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Cell separation using electric fields

Abstract

The present invention involves methods and devices which enable discrete objects having a conducting inner core, surrounded by a dielectric membrane to be selectively inactivated by electric fields via irreversible breakdown of their dielectric membrane. One important application of the invention is in the selection, purification, and/or purging of desired or undesired biological cells from cell suspensions. According to the invention, electric fields can be utilized to selectively inactivate and render non-viable particular subpopulations of cells in a suspension, while not adversely affecting other desired subpopulations. According to the inventive methods, the cells can be selected on the basis of intrinsic or induced differences in a characteristic electroporation threshold, which can depend, for example, on a difference in cell size and/or critical dielectric membrane breakdown voltage. The invention enables effective cell separation without the need to employ undesirable exogenous agents, such as toxins or antibodies. The inventive method also enables relatively rapid cell separation involving a relatively low degree of trauma or modification to the selected, desired cells. The inventive method has a variety of potential applications in clinical medicine, research, etc., with two of the more important foreseeable applications being stem cell enrichment/isolation, and cancer cell purging.

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Claims

What is claimed is:

1. A system for creating from a biological sample having a given cell population of at least a first and a second cell type, a suspension containing a selected viable subpopulation of said given cell population, said selected viable subpopulation being enriched in said second cell type by porating a substantial fraction of cells of said first cell type and selectively inactivating at least 90% of the porated cells of said first cell type while maintaining substantially viable cells of said second cell type, the system including: a generating mechanism which generates an electric field of a magnitude and duration sufficient to irreversibly porate a substantial fraction of cells of said first cell type while maintaining substantially viable cells of said second cell type; and a treatment cell electrically connected to said generating mechanism and adapted to contain a cell suspension.

2. A system as in claim 1, wherein said generating mechanism includes an electric pulse driver.

3. A system as in claim 1, wherein said generating mechanism generates a magnetic field that induces said electric field.

4. A system as in claim 1, wherein said treatment cell is constructed and arranged to provide a treatment volume for batch treatment of a cell suspension therewithin.

5. A system as in claim 1, wherein said treatment cell includes a flow path having an inlet and an outlet and is constructed and arranged to provide a treatment volume for continuous flow treatment of a cell suspension therewithin.

6. A system as in claim 1, wherein said system includes a cooling system constructed and arranged to control the operating temperature of said treatment cell.

7. A system as in claim 1, wherein said treatment cell is constructed and assembled without the need for supplemental seals.

8. A system as in claim 1, further including a mechanism operative on the suspension to remove inactivated cells and cell debris therefrom.

to poration that is sufficient to cause death, inactivation, and/or physical disruption of a discrete object without a need for a secondary inactivating step. Inactivation and cell death due to poration are believed to be caused by a loss of the permi-selective nature of the membrane leading to cell death and/or membrane disruption, or a direct physical disruption of the membrane caused by extensive poration. In the case of a loss of the permi-selective nature of the membrane, the inactivation or cell death is ultimately caused by diffusion of previously excluded molecular species, especially small ionic species such as Na.sup.+, K.sup.+, and Ca.sup.++, across the membrane followed by an uptake of water across the membrane into the cell in an attempt to achieve osmotic equilibrium with the suspending fluid medium, which can lead to colloidal osmotic lysis and irreparable (fatal) cell lysis, or to a lethal disruption of cellular metabolism. For embodiments involving a method where the applied field porates some or all of the cells to be inactivated without inactivating all of such cells, where the inactivation step is performed in a subsequent step, the poration induced by the applied field is typically less extensive and not irreversible, at least for a certain portion of the porated cells. Given sufficient time, the reversibly porated cells in such samples could seal their pores and retain long-term viability if left in the same fluid carrier or suspending media in which they were subjected to the electric field. The reversibly porated cells may, however, be effectively inactivated by resuspending them in a different post-poration media, adjusting the temperature of the poration media, and/or adding a supplemental agent to the poration media which accelerates cell death, colloidal osmotic lysis, or prevents the resealing of membrane pores. More specific techniques and conditions are discussed below.

[0096] The electric field is preferably applied to the cell suspension within a spatially defined treatment cell. The treatment cell can be designed as a static non-flow volumetric container in which the cell suspension to be treated is placed, or more preferably, the treatment cell will include an inlet and an outlet constructed and arranged to enable a cell suspension to continuously flow through the treatment volume. Systems including flow-through treatment cells may be arranged so that the cell suspension passes through the treatment cell only once (one pass) or a plurality of times (recirculating). In addition, either the flow or static systems may include multiple treatment cells. For flow systems, multiple treatment cells can be arranged in a series or parallel configuration.

[0097] The treatment cell will include at least one electrode in electrical communication with the cell suspension to be treated. Preferably the treatment cell will include two electrodes placed on either side of and in electrical communication with the cell suspension during operation to which an electric potential is applied to produce an electric field within the treatment volume. In preferred embodiments, the treatment cell and electrodes are constructed and arranged to impose an electric field that is substantially spatially uniform within the treatment volume so that all cells in the suspension are exposed to similar electric field conditions. In some embodiments, the electric field applied to the cell suspension is created by an electric signal applied to the electrodes; however, it is also contemplated that the electric field can be induced in the sample cell via induction by a magnetic field.

[0098] In order to reduce the tendency for the electrical potential applied to the electrodes to discharge by arcing, and in order to reduce the degree of electrical heating that occurs in the cell suspension, in certain preferred embodiments, the applied electric field is pulsed for short durations, such durations, except as otherwise described herein, being shorter than the residence time of the treated cell suspension in the treatment volume during the step of subjecting the suspension to the applied electric field. Such electric fields are hereinafter referred to as "pulsed electric fields" or PEFs. The shape of the electric field pulse is preferably substantially rectangular in shape, thus providing very short voltage rise and fall times and a substantially constant magnitude over the entire pulse length. Such rectangular pulse shapes yield the best performance and poration threshold resolution obtainable with the inventive method. While rectangular pulses are preferred, any pulse shape known in the art may be employed in performing the methods of the invention, especially when high resolution is not required, as, for

example, when inactivating a cell type that is substantially larger than the desired cell type.

[0099] As described previously, the electric field parameters required to effect a desired cell inactivation or isolation depend upon the nature of the cells, the suspension and suspending fluid, and the characteristics of the electric field application apparatus. The exact parameters for any given sample that will yield desired results must be found in practice via routine experimentation. What follows herein is a theoretical development and description of the inventive method, apparatus for performing the method, and important parameters affecting the performance and selectivity of the method to provide guidance to those of skill in the art in selecting parameters to develop successful cell or discrete object isolation and inactivation strategies.

[0100] The Fundamental Basis of Electric Field Cell Isolation

[0101] The mechanism by which electric fields, and particularly pulsed electric fields (PEFs), isolate cells can be best understood by examining the response of a single discrete object, as exemplified by a biological cell, to an externally applied electric field. A schematic illustration of such a system 50 is shown in FIGS. 1a-e. The externally applied electric field 57 can be established by applying a constant voltage or voltage pulse across a pair of electrodes 55 and 56 that are in electrical communication with, and preferably in physical contact with, a cellular suspension containing a plurality of cells, one of which 51 is shown in FIG. 1a. Alternatively, the electric field 57 can be applied inductively by creating a time-varying magnetic field throughout the cellular suspension. To preserve the viability of the desired target cells, the carrying fluids in which the biological cells are suspended are typically buffered saline solutions having, in some embodiments, a standard physiological osmolality (e.g. 275-300 mOs/kg-water for most mammalian cells), and a pH in the physiological range (e.g. about 7.0-7.6 for most mammalian cells). The ionic strength of the solutions in certain embodiments is essentially the same as the ionic strength of the intracellular fluid 53 (e.g. about 0.15 M NaCl equivalent for most mammalian cells). As such, these are conducting solutions. Electric field effects on cells can be estimated from the potential theory developed by Coulson (Coulson C A: Electricity, Oliver and Boyd, London, Chapter 9, 1951) incorporated herein by reference. This theory implies that induced transmembrane potentials depend on cell size and shape. Formally, the external electric field induces a potential across the cell 51, V.sub.cell, given by 1 V cell = f l E where f = l / (l - 1 3 d) Eq . 1

[0102] and where E is the field strength of the imposed electric field; d is the cell diameter 54, l is the projected length of the cell in the electric field direction 57; and f is a form factor, which is equal to 1.5 for a spherical cell (where l is equal to d) and is approximately unity for large aspect ratio cylindrically shaped cells (where l>>d). In the development to follow, the cells of interest will be assumed to be spherical so that d will be used for l in the following equations and f will be set equal to 1.5. For a more detailed discussion on the effects of non-spherical cell shape and angular orientation with the applied electric field, the reader is referred to Kinosita and Tsong (Kinosita K and Tsong T Y, Voltage-induced pore formation and hemolysis of human erythrocytes, Biochim et Biophys Acta. 471:227-242, 1977).

[0103] Biological cells have an outer, semipermeable plasma membrane 52 that allows the cell to control its internal environment by its selective permeability. The proper function of this membrane is crucial to the viability of the cell. If the function of this membrane is altered or destroyed, cell death often follows. Plasma membranes are typically lipid bilayers which behave electrically as dielectrics, i.e., they behave as electrical insulators. For eukaryotic cells, as shown in FIG. 1a, the cell nucleus 68 and accompanying nuclear membrane 69 reside within the outer membrane 52, with cytoplasm 53 filling the gap between the nuclear and outer membranes. For prokaryotic cells, there is no nucleus or nuclear membrane, so the cytoplasm, which supports the cell's genetic information (one or more DNA molecules in the form of nucleoids) fills the entire intracellular volume. Cytoplasm, which refers collectively to the

substance filling the gap 53 between the outer and nuclear membrane for eukaryotic cells, or the entire intracellular volume for prokaryotic cells, is mainly composed of cytosol, which is a semifluid concentrate having an electrical resistivity that is similar to that of aqueous solutions having a standard physiological ionic strength. As such, the cytosol is electrically conductive, which dictates that the intracellular volume of both eukaryotic and prokaryotic cells is electrically conductive. Thus, biological cells can be viewed as a conducting intracellular region surrounded by a dielectric (insulating) membrane 52. With this conceptual view of biological cells, application of an external electric field 57 causes charge separation to occur inside the biological cell 51 resulting in a nearly constant intracellular potential that has a value corresponding to the boundary average of the potential established on the outer surface of the cell's dielectric membrane 52. If the poles of the cell 51, of which there are two, are defined as the two points formed on the surface of the cell 51 by the intersection of a ray parallel to the electric field direction passing through the center of the cell, then application of an external electric field causes one half of the pole-to-pole potential drop outside of the biological cell 51 to develop across the membrane 52 at each pole of the cell. That is, the externally applied electric field 57 produces a maximum transmembrane potential, V.sub.m, at each pole of the cell 51 that scales as

V.sub.m=V.sub.cell/2 Eq. 2

[0104] or equivalently

V.sub.m=3dE/4 Eq. 3

[0105] for a spherical cell.

[0106] Since, in response to an externally applied electric field 57, the potential drop, V.sub.cell, that develops over a cell's diameter 54 or projected length is transferred approximately equally across the two poles of the cell's membrane 52, the maximum electric field thereby imparted to a cell's membrane 52 is

E.sub.m=V.sub.cell/2t.sub.m, Eq. 4

[0107] or, for spherical cells

E.sub.m=3Ed/4t.sub.m Eq. 5

[0108] or equivalently

E.sub.m=V.sub.m/t.sub.m Eq. 6

[0109] where E.sub.m is the electric field imparted to the membrane 52 for an externally applied electric field 57 of strength E; d is the diameter 54 of the biological cell 51; and t.sub.m is the thickness of the membrane. Thus it is apparent from equation 5, that the imposed electric field within the cell membrane is directly proportional to cell size and applied electric field strength and inversely proportional to the thickness of the cell membrane. Since the size of many typical biological cells falls within a range of 1<d<50 .mu.m and a typical thickness of cell membrane 52 lipid bilayer is approximately 5 nm, the electric field strength imparted to the membrane 52 can be two to three orders of magnitude greater than the strength of the externally applied electric field 57. More specifically, for a typical lipid bilayer membrane thickness of about 5 nm, a transmembrane potential, V.sub.m, of approximately one Volt will impart a 2 MV/cm electric field, E.sub.m, to the lipid bilayer membrane 52. So for a 10 .mu.m diameter spherical cell, which, for example, is about the mean size of peripheral blood cells, a 2 kV/cm externally

FIGS. 1b-e.

[0116] Electric field strength, total exposure time, and pulse duration, for PEFs, can be selected to preferentially inactivate biological cells in a suspension which are more susceptible to electric fields due, for example, to their having one or more or a combination of the following properties with respect to other cells in the suspension: a larger average size; a thinner effective dielectric membrane thickness; a more spherical shape, etc. Of particular importance for many biological samples, especially those having cells with similar shapes, such as roughly spherical, and similar dielectric membrane thickness, is selective inactivation of cells based on a difference in characteristic size. Typically, the threshold electric field required for cell inactivation is inversely proportional to the characteristic size of the cell, i.e., from Eq. 7, E.sub.c(d)=4V.sub.mc/3d, where V.sub.mc.apprxeq.1 Volt is the critical transmembrane potential for the onset of irreversible pore formation for a wide variety of cell types and d is the diameter or characteristic size of the cell. If the undesirable cells are larger in diameter than the desirable cells, then the pulsed electric field method can be used to selectively inactivate the larger cells. By operating at electric field strengths just below the characteristic electroporation threshold for inactivation of the desirable cells, yet above the characteristic electroporation threshold for the undesirable cells, a substantial fraction of the undesirable cells can be preferentially inactivated while leaving a substantial fraction of the desirable cells (primitive stem cells for example) essentially unaltered and still viable. To further illustrate the utility of the concept, a specific example related to cell isolation from hematopoietic cell suspensions will be illustrated. Table 2 lists the types of blood cells that typically will be present in bone marrow specimens during tumor cell purging and stein cell isolation processing. The cell diameters, relative abundance, and projected threshold electric field strengths (E.sub.c as calculated from Eq. 7 assuming V.sub.mc.apprxeq.1 volt) for the onset of membrane damage are also provided in the table. Similar cell sizes as those listed would be expected for hematopoietic cells derived from mobilized peripheral blood, umbilical cord blood and fetal liver tissue, although the relative abundance of each may differ. Table 2 clearly shows that the electric field damage threshold for stem cells can be significantly greater than for the other leukocytes present in bone marrow specimens. Furthermore, the electric field threshold for stem cells can be more than a factor of two greater than for breast cancer cells. Since, as will be discussed below, the fraction of cells inactivated by an applied electric field scales exponentially with electric field strength (Hulsheger 1983) the factor of two difference in the critical electroporation threshold should allow essentially complete inactivation of breast cancer cells with preservation of the viability of the cells crucial for autologous transplantation (stem cells).

2TABLE 2 Electric field damage thresholds for leukocytes and stem cells. Projected Electric Characteristic Relative Field Damage Size Abundance Threshold Cell Type (.mu.m) (%) (kV/cm) Stem .sup. 6.sup.a,b 0.001.sup.a 2.2 Lymphocyte (resting) 7.sup.b 21.sup.c 1.9 Lymphocyte 12.sup.d n/a 1.1 (active) Neutrophil 12.sup.d 73.sup.c 1.1 Eosinophil 13.sup.d 4.sup.c 1.0 Basophil 15.sup.d 0.1.sup.c 0.9 Monocyte 15.sup.d 2.sup.cc 0.9 Breast Cancer >15.sup.e n/a <0.9 .sup.aBerardi AC, et al: Functional isolation and characterization of human hematopoietic stem cells. Science, 267:104-108, 1995. .sup.bZipori D, et al: Introduction of Interleukin-3 gene into stromal cells from the bone marrow alters hematopoietic differentiation but does not modify stem cell renewal. Blood 71:586, 1988. .sup.cJandl JH, Blood: Textbook of Hematology, Little, Brown and Company, Boston/Toronto, 1987. .sup.dHenry JB: Clinical Diagnosis and Management by Laboratory Methods, 16th Ed., W.B. Saunders Company, Philadelphia, PA, Vol. 1, 1979. .sup.efrom observations by inventors

[0117] Another feature which, in the present example, further can enhance the ability to perform preferential electric field isolation of stem cells and/or to purge relatively large tumor cells, such as breast cancer cells, involves the quiescent nature of stem cells. As discussed in Berardi, et al., stem cells are quiescent and are unaffected by an anti-metabolite treatment, whereas rapidly proliferating cells are inactivated by an anti-metabolite treatment. A similar phenomenon has been observed (Hulsheger 1983)

with PEF inactivation of Escherichia coli (E. coli). The observations of Hulsheger 1983 indicate that the stationary growth phase E. coli cells (quiescent cells) are much less vulnerable to the lethal effects of PEF's than are the larger, rapidly dividing E. coli cells that are in the logarithmic growth phase. Based on these considerations, it is expected that stem cells, due to their quiescent nature and smaller size, will be much less vulnerable to the lethal effects of electric fields and PEFs and that electric field strength can be used to preferentially inactivate a substantial fraction of non-stem cell leukocytes and tumor cells while leaving a substantial fraction of the stem cells unharmed. Thus, it is expected that the inventive methods will be an effective approach for purging tumor cells from autologous transplant tissue. Similarly, the inventive methods may be applied to other cell suspensions or suspensions on non-cell discrete objects having differences in characteristic size between subpopulations in order to selectively isolate or inactivate selected subpopulations.

[0118] In addition to performing a selective cell isolation or inactivation on the basis of a difference in characteristic cell size by selecting an appropriate applied electric field strength, the method can also be employed to select cells that can be similar in size based on a difference in dielectric membrane breakdown voltage, for example, due to a difference in effective membrane thickness. For example, a variety of cells, such as some epithelial cells and cancer cells, can have a layer of mucopolysaccharide coating associated with their plasma membrane which may increase the effective thickness of the membrane and make the cells less susceptible to an applied electric field than would be predicted by Eq. 7 with V.sub.mc assumed to be 1 Volt. In fact, assuming that the critical electric field imparted to the membrane required for poration, E.sub.mc, is similar for the cells present in the suspension, Eq. 6 indicates that the critical transmembrane potential V.sub.mc will be directly proportional to the effective thickness of the dielectric layer, and, therefore, from Eq. 7, the critical applied electric field strength, E.sub.c, for poration will also be directly proportional to the effective membrane thickness. Thus, an applied electric field strength may be chosen that is sufficient to inactivate a substantial fraction of cells having an effective membrane thickness below a certain predetermined threshold without inactivating a substantial fraction of the cells having an effective membrane thickness above the threshold.

[0119] Although the threshold electric fields for the cells comprising harvested human bone marrow, as exemplified above, were theoretically estimated based on their size (see Table 1), the critical threshold electric fields for the cells listed in Table 1 have been previously measured. In addition to the importance of the magnitude of the applied electric field strength, total exposure time of the cells to the electric field is also an important parameter in determining the degree of inactivation of a given population of cells. In general, for cells that are selectively inactivated by electric fields on the basis of cell size, the electric field strength determines the size below which cells are preserved, and total electric field exposure time determines the relative reduction in cells having sizes above the critical size. Experiments in the prior art have been conducted over a wide range of pulsed electric field strengths and number of applied pulses and have led to an empirical model developed by Hulsheger 1983, herein incorporated by reference, for the surviving fraction of cells, s, following electric field treatment, as a function of the peak applied electric field strength, E, and the total time the cells are exposed to the electric field, t. The time t in the following model sums the on-time of the electric field over the total number of pulses, so that t=N.sub.p.tau..sub.p, where N.sub.p is the number of applied pulses and .tau..sub.p is the time duration of each pulse over which E.gtoreq.E.sub.c. Hulsheger 1983 demonstrated that bacterial cell surviving fraction can be roughly modeled by an empirical expression that is a power law function of time and an exponential function of electric field strength. Equation 8 provides a variant of Hulsheger's rough model that behaves correctly as the exposure time approaches zero for E>E.sub.c. 2 $s = (t t_c + 1) - (E - E_c) k$ Eq . 8

[0120] where, s is surviving fraction (ranging from 0.fwdarw.1), E.sub.c is the threshold value of the electric field strength for membrane breakdown, t.sub.c is an exposure tine normalization constant, and k

also shows that if a low ionic strength pulsing medium is used, the allowable electric field exposure time calculated for a given .DELTA.T.sub.max is directly proportional to the decrease in ionic strength; for example, reducing the ionic strength by a factor of ten would increase the allowable exposure time by about a factor of ten. Furthermore, since under low ionic strength conditions, the energy transferred to the PEF treatment volume is reduced, for a given desired total exposure time, the pulse frequency may be increased without exceeding the predefined maximum temperature rise, thus allowing for a more rapid isolation or inactivation. Accordingly, the inventive PEF strategy can be a very rapid approach to cell purging and cell isolation.

[0148] For a single- or multi-pass flow-through PEF treatment cell embodiment of the invention, Eq. 15 can be manipulated to a form that describes the temperature rise of a fluid element of the pulsing medium as it traverses from the inlet to the exit of the PEF treatment volume. This formulation represents an upper bound on the temperature rise since it neglects heat transfer to the bounding electrodes as the fluid element passes through the PEF treatment volume. If N.sub.p is the number of electric field pulses applied during the residence time of the pulsing medium in the PEF treatment volume and .tau..sub.p is the duration of each of the electric field pulses, then the total electric field exposure time is t=N.sub.p.tau..sub.p and the resulting temperature rise is given by Eq. 15a below:

.DELTA.T.sub.res=tE.sup.2/.PHI..sub.pmt.rho..sub.psc.sub.p Eq. 15a

[0149] As illustrated above for the static PEF treatment cell embodiment, reduction of the ionic strength of the pulsing medium, which results in an increase in the resistivity of the pulsing medium, can allow a corresponding increase in the number of pulses that can be applied during the residence time of the pulsing medium in the PEF treatment volume. Thus, for the flow-through PEF treatment cell embodiment, it can be beneficial to use the lowest ionic strength pulsing medium allowable in order to maximize the number of pulses that may be applied in a single pass without exceeding the temperature rise limitations beyond which thermal effects impact cell viability. The relationships given above provide guidance to the skilled practitioner for selecting reasonable pulse repetition frequencies that are appropriate, for a specific pulsing medium and electric field pulse intensity and duration, for inactivating selected cells from a cell suspension having a maximum allowable temperature rise.

[0150] Another previously mentioned factor that can influence the way in which an applied electric field interacts with a cell suspension, and the selectivity of an applied electric field at inactivating cells based on a critical electroporation threshold, is the shape and orientation of cells within the field. This factor is important for any cell inactivation involving non-spherical shaped cells. A problem arises with such samples because non-spherical cells, in a given sample, are typically randomly aligned with respect to the electric field direction. While such a random alignment is not a problem for hematopoietic stem cells or other essentially spherical cells, random orientation can reduce the effectiveness, especially for cells with large aspect ratios, of cell inactivation with applied electric fields. Eq. 1 shows that the transmembrane voltage, V.sub.m, that results from an applied electric field E is directly proportional to the projected length, l, of the cell in the electric field direction. Thus, for cells with large aspect ratios, l can be highly variable depending on the orientation of the cell. Since it is typically desirable to apply an essentially time-invariant transmembrane voltage for a predetermined length of time in order to obtain more easily predictable and controllable poration results, it is therefore desirable to align cells that are not substantially spherical so that they have a more consistent and predictable orientation with respect to the electric field direction. For embodiments of the invention where it is desired to align the axes of cylindrical or oval shaped cells to achieve maximum PEF inactivation efficiency, an AC field can be applied across the sample to accomplish this function. The AC field is preferably selected to provide an essentially uniform oscillating electric field during the PEF treatment period, and has a magnitude selected to be sufficient to align the cells with their long dimensions parallel to the PEF field direction

for optimum size selectivity by the PEF field, without porating the cells or unduly overheating the pulsing medium. The theoretical treatment of this cell alignment technique is discussed in detail by Lynch (Lynch P T and Davey M R. Electrical Manipulation of Cells, Chapman and Hall, New York, Chapter 4, 1996), herein incorporated by reference.

[0151] Some preferred embodiments of the invention include the use of an applied electric field for cell inactivation that is a bipolar electric field. A "bipolar electric field" as used herein refers to an electric field that is pulsed or otherwise applied to a sample so that the average current across the sample over the total treatment time is essentially zero. The use of bipolar electric fields in the context of the present invention provides several advantages over non-bipolar fields. When an electric field is applied across a sample, particularly a blood sample, electrochemical reactions can occur which can produce free radicals, other deleterious compounds, and species that can shift suspension pH and/or generate bubbles. Such electrochemical effects are, as previously indicated, undesirable. Within the context of the present invention, the inventors have found that undesirable electrochemical effects can be reduced or eliminated by utilizing a bipolar electric field pulsed so that the average current across the sample over the treatment time is essentially zero. Because the application of the bipolar electric field involves essentially equal current flows across the sample for each applied polarity, the reversible electrochemical reactions induced by the applied electric field component having a first polarity, can be substantially reversed by the applied electric field component having the opposite polarity, thus yielding a situation characterized by no net electrochemical reaction over the treatment time.

[0152] There are a variety of ways to apply a bipolar electric field to the sample as apparent to one skilled in the electrical engineering arts. For some embodiments, the pulses across the sample are of essentially equal magnitude, duration, and number, but alternate pulses are of opposite polarity, while for other embodiments, the pulse having a first polarity may be of greater magnitude but shorter duration while the pulse of the reverse polarity is of lower magnitude and longer duration, so that the total average current flow is essentially zero. In another embodiment, an electric field pulse having a first polarity is utilized together with a DC current having an opposite polarity selected so that the magnitude and duration of each is selected to yield an essentially zero net current in order to achieve no net electrochemical reaction within the sample. In yet another embodiment for creating a desired bipolar electric field, the pulse used to create the PEF field may also be utilized to charge a suitable capacitor. When the original PEF pulse terminates, the capacitor then discharges back through the solution containing the cells at a rate determined in part by a resistance in the discharge path to produce the desired bipolar field.

[0153] In addition to reducing undesired electrochemical reactions, bipolar PEFs can provide an additional advantage in the selective inactivation/lysing of larger cells. The additional advantage lies in that the first pulse component having a first polarity results in a charge across the membrane of the cell which remains for some period of time after the first pulse component terminates. If a second pulse component having an opposite polarity is applied across the cell during this time, the voltage across the cell can be effectively doubled for a short period of time. This doubling effect is greater for larger cells than for smaller cells because of the larger membrane charging time scale for larger cells (see Eq. 9). This effect can potentially enhance size-selective destruction of the larger cells, thereby enhancing the cell selectivity of the invention for certain applications.

[0154] Temperature may also be utilized to enhance cell inactivation by the inventive methods. In general, biological cells are less capable of repairing membrane damage at sub-physiological temperatures. This behavior can be utilized to increase inactivation of cells in response to an applied electric field. For example, in one embodiment the cells are subjected to a PEF in a solution that is maintained within a physiological range of temperatures (for most mammalian cells, approximately 33-