies, a condition of resonance is reached, and the efficiency of energy transfer is greatly enhanced. In real biological cells the resonance peak can be very broad. The pulsed radiofrequency field can be carefully varied to achieve the proper resonant frequency for the cells of interest. Consequently, the ability to induce membrane breakdown will require less power than using a direct current field and results in less risk of irreversibly damaging the cell.

Second, this invention overcomes the difficulties encountered when the conventional methods are used to fuse cells of different size. In order to produce an electrical breakdown of the cell membrane, the field-induced membrane potential must exceed a certain critical value, V_c (typically 1 volt). Such breakdown can be reversible, and the membrane will reseal after the external field is turned off if the induced membrane potential is not too much larger than V_c. The cell normally remains viable after such reversible breakdown. On the other hand, if the induced potential is much higher than V_c, the membrane breakdown is irreversible, the cell is permanently damaged, and will not remain viable.

From Eq. 1 it can be seen that when cells of different sizes are placed inside an electric field, the induced membrane potential is higher for the larger cell than for the smaller cell. This size-dependence of membrane potential causes a problem when attempting to fuse cells of different sizes using a DC field. Assume that two cells, A and B, are to be fused and that the radius of cell A, r_a, is about twice as big as the radius of cell B, r_b. In order to cause a reversible membrane breakdown in cell B, the applied external field must be sufficient so that 1.5 E r_b is greater than V_c. However, the same applied electric field will induce a much larger V_m in Cell A, and will cause an irreversible breakdown of the membrane leading to damage to this cell. Thus it is very difficult to use direct current pulses to fuse cells of significantly different sizes.

This problem can be solved by applying a pulsed radiofrequency field. When the applied field is a radiofrequency oscillating field instead of a DC field, the amplitude of the induced membrane potential is a function of the frequency. The membrane potential predicted in Eq. (1) is derived under the steady state condition. The induced potential does not arise instantaneously upon the application of the external field. If the external field is stationary, the membrane potential will reach V_m given a sufficient time. The time required to establish this steady state membrane potential is called "relaxation time", or τ, which is given by

$$1/\tau = 1/R_mC_m + 1/rC_m(R_i + 0.5R_e) \quad (3)$$

where R_m and C_m are specific resistance and specific capacitance of the membrane, and R_i and R_e are the specific resistances of the intracellular medium and the extracellular medium, respectively. (C. Holzapfel et al., *J. Membrane Biol.*, 67:13–26 (1982)). For a cell of several microns in diameter, τ is typically in the order of 1 μsec.

Since R_m in most cells is very large, for practical purposes, eq. (3) can be simplified to

$$=rC_m(R_i+0.5R_e) \quad (4)$$

Thus the relaxation time is approximately proportional to the radius of the cell.

Because the build-up of the membrane potential requires a time period characterized by the relaxation time τ,the membrane potential induced by a RF field is frequency dependent. If a radiofrequency field is applied at a frequency smaller than $1/\tau$, the membrane potential has no problem in following the external field. The applied field will produce a 100% cellular response in V_m. On the other hand, if the frequency of the applied radiofrequency field is greater than $1/\tau$, the membrane potential cannot catch up with the changes in the applied field, and the response of the membrane potential will be less than 100%. In general, the maximum membrane potential induced by a RF field is

$$V(\omega)=1.5\ rE\cos\theta\ X(\omega) \quad (5)$$

where r, E and θ have the same meaning as in Eq. (1), ω is the angular frequency, and $X(\omega)$ is a function of the frequency such that

$$X(\omega)=[1+(\omega\tau)^2]^{-\frac{1}{2}} \quad (6)$$

when $\omega<1/\tau$, $X(\omega)$ is near unity.

When $\omega>1/\tau$, $X(\omega)$ decreases very rapidly with increasing frequency.

This frequency dependent effect can be used to fuse cells of different sizes. From Eq. (4), τ of the cell is roughly proportional to r. If one applies an electrical field with a frequency $\omega>1/\tau$, $X(\omega)$ will approach $1/r$ according to Eq. (6). Then from Eq. (5), the induced membrane potential, $V(\omega)$, will no longer be sensitive to the cell radius r. Consequently, a pulsed radiofrequency field can be applied which induces a reversible breakdown of the membrane of the small cell without irreversibly damaging the larger cell.

One embodiment of a device **10** for poration and/or fusion of biological particles is shown in FIG. **1**. It is a fusion chamber which includes a non-conducting container **13** for holding the solution **16** of biological particles **19**. The container has a slightly concave bottom **22** so that biological particles **19** will congregate, under gravity, between the electrodes **25**. The electrodes **25** are a Pair of equidistant metal wires or metal bands made of nontoxic material, such as platinum or surgical stainless steel. The electrodes can be parallel wires or can be in almost any shape or design. The container **13** has an access port **28** wherein biological particles **19** can be added or removed.

To induce cell-poration or cell-fusion, a high power function generator **31** generates one or many high power RF pulses which are applied through the pair of electrodes **25**. The wave form of the high intensity electric field can be selected from the group consisting of single pulses of symmetrical oscillating fields, multiple pulses of symmetrical oscillating fields, single Pulses of direct current shifted oscillating fields and multiple pulses of direct current shifted oscillating fields. The pulse shape can include one of those shown in FIG. **2**. In FIG. **2A**, the pulse is a symmetrical RF oscillation with a single frequency. In FIG. **2B**, the RF pulse consists of a single frequency asymmetrical sinusoidal wave. In FIG. **2C**, the RF pulse contains a mixture of sinusoidal waves of multiple frequencies (in this example, two frequencies). In FIG. **2D**, alternating sinusoidal pulses of different frequency are used. In the preferred embodiment, the pulse shown in FIG. **2B** is used, because it allows the applied energy of the field to be used more efficiently in inducing cell poration or fusion. Although the preferred RF electric field wave form is sinusoidal, other wave forms with repetitive shapes can be used. For example, triangular waves, square waves, rectangular waves and sawtooth waves can be used to fuse or porate cells of different types.

One skilled in the art will readily recognize that the parameters of the high intensity pulsed electric field are changed to accommodate the characteristics of the different biological samples. The radiofrequency within the pulse may vary over the complete radiofrequency range. For example, the pulse can be in the range of 1 KHz to 100 MHz. Typically a value in the order of 0.02 to 10 MHz is used for the poration and/or fusion of biological cells.

The width of the pulse may vary from about 1 μsec to 100 msec. In the preferred embodiment approximately 20 μsec to 5 msec is used.

The field strength is controlled by varying the pulse amplitude. For fusion and poration of cells the range of 0.2 to 20 kV/cm is employed. In the preferred embodiment pulses of field strength up to about 10 KV/cm are used.

The pulse can be a single pulse, a train of pulses or multiple trains of pulses. A train of pulses are multiple pulses with an interval in between; for example, a series of ten pulses 0.5 milliseconds in width each pulse separated by 0.5 seconds. In some instances such as the fusion of HL-60 cells, the maximum fusion yield is enhanced by applying multiple pulses.

The RF pulses used for cell-poration and cell-fusion are similar. The main difference is that in cell fusion, the cells need to congregate (be brought into close proximity) before the high power RF pulse is applied. Furthermore, the cells must be maintained in close proximity after application of the RF pulse. The above described device brought the cells together by gravitational congregation. An alternative, and more efficient method of cell aggregation is dielectrophoresis, where a continuous alternating current (AC) electric field is applied across the electrodes before and/or after the application of the high-power RF pulse. The amplitude of this continuous AC field is typically in the range of 20 to 900 V/cm. Its frequency may vary from about 60 Hz to about 50 MHz. During cell fusion in the preferred embodiment the actual electric field applied across the electrodes may look like that shown in FIG. **2E**.

In order to facilitate the congregation of the cells and the use of both RF and AC fields the above described function generator can further include the capability of generating a continuous low power AC electric field.

When the function generator includes the capability of generating both a RF and an AC electric field it will preferably include a switch to automatically switch the output between the pulsed high intensity electric field

and the continuous low power alternating current electric field.

Another device for poration and/or fusion of larger volumes of cells is shown in FIG. 3. An array of equidistant electrodes 25 instead of a single pair of electrodes is used to apply the AC field and the pulsed RF field. The bottom of this fusion chamber can be either flat or slightly concave. It is made of transparent material such as glass or clear plastic. This chamber can be placed on top of an inverted optical microscope so that the events of cell fusion and/or cell poration can be directly monitored. Since the effects of different experimental conditions can be assayed in a timely manner with the design, it will be useful for establishing the optimal condition for cell fusion and/or cell poration.

The electrodes can be arranged in any pattern, as long as they are maintained equidistant from each other. In the preferred embodiments the patterns have included interdigitating array, concentric circles and double spirals.

Another preferred device 10 for cell poration and cell fusion is shown in FIG. 4. This device 10 is designed to allow observation of cell fusion under an optical microscope using a small volume of cell suspension. This device is formed by two glass plates 34 separated by spacers 37 of approximately 0.3 mm thickness, with the cell suspension 19 sandwiched between the glass plates 34. In one embodiment thin glass plates such as cover slips are used. Electrodes 25 are two parallel platinum wires which are about 0.5 mm apart. The platinum wire electrodes 25 are connected to a high-power function generator 31. The high-power function generator can generate both alternating current electric fields and pulsed radiofrequency fields. An inlet tubing 41 and an outlet tubing 44 are used to insert and remove cells from the space between the electrodes.

Another embodiment of the present invention for cell poration and cell fusion is shown in FIG. 5. The purpose of this device is to porate or fuse a very large volume of suspended biological particles; including biological cells, protoplasts, bacteria and yeasts. This device 20 is designed for ease in application, maintenance, and cleaning. The cell suspension is contained in a non-conducting cylindrical container 13. The electrode assembly 50 is attached to an insulating handle 47. To porate or fuse the suspended cells, the electrode assembly is lowered into the cell container 13 by manipulating the handle 47. The electrodes 25 are connected to the high-power function generator 31 by a connection means 49. The AC field for cell fusion and the high power RF pulses for cell poration and/or cell fusion are then applied through the electrodes 25 in the electrode assembly 50.

In this device 20 the electrode assembly 50 is a vertical cylinder 53 and metal electrodes 25 are exposed at the side (i.e., the cylindrical surface). The cylinder can be any non-conducting material, for example, glass, plastic, or teflon. When the electrode assembly 50 is lowered into the cell container 13, the suspended cells 19 are displaced and form a thin layer of cell suspension 19 surrounding the electrode assembly 50. Thus, all cells are in close proximity of the electrodes. When an electrical potential is applied across the electrodes, the cells are exposed to the electric field.

One design of the electrode assembly 50 is shown in FIG. 6. Two metal wires or bands are coiled to form a double helix electrode 25. The helices are identical in shape except one is positioned between the other. These two helices are attached to a cylindrical support 53. The spacing between these two helices 25 is kept constant. Thus, when an electrical potential is applied across the two metal wires, the amplitude of the electric field generated between the two helices is uniform along their entire length.

Another embodiment of the electrode assembly 50 for cell poration and cell fusion is shown in FIG. 7. Here the electrode 50 assembly is comprised of a stack of metal ring electrodes 25 separated by non-conducting insulating spacers 53 of fixed thickness. These ring electrodes 25 are connected together in an alternating fashion to form two sets of electrodes 25, each of which is then connected to the output terminals of the high-power function generator. The rings have an attachment means 56 and a hollow area 59 for the passage of the wire to the alternate electrode 25.

The electrodes 25 do not have to be circular, but can be any shape. Shapes which can be used include circular, rectangular as in FIG. 8 or eliptical.

Another embodiment for cell poration and cell fusion is shown in FIG. 9. The cell suspension 19 is contained in a non-conductive container 13. An electrode assembly 50 is attached to a handle 47 which can be used to manipulate the position of the electrodes. Unlike the previous devices, the electrodes of this embodiment are exposed at the bottom of the electrode assembly 50. This device is thus particularly useful in porating and/or fusing cultured cells that attach to the bottom of culture dishes.

One design of the bottom-contact electrode assembly 50 is shown in FIG. 10. The electrode assembly 50 consists of two spirals of metal bands, which serve as the "ground" (−) and "high voltage" (+) electrodes 25. The two spirals are positioned in such a way that the spacing between each spiral is maintained constant. The equal spacing arrangement ensures that an applied electric field across the two electrodes 25 is uniform in strength throughout the entire area covered by the electrode assembly.

In addition to the spiral design, other configurations including, multiple concentric rings, rectangular shapes, interdigitating arrays, parallel plates or eliptical shapes can be used (see FIGS. 11 and 12). The rings or shapes connected in alternating fashion into two groups. One group of these rings or shapes is connected to the "ground" (−) terminal, while the other group of rings or shapes are connected to the "high voltage" (+) terminal of the high-power function generator. The spacing between the rings or shapes is constant so that the strength of the electric field generated between the adjacent rings or shapes is uniform throughout the entire assembly. In the bottom-contact electrode assemblies, the electrodes can be wires, plates or bands. In the preferred embodiment, the width of the electrodes is greater than the depth of the cell suspension.

Another embodiment of the present invention for cell poration and cell fusion is shown in FIGS. 13A–C. The probe 20 allows cell fusion or gene transfection for a small volume of cell suspension. The probe 20 will fit into a flat-bottomed 96-well cell culture plate, for example Corning model 25860. The probe 20 includes two coaxial electrodes 25. The inner electrode 25*a* is a solid cylinder and the outer electrode 25*b* is a hollow tube. The coaxial electrodes 25 can be made of a variety of conductive materials. In the preferred embodiment, the coaxial electrodes 25 are made of stainless steel. The