

APPARATUS FOR ELECTROPORATION WITH COOLING SYSTEM

Field of the Invention

[0001] The present invention relates to electroporation technology, in particular to electroporation apparatuses with a cooling system.

Background of the Invention

[0002] Electroporation is a well-established technique for moving exogenous molecules, such as nucleic acids, drugs, and other compounds, across membranes including cell membranes and the membranes that form liposomes and other lipid-encapsulated vesicles. It involves the external application of electric field of suitable strength across a biological sample, for instance, a suspension of cells. While the mechanism by which electroporation functions is not fully understood, it is generally agreed that in context of living cells, the electric field induces reversible/irreversible structural changes in the cell membrane's lipid bilayer, leading to the formation of transient/permanent pores in the membrane that permit exogenous molecules to enter the cell by diffusion. Under optimal electroporation conditions, these pores reseal after a short period of time and cells will recover and continue to grow.

[0003] Electroporation is executed with an electroporator, an apparatus that applies pulses of a high-voltage electric field to the biological sample via a pair of electrodes, namely a cathode and an anode. It is believed that pore formation in the membrane occurs at the cell poles, the sites on the cell membranes that directly face the electrodes and thus experience the highest transmembrane electric field potential. The outcome of pore formation is dependent on energizing parameters such as pulse amplitude, duration, wave form shape, and repetition rate, in addition to the type and inherent nature of the biological sample.

[0004] Although electroporation is able to increase membrane permeability to exogenous molecules, it may also cause detrimental side effects on the cells, which can be extensive and irreversible. Permanent permeabilization of cell membrane due to excessive exposure to the electric field results in changes in cell homeostasis and cell death. In the

context of therapeutic gene transfer, such deleterious impact seriously hampers the clinical efficacy, because of the resulting low cell viability.

[0005] One of the contributing factors to the electroporation-induced cell damage is the thermal effect due to Joule heating. Cells are sensitive to the change of temperature. The effects of elevated temperatures on biological materials are reviewed in the reference titled 'Bioengineering Heat Transfer, Advances in Heat Transfer' volume 32, published by Academic Press in 1992. The amount of heat produced during an electroporation procedure is not negligible. Intensive research has been performed to examine the thermal effects and to investigate the relevant parameters during electroporation including electrode geometry, pulse frequency and heat dissipation through the electrodes. See 'Thermal effects during electroporation: theoretical and experimental considerations', Roumanian Biotechnological Letters Vol. 9, No. 2, 2004, pp1587-1590; and 'Theoretical analysis of the thermal effects during in vivo tissue electroporation', Bioelectrochemistry Vol. 61, 2003, pp99-107.

[0006] Aware of the thermal effects associated with electroporation, scientists have made great efforts to optimize the experimental electric pulse parameters in order to maximize the amount of molecules transferred across the cell membrane while minimizing the damage to the cells or tissues. However, little has been done to address the problem by improving the functionality of the conventional electroporator, in particular by speeding up the dissipation of the generated heat.

Summary of the Invention

[0007] The present invention provides an apparatus for electroporation with a thermal cooling system. The apparatus comprises an electroporation module for receiving a sample and executing electroporation thereon; and a cooling system for enhancing dissipation of the heat generated by the electroporation module so as to minimize thermal effect on the sample during electroporation.

[0008] According to one aspect of the present invention, the electroporation module comprises a pair of spaced electrodes operable to be connected to an energy source, for directing an electric current through the sample. The cooling system comprises a first and second cooling units, each attached to one of the electrodes for conducting heat therefrom and dissipating heat into ambient air.

[0009] In one embodiment, each of the cooling units comprises a solid-state cooling element.

[0010] In another embodiment, the solid-state cooling element is a Peltier element.

[0011] In yet another embodiment, each of the cooling units further comprises a heat sink thermally coupled to the solid-state cooling element for transferring heat away therefrom.

[0012] According to another aspect of the present invention, the apparatus further comprises a base for detachably supporting a chambered slide and the pair of electrodes, whereby the pair of electrodes is operable to execute electroporation on the sample loaded in the chambered slide.

[0013] In one embodiment, the cooling system further comprises a third cooling unit disposed on the base for conducting heat from the supported chambered slide.

[0014] In other embodiment, the apparatus further comprises an adjusting means for controlling the space between the electrodes.

[0015] According to yet another aspect of the present invention, there is provided an apparatus for large-scale electroporation. The apparatus further comprises a housing that defines an enclosure for accommodating the electroporation module. The electroporation module comprises a plurality of wells for receiving and energizing multiple electroporation cuvettes so as to execute electroporation on the sample loaded therein; and an circuit for electronically connecting each well to an external energy source. The cooling system is operable to control the ambient temperature within the enclosure.

[0016] In one embodiment, the housing comprises a first and second side ends in communication with the external air, and a first and second vertical structures disposed in the housing for creating the enclosure between the first and second side ends. The cooling system comprises a first and second cooling units embedded in the first and second vertical structures respectively.

[0017] In another embodiment, each of the first and second cooling units comprises a solid-state cooling element that has a first surface facing the enclosure for transferring heat away therefrom, and a second surface facing one of the side ends of the housing for dissipating heat to the external air.

[0018] In yet another embodiment, each of the first and second cooling units further comprises a first heat sink having a first end in contact with ambient air in the

enclosure and a second end thermally coupled to the first surface of the solid-state cooling element, thereby being operable to transfer heat from the ambient air within the enclosure to the solid-state cooling element; and a second heat sink having a first end in contact with the external air and a second end thermally coupled to the second surface of the solid-state cooling element, thereby being operable to transfer the heat from the solid-state cooling element to the external air.

[0019] In a further embodiment, each of the first and second cooling units further comprises a first radial fan attached to the first end of the first heat sink; and a second radial fan attached to the first end of the second heat sink; both of which are operable to create air convection to facilitate heat exchange.

[0020] In yet another embodiment, the cooling system further comprises a third cooling unit located within the housing and below the electroporation module. In one embodiment, the third cooling unit is a radial fan for creating air convection below the electroporation module.

[0021] The objectives and advantages of the present invention will become apparent from the following detailed description of embodiments thereof in connection with the accompanying drawings.

Brief Description of the Drawings

[0022] Preferred embodiments according to the present invention will now be described with reference to the Figures accompanied herein, in which like reference numerals denote like elements.

[0023] FIG. 1A shows an isometric perspective view of an apparatus for electroporation in accordance with one embodiment of the present invention.

[0024] FIG. 1B shows a cross sectional perspective view of the apparatus in FIG. 1A.

[0025] FIG. 2A shows an assembled view of an apparatus for electroporation in accordance with another embodiment of the present invention.

[0026] FIG. 2B shows an exploded perspective view of the apparatus in FIG. 2A.

[0027] FIG. 2C shows a top plane view of the apparatus in FIG. 2A.

[0028] FIG. 2D shows a cross sectional view of the apparatus in FIG. 2C.

[0029] FIG. 3A shows an assembled perspective view of an apparatus for electroporation in accordance with yet another embodiment of the present invention.

[0030] FIG. 3B shows a partially exploded perspective view of the apparatus in FIG. 3A.

[0031] FIG. 3C shows a top plane view of the apparatus in FIG. 3A.

[0032] FIG. 3D shows a cross section view of the apparatus in FIG. 3C along axis J-J.

[0033] FIG. 4A shows an exploded perspective view of the electroporation array assembly and the first cooling units of the apparatus in FIG. 3A.

[0034] FIG. 4B shows a partial enlarged perspective view of FIG. 4A.

[0035] FIG. 5A shows an enlarged isometric perspective view of one of the cooling units in FIG. 3A.

[0036] FIG. 5B shows an exploded perspective view of the cooling unit in FIG. 5A.

Detailed Description of the Invention

[0037] The present invention may be understood more readily by reference to the following detailed description of certain embodiments of the invention.

[0038] Now referring to FIG. 1, there is provided an apparatus 1 for electroporation, particularly for *in vivo* application, in accordance with one embodiment of the present invention. FIG. 1A shows an isometric perspective view of the apparatus 1; FIG. 1B a cross sectional perspective view. The apparatus 1 comprises a pair of spaced electrodes 11 for applying electric pulses to a sample positioned therebetween, and a first and second cooling units 12 attached to one of the electrodes 11 respectively for dissipating heat therefrom into ambient environment.

[0039] Each electrode 11 has a stretching body 112, a first end 114 and a second end 116. The first ends 114 of electrodes are housed in an electronically insulated holder 14, and are kept apart by an electrically insulated spacer 15. Three electronically insulated pins 142 are lodged to fix the electrodes 11, spacer 15 and holder 14 together for preventing displacement thereof. A corresponding female socket (not shown in FIG. 1A and 1B) with electrical connection outlets is attached onto the distal end of the holder 14

for detachably connecting the electrodes 11 to an external power source via electrical wiring of appropriate length. The second ends 116 of electrodes are operable to be in direct contact with the biological sample, e.g. animal tissues, and are configured to be substantially parallel to one another, so as to create a uniform electric field in between when energized. The bodies 112 of electrodes are coated with an electrical insulating material.

[0040] In one embodiment as shown in FIG. 1A, the second end 116 of each electrode 11 is of a disk shape. In another embodiment, the second end 116 of each electrode 11 is of a needle shape. In yet another embodiment, the second end 116 of each electrode 11 is oval shaped.

[0041] In one embodiment, the distance between the second ends 116 of the electrodes is adjustable.

[0042] As shown in FIG. 1B, the cooling units 12 are attached to the outer surfaces of the electrode bodies 112, being away from the second ends 116 for a pre-determined distance so as to avoid any contact with the biological sample. Each cooling unit 12 includes a solid-state cooling element 122 and a heat sink 124. The solid-state cooling element 122 has a first surface in direct contact with the electrode body 112, and a second surface with the base 124a of the heat sink 124. A plurality of fins 124b extends from the heat sink base 124a for increasing the heat exchange surface with the ambient environment.

[0043] In one embodiment, the solid-state cooling 122 is a Peltier element, which includes two lead wires (not shown in FIG. 1A and 1B) for electronically connecting to an external power source. In operation, when a DC voltage is applied to the Peltier element 122, the first surface of the Peltier element will cool to a pre-set temperature, e.g. room temperature, and the second surface will heat. Heat generated in the biological sample during the electroporation process will be transferred to the second end 116 of each electrode 11, conducted along the electrode body 112 to the coupled cooling unit 12, where the heat is transformed from the first surface of the Peltier element 122 to the second surface against the temperature gradient, and eventually dissipated to the ambient air via the heat sink 124. Through instant removal of the generated heat, the detrimental thermal effect of the electroporation on the treated biological sample is minimized.

[0044] It is to be understood that the solid state cooling element 122 is not limited to Peltier element. Other cooling devices, such as thermotunneling heat pump, may be applicable as well.

[0045] In one embodiment, the cooling units 12 are coupled to the electrodes 11 via heat conducting adhesive means. In another embodiment, the cooling units 12 are fixed to the electrodes 11 via mechanical means such as screws and nuts.

[0046] In yet another embodiment, the cooling units 12 do not have the heat sinks 124 attached to the solid-state cooling elements 122. Instead, the second surface of each solid-state cooling element 122 is directly exposed to the ambient environment.

[0047] Now referring to FIG. 2, there is provided an apparatus 2 for electroporation, particular for applying electroporation to cells grown in a culture chamber, in accordance with another embodiment of the present invention. FIG. 2A shows an assembled perspective view of the apparatus 2; FIG. 2B an exploded perspective view; FIG. 2C a top plane view; FIG. 2D a cross sectional view. The apparatus 2 comprises a planar base 24, a pair of electrodes 22 detachably disposed thereon, adjusting means 25 for controlling the inter-electrode distance, a first and second cooling units 22 attached to one of the electrodes respectively, and a third cooling unit 23 (not shown in FIG. 2A) installed on the base 24.

[0048] The base 24 is rectangular and dimensioned to house the electrodes 22 and at least one chambered slide 20 simultaneously. A typical chambered slide 20, as shown in FIG. 2A and 2B, comprises a rectangular glass slide 202, and two leak-proof cell culture chambers 204 formed thereon. The upper surface of the glass slide 202 within the chamber 204 may be chemically pre-treated to facilitate cell adhesion.

[0049] The base 24 further has a mounting plate 242 fixed thereon for providing an elevated stage, and two vertical supporting structures 244 disposed at two ends of the mounting plate 242 for supporting the adjusting means 25. The adjusting means 25 comprises a guide rod 252 and a threaded shaft 254, which are parallel to each other in the horizontal direction. The thread shaft 254 is rotably connected to the vertical supporting structures 244 via bearings 256. A driving knob 258 is coupled to one end of the shaft 254 for actuating the rotation thereof. The outer thread along the longitudinal axis of the shaft 254 is so configured that a first half of which is in a first direction and a second half of which is in the opposite direction.

[0050] Two electronically insulated holders 246 are arranged to engage the electrodes 21 with the adjusting means 25. Each holder 246 has a first through hole 246a for engaging with the guide rod 25, a second through hole 246b with inner thread matching with the outer thread on one half of the thread shaft 254, and a planar top surface 246c for connecting to and supporting the first end 214 of electrode 21. The inter-electrode distance is adjustable through controlling linear displacement of the two holders 246 along the longitudinal axes of the threaded shaft 254 and guide rod 252 by rotating the thread shaft 254.

[0051] The configuration of each electrode 21 is similar to the earlier embodiment shown in FIG. 1, except that the first end 214 is so configured to be detachably mounted onto the top surface 246c of the holders, and that the second end 216 is so dimensioned as to apply an electric field cross the width and depth of the chambers 204. A user is able to choose electrodes 21 of optimal configuration, e.g. geometry, size, material, tip surface size, to match with chambers 204 of distinct size and shape.

[0052] Still referring to FIG. 2A and 2B, the first and second cooling units 22 are attached to the outer surfaces of the electrodes 21 respectively, and have identical configurations to the ones 11 in the earlier embodiment shown in FIG. 1. The position of each cooling unit 22 relative to the second end 216 of electrode is so configured that the cooling unit 22 is able to instantly and optimally absorb electroporation-generated heat from the second end 216 of electrode without contaminating the biological sample in the chamber 204.

[0053] Now referring to FIG. 2B and 2D, a recess 246 is carved in the base 24 at the position for mounting the chambered slide 20. The third cooling unit 23 is disposed therein, which forms a planar surface with the base 24 to support the chambered slide, and functions to absorb and dissipate heat from the chambered slide 20 during electroporation.

[0054] In one embodiment, the third cooling unit 23 is a Peltier element. In another embodiment, the size of the third cooling unit 23 is the same as the chambered slide 20.

[0055] Now referring to FIG. 3, there is provided an apparatus 3 for high throughput *ex vivo* electroporation, in accordance with another embodiment of the present invention. FIG. 3A shows an assembled view of the apparatus 3; FIG. 3B a partially exploded perspective view; FIG. 3C a top plane view; FIG. 3D a cross sectional view. The apparatus 3 comprises a housing 36 defining an internal enclosure 362, an electroporation

array assembly 37 disposed in the enclosure 362 for receiving multiple electroporation cuvettes 30 and carrying out electroporation therein; and a plurality of cooling units 32, 33 for controlling ambient temperature in the enclosure 362.

[0056] The housing 36 is of a rectangular configuration, having two side openings 364 in communication with the surrounding air and a pivotal lid 366 installed in the top surface. Two vertical structures 368 are disposed in the housing 36 for creating the internal enclosure 362 between the two side openings 364 and beneath the lid 366, where the electroporation array assembly 37 is accommodated. The array assembly 37 has twenty-five sample wells 373 arranged in 5 by 5 grid for holding multiple electroporation cuvettes 30. One cooling unit 33 is installed within the enclosure 362 and beneath the array assembly 37, whilst two cooling units 32 are mounted on each vertical structure 368.

[0057] Now referring to FIG. 4A, there are provided an exploded perspective view of the array assembly 37 and the cooling units 33. The array assembly 37 comprises five pairs of side blocks 371 and one bottom block 372 to form the sample wells 373 (not shown in FIG. 4A), and an operational circuit 374 (now shown in FIG. 4A) for electronically connecting each sample well 373 to an external power source (not shown in FIG. 4A).

[0058] Each side block 371 has five recesses 375 carved in a row. FIG. 4B provides an enlarged view of one of such recesses 375. The recess 375 has a leaf spring 376 disposed therein. The leaf spring 376 has a first end 376a electronically connected to the operational circuit 374 (not shown in FIG. 4B), and a second end 376b for biasing against the electrode 31 (not shown in FIG. 4B) embedded in the sidewall of the loaded conventional electroporation cuvette 30 (not shown in FIG. 4B) in order to supply the electric power thereto.

[0059] It is to be understood by a person skilled in the art that the number of sample wells 373 can be varied and scalable depending on the specific application requirements.

[0060] Referring back to FIG. 4A, the operational circuit 374 (not shown in FIG. 4A) is electronically connected to the sample wells 373 by a matrix of link bars 377a and link rods 377b that are integrated with the side blocks 371 and bottom blocks 372 via a plurality of tie bars 378a and lock plates 378b.

[0061] In accordance with one embodiment, a controller is provided to regulate the operational circuit 374 so that the sample wells can be energized independently from one other. In other embodiment, the controller is operable to direct a series of pulse trains generated by the external energy source to pre-selected sample wells 313 in a pre-determined sequence.

[0062] Now referring back to FIGs. 3B and 4A, the array assembly 37 is supported and elevated by a pair of brackets 365 disposed in the housing 36, thereby creating a space beneath the bottom block 372. The cooling unit 33 is installed therein. In one embodiment, the cooling unit 33 is a radial fan, the rotation of which creates an artificially induced air convection within the space to enhance heat dissipation.

[0063] The four cooling units 32 are identical. FIG. 5A and 5B provide an enlarged isometric view and an exploded view of one of the cooling unit 32. The cooling unit 32 comprises a solid-state cooling element 322, two heat sinks 324 sandwiching the solid-state cooling element 322; and two radial fans 326 connected to the heat sinks 324 at distal ends. The configuration of the heat sinks 324 is identical to the one in the earlier embodiments shown in FIG. 1 and FIG. 2.

[0064] Now referring back to FIG. 3D, each solid-state cooling element 322 is embedded in one of the vertical structures 368, having a first surface 322a facing towards the enclosure 362 and a second surface 322b opposite thereto. In one embodiment, the solid-state cooling element 322 is a Peltier element. When electrical current is supplied thereto via two lead wires, heat will be transferred from the first 322a to the second 322b surface. Radial fans propel ambient air towards the heat sinks 324 that are in turn thermally coupled to the Peltier elements 322. In this way, heat generated within the enclosure 362 during electroporation process will be instantly dissipated to the surrounding air outside the housing 36.

[0065] It is to be understood by a person skilled in the art that the number of cooling units 32 can be varied depending on the specific application requirements.

[0066] While the present invention has been described with reference to particular embodiments, it will be understood that the embodiments are illustrative and that the invention scope is not so limited. Alternative embodiments of the present invention will become apparent to those having ordinary skill in the art to which the present invention pertains. Such alternate embodiments are considered to be encompassed within the spirit

and scope of the present invention. Accordingly, the scope of the present invention is described by the appended claims and is supported by the foregoing description.

Claims

1. An apparatus comprising:
 - an electroporation module for receiving a sample and executing electroporation thereon; wherein the electroporation module comprises a pair of spaced electrodes operable to be connected to an energy source, for directing an electric current through the sample;
 - a cooling system for enhancing dissipation of the heat generated by the electroporation module so as to minimize thermal effect on the sample during electroporation; wherein the cooling system comprises a first and second cooling units, each attached to one of the electrodes for conducting heat therefrom and dissipating heat into ambient air; and
 - a base for detachably supporting a chambered slide and the pair of electrodes, whereby the pair of electrodes are operable to execute electroporation on the sample loaded in the chambered slide.
2. The apparatus in accordance with claim 1, wherein each of the cooling units comprises a solid-state cooling element.
3. The apparatus in accordance with claim 2, wherein the solid-state cooling element is a Peltier element.
4. The apparatus in accordance with claim 2, wherein each of the cooling units further comprises a heat sink thermally coupled to the solid-state cooling element for transferring heat away therefrom.
5. The apparatus in accordance with claim 1, wherein the space between the electrodes is adjustable.
6. The apparatus in accordance with claim 1, wherein the cooling system further comprises a third cooling unit disposed on the base for conducting heat from the supported chambered slide.

7. The apparatus in accordance with claim 6, wherein the base has a recess for housing the third cooling unit; and
wherein the third cooling unit is so dimensioned to form a planar supporting surface with the base for supporting the chambered slide, and so positioned to be beneath the chambered slide.
8. The apparatus in accordance with claim 6, wherein the third cooling unit comprises a solid-state cooling element.
9. The apparatus in accordance with claim 8, wherein the solid-state cooling element is a Peltier element.
10. The apparatus in accordance with claim 1, which further comprises an adjusting means for controlling the space between the electrodes, the adjusting means having:
a first and second support structures mounted on the base,
a threaded shaft and a guide rod, both being supported by the first and second support structures, and aligned with each other in the horizontal direction; wherein the threaded shaft has a first half that is threaded in a first direction and a second half that is threaded in a second direction; and
a first and second holders for releasably holding the pair of electrodes in parallel, wherein each holder has a first bore that is internally threaded so as to engage with and maneuver on one half of the threaded shaft, and a second bore for engaging with the guide rod.
11. The apparatus in accordance with claim 1, which further has a housing that defines an enclosure for accommodating the electroporation module; and
wherein the cooling system is operable to control the ambient temperature within the enclosure.
12. An apparatus comprising:

an electroporation module for receiving a sample and executing electroporation thereon;

a cooling system for enhancing dissipation of the heat generated by the electroporation module so as to minimize thermal effect on the sample during electroporation; and

a housing that defines an enclosure for accommodating the electroporation module; wherein the housing comprises

a first and second side ends in communication with the external air; and

a first and second vertical structures disposed in the housing for creating the enclosure between the first and second side ends; and

wherein the cooling system comprises:

a first and second cooling units embedded in the first and second vertical structures respectively,

wherein each of the first and second cooling units comprises a solid-state cooling element that has a first surface facing the enclosure for transferring heat away therefrom, and a second surface facing one of the side ends of the housing for dissipating heat to the external air.

13. The apparatus in accordance with claim 12, wherein each of the solid-state cooling elements is a Peltier element.

14. The apparatus in accordance with claim 12, wherein each of the first and second cooling units further comprises:

a first heat sink having a first end in contact with ambient air in the enclosure and a second end thermally coupled to the first surface of the solid-state cooling element, thereby being operable to transfer heat from the ambient air within the enclosure to the solid-state cooling element; and

a second heat sink having a first end in contact with the external air and a second end thermally coupled to the second surface of the solid-state cooling element, thereby being operable to transfer the heat from the solid-state cooling element to the external air.

15. The apparatus in accordance with claim 14, wherein each of the first and second cooling units further comprises:
- a first radial fan attached to the first end of the first heat sink; and
 - a second radial fan attached to the first end of the second heat sink;
 - both of which are operable to create air convection to facilitate heat exchange.
16. The apparatus in accordance with claim 12, wherein the cooling system comprises a third cooling unit located within the housing and below the electroporation module.
17. The apparatus in accordance with claim 16, wherein the third cooling unit is a radial fan for creating air convection below the electroporation module.
18. The apparatus in accordance with claim 12, wherein the electroporation module comprises:
- a plurality of wells for receiving and energizing multiple electroporation cuvettes so as to execute electroporation on the sample loaded therein; and
 - an circuit for electronically connecting each well to an external energy source.
19. The apparatus in accordance with claim 18, which further comprises a controller for regulating the circuit so that each well is operable to be independently energized with pre-set electric parameters and in a pre-determined order.
20. An apparatus comprising:
- an electroporation module for receiving a sample and executing electroporation thereon; wherein the electroporation module comprises a pair of spaced electrodes operable to be connected to an energy source, for directing an electric current through the sample; and
 - a cooling system for enhancing dissipation of the heat generated by the electroporation module so as to minimize thermal effect on the sample during electroporation; wherein the cooling system comprises a first and second cooling units;
- wherein each of the electrodes comprises:
- a first end electronically connected to the energy source;

a body covered with electrically insulated coating; and

a second end in contact with the sample for directing the electric current therethrough; and

wherein each of the cooling units is attached to the body at a pre-determined distance from the second end, so as to optimally conduct heat without contaminating the sample.

21. The apparatus in accordance with claim 20, wherein each of the first and second cooling units comprises a solid-state cooling element.

22. The apparatus in accordance with claim 21, wherein each of the solid-state cooling elements is a Peltier element.