Annals of Biomedical Engineering, Vol. 33, No. 2, February 2005 (©2005) pp. 223–231
DOI: 10.1007/s10439-005-8981-8

Tissue Ablation with Irreversible Electroporation

R. V. DAVALOS,[1] L. M. MIR,[2] and B. RUBINSKY[3]

[1]Microsystems and Advanced Concepts Engineering, Sandia National Laboratories, Livermore, CA 94550; [2]Vectorology and gene transfer, UMR 8121 CNRS, Institut Gustave-Roussy, Villejuif Cédex, France; and [3]Department of Mechanical Engineering, Biomedical Engineering Laboratory, University of California, Berkeley, CA 94720-1740

(Received 1 April 2004; accepted 7 September 2004)

Abstract—This study introduces a new method for minimally invasive treatment of cancer—the ablation of undesirable tissue through the use of irreversible electroporation. Electroporation is the permeabilization of the cell membrane due to an applied electric field. As a function of the field amplitude and duration, the permeabilization can be reversible or irreversible. Over the last decade, reversible electroporation has been intensively pursued as a very promising technique for the treatment of cancer. It is used in combination with cytotoxic drugs, such as bleomycin, in a technique known as electrochemotherapy. However, irreversible electroporation was completely ignored in cancer therapy. We show through mathematical analysis that irreversible electroporation can ablate substantial volumes of tissue, comparable to those achieved with other ablation techniques, without causing any detrimental thermal effects and without the need of adjuvant drugs. This study suggests that irreversible electroporation may become an important and innovative tool in the armamentarium of surgeons treating cancer.

Keywords—Electropermeabilization, Cancer therapy, Bioheat equation.

INTRODUCTION

In many medical procedures, such as the treatment of benign or malignant tumors, it is important to be able to ablate undesirable tissue in a controlled and focused way without affecting the surrounding desirable tissue. Over the years, a number of minimally invasive methods have been developed to selectively destroy specific areas of undesirable tissues as an alternative to resection surgery. Each technique has specific advantages and disadvantages, which are indicated and contraindicated for various applications. For example, cryosurgery is a low temperature minimally invasive technique in which tissue is frozen on contact with a cryogen-cooled probe inserted into the undesirable tissue.[37] The area affected by low temperature therapies can be easily controlled through imaging; however, the probes are large and difficult to use. Nonselective chemical ablation is a technique in which chemical agents, such as ethanol, are injected into the undesirable tissue to cause ablation.[38] Nonselective chemical therapy is easy to apply. Unfortunately, the affected area cannot be controlled because of the local blood flow and transport of the chemical species. Focused ultrasound is a high temperature noninvasive technique in which the tissue is heated to coagulation using high-intensity ultrasound beams focused on the undesirable tissue.[14,22] Radiofrequency ablation (RF) is another high temperature minimally invasive technique in which an active electrode is introduced into the undesirable area and a high frequency alternating current of up to 500 kHz is used to heat the tissue to coagulation.[34] Interstitial laser coagulation is a high temperature thermal technique in which tumors are slowly heated to temperatures exceeding the threshold of protein denaturation using low power lasers delivered through optical fibers.[3] High temperature thermal therapies have the advantage of ease of application. The disadvantage is that the extent of the treated area is difficult to control because blood circulation has a strong local effect on the temperature field that develops in the tissue. The armamentarium of surgery is thus enhanced by the availability of several different minimally invasive surgical techniques in existence, each with their own advantages and disadvantages and particular applications. The goal of this study is to introduce a new minimally invasive surgical technique for tissue ablation-irreversible electroporation. This technique has the advantages that it is easy to apply, can be monitored and controlled, is not affected by local blood flow, and does not require the use of adjuvant drugs. We will describe the technique and demonstrate its feasibility through mathematical modeling.

Electroporation is a phenomenon that increases the permeabilization of the cell membrane by exposing the cell to electric pulses.[43] The external electric field to which the tissue is exposed is the primary parameter affecting the transmembrane potential, the potential difference across the plasma membrane. As a function of the

Address correspondence to Rafael V. Davalos, Microsystems and Advanced Concepts Engineering, Sandia National Laboratories, 7011 East Ave, MS 9036, Livermore, CA 94550. Electronic mail: rvdaval@sandia.gov

transmembrane potential, the electroporation pulse can either have no effect on the cell membrane, reversibly open the cell membrane after which cells can survive, or irreversibly open the cell membrane, after which the cells die. Dielectric breakdown of the cell membrane due to an induced electric field was first observed in the early 1970s.[6,28,44] The ability of the membrane to reseal was discovered during the late 1970s.[1,21,15] The mechanism for electroporation is not yet completely understood. It is thought that the electric field changes the electrochemical potential across the cell membrane and induces instabilities in the polarized cell membrane lipid bilayer. The unstable membrane then alters its shape, forming aqueous pathways that possibly are nanoscale pores through the membrane.[41] Mass transfer can now occur through these channels under electrochemical control. Electroporation is commonly used in medicine and biotechnology to introduce nonpermeable chemical species across the cell membrane, ranging from small molecules such as fluorescent dyes, drugs, and radioactive tracers to high molecular weight molecules such as antibodies, enzymes, nucleic acids, HMW dextrans, and DNA.[5,29,30]

Therapeutic electroporation is becoming increasingly popular as a minimally invasive surgical technique for introducing small drugs and macromolecules into cells in specific areas of the body. Once the nonpermeable substance is injected into the body, electrodes placed into or around the targeted tissue are used to generate reversibly permeabilizing electric fields throughout the targeted tissue that facilitate the entry of the agent into the cells of the targeted area.[24] The use of electroporation to increase the permeability of the cell membrane in tissue was introduced by Okino and Mohri in 1987 and by Mir *et al*. in 1991, who independently discovered that combining an impermeant anticancer drug with reversibly permeabilizing electric pulses greatly enhanced the effectiveness of the treatment rather than either one alone.[26,31] This combination is a form of cancer treatment now known as electrochemotherapy (ECT).[19] ECT is a promising minimally invasive surgical technique to locally ablate tissue and treat tumors, regardless of their histological type, with minimal adverse side effects and a high response rate.[16,17,27] ECT benefits from the ease of application, and results in outcomes comparable to both high temperature treatment therapies and nonselective chemical therapies. In addition, because the cell membrane's permeabilizing electric field is not affected by local blood flow, control over the extent of the affected tissue is possible unlike in many other modes such as thermal and nonselective chemical therapies. ECT is beneficial due to its selectivity; however, a disadvantage is that by its nature, it requires the combination of chemical agents with an electric field. Currently, the primary therapeutic *in vivo* applications of electroporation are ECT, electrogenetherapy (EGT) as a form of nonviral gene therapy, and transdermal drug delivery.[24] The studies on ECT and EGT have been recently summarized in several publications.[17,19,24]

Electroporation can either be reversible or irreversible depending on the induced transmembrane potential from the applied electric pulses. Irreversible electroporation is clearly detrimental in certain applications because it is nonselective and can lead to instantaneous necrosis of the entire tissue affected by the electric field, whether diseased or healthy.[25] In therapeutic tissue electroporation, the sole purpose of the electric pulses has been to facilitate the introduction of the molecules into the tissue. This approach has affected the thinking in ECT where irreversible electroporation has been considered undesirable.

We propose in this study that irreversible electroporation can be used as a minimally invasive surgical procedure to ablate undesirable tissue without the use of adjuvant drugs. Although not as selective as ECT, its nonselective mode of ablation is acceptable in the field of minimally invasive surgery and more comparable to cryosurgery, nonselective chemical ablation and high temperature thermal ablation. Irreversible electroporation has been studied extensively with *in vitro* cellular systems. For instance, it is considered an effective means to destroy both gram positive and gram negative bacteria and amoebae with regards to water decontamination.[20,36,42] However, to the best of our knowledge, irreversible electroporation has yet to be evaluated for its potential in minimally invasive surgery for the ablation of tissue. We suggest that irreversible electroporation ablation of tissue may have the benefits of ECT without the reliance on potentially harmful chemotherapeutic drugs.

Electroporation protocols involve the generation of electric fields in tissue and are affected by the Joule heating of the electric pulses. When designing tissue electroporation protocols, it is important to determine the appropriate electrical parameters that will maximize tissue permeabilization without inducing deleterious thermal effects. Our previous study showed that substantial volumes of tissue can be reversibly electroporated without inducing damaging thermal effects to cells.[8] The electric pulses required to induce irreversible electroporation in tissue are larger in magnitude and duration than the electric pulses required for reversible electroporation. Therefore, when we propose using irreversible electroporation for tissue ablation, there is concern that irreversible electroporation pulses will be so large as to cause thermal damaging effects to the tissue and the extent of the tissue ablated by irreversible electroporation will not be significant relative to the amount ablated by thermal effects. Under such circumstances, irreversible electroporation could not be considered an effective tissue ablation modality, as it will act in superposition with thermal ablation. Consequently, the goal of this study is to evaluate, through mathematical modeling, the maximal extent of tissue ablation that could be accomplished by irreversible electroporation prior to the onset of thermal effects. Our models focus on electroporation of liver tissue using two and four needle electrodes and available experimental data. The liver was chosen as a potential candidate for

irreversible electroporation ablation because in other tissues, such as skin and muscle, the larger electric pulses could cause muscle and nerve excitation. Our results show that the area ablated by irreversible electroporation prior to the onset of thermal effects is comparable to that which can be ablated by ECT, validating the potential use of irreversible electroporation as a minimally invasive surgical modality. Our earlier studies have shown that the extent of electroporation can be imaged in real-time with electrical impedance tomography.[7,9] Irreversible electroporation, therefore, has the advantages of being a tissue ablation technique that does not require adjuvant chemicals, is as easy to apply as high temperature ablation, and has the capability of being monitored and controlled with electrical impedance tomography.

METHOD AND MODEL

Method

The goal of this study is to determine through a mathematical model whether irreversible tissue ablation can affect substantial volumes of tissue without inducing thermally damaging effects. To this end, we have employed the Laplace equation to calculate the electrical potential distribution in tissue during typical electroporation pulses and a modified Pennes (bioheat)[35] equation to calculate the resulting temperature distribution. It is important to note that there are several forms of the bioheat equation which have been reviewed in Carney (1992)[4] and Eto and Rubinsky (1996).[13] While the Pennes equation is controversial, it is, nevertheless, commonly used because it can provide an estimate of the various biological heat transfer parameters, such as blood flow and metabolism. The modified Pennes equation in this study contains the Joule heating term in tissue as an additional heat source.

The electrical potential associated with an electroporation pulse is determined by solving the Laplace equation for the potential distribution:

$$\nabla(\sigma \nabla \phi) = 0 \tag{1}$$

where ϕ is the electrical potential and σ is the electrical conductivity. The electrical boundary condition of the tissue that is in contact with the leftmost electrode(s) on which the electroporation voltage, V_0, is applied is

$$\phi = V_0 \tag{2}$$

The electrical boundary condition at the interface of the rightmost electrode(s) is

$$\phi = 0 \tag{3}$$

The boundaries where the analyzed domain is not in contact with an electrode are treated as electrically insulative to provide an upper limit to the temperature distribution that results from electroporation:

$$\frac{\partial \phi}{\partial n} = 0 \tag{4}$$

Solving the Laplace equation enables one to calculate the associated Joule heating (p), which is the heat generation rate per unit volume caused by the electrical field:

$$p = \sigma |\nabla \phi|^2 \tag{5}$$

This term is added to the original Pennes equation[35] to represent the heat generated from the electroporation procedure:

$$\nabla(k \nabla T) + w_b c_b (T_a - T) + q''' + p = \rho c_p \frac{\partial T}{\partial t} \tag{6}$$

where k is the thermal conductivity of the tissue, T is the temperature, w_b is the blood perfusion, c_b is the heat capacity of the blood, T_a is the arterial temperature, q''' is the metabolic heat generation, ρ is the tissue density, and c_p is the heat capacity of the tissue.

The intent of the analysis is to determine the extent of the region in which reversible or irreversible electroporation is induced in tissue for various electroporation voltages and durations while the maximal temperature in the tissue is below 50°C. Thermal damage, Ω, is a time-dependent process described by an Arhenius type equation:

$$\Omega = \int \xi e^{-E_a/RT}\, dt \tag{7}$$

where ξ is the frequency factor, E_a is the activation energy, and R is the universal gas constant.[11,18] There are several reasons, however, why 50°C is generally chosen as the target temperature. Thermal damage begins at temperatures higher than 42°C, but only for prolonged exposures on the order of several seconds to hours. Damage is relatively low until 50–60°C at which the rate of damage dramatically increases.[11] Since the Laplace and bioheat equations are linear, the information produced in this paper can be extrapolated and considered indicative of the overall thermal behavior.

Model

The models are two-dimensional with conditions typical to an electroporation procedure in the liver. The liver was chosen because it is the organ that most minimally invasive ablation techniques treat since cancer in the liver can be resolved by extirpation of the diseased area while surgical resection is not possible in many cases for this organ.[32] Furthermore, the liver will not experience the possible muscle contractions and the sensations that the patients may feel due to electric pulses as compared to when this method is applied to other organs. The electroporation parameters, i.e., pulse parameters for reversible and irreversible electroporation, were obtained from rat liver data,[23,40] but referenced biological properties of human

TABLE 1. Electrical and thermal properties of liver tissue.

Quantity	Symbol	Units	Value	Ref.
Electrical conductivity	σ	S m^{-1}	0.286	2
	$\Delta\sigma/\sigma/\Delta$T	% °C^{-1}	1.5	12
Thermal conductivity	k	W m^{-1} K^{-1}	0.512	12
	$\Delta k/(k/\Delta$T)	% °C^{-1}	0.25	12
Heat capacity	c_p	J kg^{-1} K^{-1}	3600	12
Density	ρ	kg m^{-3}	1050	12
Initial temperature	T_0	°C	37	—

liver were actually used in the analysis. Tissue electrical properties are taken from Boone *et al.*,[2] and tissue thermal properties from Duck.[12] The dependence of electrical conductivity and thermal conductivity on temperature were taken from Duck[12]; however, the increase in electrical conductivity due to electroporation[9] was not included in this preliminary study. The tissue is assumed to be isotropic and macroscopically homogeneous. Biophysical properties such as metabolic heat and blood perfusion are taken from Deng and Liu.[10] A summary of the material properties used in the study can be found in Tables 1 and 2.

The analyzed configurations have either two or four needle electrodes embedded in a large enough square model of the liver to avoid outer surface boundary effects and to produce an upper limit for the temperature that develops. Needle electrodes are most commonly used in tissue electroporation of the liver.[39] For each configuration, the surface of one electrode is assumed to have a prescribed voltage, and the other electrode is set to ground. The effect of the spacing between the electrodes was investigated by comparing typical distances of 5, 7.5, and 10 mm. The electrodes were also modeled with typical dimensions of 0.5, 1, and 1.5 mm in diameter. A summary of the parameters varied can be found in Table 3.

To solve Eq. (6), it is assumed that the entire tissue is initially at the physiological temperature of 37°C:

$$T(x, y, 0) = 37 \tag{8}$$

The outer surface of the analyzed domain and the surfaces of the electrodes are taken to be adiabatic to produce an upper limit to the calculated temperature distribution in the tissue:

$$\frac{\partial T}{\partial n} = 0 \text{ on the electrodes boundary and the outer surface domain} \tag{9}$$

TABLE 2. Biophysical properties used in study.

Quantity	Symbol	Units	Value	Ref.
Blood perfusion term	w_b	kg m^{-3} s^{-1}	1	10
Metabolic heat	q'''	W m^{-3}	33800	10
Blood heat capacity	c_b	J kg^{-1} K^{-1}	3640	12
Arterial temperature	T_a	°C	37	—

TABLE 3. Geometric parameters.

Quantity	Symbol	Units	Values		
Electrode diameter	d	mm	0.5	1	1.5
Electrode spacing	L	mm	5	7.5	10

The calculations were made using an electroporation pulse of 800 μs. This pulse duration was chosen because typically, reversible electroporation is done with eight separate 100-μs pulses,[23] therefore, the value we chose is an upper limit of the thermal effect in a pulse time frame comparable to that of reversible electroporation. Consequently, the results obtained are the lower limit as to the possible size of the irreversibly electroporation lesion. It should be emphasized that irreversible electroporation tissue ablation can be accomplished with much shorter pulses than these. To evaluate the thermal effect in our mathematical model, we gradually increased the applied pulse amplitude for the 800-μs pulse length until our calculations showed that the maximum temperature in the domain reached 50°C, which we considered to be the thermal damage limit. Since the configurations presented in this study contain needle electrodes, the peak temperature is located near the tissue electrode interface, the area with the highest electric field. The voltage applied when the thermal limit is reached is presented in the results, and the corresponding electric field distribution throughout the liver is then used to determine the amount of tissue irreversibly electroporated.

A transmembrane potential on the order of 1 V is sufficient to induce irreversible electroporation. This induced potential is dependent on a variety of conditions such as tissue type, cell size, and other external conditions and pulse parameters. The primary electrical parameter affecting the transmembrane potential for a specific tissue type is the amplitude of the electric field to which the tissue is exposed. The electric field thresholds used in estimating the extent of the region that was irreversibly electroporated were taken from the fundamental studies of Miklavcic, Mir, and their colleagues performed with rabbit liver tissue.[23] In this study correlating electroporation experiments with mathematical modeling, they found the electric field threshold for reversible electroporation is 362 ± 21 V cm^{-1} and the threshold for irreversible electroporation is 637 ± 43 V cm^{-1} for rat liver tissue using eight 100-μs pulses at a frequency of 1 Hz. Therefore, in our analysis, we have taken an electric field of 360 V cm^{-1} to represent the delineation between no electroporation and reversible electroporation, and 680 V cm^{-1} to represent the delineation between reversible and irreversible electroporation.

All calculations were performed using MATLAB's finite element solver, Femlab v2.2 (The MathWorks, Inc. Natick, MA). To ensure mesh quality and validity of solution, meshes were refined until there was less than a 0.5% difference in solution between refinements. The baseline mesh

containing two 1-mm electrodes with 10-mm spacing had 4035 nodes and 7856 triangles. The simulations were conducted on a Dell Optiplex GX240 with 512 MB of RAM operating on Microsoft Windows 2000.

RESULTS AND DISCUSSION

Figure 1 examines the effect of the electrode size and spacing on the electric field distribution at 800 μs and the ensuing ablated area for a two-needle electroporation configuration. Figures 1(A)–1(C) compare the extent of irreversible electroporation for electrode sizes of 0.5, 1, and 1.5 mm in diameter and a center-to-center electrode spacing of 10 mm. The strong effect of the electrode size is evident. It is seen that for the smaller 0.5-mm electrode, the two irreversible electroporated areas are not contiguous, while for a 1.5-mm electrode, the area of potential tissue ablation has an elliptical shape with dimensions of about 15 mm by 10 mm. In the parentheses, we give the electroporation voltage at which the probe temperature reaches 50°C in 800 μs for these three configurations. It is seen that the range is from 888 V for the 0.5 mm probe to 1613 V for the 1.5 mm probe. This is within the typical range of tissue electroporation pulses. Figures 1(D)–1(F) evaluate the effect of the spacing between the electrodes. It is observed that in the tested range the minor axis of the contiguous elliptical shape of the ablated lesion remains the same, while the major axis seems to scale with the distance between the electrodes. Figure 1 demonstrates that the extent of tissue ablation with irreversible electroporation is comparable to that of other typical minimally invasive methods for tissue ablation, such as cryosurgery.[32,33] It also shows that varying electrode size and spacing can control lesion size and shape.

Figure 2 illustrates the transient thermal response of the tissue due to an 800-μs, 1331-V pulse for the two-electrode configuration with 10-mm center-to-center spacing, 1 mm in diameter. Figures 2(A)–2(C) are surface plots illustrating the symmetric temperature distribution near the electrodes at 200, 400, and 800 μs, respectively. Figures 2(D)–2(F) are contour plots of the temperature distribution near the rightmost electrode at 200, 400, and 800 μs, respectively. It can be seen that the temperature rise is most pronounced near the electrode-interface, specifically in between the electrodes.

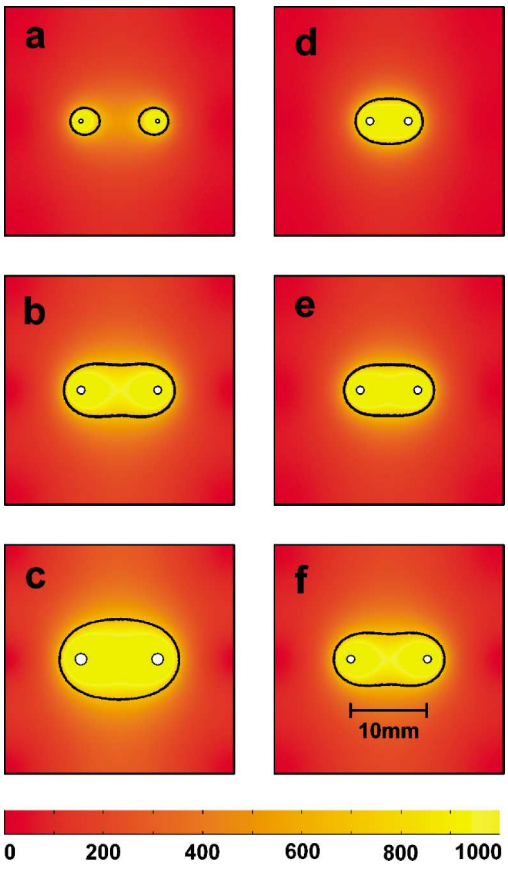

FIGURE 1. The effect of electrode geometry on the electric field distribution (V cm^{-1}) and the amount of irreversible electroporation attainable for a two-electrode configuration. Assuming a 680 V cm^{-1} threshold, the amount of irreversible electroporation achievable is delineated by the solid line. The effect of needle diameter using 10-mm center-to-center spacing and (A) 0.5 mm (888 V), (B) 1.0 mm (1331 V), and (C) 1.5 mm (1613 V) diameter electrodes. The effect of electrode spacing using 1-mm diameter electrodes and (D) 5 mm (891 V), (E) 7.5 mm (1143 V), and (F) 10 mm (1331 V) center-to-center spacing. The value in parentheses is the maximum applied voltage achievable with 800-μs pulse without reaching 50°C for each configuration.

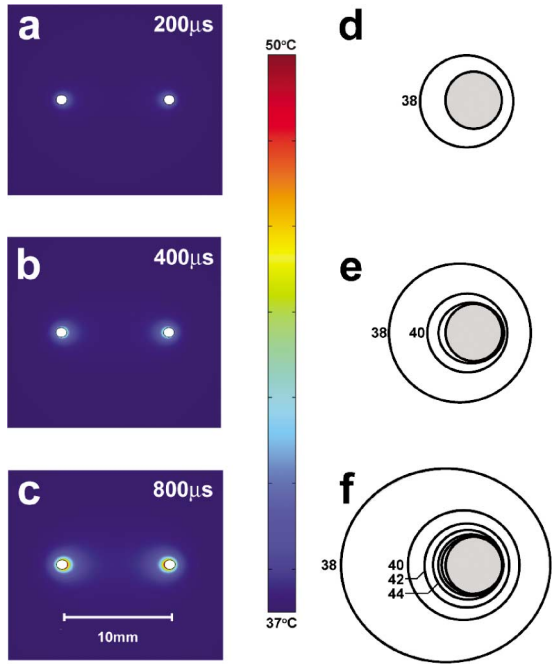

FIGURE 2. The transient temperature distribution due to an 800-μs, 1331-V pulse for the two-electrode configuration, 1 mm in diameter with 10-mm center-to-center spacing. Surface plots illustrating the distribution at (A) 200, (B) 400, and (C) 800 μs. Contour plots detailing the temperature distribution near the rightmost electrode at (D) 200, (E) 400, and (F) 800 μs.

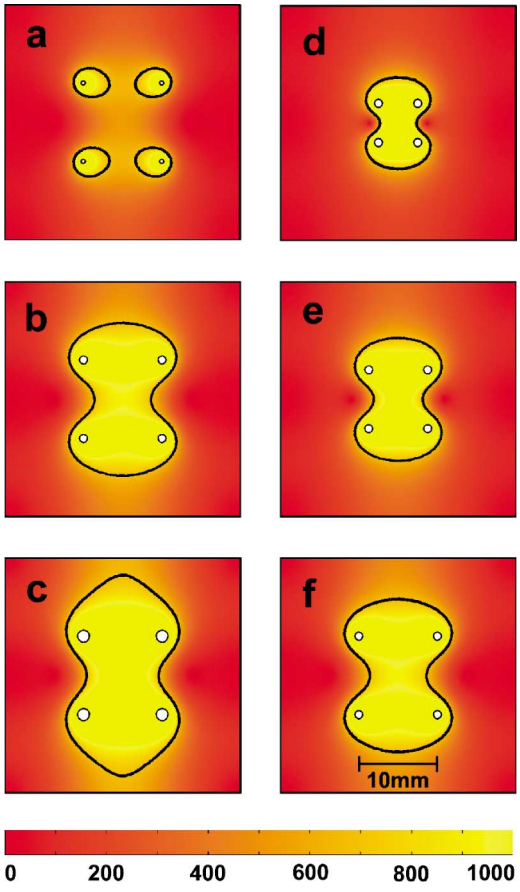

FIGURE 3. The effect of electrode geometry on the electric field distribution (V cm^{-1}) and the amount of irreversible electroporation attainable for a four-electrode configuration. Assuming a 680 V cm^{-1} threshold, the amount of irreversible electroporation achievable is delineated by the solid line. The effect of needle diameter using 10-mm center-to-center spacing and (A) 0.5 mm (971 V), (B) 1.0 mm (1438 V), and (C) 1.5 mm (1716 V) diameter electrodes. The effect of electrode spacing using 1-mm diameter electrodes and (D) 5 mm (928 V), (E) 7.5 mm (1212 V), and (F) 10 mm (1438 V) center-to-center spacing. The value in parenthesis is the maximum applied voltage achievable with an 800-μs pulse without reaching 50°C for each configuration.

Varying the number of electrodes used can also control the shape and size of the ablated lesion. This is shown in Figure 3 using a four-electrode configuration. For these configurations, the two left electrodes have a prescribed voltage and the two right electrodes are set to ground. These figures also compare the effect of probe size and spacing. Again, it is seen that larger electrodes have a substantial effect on the extent of the ablated region and that the extent of ablation scales with the spacing between the electrodes. In obtaining the results in Figures 1 and 3, it was observed that metabolism and blood perfusion had a negligible effect on the possible extent of irreversible electroporation achievable, and that the temperature dependence of electrical and thermal conductivity was more considerable.

A comparison between reversible and irreversible electroporation protocols is achieved in Figures 4 and 5. In

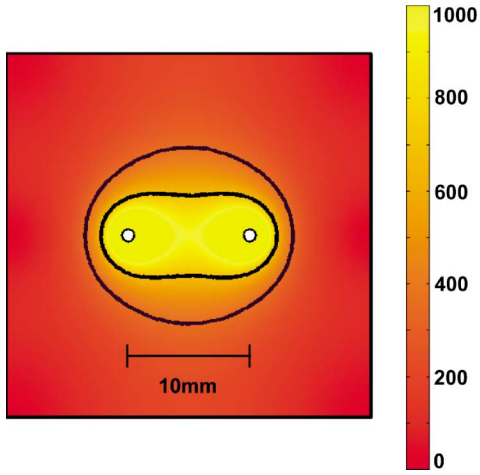

FIGURE 4. The amount of reversible electroporation achievable (360 V cm^{-1} threshold, outer contour line) as compared to the amount of irreversible electroporation achievable (680 V cm^{-1} threshold, inner contour line) with the electric field (V cm^{-1}) superimposed due to an 800-μs, 1331-V pulse for a two-electrode configuration, 1-mm diameter and 10-mm center-to-center spacing.

Figure 4, an 800-μs, 1331-V pulse was applied between two 1-mm diameter electrodes placed 10-mm apart. As shown in Figure 2, this produced a tissue temperature lower than 50°C. The figure compares the margin of the irreversibly electroporated region, which uses 680 V cm^{-1} electric fields, to that of the reversible electroporated region, which uses 360 V cm^{-1} fields. The most commonly used voltage parameters for ECT are eight 100-μs pulses at a 1-Hz frequency, and 1300 V is often applied across the two electrodes. These frequently used values

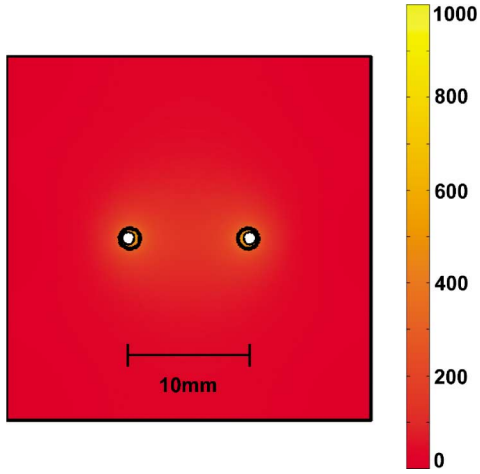

FIGURE 5. The maximum amount of reversible electroporation achievable without inducing irreversible electroporation for a two-electrode configuration, 1-mm diameter and 10-mm center-to-center spacing. A voltage of 189 V applied between the electrodes does not breach the 680 V cm^{-1} irreversible electroporation threshold. The electric field distribution (V cm^{-1}) and the 360 V cm^{-1} contour line, indicating reversible electroporation, are shown.

are comparable to those used in Figure 4. The reversible electroporation margin extends beyond the irreversibly electroporated margin, and suggests that by combining irreversible electroporation with cytotoxic drugs incorporation by reversible permeabilization, it is possible to increase the extent of the region ablated by electroporation. Adding the cytotoxic drug requires an additional step in the ablation procedure. Results such as those in Figure 4 should be utilized to determine whether physicians prefer to use ECT or irreversible electroporation for a particular application.

Figure 5 was also obtained for two 1-mm electrodes placed 10-mm apart. In this figure, we produced a reversibly electroporated region with electric fields lower than 360 V cm^{-1} using an unconventionally low applied voltage of 189 V such that no irreversible electroporation is produced. Figure 5 shows that using reversible electroporation alone is not practical because the area affected would be dramatically reduced. In addition, Figure 5 shows that irreversible electroporation near the electrodes may actually be attractive in ECT because the electrodes would not need to be sterilized when being reused in a secondary location since all of the cells near the electrodes would be killed. Nevertheless, in comparing Figures 1(B) and 5, it is evident that the extent of the ablated area possible through ECT alone is substantially smaller than that which is possible through irreversible electroporation alone.

The thermal damage can be conservatively approximated by assuming that the tissue reaches 50°C instantaneously, such that the damage is defined as

$$\Omega = t_{\mathrm{p}}\, \xi e^{-\Delta E/RT} \tag{10}$$

Several values taken from the literature for activation energy and frequency factor were applied to Eq. (10). Because the application of the pulse is so short, the damage would be near zero, many times less than the value ($\Omega = 0.53$) to induce a first degree burn,[11] regardless of the values used for activation energy and frequency factor.

The results from this study demonstrate that irreversible electroporation is not electrically induced thermal coagulation, but rather a more benign method to destroy only the targeted tissue. These results are theoretical and must be verified experimentally to show that irreversible electroporation does destroy undesirable tissue. Since the field strengths associated with this procedure are more significant than those associated with reversible electroporation, there are issues regarding the pulse delivery that need to be reinvestigated in future studies. These include whether the sensations, which may be painful, and the muscle contractions often associated with reversible electroporation, will be bearable with this technique or whether they need to be alleviated using an aesthetic and a muscle relaxant. As with reversible electroporation, but enhanced with irreversible electroporation, applying the field over the skin may lead to necrotic lesions because of its large impedance.

In summary, this study introduces a new unique method for the ablation of undesirable tissue. This method involves the placement of electrodes into or near the vicinity of the undesirable tissue with the application of electric pulses to cause irreversible electroporation of the cells throughout the entire undesirable region. The electric pulses irreversibly permeate the membranes, thereby invoking cell death. The irreversibly permeabilized cells are left *in situ* and are removed by the immune system. The amount of tissue ablation achievable through the use of irreversible electroporation without inducing thermal damage is considerable, as illustrated in this paper.

CONCLUSION

Currently, tissue ablation by electroporation is achieved through the use of cytotoxic drugs injected in tissue combined with reversible electroporation in a procedure known as electrochemotherapy. The goal of this study was to determine whether irreversible electroporation alone could produce substantial tissue ablation for the destruction of undesirable tissues in the body. The concern was that the higher voltages required for irreversible electroporation would cause Joule heating, thereby inducing thermal tissue damage to a degree that would make irreversible electroporation a marginal effect in tissue ablation. Using a mathematical model for calculating the electrical potential and temperature field in tissue during electroporation, we have shown that the area ablated by irreversible tissue electroporation prior to the onset of thermal effects is substantial and comparable to that of other tissue ablation techniques, such as cryosurgery. We therefore claim that for certain medical applications irreversible electroporation alone could be used as an effective technique for tissue ablation without the use of cytotoxic drugs. Our earlier studies have shown that the extent of electroporation can be imaged in real-time with electrical impedance tomography.[7,9] Irreversible electroporation, therefore, has the advantage of being a tissue ablation technique, which is as easy to apply as high temperature ablation, without the need for adjuvant chemicals as required in electrochemical ablation and electrochemotherapy. Additionally, a unique aspect of irreversible electroporation is that the affected area can be controlled and monitored with electrical impedance tomography.

ACKNOWLEDGMENTS

Rafael Davalos acknowledges Sandia National Laboratories for their support. L.M. Mir acknowledges the CNRS, and the Institut Gustave-Roussy for their general support of his research. B. Rubinsky was supported by NIH grant #ROI –RR018961.

REFERENCES

[1]Baker, P. F., and D. E. Knight. Calcium-dependent exocytosis in bovine adrenal medullary cells with leaky plasma membranes. *Nature* 276:620–622, 1978.

[2]Boone, K., D. Barber, and B. Brown. Review—Imaging with electricity: Report of the European Concerted Action on Impedance Tomography. *J. Med. Eng. Technol.* 21:201–232, 1997.

[3]Bown, S. G. Phototherapy of tumors. *World J. Surgery* 7:700–709, 1983.

[4]Carney, C. K. Mathematical models of bioheat transfer. In: Bioengineering Heat Transfer, edited by Y. I. Choi. Boston: Academic Press, 1992, pp. 19–152.

[5]Chang, D. C., B. M. Chassy, J. A. Saunders, and A. E. Sowers. Guide to Electroporation and Electrofusion. San Diego, CA: Academic Press, 1992, 569 pp.

[6]Crowley, J. M. Electrical breakdown of biomolecular lipid membranes as an electromechanical instability. *Biophys. J.* 13:711–724, 1973.

[7]Davalos, R. V., B. Rubinsky, and D. M. Otten. A feasibility study for electrical impedance tomography as a means to monitor tissue electroporation for molecular medicine. *IEEE Trans. Biomed. Eng.* 49:400–403, 2002.

[8]Davalos, R. V., B. Rubinsky, and L. M. Mir. Theoretical analysis of the thermal effects during in vivo tissue electroporation. *Bioelectrochemistry* 61:99–107, 2003.

[9]Davalos, R. V., D. M. Otten, L. M. Mir, and B. Rubinsky. Electrical impedance tomography for imaging tissue electroporation. *IEEE Trans. Biomed. Eng.* 51(5):761–767, 2004.

[10]Deng, Z. S., and J. Liu. Blood perfusion-based model for characterizing the temperature fluctuations in living tissue. *Phys. A STAT Mech. Appl.* 300:521–530, 2001.

[11]Diller, K. R. Modeling of bioheat transfer processes at high and low temperatures. In: Bioengineering Heat Transfer, edited by Y. I. Choi. Boston: Academic Press, 1992, pp. 157–357.

[12]Duck, F. A. Physical Properties of Tissues: A Comprehensive Reference Book. San Diego: Academic Press, 1990.

[13]Eto, T. K., and B. Rubinsky. Bioheat transfer. In: Introduction to Bioengineering, edited by S. A. Berger, W. Goldsmith, and E. R. Lewis. Oxford: Oxford Press, 1996.

[14]Foster, R. S., R. Bihrle, N. T. Sanghvi, F. J. Fry, J. P. Donohue. High-intensity focused ultrasound in the treatment of prostatic disease. *Eur. Urol.* 23:44–47, 1993.

[15]Gauger, B., and F. W. Bentrup. A study of dielectric membrane breakdown in the Fucus egg. *J. Membr. Biol.* 48:249–264, 1979.

[16]Gothelf, A., L. M. Mir, and J. Gehl. Electrochemotherapy: Results of cancer treatment using enhanced delivery of bleomycin by electroporation. *Cancer Treat. Rev.* 29:371–387, 2003.

[17]Heller, R., R. Gilbert, and M. J. Jaroszeski. Clinical applications of electrochemotherapy. *Adv. Drug Deliv. Rev.* 35:119–129, 1999.

[18]Henriques, F. C., and A. R. Moritz. Studies in thermal injuries: The predictability and the significance of thermally induced rate processes leading to irreversible epidermal damage. *Arch. Pathol.* 43:489–502, 1947.

[19]Jaroszeski, M. J., R. Gilbert, C. Nicolau, and R. Heller. *In vivo* gene delivery by electroporation. *Adv. Applic. Electrochem.* 35:131–137, 1999.

[20]Joshi, R. P., and K. H. Schoenbach. Mechanism for membrane electroporation irreversibility under high-intensity, ultrashort electrical pulse conditions. *Phys. Rev. E* 66:052901:1–4, 2002.

[21]Kinosita, K., Jr., and T. Y. Tsong. Hemolysis of human erythrocytes by a transient electric field. *Proc. Natl. Acad. Sci. U.S.A.* 74:1923–1927, 1977.

[22]Lynn, J. G., R. Zwemer, A. J. Chick, and A. E. Miller. A new method for the generation of use of focused ultrasound in experimental biology. *J. Gen. Physiol.* 26:179–193, 1942.

[23]Miklavcic, D., D. Semrov, H. Mekid, and L. M. Mir. A validated model of *in vivo* electric field distribution in tissues for electrochemotherapy and for DNA electrotransfer for gene therapy. *Biochim. Biophys. Acta* 1523:73–83, 2000.

[24]Mir, L. M. Therapeutic perspectives of *in vivo* cell electropermeabilization. *Bioelectrochemistry* 53:1–10, 2001.

[25]Mir, L. M., and S. Orlowski. Mechanisms of electrochemotherapy. *Adv. Drug Deliv. Rev.* 35:107–118, 1999.

[26]Mir, L. M., S. Orlowski, J. Belehradek Jr., and C. Paoletti. Electrochemotherapy potentiation of antitumour effect of bleomycin by local electric pulses. *Eur. J. Cancer* 27:68–72, 1991.

[27]Mir, L. M., L. F. Glass, G. Sersa, J. Teissie, C. Domenge, D. Miklavcic, M. J. Jaroszeski, S. Orlowski, D. S. Reintgen, Z. Rudolf, M. Belehradek, R. Gilbert, M. P. Rols, J. Belehradek, J. M. Bachaud, R. Deconti, B. Stabuc, M. Cemazar, P. Coninx, and R. Heller. Effective treatment of cutaneous and subcutaneous malignant tumours by electrochemotherapy. *Br. J. Cancer* 77:2336–2342, 1998.

[28]Neumann, E., and K. Rosenheck. Permeability changes induced by electric impulses in vesicular membranes. *J. Membr. Biol.* 10:279–290, 1972.

[29]Neumann, E., A. E. Sowers, and C. A. Jordan. Electroporation and Electrofusion in Cell Biology. New York: Plenum Press, 1989.

[30]Neumann, E., M. Schaefer-Ridder, Y. Wang, and P. H. Hofschneider. Gene transfer into mouse lyoma cells by electroporation in high electric fields. *J. EMBO* 1:841–845, 1982.

[31]Okino, M., and H. Mohri. Effects of a high-voltage electrical impulse and an anticancer drug on *in vivo* growing tumors. *Jpn. J. Cancer Res.* 78:1319–1321, 1987.

[32]Onik, G., B. Rubinsky, and E. Al. Ultrasound-guided hepatic cryosurgery in the treatment of metastatic colon carcinoma. *Cancer* 67:901–907, 1991.

[33]Onik, G. M., J. K. Cohen, G. D. Reyes, and B. Rubinsky. Transrectal ultrasound-guided percutaneous radical cryosurgical ablation of the prostate. *Cancer* 72:1291–1299, 1993.

[34]Organ, L. W. Electrophysiological principles of radiofrequency lesion making. *Appl. Neurophysiol.* 39:69–76, 1976.

[35]Pennes, H. H. Analysis of tissue and arterial blood flow temperatures in the resting forearm. *J. Appl. Physiol.* 1:93–122, 1948.

[36]Rowan, N. J., S. J. Macgregor, J. G. Anderson, R. A. Fouracre, and O. Farish. Pulsed electric field inactivation of diarrhoeagenic Bacillus cereus through irreversible electroporation. *Lett. Appl. Microbiol.* 31:110–114, 2000.

[37]Rubinsky, B. Cryosurgery, In: Annual Review of Biomedical Engineering v. 2, edited by M. L. Yarmush, K. R. Diller, M. T. Toner, Palo Alto: Annual Reviews, pp. 157–187, 2002.

[38]Shiina, S., K. Tagawa, Y. Niwa, and E. Al. Percutaneous ethanol injection therapy for hepatocellular carcinoma: Results in 146 patients. *Am. J. Roentgenol.* 160:1023–1028, 1993.

[39]Somiari, S., J. Glasspool-Malone, J. J. Drabick, R. A. Gilbert, R. Heller, M. J. Jaroszeski, and R. W. Malone. Theory and *in vivo* application of electroporative gene delivery. *Molec. Ther.* 2:178–187, 2000.

[40]Suzuki, T., B. Shin, K. Fujikura, T. Matsuzaki, and K. Takata. Direct gene transfer into rat liver cells by *in vivo* electroporation. *FEBS Lett.* 425:436–440, 1998.

[41]Tieleman, D. P., H. Leontiadau, A. E. Mark, and S. J. Marrink. Simulation of pore formation in lipid bilayers by mechanical stress and electric fields. *J. Am. Chem. Soc.* 125:6382–6383, 2003.

[42]Vernhes, M. C., A. Benichou, P. Pernin, P. A. Cabanes, and J. Teissie. Elimination of free-living amoebae in fresh water with pulsed electric fields. *Water Res.* 36:3429–3438, 2002.

[43]Weaver, J. C., and Y. A. Chizmadzhev. Theory of electroporation: A review. *Bioelectrochem. Bioenerg.* 41:135–160, 1996.

[44]Zimmermann, U., J. Vienken, and G. Pilwat. Dielectric breakdown of cell membranes. *Biophys. J.* 14:881–899, 1974.