ELECTROPORATION

DAMIJAN MIKLAVČIČ
MARKO PUC
University of Ljubljana
Ljubljana, Slovenia

1. BRIEF HISTORICAL OVERVIEW OF ELECTROPORATION DEVELOPMENT

Reversible "electrical breakdown" of the membrane has first been reported by Stampfli in 1958, but for some time this report has been mostly unnoticed. Nearly a decade later, Sale and Hamilton reported on nonthermal electrical destruction of micro-organisms using strong electric pulses. In 1972, Neumann and Rosenheck showed that electric pulses induce a large increase of membrane permeability in vesicles (11). Following these pioneering studies were two major breaking points that have motivated a series of further investigations. In the first study in 1982, Neumann et al. showed that genes can be transferred into the cells by using exponentially decaying electric pulses (12). A few years later, in 1987, Okino and Mohri and, in 1988, Mir et al. showed that definite amounts of molecules are introduced into the cells thus increasing cytotoxicity of bleomycin (chemotherapeutic drug), in either *in vivo* or *in vitro* conditions, by using electric pulses (13,14). From this time on, the data started to accumulate more rapidly and systematically. Most of the early work was done on isolated cells in conditions *in vitro*, but it is now known that many applications are also successful in *in vivo* situation. Using electroporation, small and large molecules can be introduced into cells and extracted from cells, and proteins can be inserted into the membrane and cells can be fused. As a result of its efficiency, electroporation has rapidly found its application in gene transfection, preparation of monoclonal antibodies, and electrochemotherapy of tumors. Today, it is paving its way into many fields of biochemistry, molecular biology, and medicine and is becoming an established method used in oncology for treatment of solid tumors. It also holds great promises for gene therapy as an efficient and safe nonviral vector (2).

2. THEORETICAL BACKGROUND OF ELECTROPORATION

Permeabilization of cell plasma membrane is achieved by exposure of the cell to short but intense electric pulses (Fig 1) (1–10). The basic quantity underlying the process of electroporation is presumably the induced transmembrane voltage generated by external electric field because of the difference in the electric properties of the plasma membrane and the external medium, known as the Maxwell–Wagner polarization. For a cell with spherical shape (Fig. 2a, which is acceptable approximation for most suspended cells), the induced transmembrane voltage $\Delta\Phi_m$ is described by:

$$\Delta\Phi_m = f_s r E \cos\theta \left[1 - \exp\left(-\frac{t}{\tau}\right)\right], \tag{1}$$

where r is the cell radius, E is intensity of the electric field, θ is the polar angle measured with respect to the direction of the electric field, t is time elapsed from the onset of the electric field, whereas function f_s, which reflects the geometrical, material, and electrical properties of the cell and its surroundings, and time constant τ of the inducement of transmembrane voltage are given by:

$$f_s = \frac{3\lambda_o[3dr^2\lambda_i + (3d^2r - d^3)(\lambda_m - \lambda_i)]}{2r^3(\lambda_m + 2\lambda_o)(\lambda_m - \frac{1}{2}\lambda_i) - 2(r-d)^3(\lambda_o - \lambda_m)(\lambda_i - \lambda_m)}, \tag{2}$$

$$\tau = \frac{rC_m}{\dfrac{2\lambda_o\lambda_i}{2\lambda_o + \lambda_i} + \dfrac{r}{d}\lambda_m}, \tag{3}$$

with C_m the membrane capacitance, d the membrane thickness, and λ_i, λ_o, λ_m the conductivities of the cytoplasm, extracellular medium, and cell membrane, respectively (4,15,16). For effective permeabilization of the plasma membrane, the induced transmembrane voltage $\Delta\Phi_m$ must exceed a certain threshold value (reversible threshold) that ranges from 200 mV to 1 V (17–20).

Parameters of electric field and parameters that define the state of cells, their surroundings, and their cell geometry are very important for the effectiveness of permeabilization and were the subject of many systematic studies over the past few decades, which eventually brought to a better understanding of the phenomenon. Probably the most important parameter is electric field strength E (i.e., pulse amplitude over electrode distance ratio in case of homogenous electric field) that has to exceed a certain threshold to initiate the process of permeabilization (induction step) (4,21–23). Also, the electric field strength will control the geometry of the part of the cell surface that is affected (i.e., permeabilized) (4,6,8). The intensity of permeabilization that occurs in the affected cell surface, however, is controlled predominantly by pulse duration T and number of pulses N (4,8,24,25). It is important that cells in suspension or in tissue are exposed to the electric field for some time because this exposure influences the intensity of permeabilization (expansion step) (4,8) of the affected cell surface. After the field intensity becomes lower than threshold value, stabilization is taking place, which brings the membrane to the permeabilized state for small molecules (stabilization step) (4). Furthermore, if no successive pulses occur, a resealing process begins, which returns the plasma membrane into its initial state (i.e., state before the electropermeabilization) (4,8,26,27). Yet if several pulses follow, the pulse repetition frequency f plays a substantial role because, if it is high enough (i.e., 1 Hz or more), the resealing process between the pulses is negligible and successive pulses contribute to a higher intensity of permeabilization in the affected regions (4,24,28). One of the

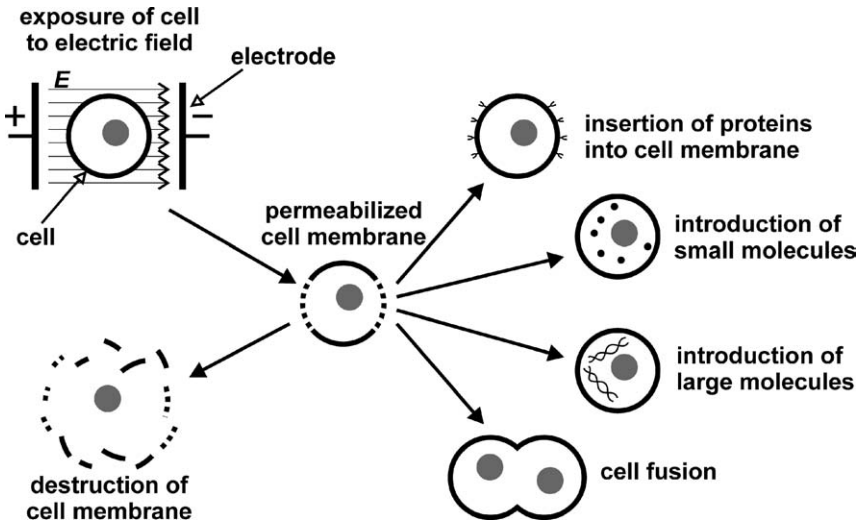
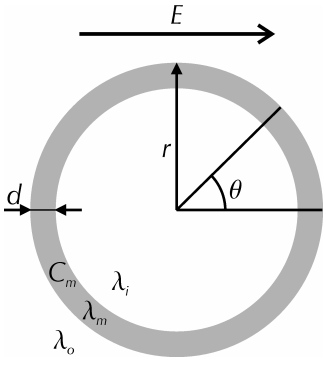


Figure 1. Exposure of a cell to an electric field may result either in permeabilization of cell membrane or its destruction. In this process, the electric field parameters play a major role. If these parameters are within certain range, the permeabilization in reversible; therefore, it can be used in applications such as introduction of small or large molecules into the cytoplasm, insertion of proteins into cell membrane, or cell fusion.



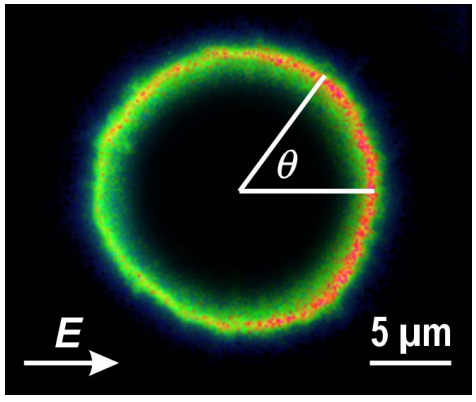


Figure 2. The model of spherical cell on the left side. Symbols represent: E-electric field, r-cell diameter, θ-the angle between the direction of E and a point on the membrane, d-membrane thickness, λ_i, λ_o, λ_m-intracellular, extracellular, and membrane conductivity, and C_m-membrane capacitance. Figure on the right is an experimental result where transmembrane voltage was observed under the microscope using potenciometric fluorescent dye. B16F1 (mouse melanoma) cells were stained for 12 min at 4°C with 40 mM di-8-ANEPPS and 0.05% Pluronic (both Molecular Probes, USA) in SMEM medium (Gibco, USA). The cells were exposed to an electric field of ~63 V/cm during the excitation with 460 nm and 510 nm wavelengths (150 ms each), and the emission was detected at 605 nm. The ratio image was obtained by dividing the fluorescence of corresponding pixels in the images obtained at 460 nm and 510 nm excitations. Pseudocolors were then assigned to the ratio values (red-higher voltage, blue-lower voltage). The images were acquired with a cooled CCD camera (Visicam 1280, Visitron Systems Germany) connected to the fluorescence microscope (Zeiss, Axiovert 200, objective ×100, oil immersion), and processed with Metafluor imaging software (Visitron Systems, Germany).

parameters that has not been so comprehensively studied is time variation of electric field (i.e., shape of the signal delivered from the electroporator). Two groups of signals exist that can be used for electropermeabilization: unipolar and bipolar. The two most commonly used unipolar signals that have been used for decades and are still used today in different applications are exponentially decaying and square wave pulses. Furthermore, it has been shown

that increase of efficiency of electroporation can be obtained only by optimization of time, during which the pulse or signal exceeds a certain threshold value and not by changing parameters that describe shape of signals (e.g., rise/fall time, modulation, etc.) (29–32). Symmetrical bipolar pulses have also been used for electropermeabilization in spite of scarcity of electroporators that are able to produce such signals. However, it was shown that effective electropermeabilization is achieved at electric field strengths that are 20% lower than strengths of unipolar signals. Again, the major role in the efficiency of electropermeabilization with symmetrical bipolar pulses was ascribed to the time during which the pulse amplitude exceeds a certain threshold value (32,33). Another potential advantage of symmetrical bipolar pulses is reduced electrolytic contamination of the sample by metal deposits from the electrodes (7).

The electric field parameters are the most influential parameters for efficacy of the electropermeabilization. Yet when experiments are performed, parameters of experiment also become very important. The level of control over these parameters varies in different experimental conditions [i.e., *in vitro*, (*in ovo*, *in situ*), *ex vivo*, and *in vivo*]. In *in vitro* conditions, practically all parameters (i.e., cell size, shape, density and orientation, conductivity of cell suspension, osmotic pressure, and temperature) can be very well controlled in contrast to *in vivo* conditions where experiments are performed on animals, whose histological and anatomical structure of tissues and physiological states vary even though treated animals are of the same species.

Different types of cells are usually irregularly shaped and different in size, thus approximation with spheres can only be made for some cell types. In addition, cells differ in size even though they belong to the same culture. The two parameters, cell size and shape, reflect on the value of induced transmembrane voltage, which is proportional to the cell radius r and f_s function that also reflect geometrical properties (4,34,35). It is evident from Equation 1 that large cells are more sensitive to the same electric field strengths than small cells (4,6). By changing the orientation of cells in the electric field (or vice versa), the induced transmembrane potential will decrease from its maximum value when the longest axis of the cell is parallel to the electric field to its minimum value when the longest axis of the cell is perpendicular to the electric field (34,35). Furthermore, the induced transmembrane voltage is also affected by variation of density of cells (i.e., number of cells per volume unit) in the sample that is exposed to the electric field (36,37).

In *in vitro* conditions, conductivity of cell suspension and osmotic pressure can be altered by using different media in which cells are suspended during the experiment. By changing conductivity of the medium, we influence the percentage of survived cells that were exposed to the electric field, while percentage of permeabilized cells is unaffected. If the conductivity of the cell suspension is decreased, the percentage of survived cells increases (38,39). Osmotic pressure can be altered by adding a hypoosmotic medium (i.e., osmotic stress), which causes swelling of the cells within a minute or two. The process is reversible and cells regain almost the same size after ten to twenty minutes after the osmotic stress. But during the period of increased diameter of swollen cells, lower electric field strengths can be used to achieve the same effect of permeabilization as if the equivalent cells were stored in an isoosmotic medium (40–42).

Application of electric pulses to the sample causes Joule heating caused by a current that flows through the sample (4,43). If we assume a total conversion of the electric energy into heat, the change of temperature ΔT in the sample is described by:

$$\Delta T = \int_0^{t_{END}} \left(E(t)^2 \frac{\lambda(t)}{C_p \rho} \right) dt, \tag{4}$$

where E is electric field, λ is electrical conductivity of the exposed sample, C_p is the specific heat capacity (J/g°C), ρ is the density of the sample (g/cm^3), t is time, and t_{END} is total duration of exposure to the electric field (43). This side effect can be well controlled in *in vitro* conditions where a low conducting media can be used in contrast to *in vivo* conditions where control can be only applied by limiting the electric field parameters (i.e., pulse amplitude and duration, pulse repetition frequency and number of pulses). In any case, Joule heating must be taken into account, especially when highly conductive pathways are present or longer pulses are used (4,44).

However, controlled temperature changes of treated sample during experiment have a positive influence on the efficacy of electropermeabilization and uptake of molecules. It has been shown that low temperature (i.e., 4°C) of cell suspension before the application of electric pulses (i.e., preincubation temperature) and high temperature (i.e., 37°C) after exposure (i.e., postincubation temperature) yielded the highest survival rate and transfection efficacy (45).

3. THERAPEUTIC AND TECHNOLOGICAL APPLICATIONS OF ELECTROPORATION

Today, electropermeabilization is widely used in various biological, medical, and biotechnological applications. Applications can be divided in numerous ways, but let us consider the following one. According to the type of electropermeabilization (i.e., reversible or irreversible), two groups of applications exist: functional, where functionality of cells, tissues, or micro-organisms must be sustained, and destructive, where electric fields are used to destroy plasma membranes of cells or micro-organisms (Fig. 1).

Functional applications are currently more widespread and established in different experimental or practical protocols. Probably the most important functional application is the introduction of a definite amount of small or large molecules to the cytoplasm through the plasma membrane (2,46). Furthermore, a slight variation of electric field parameters results in an application where molecules can be directly inserted into the plasma membrane (47). Also, permeabilization can be effectively used for cell fusion (48–50). In contrast, destructive applications are less than a decade old, but their efficacy is promising, especially in the

field of water treatment where efficacy of chemical treatment is enhanced with electropermeabilization (51,52) or in food preservation where electropermeabilization has proven, in some cases, to be as effective as pasteurization (53–55).

3.1. Electrochemotherapy

The most representative application of delivery of small molecules through electroporated membrane is electrochemotherapy, which is a therapeutic approach in cancer treatment where cytotoxicity of a nonpermeant drug is enhanced by means of locally delivered permeabilizing electric pulses. The earliest report dates back to 1987 when Okino and Mohri performed the first *in vivo* experiments in which a single exponential pulse of 5000 V/cm was delivered to the tumor after administration of bleomycin. This combined treatment resulted in a 17% decrease of the initial mass of tumor four days after the treatment (13). Independently of this report, systematic *in vitro* experiments were performed by Mir et al. In their experiments, eight square wave pulses of 100 μs were delivered at the frequency of 1 Hz and with the electric field strength ranging from 0 to 2000 V/cm to cell suspension. They demonstrated that increase of electric field intensity increases the uptake of molecules while the cell survival decreases (14). The main objectives of the following studies were optimization and introduction of the method into the clinical environment. Optimization of the method resulted in introduction or revival of drugs (56,57), improvements in electric field delivery and distribution in the tissues by changing electrode orientation (58,59), and novel electrode designs (60). In several preclinical and clinical studies (Fig. 3), either on humans or animals, it was demonstrated that electrochemotherapy can be used as the treatment of choice in local cancer treatment (61–64).

3.2. Gene Transfer by Electroporation

Exogenous genetic material can be delivered to cells by using different viral and nonviral methods. Although viral methods enhance delivery efficiency, use of viral vectors is associated with possible complications that originate from highly evolved and complex viral biology and host-parasite interactions (65,66). These problems can be avoided by using nonviral methods such as electropermeabilization (3,21,22,67–69). One of the first reports of such a gene transfer was published in 1982 by Neumann et al. In their experiments they transferred genes into mouse lyoma cells using exponential electric impulses of 8000 V/cm (12). This nonviral method of gene transfer has been termed electrotransfection. Therefore, in the following studies of electrotransfection different parameters of electric fields were tested. *In vitro* electrotransfection can be achieved by using exponentially decaying pulses (1,12); square wave pulses with superimposed RF signals (70); and long square wave pulses up to 20 ms and with amplitudes up to 800 V/cm (21). In general, it can be stated that longer pulses are used in gene transfection than in electrochemotherapy. In the first *in vivo* studies of the electrotransfection long square wave pulses were used up to several milliseconds, with amplitudes up to 300 V/cm for insertion into skeletal muscle (71) and from 400 V/cm to 600 V/cm for insertion into tumors (72). Recently, a novel approach was introduced where combination of high- and low-voltage pulses is used for treatment. The new method is based on application of several short high-voltage pulses (e.g., 8 × 100 μs of 1300 V/cm), which are followed by long low-voltage pulses (e.g., 1 × 100 ms of 100 V/cm) (68). It was suggested that short high-voltage pulses are permeabilizing the membrane while the longer lower-voltage pulses have an electrophoretic effect on DNA itself, facilitating interaction of plasmid with the membrane.

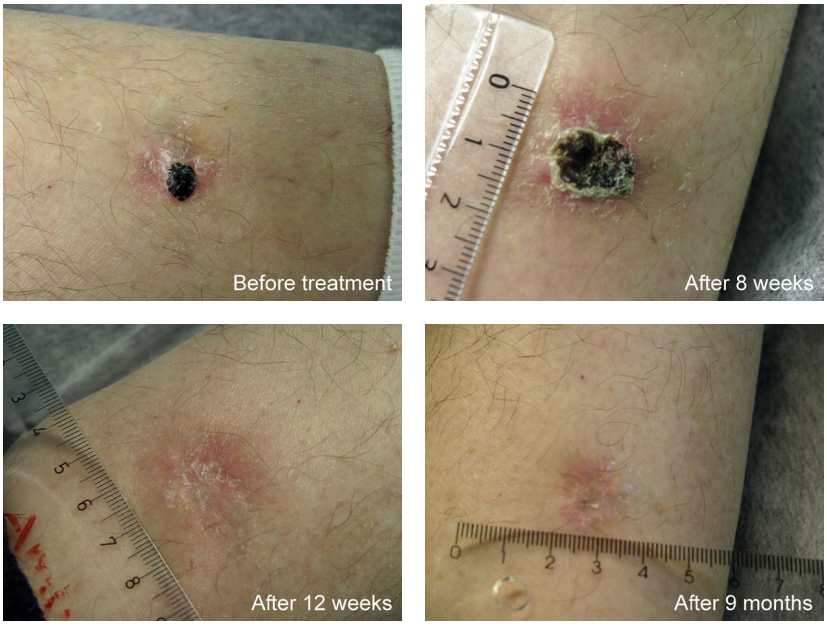


Figure 3. Cutaneous tumor nodule of malignant melanoma (1.8 × 1.6 cm in diameter) was treated by electrochemotherapy with bleomycin. Bleomycin was injected intratumorally, and immediately thereafter electroporation of the tumor nodule was performed by four applications of electric pulses using needle electrodes. The tumor nodule responded with complete regression. Superficial scab was present up to 8 weeks after treatment, and the tumor nodule is in complete response 9 months after treatment.

3.3. Electroinsertion

To achieve uptake of ions or molecules through cell plasma membrane to the cytosol with electroporation, electric field intensity must exceed critical value. If the field intensity is just below the critical value, it is possible to insert different transmembrane proteins, such as CD4 receptors and glycophorin, directly into the membrane of erythrocyte (73–75). The great adventure of this biological feature is that it is possible to graft viral receptors on erythrocyte surface to lure AIDS virus and to decrease the viral charge (74).

Further studies have shown that insertion of proteins can also be performed on nucleated cells but, in that case, electric field must trigger electropermeabilization of cells. The process of insertion is a two-step phenomenon where, in the first step just after the pulse, spontaneous insertion of proteins occurs in the permeabilized region of cell membrane. In the next step, the proteins diffuse slowly in the membrane to give a homogeneous distribution (47).

3.4. Electrofusion

So far we have presented applications of electroporation that are used to introduce different molecules either to the cytosol or into the cell plasma membrane. But electroporation of cell plasma membrane can also result in fusion of cells. This process has been termed electrofusion. First reports of *in vitro* electrofusion of cells date back to the 1980s. In the reports, it has been shown that fusion between two cells can proceed only if the cells are in contact prior or immediately after electroporation (76–78). The contact between the cells can be achieved either by dielectrophoretic collection of neighboring cells, which is followed by electropermeabilization or by centrifugation of cell suspension after exposure to electric field (79,80). In both cases, cells must be reversibly permeabilized, otherwise they lose viability and there is no electrofusion. Electrofusion in *in vitro* environment is possible because of a high possibility of cell movement (Fig. 4), whereas cells in tissues are more or less fixed, nevertheless *in vivo* electrofusion has been observed in B16 melanoma tumors (81) as well as cells to tissue fusion (82–84). Electrofusion has proved to be a successful approach in production of vaccines (85,86) and antibodies (87,88).

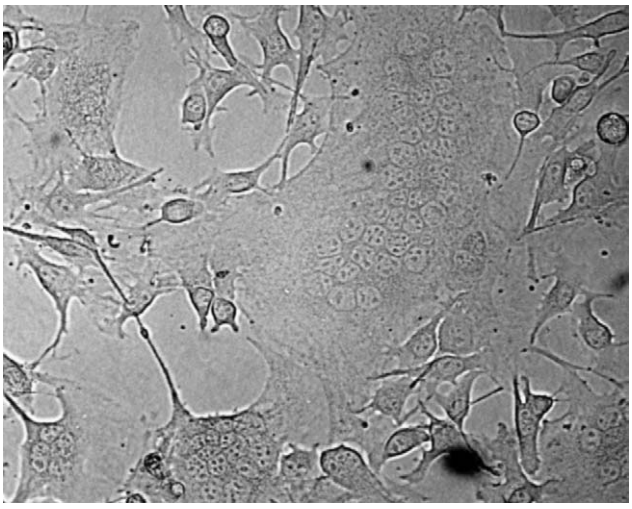


Figure 4. *In vitro* electrofusion of B16F1 cells. Cells were exposed to eight square wave pulses of 1 ms duration and electric field strength of 600 V/cm. The pulses were delivered using a custom-made electroporation device and electrodes that allowed delivery of rotational electric field (i.e., the direction of electric field was rotated by 90 degrees according to the predeceasing pulse). After exposure to the electric field, cells were incubated at room temperature for 15 minutes, then cells were transferred into the culture medium that consisted of Eagle minimum essential medium (EMEM) with 10% fetal bovine serum, and incubated at 37°C and 5% CO_2 in a Universal Jacketed Incubator. After 24 hours, the images were acquired with a cooled CCD camera (Visicam 1280, Visitron Systems, Germany) connected to the microscope (Zeiss, Axiovert 200, objective × 20, oil immersion), and processed with Metafluor imaging software (Visitron Systems, Germany).

3.5. Transdermal Drug Delivery

A mammalian skin represents a remarkable barrier because of its outermost and dead layer, the *stratum corneum*. Therefore, conventional transdermal drug delivery is limited only to lipophilic molecules while charged polar molecules cannot pass this barrier. To overcome this problem, in addition to iontophoresis, electroporation has been presented as a new method for transdermal drug delivery. Both methods use the electric field either as a direct or indirect mediator to introduce the drugs into the body (89). The basic difference between these two methods, however, is that the electric field used in iontophoresis acts directly on the drug, whereas in electroporation the electric field acts on the barrier by creating new pathways called local transport regions (90) through which the drug can now diffuse across the skin and reach the lower parts of dermis. Electric fields that are used in electroporation thus cause transient changes in the structure of the skin. Electroporation increases the transport by orders of magnitude on a timescale of minutes, but the transport can even be greater and faster if after electroporation iontophoresis is used to drag the drug through the established paths in the permeabilized skin (89–92). By now, electroporation has been used for transdermal drug delivery only in experimental conditions; however, some trends exist that might move these studies into the clinical environment.

3.6. Electrosterilization

Irreversible electroporation can be used in applications where permanent destruction of micro-organisms is required [i.e., food preservation (53) and water treatment (51,52)]. Still, using irreversible electropermeabilization in these applications means that the substance under treatment is exposed to a limited electric field because it is desirable that changes in treated substance do not occur (e.g., change of food flavor) and that no byproducts emerge because of electric field exposure (e.g., byproducts caused by electrolysis) (54,55).

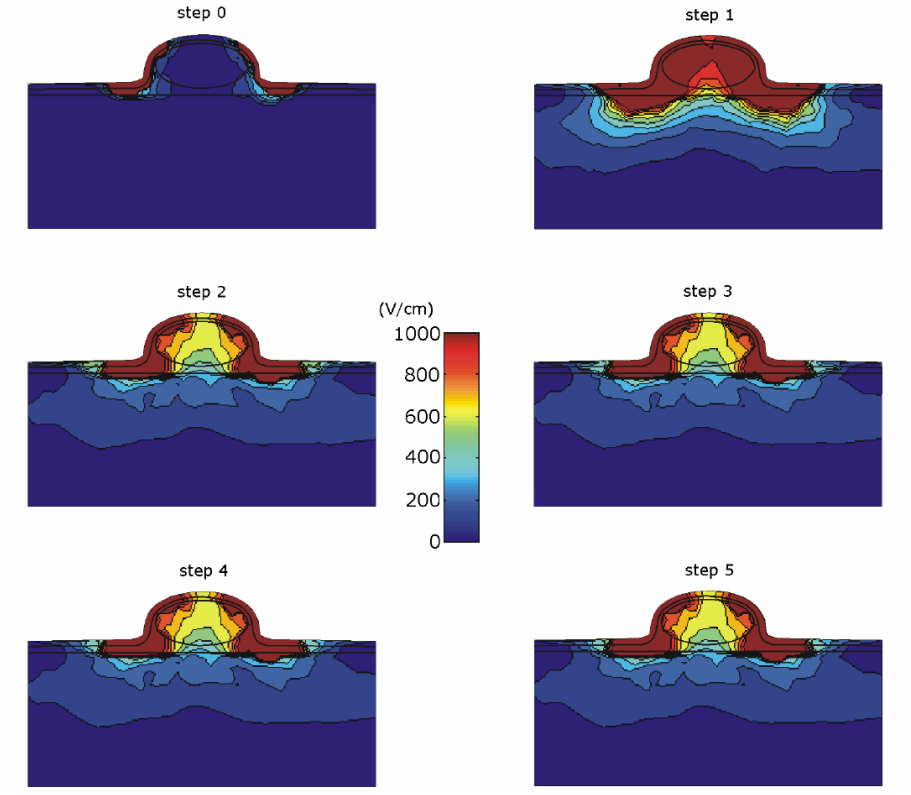


Figure 5. Six steps of the sequential analysis of the electroporation process in the subcutaneous tumor model at 1000 V between two plate electrodes with distance of 8 mm. The electric field distribution is shown in V/cm.

4. ELECTRIC FIELD DISTRIBUTION *IN VIVO*

In most applications of tissue permeabilization, it is required to expose the volume of tissue to electric field intensities between the two thresholds (i.e., to choose in advance a suitable electrode configuration and pulse parameters for the effective tissue permeabilization). Therefore, electric field distribution in tissue has to be estimated before the treatment, which can be achieved by combining results of rapid tests (93,94) with models of electric field distribution (20,59). However, modeling of electric field distribution in tissue is demanding because of heterogeneous tissue properties and usually complex geometry. Analytical models can be employed only for simple geometries. Usually, they are developed for 2-D problems and tissue with homogenous electrical properties (95). Therefore, in most cases, numerical modeling techniques are still more acceptable as they can be used for modeling 3-D geometries and complex tissue properties. For that purpose, mostly finite element method and finite difference method are applied. Both numerical methods have been successfully applied and validated by comparison of computed and measured electric field distribution (20,59,93). Furthermore, a few advanced numerical models were built, which also took into consideration tissue conductivity increase because of tissue or cell electroporation (96,97). These advanced models consist of a sequence of static models (steps), which describe E distribution in discrete time intervals during permeabilization (Fig. 5). In this way, models present dynamics of electroporation because in each step the tissue conductivity is changed according to distribution of electric field intensities from the previous step.

5. ELECTRODES FOR *IN VITRO* AND *IN VIVO* APPLICATIONS

Effectiveness of electroporation in either *in vitro*, *in vivo*, or clinical environment depends on the distribution of electric field inside the treated sample. Namely, the most important parameter governing cell membrane permeabilization is local electric (98) field exceeding critical threshold. To achieve these results, we have to use an appropriate set of electrodes (Fig. 6) and an electroporation device—electroporator that generates the required voltage or current signals. Although both parts of the mentioned equipment are important and necessary for effective electroporation, electroporator has a substantially more important role because it has to be able to deliver the required signal to its output loaded by impedance of the sample between electrodes.

Today, numerous types of electrodes exist that can be used for electroporation in any of the existing applications. According to the geometry, electrodes can be classified into several groups (i.e., parallel plate electrodes, needle arrays, wire electrodes, tweezers electrodes, coaxial electrodes, etc.) (Fig. 6). Each group comprises several types of electrodes that can be further divided according to the applications, dimensions, electrode material, etc. In any case, selection of electrode type plays an important role in characterization of the load that is connected to the output of the electroporator. During the design of the

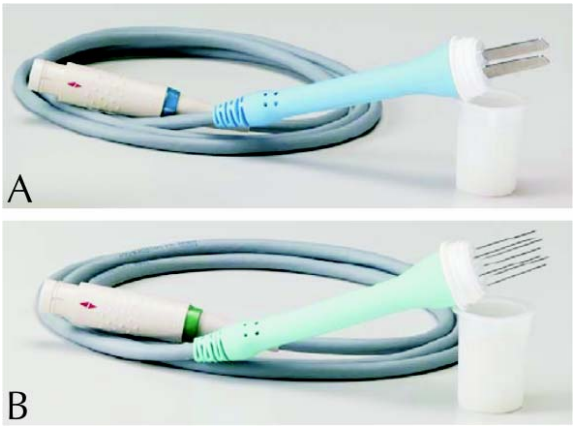


Figure 6. Examples of commercially available electrode for clinical applications of electrochemotherapy and electrotransfection electropermeabilization, which are produced by IGEA, Italy.

electroporator, load characterization represents the greatest engineering problem, because electrical characteristics of substance between electrodes (e.g., cell suspension, tissue, etc.) vary from experiment to experiment and even during the same experiment. In general, the load between electrodes has a resistive and a capacitive component. The value of each component is defined by geometry and material of electrodes and by electrical and chemical properties of the treated sample. In *in vitro* conditions, these parameters that influence the impedance of the load can be well controlled because size and geometry of the sample are known, especially if cuvettes are used. Furthermore, by using specially prepared cell media, electrical and chemical properties are defined or can be measured (38,39). On the other hand, in *in vivo* conditions, size and geometry can still be controlled to a certain extent, but electrical and chemical properties can only be estimated, especially if needle electrodes are used that penetrate different tissues. However, even if we manage to reliably define these properties during the development of the device, it is practically impossible to predict changes in the electrical and chemical properties of the sample because of exposure to high-voltage electric pulses. Besides, electropermeabilization of cell membranes increases electrical conductivity of the sample (99,100), electric pulses also cause side effects like Joule heating and electrolytic contamination of the sample (2,6,43,44), which further leads to increased sample conductivity.

6. ELECTROPORATORS—THE NECESSARY PULSE GENERATORS

Electroporator is an electronic device that generates signals, usually square wave or exponentially decaying pulses, required for electroporation. Parameters of the signal delivered to electrodes with the treated sample vary from application to application. Therefore, it is very important that electroporator is able to deliver signals with the widest possible range of electrical parameters if used in research. If, however, used for a specific application only (e.g., clinical treatment such as electrochemotherapy), pulse generator has to provide exactly the required pulse parameters. Moreover, electroporator must be safe and easy to operate and should offer some possibilities of functional improvements. In principle, electroporators can be divided in several groups depending on biological applications, but from the electrical point of view they can be grouped in electroporators with voltage output [output is voltage signal $U(t)$] and electroporators with current output [output is current signal $I(t)$]. Both types of electroporators have their advantages and disadvantages, but one point definitely speaks in favor of devices with voltage output. For example, if we perform *in vitro* experiments with stainless-steel parallel-plate electrodes with plate sides substantially larger than the distance between them, the electric field strength E that is applied to the sample can be approximated by the voltage-to-distance ratio U/d, where d is the electrode distance and U the amplitude of applied signal obtained from an electroporator with voltage output. On the other hand, if an electroporator with current output is used, the same approximation could be used only if additional measurement of voltage difference between electrodes is performed or if the impedance Z of the sample is known, measured, or approximated and voltage difference between electrodes is estimated using Ohm's law $U = IZ$. Nevertheless, several commercially available electroporators exist that fulfill different ranges of parameters and can be used in different applications. A list of commercially available electrodes and electroporators has been presented in 2004 by Puc et al. in a paper that describes techniques of signal generation required for electropermeabilization (101).

The choice/selection of electroporator clearly depends on the application that is to be performed (e.g., for small molecules, shorter pulses are used than for DNA). In principle, we can say that pulse amplitude (voltage-to-distance ratio) should typically be in the range from 200 V/cm up to 2000 V/cm. Pulse durations should be in the range of hundreds of microseconds for smaller molecules and from several milliseconds up to several tens of milliseconds for macromolecules such as DNA fragments (in the latter case, because of the very long pulse duration, optimal pulse amplitude can even be lower than 100 V/cm). If any possibility exists to obtain the equipment that generates bipolar pulses, this type of pulses should be used because bipolar pulses yield a lower poration threshold, higher uptake, and an unaffected viability compared with unipolar pulses of the same amplitude and duration. They also reduce electrolytic contamination of the sample. This general overview of electrical parameters to be provided by electroporators are only indicative and should be a starting point for a design of experiments or treatments with electroporation. Optimal values of parameters strongly depend, as stated earlier, on the cell type used, molecules to be introduced, and specific experimental conditions.

7. CONCLUSIONS

Electroporation has been studied extensively until now, and a number of applications have been suggested. Elect-

rochemotherapy has been demonstrated as an effective local treatment of solid tumors and is the most mature therapeutic application right now. Electroporation for gene transfection, however, has been long used in *in vitro* situation. With a hold on viral vectors, electroporation represents a viable nonviral alternative also for *in vivo* gene transfection. Clinical applications and expansion of electrochemotherapy have been hindered by the lack of adequate electroporators and their certification in Europe (CE Medical Device) and limited approval by the FDA in the United States. Recently, Cliniporator (IGEA, s.r.l. Carpi, Italy) was certified as a medical device and is offered on the market along with standard operating procedures. It has to be stressed also that Cliniporator has an important feature that allows monitoring of voltage and current delivery through the electrodes to the patient. Other applications of electroporation are less mature and remain to be further elucidated. One of the recent developments and a novel approach is also the use of ultrashort electrical pulses (nano seconds) to influence intracellular organelles, which opens new applications in apoptosis, gene delivery to the nucleus, altered cell functions, etc. (102).

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