

Instrumentation and Electrodes for In Vivo Electroporation

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1. Introduction

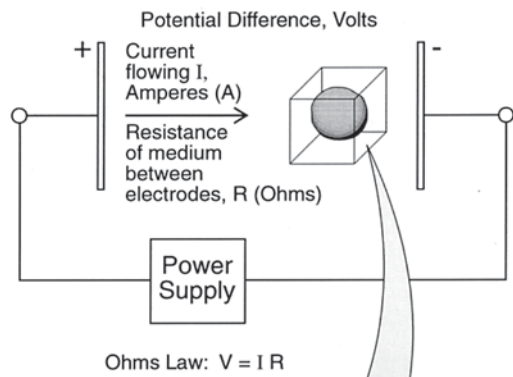
Electroporation (EP) of drugs and genes into cells in vitro became a standard procedure in molecular biology laboratories in the last decade. Numerous protocols aid the researcher in selecting appropriate procedures; commercial instrumentation is readily available and discussed (*1*). The more recent transition to applying EP to living tissue poses a new set of requirements and few practical guidelines are available.

In general, the requirements for successful in vivo electroporation for delivery of drugs or genes are twofold: the molecules need to be present at the site to be treated, and an appropriate electrical field needs to be applied to this site within a time window. For the choice of electrical parameters, the type of tissue appears to be of less importance than the molecule to be delivered: drug versus genes.

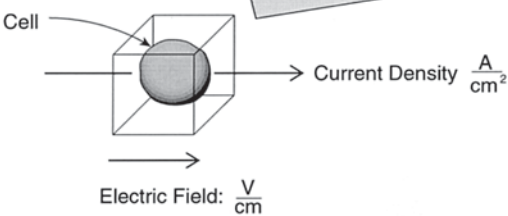
In vivo EP requires techniques for the delivery of the drug/gene to the tissue site, and techniques for the delivery of the field. The delivery of the field is done by a voltage pulse generator and applicators that transform the voltage into an efficacious electric field in the tissue. **Figure 1** shows the relationship between the macroscopic parameters of voltage, current, and resistance and the microscopic, effective, parameter, the electric field strength as well as the current density, which is a function of the medium specific resistivity.

The generator provides a voltage output to the electrodes. This voltage, or potential difference, between electrodes results in the generation of an electric field in the volume between the electrodes and extending somewhat beyond. The voltage needs to be selected so that in the volume between the electrodes

1. Macroscopic Parameters



2. Microscopic (Local) Effects



Specific Resistivity (Medium Property): ρ Ohm x cm

Microscopic Ohms Law: $E = i \rho$

Macroscopic	Microscopic
I	i
V	E
R	ρ

E is the efficacious parameter for electroporation

Fig. 1. Important electrical parameters for electroporation.

the efficacious field strength is achieved or exceeded. It is desirable to provide a field amplitude that has a safety margin above the marginally efficacious field strength. These issues are the subject of the following sections.

The process of developing a new in vivo therapeutic application of EP generally proceeds in the following steps: Uptake of the drug or gene is demonstrated in vitro, then efficacy shown in vivo in an appropriate animal model,

then, if possible, *in situ* in an animal model, and, finally, in human clinical trials. We will discuss only *in vivo* and comment briefly on hardware issues relating to the steps from animal experimental trials to human clinical trials. A large variety of drugs or genes can be electroporated into widely differing tissues *in vivo*. In the following, we will focus on a few representative examples.

2. Delivery of Drug/Gene to the Tissue

In vivo EP is a process of delivering drugs and genes from the interstitial tissue space into cells by temporary permeabilization of cell membranes. As a first step, the molecule of interest is typically brought into the tissue before EP. Several techniques are being used: systemic delivery by intravenous injection (IV) or intratumoral injection (IT). Tumors differ from normal tissue by elevated interstitial pressure which is typically between 10 and 40 mmHg, whereas normal skin has 0.4 mmHg pressure (2). This high pressure and gradient towards normal tissue makes systemic delivery less effective than IT. When IT is used, a technique of fanning the syringe throughout the tumor aids in the distribution of the drug. IT delivery of bleomycin into tumors and subsequent EP gave superior results over the IV route (3). Iontophoresis might be employed as a transport mechanism of charged molecules across tissue to the site of EP.

The transport of molecules through the skin is made difficult by the presence of the *stratum corneum* (SC), the outermost layer of the skin made up of dead cells. Iontophoresis can be used to transport charged molecules through existing pathways such as sweat glands and hair follicles through the skin; brief electrical pulses across the SC can create additional pathways by breakdown and formation of aqueous pores. Ultrasound can enhance the transport of molecules across skin (4,5).

3. Electric Field Configurations

The voltage delivered from the EP pulse generator needs to be transmitted to the tissue so an efficacious electric field can be generated at the desired tissue site. A variety of possible basic electrode configurations are shown in **Fig. 2**.

If the tissue is easily accessible, not too large in volume and raised, outside electrodes (**Fig. 2-1**) in form of parallel plates can be utilized. Early gene EP experiments (6) and tumor treatments by EP (7,8) used parallel plate type electrodes. If it is desirable to confine the electric field to a shallow layer of tissue, as in transdermal drug delivery, then closely spaced surface electrodes as shown in **Fig. 2-2** are useful. Deeper-seated tissue can be reached with insertion electrodes or needles (**Fig. 2-3**). The resulting electric field distribution can be improved by arranging needles in arrays of different geometries (**Fig. 2-4**).

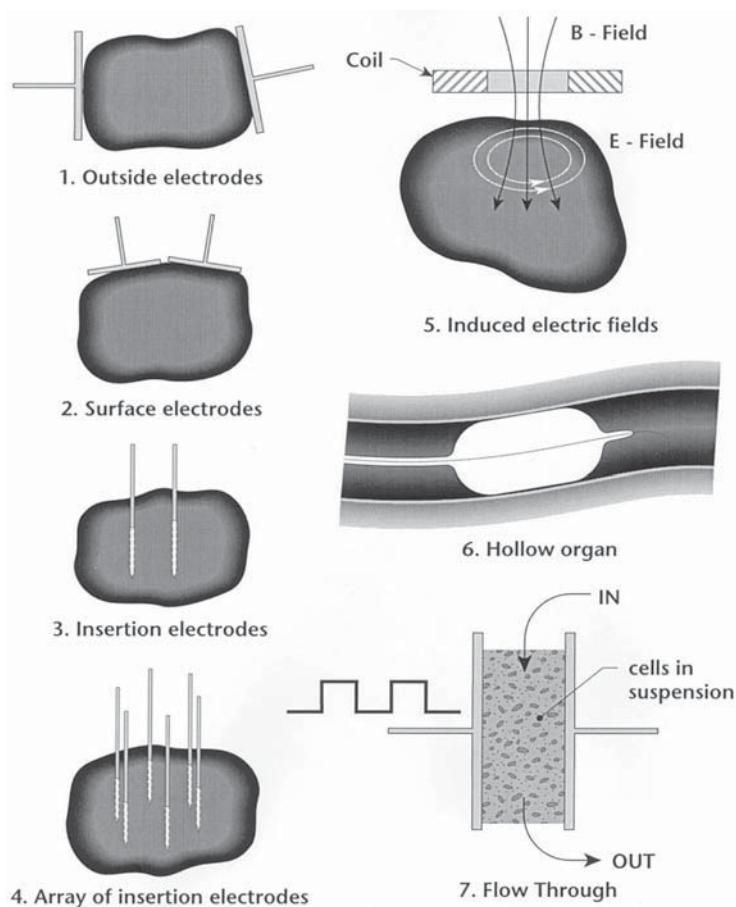


Fig. 2. Basic field applicator configurations.

In principle, an electric field can be generated by induction according to Faraday's law from a coil with a fast varying electrical current. Though this approach allows for an electrodeless creation of the electric field in tissue, it is not very practical. Very high currents at high frequency are needed in order to create induced fields of an amplitude sufficient to induce EP. A tumor response effect was demonstrated with this technique even without addition of a drug (9).

Hollow organs and cardiovascular applications of EP require catheter-type configurations (**Fig. 2–6**). Some cardiovascular implementations are described in (10–12). A flow-through EP system (**Fig. 2–7**) can be used either for ex vivo EP therapy or, in a shunt mode, to electroporate bodily fluids extracorporeally. Practical implementations of some of these electrode configura-

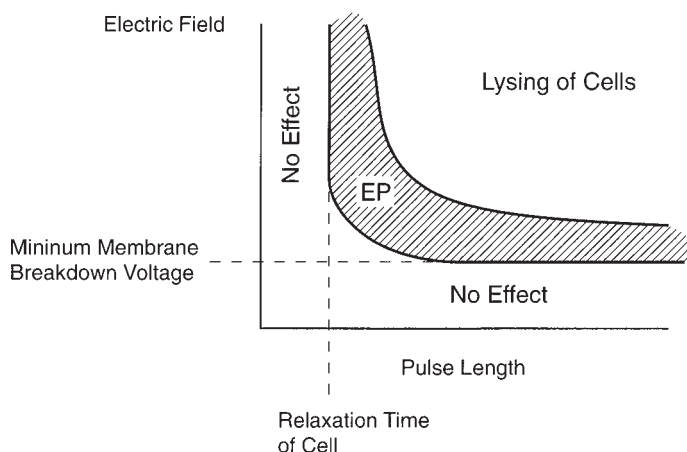


Fig. 3. General electroporation relationships.

tions will be discussed in **Subheading 6**. The determination of the minimum efficacious field strength for EP will be discussed in **Subheading 4**.

4. Minimum Efficacious Field Strength

The parameters which determine the efficacy of the delivery of molecules into cells are field strength, pulse length, wave shape of the pulse and number of pulses. The combination of all of these is too large to address their specific importance, especially when one considers that the molecule to be delivered may change the optimal parameter combination. However, some general statements can be made which will help in trade-off considerations of the most important parameters which are field strength and pulse length. **Figure 3** shows the area of EP efficacy. If the field strength is too low, the transmembrane potential required to permeabilize the cell membrane (typically 0.7 V) can not be reached. Similarly, if the pulse length is too short (microseconds), the membrane capacitance does not charge up high enough to reach the required transmembrane potential. If the delivery of genes is intended, the parameters need to be selected within the EP effective area. However, if the goal is the destruction of tumors by delivery of chemotherapeutic agents with EP, lysing of cells, which results from excessive field strength or pulse length, is not detrimental to the ultimate goal of tumor destruction.

There appears to be a difference in effective parameters between drug and gene for delivery by EP. High field strength, short pulse length gives good results at least with some of the drugs investigated (i.e., bleomycin), whereas gene EP benefits more from a combination of low electric field and long pulse length. The majority of presently ongoing drug EP is applied to the treatment

of tumors, and the drug of choice is mostly bleomycin. We will therefore discuss the determination of the minimum efficacious field strength for this application.

Belehradec and colleagues (*13*) injected bleomycin into nude mice bearing subcutaneously T-DC-3F clone 4 tumors (Chinese hamster lung transformed fibroblast cell line). Tumors were removed and sliced. Slices were electroporated in the presence of bleomycin with 100 μ sec pulses between parallel electrodes, which provided a reasonable homogeneous field. The lower limit of effective field was between 400 V/cm and 600 V/cm. In a study comparing the efficacy of different needle arrays and voltages in a human prostate cancer model in mice (*14*), a good tumor response was found in the center of a needle array at a field strength of 780 V/cm, which was the lowest field strength investigated.

Does this critical minimum field vary much with the tumor cell type? The critical parameter for the electroporation of mammalian cells is the achievement of a transmembrane potential of about 0.7–1 V. For a given field strength, the induced voltage V is inversely proportional to the cell diameter: $V = 1.5Er \cos \theta$, where E is the field strength, r is the cell radius, and θ is the angle between the direction of the field and the cell surface vector. If the cells are of similar size, similar minimum efficacious field strength can be expected.

5. Effect of Electroporation on Normal Tissue

The following issues are of importance when considering inserting electrodes into a tumor and transversing healthy tissue: What is the safe level of the electric field and what is the effect of EP of a drug into healthy tissue?

5.1. Electric Field Effects

Reilly (*15*) offers some comments on the effect of electric fields in tissue. Only the field strength is given but, unfortunately, no pulse length. The effects listed may not occur at the very short pulse length (100 μ s) used in most EP drug delivery experiments. The minimum field to stimulate nerves is 6 V/m = 0.06 V/cm. No significant alterations in the evoked response in the peripheral nerves of hogs occur up to 33 V/cm. To generate lesions in tissue requires field strengths above 100 V/cm. Similar levels are probably necessary for neural damage as well. In vitro muscle cells rupture between 50 and 300 V/cm. Fibroblasts rupture above 1 kV/cm.

5.2. Electroporation of Healthy Tissue

Recently Hasegawa and coworkers (*16*) have published an interesting paper that discusses results of electrochemotherapy (ECT) of squamous cell carci-

noma and hepatocellular carcinoma transplanted into the tongues of rats. They used bleomycin as the agent and electrical parameters typical for those used for parallel plates, 1200 V/cm and $8 \times 100 \mu\text{s}$ pulse length at 1 Hz. The authors made detailed observation of (i) not only the tumor but also the surrounding normal tissue, (ii) the epithelium in or not in contact with the electrodes, (iii) the nuclei of the endothelial cells and also changes in the muscle on day 1, and (iv) the nature of the healing process throughout day 14 after the treatment.

The readers are referred to the actual paper for details, but the following features are notable and summarized. On day 1, they observed massive destruction of the tumor and edema which by now are well established phenomena following ECT. The epithelium in contact with the electrodes peeled off but the tissue surrounding the tumor, not in contact, was found to be normal. Nuclei of the endothelial cells were enlarged and some skeletal muscle lost the striation pattern. As days progressed, several important features became evident; the epithelium close to the necrotic tissue was regenerated and the granulation tissue proliferated. Eventually, the necrosis fell off, the wound healing was nearly complete and the tongue was covered with stratified epithelium.

We point out that because of the presence of the mucosal component in the tongue, some of the features seen by the authors are not necessarily the same as are seen in the xenografted subcutaneous tumors. The bleomycin dose the authors use for subcutaneous injection is very high, $\sim 7\text{--}10$ times of what is normally used intratumorally (0.5 U [0.5 mg] for a tumor close to 8 mm in the maximum dimension) (17–19). The authors conclude, “The healing process following ECT progressed smoothly, including that of normal tissue within the electrical field that was seriously damaged.”

6. Applicators

The role of the applicators is to act as a conduit to transform the voltage pulse from a pulse generator into local electric fields in tissue.

6.1. Plate Electrodes

The simplest configuration and the one best suited to generate a more or less uniform electric field are parallel plate electrodes in the form of calipers (Fig. 4). The use of parallel plates is facilitated if a scale is attached so that the distance between the electrodes can be measured. These plates are often mounted on a Vernier caliper as shown in the figure. The voltage can then be determined from the desired field strength and the distance ($V = E \times \text{distance}$). Parallel plate electrodes produced good results in human clinical trials (7,8) with tumors close to the surface. However, superficial skin burning was observed as a consequence of the breakdown of the SC. Though the breakdown

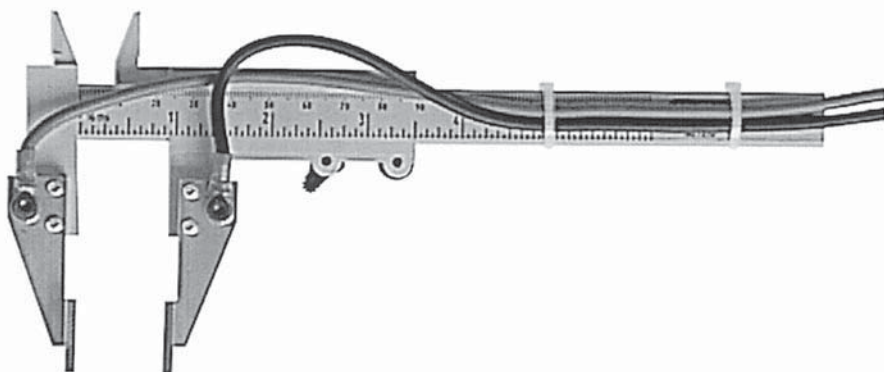


Fig. 4. Caliper electrodes for the treatment of subcutaneous tumors. The scale on the caliper allows measurement of the electrode distance so that the generator voltage can be set according to the desired field strength: $E = V/\text{distance}$.

occurs at low voltages of about 60 V, the high-current density after the breakdown is detrimental to the structure of the skin. Plate electrodes are less efficacious for deeper-seated tumors (20); needles in the form of arrays are better suited. Improved efficacy with parallel plate electrodes can be obtained by rotating the field (position of electrodes) 90° between pulses (21).

6.2. Needle Electrodes

6.2.1. Computer Simulation of Electric Field Distribution

The electric field between needles has been calculated with a three-dimensional computer program (E3 Electrostatic Field Solutions in Three Dimensions, produced by Field Precision of Albuquerque, New Mexico). For needle arrays, the field was calculated in a first step between one pair of needles with the appropriate geometry. The contour lines of constant electric field (absolute amplitude) were plotted and then plots were superposed after rotating by 60° (for the six-needle array) or 90° for the square needle array. Ultimately, lines were drawn around areas where the electric field amplitude was equal to 600 V/cm and 100 V/cm. Inside the shaded area, the electric field is everywhere above 600 V/cm.

6.2.2. Needle Pair

Early experiments in animals were performed with single pairs of needles (22–25). **Figure 5** shows a typical experiment with needle pairs. The field is highly divergent, with a high field strength at the needle surface. **Figure 6** shows the 600 V/cm iso field contour line in a cross section of two parallel needles 0.65 cm apart and with an applied voltage of 942 V. The nominal field

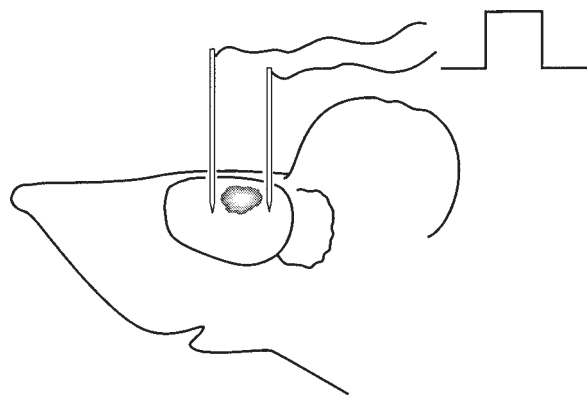


Fig. 5. Electroporation of tissue (tumors) with two needles.

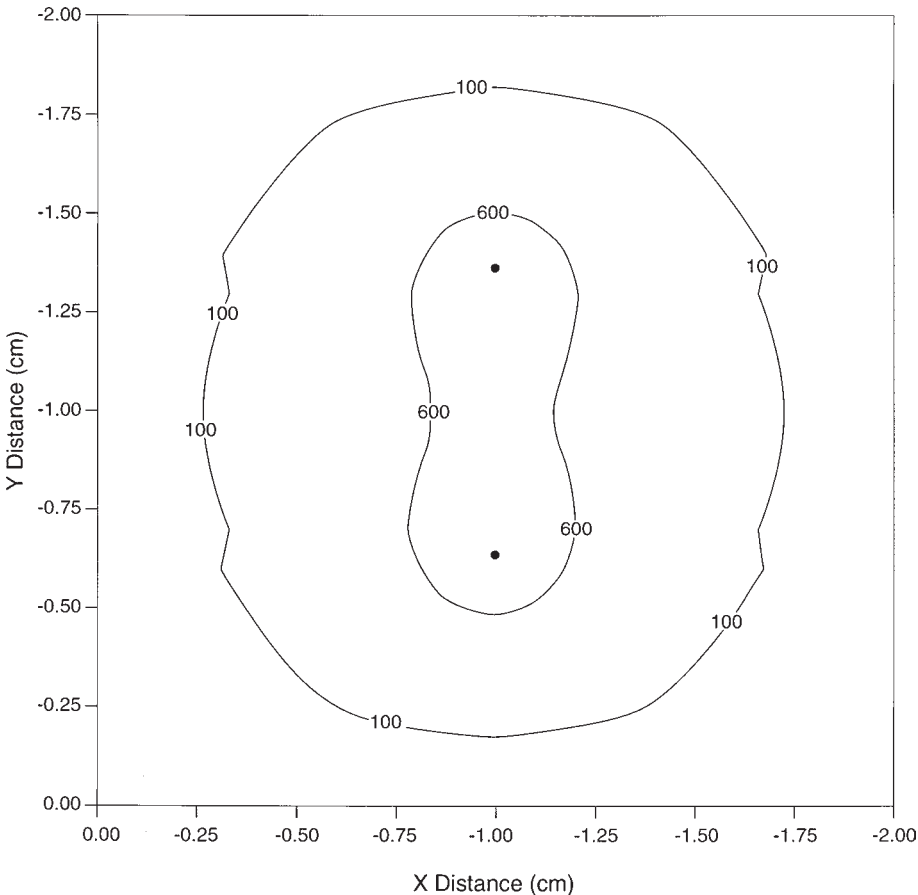


Fig. 6. Isoelectric field lines around a two-needle array. Field strength: V/cm; potential applied: 942 V; distance between needles: 0.65 cm.

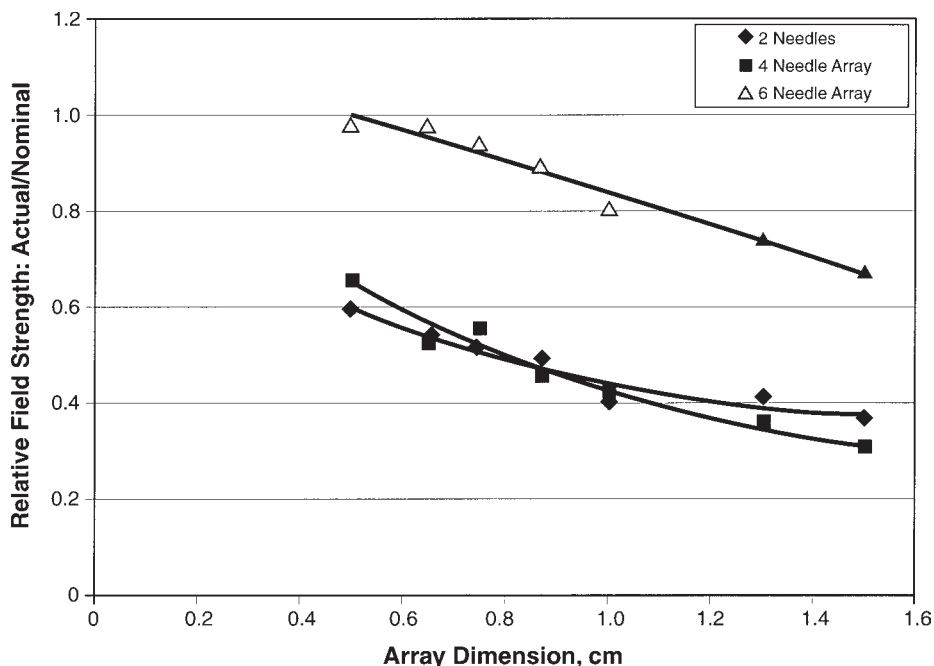


Fig. 7. Ratio of actual versus nominal field strength.

strength in the center between needles is often given as the voltage on the needles divided by the needle distance. However, the actual center field strength is quite different (lower) as shown in **Fig. 7**. A better field configuration results from multiple needles or needle arrays. Gilbert and colleagues (26) investigated several needle configurations; the needle pair showed the lowest efficacy, parallel plates were better. The highest efficacy resulted from the use of a six-needle array.

6.2.3. Six-Needle Arrays

In a six-needle array configuration, six needles are placed equidistant in a circle. Six pulses are applied to consecutive pairs of needles around the circle. **Figure 8** shows the six-needle array concept and the switching scheme. By switching the field between different pairs of needles, a good coverage of the area within the needle array is achieved (**Fig. 10**). As shown in **Fig. 7**, the actual field strength in the center is closer to the nominal field strength (voltage divided by the array diameter) than with two needles. Six-needle arrays were used (27,28) in clinical trials with good efficacy. A variety of needle array diameters, angles of the tip, and needle lengths is needed to reach tumors

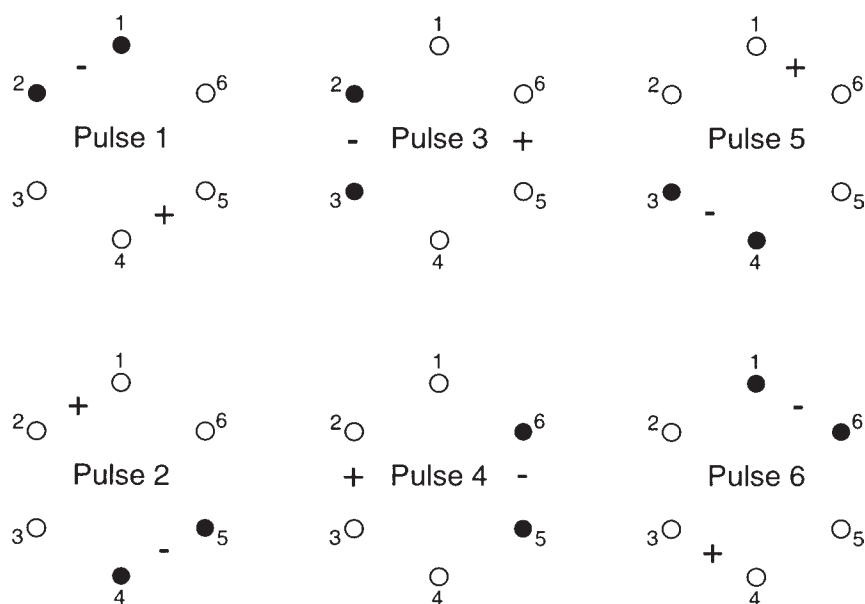


Fig. 8. Sequence used to energize individual needles for a six-needle array around the treatment site.

at different locations in the body (**Fig. 11**). Though the six-needle arrays showed good efficacy, the disadvantage of this configuration is the requirement to increase the array voltage with the array diameter. Some tumors can be quite large; though it is possible to use an overlapping multiple entry treatment strategy, the accuracy of the array placement is not assured. Furthermore, the voltage between needles can not be increased indefinitely. Above about 1500 V, arcing occurs at the needle tips because of the high field enhancement factor. It is therefore desirable to restrict the voltage to lower values. A very good solution to this problem is the subdivision of an array into treatment zones, with square needle arrays as the most practical approach (29).

6.2.4. Square Needle Arrays

An arrangement of 4 needles in a square (a treatment zone) and pulsing between opposing pairs, provides a good area of efficacious field (**Figs. 9 and 12**). Uptake of agents is increased by delivering 4 pulses in a sequence between opposing pairs, changing the orientation by 90° between pulses. The total treatment area can be increased by adding treatment zones. **Figure 13** shows the field distribution in a 9-needle array. This configuration allows the electrically paralleling of zones so that the number of switching steps is vastly reduced.

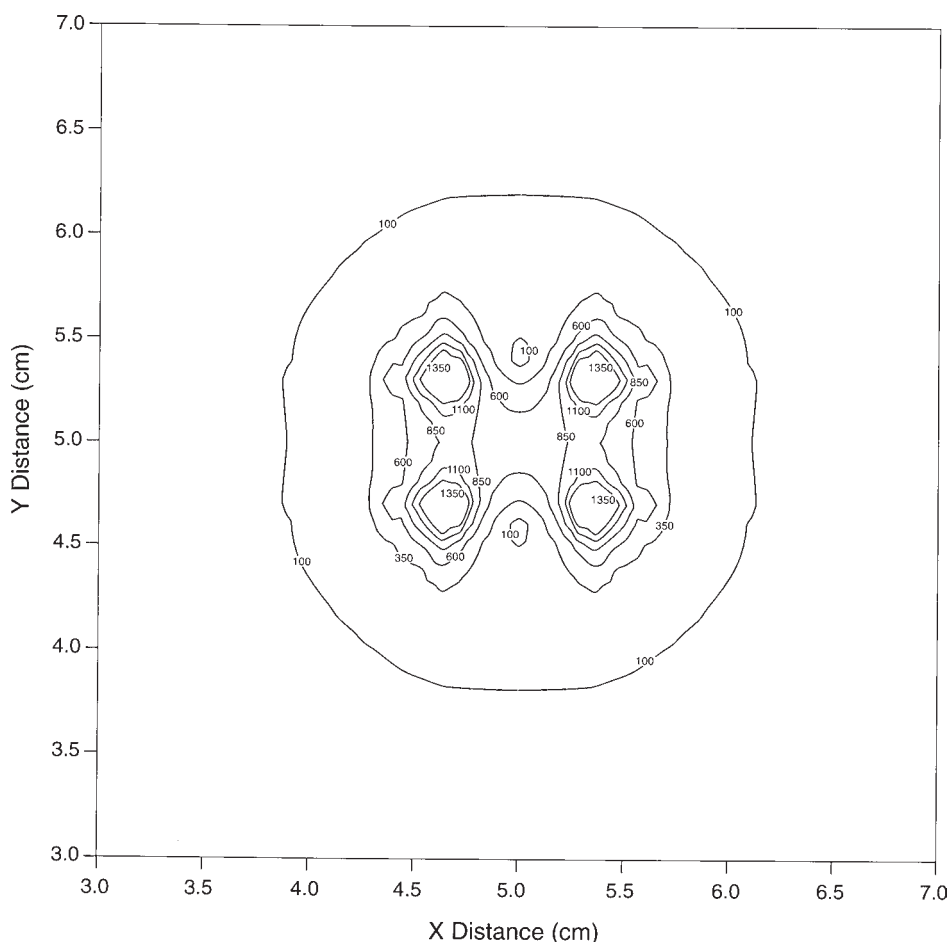


Fig. 9. Isoelectric field lines around a four-needle array; 0.65 cm base length. Cross section is taken in the middle plane of the array. Field strength: V/cm; electrode spacing: 0.65 cm; needle diameter: 0.04 cm; potential applied: 845 V.

The required voltage remains the same as for one square; the current and power requirements increase with the addition of treatment zones. **Figure 14** shows the electrical arrangement of a 25-needle array covering an area of 2.6×2.6 cm with a 0.65 cm zone base length. All needles with the same number are connected electrically in parallel. The required voltage is only 940 V; the minimum field in the treatment zones is 780 V/cm, which reflects a margin of efficacy of 30% over the assumed minimum efficacious field of 600 V/cm. Only 4 pulses are required which can be delivered by the MedPulser™ (28)

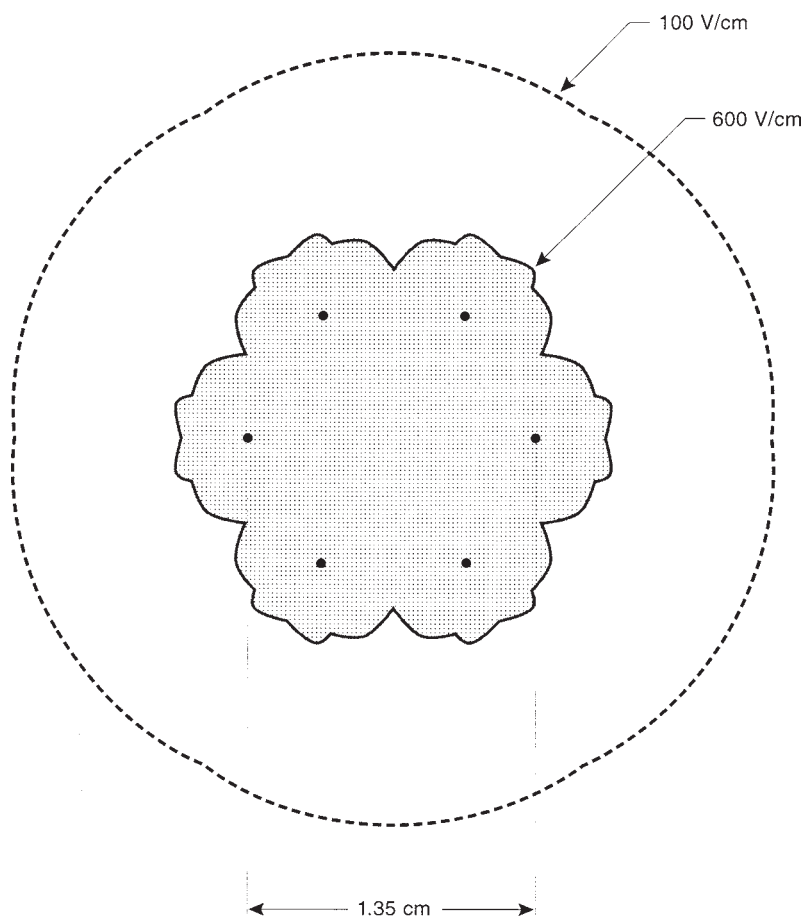


Fig. 10. Isoelectric field lines generated by a six-needle array (four are active), after switching 60°; 1.35 cm diameter; 1500 V between needle pairs.

generator in one second: first pulse: Needles 1–2 pulsed against 3–4; second pulse: reversed polarity; third pulse: 1–3 pulsed against 2–4; fourth pulse: reversed polarity.

6.3. Surface (Meander) Electrodes for the Electroporation of Skin

The biophysical phenomenon of electroporation is pronounced when a thin, highly resistive membrane surrounds or shields a conductive medium. A very strong field enhancement will take place in the membrane which can lead to permeabilization or electroporation. The field enhancement is in first order proportional to the ratio of the thickness of the conductive medium to the

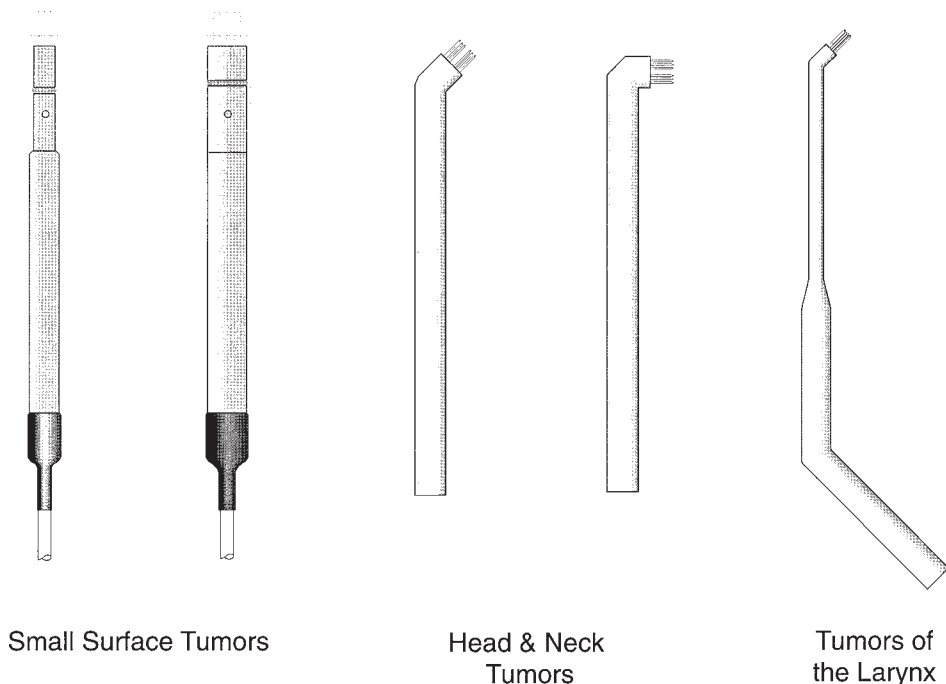


Fig. 11. Various six-needle array applicators.

membrane thickness. In the classical case of cells, the cell membrane and the cytoplasm play this role.

The skin presents a similar biophysical model. A very thin (typically 15 μm), highly resistive layer, the SC is surrounding the highly conductive epidermis and dermis (30). A very low potential difference applied to the skin, for example, 60 V across two layers of SC (representative for adjacent surface electrodes), results in very high fields in the SC of about 20 kV/cm. Such a high field appears to suffice for the breakdown and formation of aqueous pores in the SC. The SC is normally a very effective barrier against penetration of outside agents; electroporation of the SC allows the transport of agents across this barrier by several methods (31,32).

In tissue electroporation, the goal is an even distribution of efficacious field. In the electroporation of skin for transdermal delivery of agents, it is desirable to contain the electric field to a shallow skin surface layer so that underlying nerves and muscles are not subjected to a strong electrical stimulus. This objective can be realized by meander electrodes, which consist of closely spaced opposing finger electrodes (Fig. 15). Figure 16 shows the field and potential distribution around a meander electrode. As can be seen, the potential

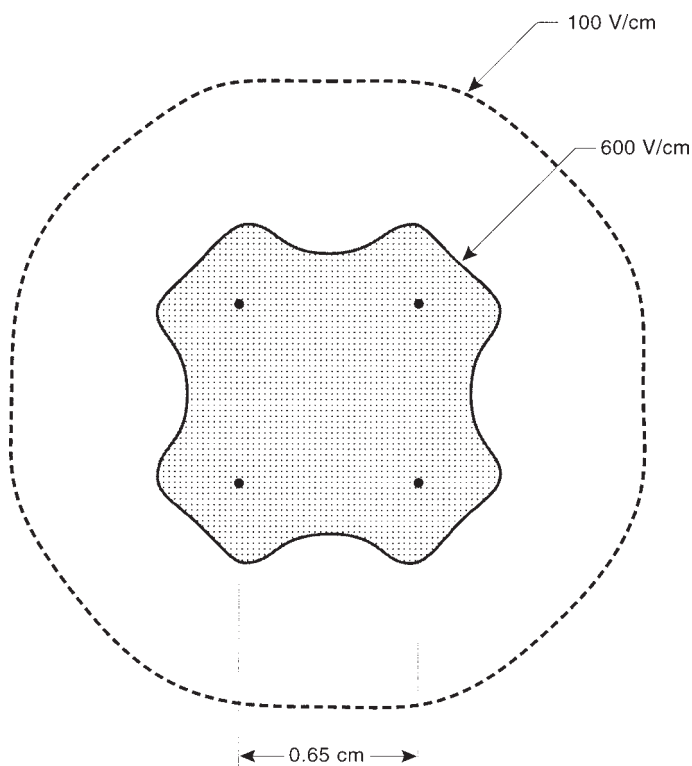


Fig. 12. Isoelectric field lines generated by a four-needle square array after switching 90°; 0.65 cm base length.

drop between the electrodes is mainly confined to the SC. After breakdown, the range in depth of the electric field is related to the electrode spacing; a narrow spacing will confine the field to a shallow surface region.

6.4. Electroporation in Vessels: Catheters

In vivo electroporation of agents into the walls of vessels requires first positioning of the agents proximal to the vessel wall without being washed away. A double-balloon catheter as shown in **Fig. 17** blocks the blood flow after inflation of the two balloons. Infusion of the agent occurs into the volume between the balloons. The electrical pulse is applied between an electrode located between the balloons and the catheter guide wire as the current return path. **Figure 18** shows the field distribution around the double-balloon catheter. A strong electric field exists between the center electrode and the adjacent tissue. Double balloons were successfully employed for the intravascular delivery of heparin (**12**) and DNA-binding propidium iodide (**10**).

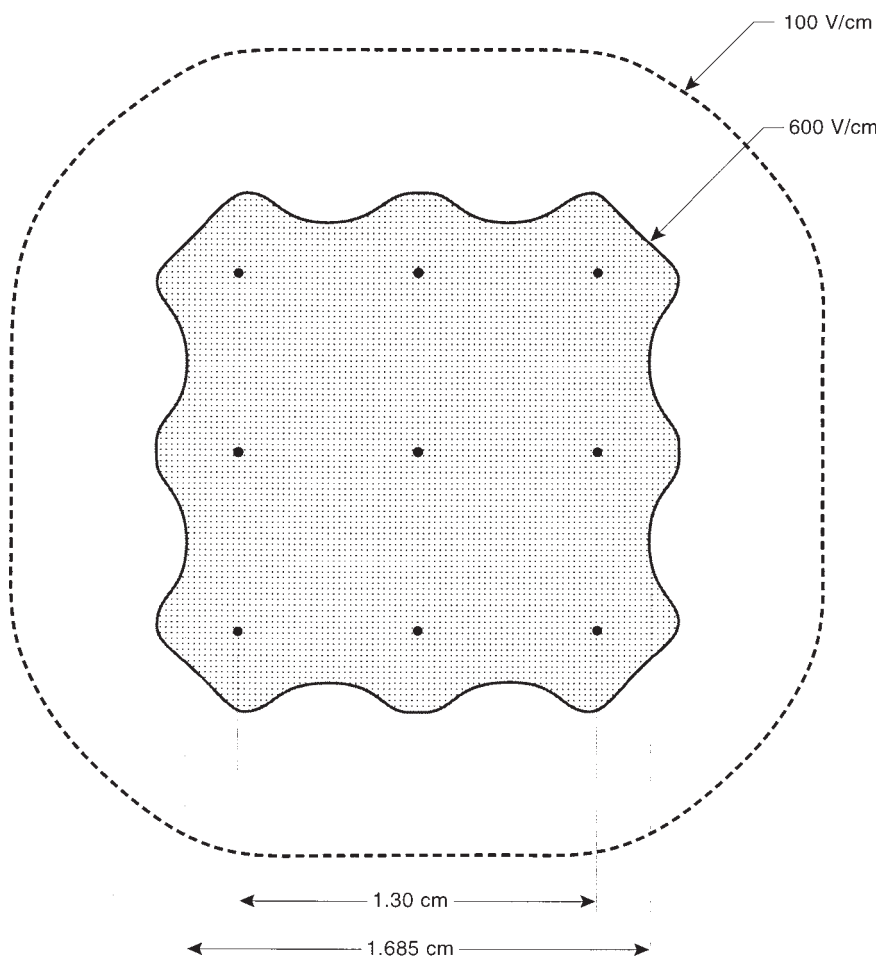


Fig. 13. Isoelectric field lines generated by array of four-needle squares after switching 90° ; 0.65 cm base length; 940 V between needle pairs.

6.5. Other Electrode Configurations

An interesting application of EP *in vivo* is the delivery of bleomycin into the eye for the treatment of high intraocular pressure (33). A special cup shaped applicator was developed to fit around the eye of rabbits. Earlier *in vivo* work described the fusing of cells to the cornea of rabbits (34) by means of pulsed electric fields with a cup-shaped applicator.

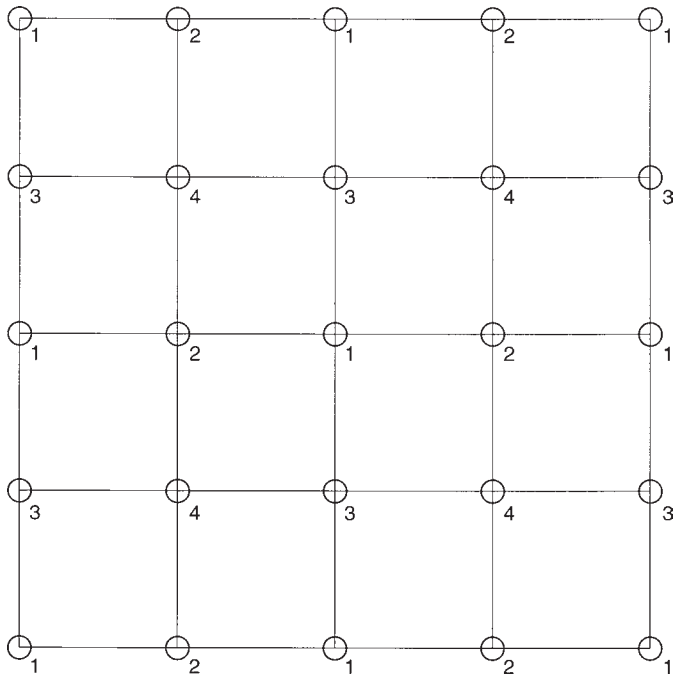


Fig. 14. Square needle array (2.6 cm \times 2.6 cm) with paralleling all switching zones.

7. Pulse Generators

The pulse generators for in vivo drug and gene delivery need to deliver on command a voltage wave form which the electrodes transform into an efficacious electric field in the tissue. The important parameters are voltage amplitude, length of the pulse, shape of the pulse, and the number of repeat applications.

In vitro, a variety of different wave shapes are used with varying effectiveness: square pulses, exponentially decaying pulses or bursts of radiofrequency waveforms. In the overwhelming majority of in vivo applications, square waves are being used. They have the advantage of allowing the user to preset an exact amplitude and pulse length, provided that the generator was designed to supply the tissue with the required current. The design requirements for a square pulse generator are described in detail by Hofmann and associates (29). **Table 1** compares the specifications of the generators which were used in the majority of animal work, preclinical and clinical human trials.

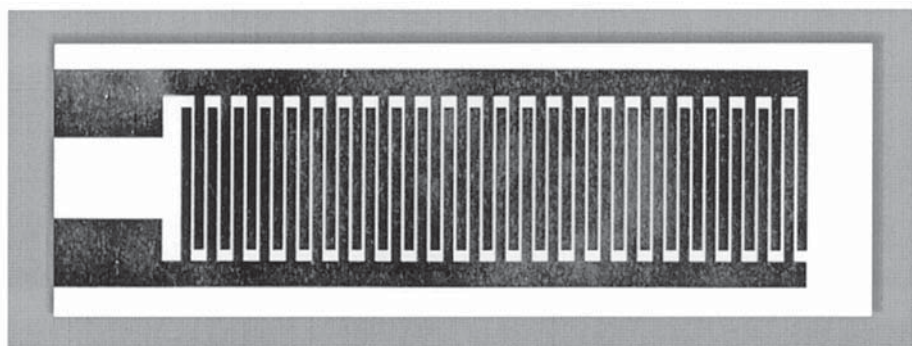


Fig. 15. Meander electrodes: electrode gap, 0.2 mm; electrode width, 0.2 mm.

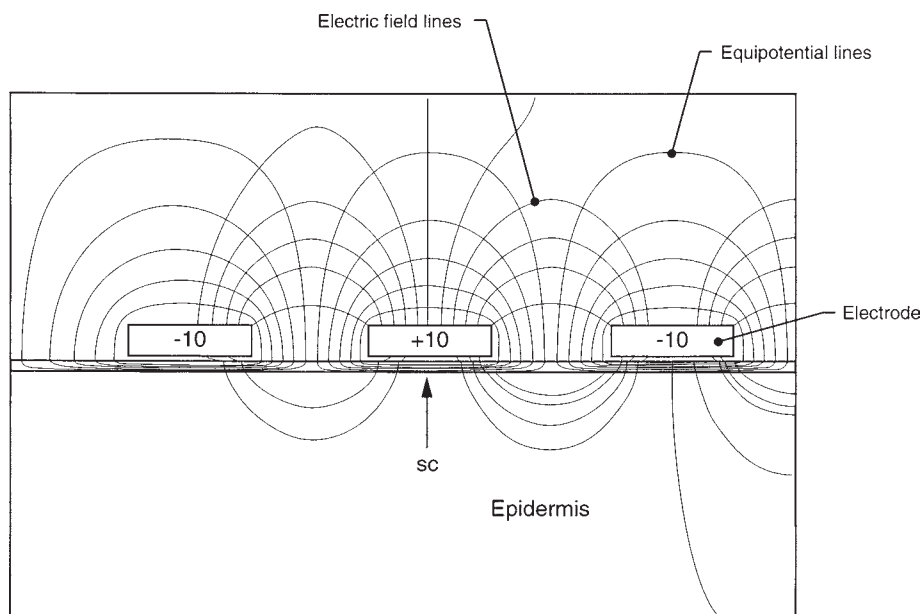


Fig. 16. Plot of equipotential and electrostatic field lines of meander electrodes on top of the SC with a distance of 10 μm . Specific resistivities: medium surrounding the meander electrodes, $1000 \Omega \times \text{cm}$; SC, $6 \times 10^8 \Omega \times \text{cm}$; viable epidermis, $10^5 \Omega \times \text{cm}$. Potential difference between meander electrodes: $\pm 10 \text{ V}$.

High electric fields and short pulse length are generally used for the delivery of chemotherapeutic drugs into tumors, typically 1000 V/cm and 100 μs . Genes, however, were shown to be delivered at high efficiency (35,36) with low field strength (100 V/cm) and long pulse lengths (10–50 msec).

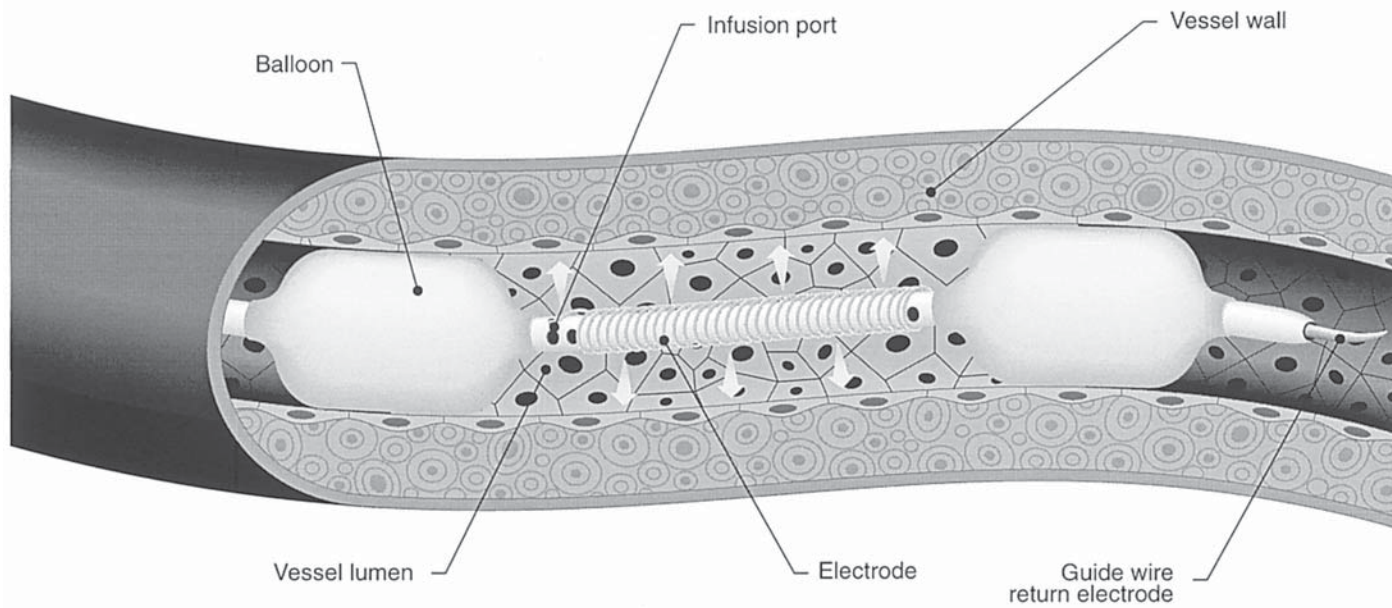


Fig. 17. Double-balloon electroporation catheter.

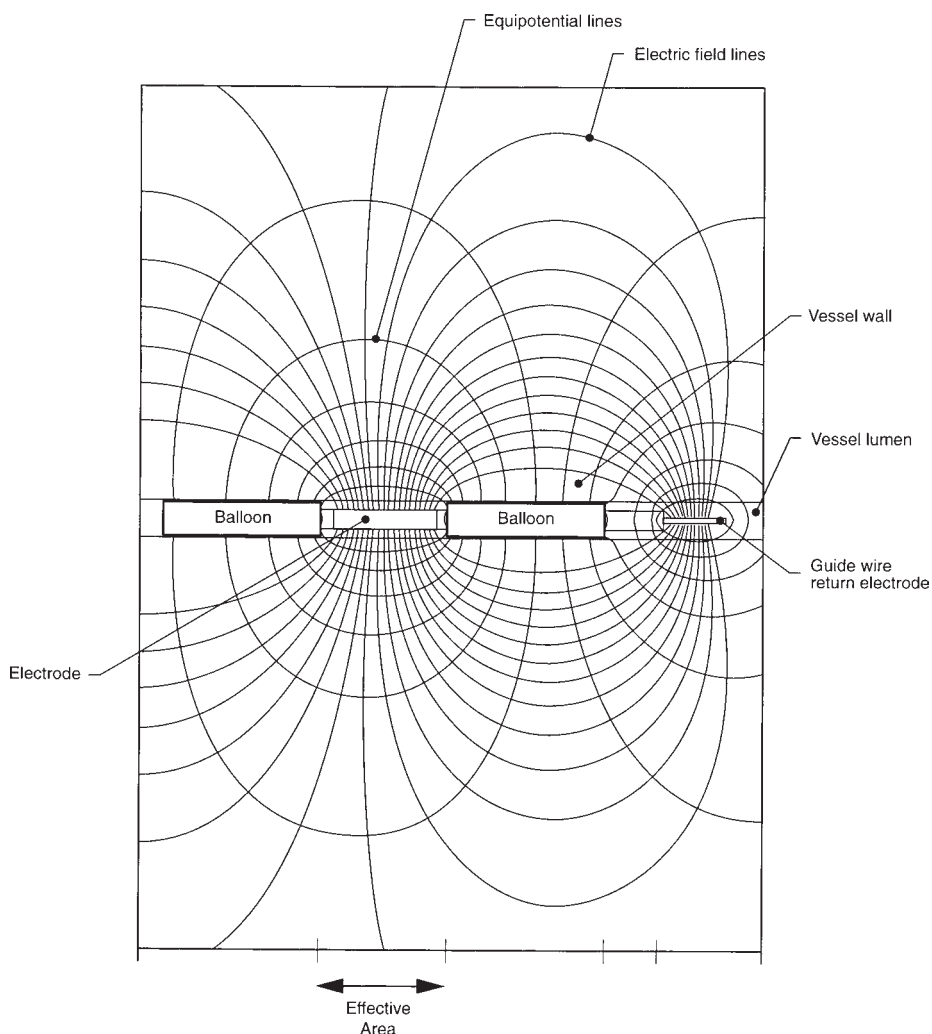


Fig. 18. Electric field and equipotential lines around a double-balloon catheter.

Though a researcher in most cases is not involved in bringing a therapeutic EP system to the market, the step from *in vivo* experiments in animals to initial preclinical trials should be understood. For animal electroporation experiments and preclinical human trials, commercially available generators, which are available for *in vitro* electroporation, were generally used. The following section discusses the additional steps in generator design which will make it perform to regulatory requirements. These requirements must be met in order to be able to perform large-scale multi-center clinical trials.

Table 1
Square Wave Generators for In Vivo Drug/Gene Delivery

Manufacturer	Ref.	Voltage Range	Pulse Length	Electrodes
Jouan, France Electropulsator PS 15 Commercially available	7	0–1500 V Variable	5 μ s–24 msec Variable	Parallel plates
BTX, a division of Genetronics, Inc. San Diego, CA ElectroSquare Porator T 820 Commercially available	29	0–500 V 20–3000 V Variable	0.3 ms–99 msec 5 μ s–99 μ sec Variable	Parallel plates; needle electrodes with auxiliary switch
Genetronics, Inc. San Diego, CA MedPulser In use for multicenter clinical trials	14, 28	1500 Vmax; preset for each needle array applicator type	100 μ s fixed	Needle electrodes only

8. Regulatory Requirements for Clinical Applications

To encourage the discovery and development of useful medical devices, the Food and Drug Administration (FDA) provides exemptions for investigational devices from the premarket approval process. An Investigational Device Exemption (IDE) permits a device to be shipped in interstate commerce for clinical investigation to determine its medical safety and effectiveness. Although the IDE regulation exempts the device from certain requirements, it requires safeguards for humans who are subjects of investigations: maintenance of sound ethical standards, and procedures to ensure development of reliable scientific data.

Certain device investigations are exempt from the requirements of the IDE regulation. This determination is based upon the risk presented to the patient either directly from device use or indirectly from medical decisions made with data from the device. Depending on the device, an IDE may be approved either by an Institutional Review Board (IRB) or by both an IRB and FDA; informed consent for all patients, adequate monitoring and necessary records and reports are required in either case.

If the device has patient contact, it should be constructed with safe, biocompatible materials and their evaluation should be consistent with the International Organization for Standardization (ISO)–10993 guidelines, “Bio-

logical Evaluation of Medical Devices.” Potential risks should be addressed in a formal risk analysis and measures should be taken to limit patient risk whenever possible and feasible.

The products used for investigations should be designed with the clinical researcher in mind. The following design objectives should be considered in the clinical product design:

1. The design should preclude the opportunity for operator mistakes.
2. If that is not feasible, then reduce the likelihood of mistakes (e.g. interlocks).
3. If reduction cannot be achieved, then mitigate or limit the adverse consequences when a mistake is made.

Relying on the user to adjust and compensate for problems presented by a poorly designed user interface is the least desirable alternative. The goal is to design devices that are easy to use (user-friendly) and minimize the chance for users to make mistakes. In addition, since it is not possible to predict and prevent all errors, the design must also be error tolerant.

The most common cause of human factors problems is the failure of the device designers and developers to anticipate and deal with the characteristics of the people who interact with the device and the nature of these interactions. Common problems include:

1. Unusual or unexpected device operation.
2. Lack of protection against incorrect use.
3. Confusing or complex controls, labeling or operation.
4. Defeatable or ignorable safety features.

Although Investigative Devices are exempt from most FDA Quality System Requirements (QSR), they are not exempt from the Design Control regulations. At a minimum, the clinical product should be designed based on a product development plan, formal design input and output specifications, risk analysis, and verification tests that demonstrate that the product performs as designed and intended. The product should be manufactured under a controlled condition with a stable and repeatable process. The design and manufacturing revision and history records must be documented and maintained. Acceptance test and inspection procedures must be developed.

9. Summary and Conclusions

In vivo delivery of drugs and genes to cells in tissue is becoming a powerful tool, which compares favorably to other delivery modalities (37). Efficacy depends mainly on two requirements: to have the drug or gene at the tissue site and to apply the appropriate field pulse to the site. For the treatment of tumors, it is desirable to investigate a lower drug dosage of bleomycin than currently

used, while maintaining tumor response, in order to minimize the effect of ECT on healthy tissue. Some future developments include the selection of the optimum drug or gene; determination of the most efficacious electrical parameters: amplitude, pulse length, waveform (it is desirable to minimize muscle reactions and pain caused by the EP treatment); and development of the most appropriate applicator: catheter type applicators for hollow organs, minimally invasive laparoscopic applicators, and possibly implantable EP applicators.

As the medical field moves from treatment of diseases with drugs to treatment with genes, the EP delivery systems being developed now will be able to make this transition with ease.

Acknowledgment

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References

1. Hofmann, G. A. (1995) Instrumentation. *Methods Mol. Biol.* **48**, 41–59.
2. Jain, R. K. (1994) Barriers to drug delivery in solid tumors. *Sci. Am.* **271**, 58–65.
3. Heller, R., Jaroszeski, M. J., Reintgen, D. S., Puleo, C. A., DeConti, R. C., Gilbert, R. A., and Glass, L. F. (1998) Treatment of cutaneous and subcutaneous tumors with electrochemotherapy using intralesional bleomycin. *Cancer* **83**, 148–157.
4. Prausnitz, M. R. (1997) Reversible skin permeabilization for transdermal delivery of macromolecules. *Crit. Rev. Ther. Drug Carrier Sys.* **14**, 455–483.
5. Banga, A. K. (1998) Electrically assisted transdermal and topical drug delivery. Taylor & Francis Ltd., London.
6. Titomirov, A. V., Sukharev, S. I., and Kistanova, E. (1991) In vivo electroporation and stable transformation of skin cells of newborn mice by plasmid DNA. *Biochim. Biophys. Acta* **1088**, 131–134.
7. Mir, L. M., Belehradek, M., Domenge, C., Orlowski, S., Poddevin, B., Belehradek, J. J., Schwaab, G., Luboinski, B., and Paoletti, C. (1991) Electrochemotherapy, a novel antitumor treatment: first clinical trial. *C.R. Acad. Sci. III* **313**, 613–618.
8. Heller, R., Jaroszeski, M. J., Glass, L. F., Messina, J. L., Rapaport, D. P., DeConti, R. C., Fenske, N. A., Gilbert, R. A., Mir, L. M., and Reintgen, D. S. (1996) Phase I/II trial for the treatment of cutaneous and subcutaneous tumors using electrochemotherapy. *Cancer* **77**, 964–971.
9. Malignancy Treatment (1997) U.S. Patent 4,665,898.
10. Cui, J., Robinson, K. A., Brown, J. E., Chronos, N. A. F., Cipolla, G. D., Dev, S. B., Hofmann, G. A., Crawford, N., and King III, S. B. (1997) Local drug delivery to pig carotid arteries by direct vessel wall electroporation using a novel catheter. [Abstract]. *Proc. Am. Coll. Cardiol.* **29**, 201A.

11. Dev, N. B., Preminger, T. J., Hofmann, G. A., and Dev, S. B. (1998) Sustained local delivery of heparin to the rabbit arterial wall with an electroporation catheter. *Cathet. Cardiovasc. Diagn.* **45**, 337–345.
12. Dev, S. B., Hofmann, G. A., Preminger, T. J., and Dev, N. B. (1997) In vivo delivery of heparin into arterial wall with electroporation catheters. *Proc. World Congr. Electr. Magn. Biol. Med.* Bologna, Italy, **C4**, 95–96.
13. Belehradek, J. J., Orlowski, S., Ramirez, L. H., Pron, G., Poddevin, B., and Mir, L. M. (1994) Electroporabilization of cells in tissues assessed by the qualitative and quantitative electroloading of bleomycin. *Biochim. Biophys. Acta* **1190**, 155–163.
14. Nanda, G. S., Merlock, R. A., Hofmann, G. A., and Dev, S. B. (1998) A novel and effective therapy for prostate cancer. [Abstract]. *Proc. Am. Assoc. Cancer Res.* **39**, 2911.
15. Reilly, J. P., (1992) *Electrical Stimulation and Electropathology*. Cambridge University Press, Victoria, Australia, pp.120, 388, 417.
16. Hasegawa, H., Kano, M., Hoshi, N., Watanabe, K., Satoh, E., Nakayama, B., and Suzuki, T. (1998) An electrochemotherapy model for rat tongue carcinoma. *J. Oral Pathol. Med.* **27**, 249–254.
17. Dev, S. B., Nanda, G. S., An, Z., Wang, X., Hoffman, R. M., and Hofmann, G. A. (1997) Effective electroporation therapy of human pancreatic tumors implanted in nude mice. *Drug Deliv.* **4**, 293–299.
18. Nanda, G. S., Sun, F. X., Hofmann, G. A., Hoffman, R. M., and Dev, S. B. (1998) Electroporation therapy of human larynx tumors Hep-2 implanted in nude mice. *Anticancer Res.* **18**, 999–1004.
19. Nanda, G. S., Sun, F. X., Hofmann, G. A., Hoffman, R. M., and Dev, S. B. (1998) Electroporation enhances therapeutic efficacy of anticancer drugs: Treatment of human pancreatic tumor in animal model. *Anticancer Res.* **18**, 1361–1366.
20. Domenge, C., Orlowski, S., Lubinski, B., DeBaere, T., Schwaab, G., Belehradek, J., and Mir, L. M. (1996) Antitumor electrochemotherapy: New advances in the clinical protocol. *Cancer* **77**, 956–963.
21. Serša, G., Čemažar, M., Šemrov, D., and Miklavčič, D. (1996) Changing electrode orientation improves the efficacy of electrochemotherapy of solid tumors in mice. *Bioelectrochem. Bioenerg.* **39**, 61–66.
22. Okino, M. and Mohri, H. (1987) Effects of a high-voltage electrical impulse and an anticancer drug on in vivo growing tumors. *Jpn. J. Cancer Res.* **78**, 1319–1321.
23. Okino, M. and Esato, K. (1990) The effects of a single high voltage electrical stimulation with anticancer drug on in vivo growing malignant tumors. *Jpn. J. Surg.* **20**, 197–204.
24. Salford, L. G., Persson, B. R. R., Brun, A., Ceberg, C. P., Kongstad, P., and Mir, L. M. (1993) A new brain tumor therapy combining bleomycin with in vivo electroporabilization. *Biochem. Biophys. Res. Commun.* **194**, 938–943.
25. Nishi, T., Yoshizato, K., Yamashiro, S., Takeshima, H., Sato, K., Hamada, K., Kitamura, I., Yoshimura, T., Saya, H., Kuratsu, J., and Ushio, Y. (1996) High

- efficiency in vivo gene transfer using intra arterial plasmid DNA injection following in vivo electroporation. *Cancer Res.* **56**, 1050–1055.
26. Gilbert, R. A., Jaroszeski, M. J., and Heller, R. (1997) Novel electrode designs for electrochemotherapy. *Biochim. Biophys. Acta* **1334**, 9–14.
 27. Glass, L. F., Pepine, M. L., Fenske, N. A., Jaroszeski, M. J., Reintgen, D. S., and Heller, R. (1996) Bleomycin-mediated electrochemotherapy of metastatic melanoma. *Arch. Dermatol.* **132**, 1353–1357.
 28. Panje, W. R. (1998) Electroporation therapy of head and neck cancer. *Ann. Otol. Rhinol. Laryngol.* **107**, 779–785.
 29. Hofmann, G. A., Dev, S. B., and Nanda, G. S. (1996) Electrochemotherapy: Transition from laboratory to the clinic. *IEEE Eng. Med. Biol.* **15**, 124–132.
 30. Chien, Y. W. (1993) *Dermal and Transdermal Drug Delivery: New Insights and Perspectives*. (Gurny, R. and Teubner, A., eds.), Wissenschaftliche Verlagsges., Stuttgart, p. 136.
 31. Hofmann, G. A., Rustrum, W. V., and Suder, K. S. (1995) Electro-incorporation of microcarriers as a method for the transdermal delivery of large molecules. *Bioelectrochem. Bioenerg.* **38**, 209–222.
 32. Prausnitz, M. R. (1996) The effects of electric current applied to skin: A review for transdermal drug delivery. *Adv. Drug Deliv. Rev.* **18**, 395–425.
 33. Sakamoto, T., Oshima, Y., Sakamoto, M., Kawano, Y. I., Ishibashi, T., Inomata, H., and Ohnishi, Y. (1997) Electroporation and bleomycin in glaucoma-filtering surgery. *Invest. Ophthalmol. Vis. Sci.* **38**, 2864–2868.
 34. Grasso, R. J., Heller, R., Cooley, J. C., and Haller, E. M. (1989) Electrofusion of individual animal cells directly to intact corneal epithelial tissue. *Biochim. Biophys. Acta* **980**, 9–14.
 35. Muramatsu, T., Shibata, O., Ryoki, S., Ohmori, Y., and Okumura, J. (1997) Foreign gene expression in the mouse testis by localized in vivo gene transfer. *Biochem. Biophys. Res. Commun.* **233**, 45–49.
 36. Nishi, T., Goto, T., Yoshizato, K., Takeshima, H., Kuratsu, J., Ushio, Y., Hofmann, G. A., and Dev, S. B. (1997) High efficiency gene transfer in solid tumors by in vivo electroporation. [Abstract]. *Proc. Sixth Intl. Conf. Gene Ther. Cancer* **4**, P-56, S27.
 37. Muramatsu, T., Nakamura, A., and Park, H. M. (1998) In vivo electroporation: A powerful and convenient means of nonviral gene transfer to tissues of living animals. *Int. J. Mol. Med.* **1**, 55–62.

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