Chapter 3

NON-THERMAL IRREVERSIBLE ELECTROPORATION FOR TISSUE ABLATION

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ABSTRACT

Non-thermal irreversible electroporation (IRE) is a promising new technique for the ablation of undesirable tissues, particularly tumors and arrhythmogenic regions in the heart (Davalos, Otten et al. 2002; Davalos, Mir et al. 2005; Edd, Horowitz et al. 2006; Al-Sakere, André et al. 2007; Al-Sakere, Bernat et al. 2007; Edd and Davalos 2007; Onik, Mikus et al. 2007; Rubinsky 2007). The procedure involves placing electrodes around the targeted tissue and delivering a series of low energy (intense but short) electric pulses. These pulses induce irrecoverable structural changes in the cell membranes of the targeted tissue, ultimately leading to cell death. IRE is a form of molecular surgery since it only affects a single molecular component of the treated volume, the cell membrane. In addition, the procedure is minimally invasive, requires only a few minutes for administration, promotes an immune response, supports rapid lesion resolution, and may be monitored in real-time with ultrasound (Al-Sakere, Bernat et al. 2007; Lee, Loh et al. 2007; Maor, Ivorra et al. 2007; Onik, Mikus et al. 2007). IRE has the ability to create complete and predictable cell ablation with sharp transition between normal and necrotic tissue, while preserving important components of the tissue such as the extracellular matrix, major blood vessels, myelin sheaths, and nerves. This chapter introduces IRE, then describes the relevant issues to consider and how to account for them when planning IRE therapies.

INTRODUCTION

Non-thermal irreversible electroporation (IRE) is a new surgical technique to ablate undesirable tissue (Davalos, Mir et al. 2005). The technique 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. The minimally invasive procedure involves placing needle electrodes into or around the targeted area to deliver a series of short and intense electric pulses that induce structural changes in cell membranes that promote cell death.

Electroporation, which results in an increase in the permeability of the cell membrane, is initiated by exposing cells or tissues to electric pulses (Weaver and Chizmadzhev 1996; Weaver 2003). As a function of the induced transmembrane potential (the electric potential difference across the plasma membrane), the electroporation pulse can either: have no effect on the cell membrane, reversibly permeabilize the cell membrane, after which the cells can survive (reversible electroporation), or permeabilize the cell membrane in a manner that leads to cell death (irreversible electroporation), presumably through a loss of homeostasis if not from other superimposed damage modes. This increase in transmembrane potential is dependent on a variety of conditions such as tissue type, cell size, and pulse parameters including pulse shape, duration, number, and repetition rate. However, for a specific tissue type and set of pulse conditions, the primary parameter determining the extent of electroporation is the electric field to which the tissue is exposed (Edd and Davalos 2007).

Recently, IRE was shown to be an effective method to treat tumors through studies with aggressive cutaneous mouse sarcoma tumors in vivo in preclinical mouse models (Al-Sakere, André et al. 2007). The electrical pulses were delivered through two plate electrodes placed across the tumors. Complete regression was achieved in 12 out of 13 treated tumors when eighty 100 µs pulses were delivered at a repetition rate of 1 pulse every 3.3 seconds using an applied electric field of 2500 V/cm (Figure 1). Histology verified that ablation occurred as a direct result of irreversible membrane permeabilization (Al-Sakere, André et al. 2007). These results were achieved with a single treatment that lasted less than five minutes.

![Figure 1. Tumor volume (mm$^3$) within nude mice after IRE treatment as a function of days after treatment. A: No treatment – tumors continue to grow. B: Eight 1000 µs pulses at 0.03 Hz deterred growth tumors and 4/13 tumors completely regressed. C: Eighty 100 µs pulses at 0.3 Hz deterred growth and 12/13 tumors completely regressed. Parameters were characterized by the same total energized (8 ms) and treatment durations (267 s). Adapted from (Al-Sakere, André et al. 2007).](image-url)

The goal of this chapter is to introduce readers to the field of non-thermal IRE for tissue ablation, with particular application to cancer therapy, and to supply readers with the tools and understanding necessary to design appropriate treatment protocols. To this end, after providing a historical perspective, we present the fundamental theory that determines how electric field and temperature distributions will result from a chosen electrode configuration, pulse characteristics, and the electrical and thermal properties of the tissue.
IRREVERSIBLE ELECTROPORATION FOR NON- THERMAL TISSUE ABLATION

In many medical procedures, such as the treatment of benign or malignant tumors, it is important to ablate undesirable tissue in a controlled and focused way. Over the years, several minimally invasive methods have been developed to selectively destroy specific areas of undesirable tissues as an alternative to resection surgery. Cryosurgery, for example, is a low temperature thermal technique in which tissue is frozen on contact with a cryogen-cooled probe (Rubinsky 2000; Davalos, Mir et al. 2005). The area affected by low temperature therapies can be easily monitored through imaging. However, the probes are large and difficult to use, there is a discrepancy between the visualized cooled regions, and the outcome is affected by blood flow (the “cold sink” effect). Nonselective chemical ablation uses agents, such as ethanol, to cause the tissue ablation (Shiina, Tagawa et al. 1993; Davalos, Mir et al. 2005). This therapy is easy to apply, but the affected area cannot be controlled because of local blood flow transport of the chemical species. Focused ultrasound uses high-intensity ultrasound beams to heat the undesirable tissue to coagulation (Lynn, Zwemer et al. 1942; Foster, Bährle et al. 1993; Davalos, Mir et al. 2005). Radiofrequency ablation (RF) is a technique in which an active electrode is introduced into the undesirable area to heat the tissue to coagulation (Organ 1976; Davalos, Mir et al. 2005). Interstitial laser coagulation is yet another thermal technique in which tumors are slowly heated to temperatures exceeding the threshold of protein denaturation using low power lasers (Bown 1983; Davalos, Mir et al. 2005). 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. In addition, damage to regions outside the target area, such as the blood vessels, extracellular matrix, and other vital physiologic structures is inevitable. Consequently, when destroyed, the possible regeneration of the tissue might take months to years, and scarring is unavoidable.

Davalos, Mir and Rubinsky postulated that IRE could be induced in vivo to destroy substantial volumes of targeted tissue prior to the onset of thermal damage (Davalos, Mir et al. 2005). It had been shown on cells in vitro that IRE is an effective means to kill mammalian cells, including cancer cells (Pinero, Lopez-Baena et al. 1997; Krassowska, Nanda et al. 2003). However, if IRE could not ablate a significant amount of tissue prior to the onset of thermal damage, there would be no benefit to using IRE over thermal ablation techniques since it would act in superposition. Alternatively, there would be tremendous advantages in using IRE if it could non-thermally kill the targeted area while sparing major blood vessels, connective tissue, nerves, and the surrounding tissue. The integrity of these structures is vital for the healing of the tissue after surgery. Their hypothesis that IRE could be used as an independent modality for tissue ablation was confirmed in small animal models in the liver (Edd, Horowitz et al. 2006), and on tumors (Al-Sakere, André et al. 2007), as well as in large animal models in the liver (Rubinsky, Onik et al. 2007), the prostate (Onik, Mikus et al. 2007), and the heart (Lavee, Onik et al. 2007). These in vivo studies yielded a wealth of information pertaining to the additional benefits of IRE ablation procedures as described below.
An original *in vivo* study conducted by Edd *et al.* used a single 20-ms-long square wave pulse of 1000 V/cm (chosen to have no significant thermal effect) applied across rat livers using two plate electrodes three hours before sacrificing the animals and performing histology on the liver (Edd, Horowitz *et al.* 2006). It was found that the livers experienced microvascular occlusion while large vessel architecture was preserved as well as a strong demarcation between regions that were unaffected, and those that experienced IRE induced cell death.

The preservation of vasculature was more thoroughly investigated by Maor *et al.* (Maor, Ivorra *et al.* 2007), where ten 100 µs pulses of 3800 V/cm at a frequency of 10 pulses per second were administered across the carotid artery of six rats 28 days before histology. This study found a large decrease in the number of vascular smooth muscle cells without evidence of aneurysm, thrombus formation, or necrosis. In addition to promoting the efficacy of performing IRE procedures on regions near major blood vessels and the potential application of IRE to treat pathological processes involving excessive proliferation of vascular smooth muscle cells, such as restenosis, an increase in conductivity of the tissue during treatment was found, resulting from the increased permeability of the cell membranes.

In a study on swine liver from (Lee, Loh *et al.* 2007), a total of 11 lesions were created using monopolar and bipolar electrodes with ninety 100 µs pulses ranging from 1000 to 1667 V/cm. This study showed complete hepatic cell death without structural destruction as well as hypoechoic properties to the ablated regions during procedure administration, allowing real-time procedure monitoring using ultrasound.

A preclinical study on the implications of IRE for the ablation of prostate tissue (both cancerous and regions exhibiting prostatitis) was performed by (Onik, Mikus *et al.* 2007), where one or four bipolar or monopolar electrodes were placed in the prostates of six beagle dogs before administering eighty pulses of 100 µs ranging from 1000 to 3000 V/cm. This study found IRE lesions with a narrow transition between complete necrosis and unaffected tissue, with complete destruction of the IRE lesion and resolution within two weeks, as observed by marked shrinkage. The shrinking regions resulting from the procedure show strong adaptability of IRE protocols for the treatment of pathologies involving swollen tissues and organs. This study also examined the effects on sensitive peripheral structures such as the urethra, blood vessels, nerves, and rectum that have experienced problems with thermal techniques and found all to be unaffected by the IRE treatment application. The preservation of the microvasculature experienced in this study also raises the question for the possibility of normal tissue regeneration within the ablated regions.

A unique observation in the IRE lesions from (Onik, Mikus *et al.* 2007) was evidence of an immunologic reaction, prompting the possibility of a tumor specific immunological reaction that may be promoted by IRE, further enhancing treatment outcome. This also shows the potential for destruction of micro-metastasis in affected lymph nodes, possibly reducing the risk for recurrence. Furthermore, a study was performed on the immunologic response by (Al-Sakere, Bernat *et al.* 2007) on implanted tumors from a mouse sarcoma cell line. They used IRE parameters previously found in (Al-Sakere, André *et al.* 2007) to yield complete tumor regression (plate electrodes with four trains of 16 pulses 100 µs in length at 2500V/cm with a 90° electrode rotation between trains) on mice sacrificed 1-72 hours after treatment. Immunohistochemistry was performed, and it was determined that IRE from this procedure did not require an immunological response to produce ablation, suggesting the efficacy of using IRE on immunodepressed patients.
These IRE animal experiments verified the many beneficial effects resulting from this special mode of non-thermal cell ablation. With major structures such as the extracellular matrix, major blood vessels, and myelin sheaths preserved, there is extremely rapid lesion resolution with healthy tissue (Rubinsky, Onik et al. 2007), preventing scar formation and promoting a beneficial immune response (Rubinsky, Onik et al. 2007). Preventing scar formation is especially important because it allows the determination of treatment success or failure through imaging, something not possible when using thermal techniques (Sickles and Herzog 1980; Onik, Mikus et al. 2007). This method also allows treatment in the heart (Lavee, Onik et al. 2007) and blood vessels (Maor, Ivorra et al. 2007) without the danger of coagulation in the blood stream and subsequent emboli.

**HISTORY**

It is difficult to discern when IRE was first observed. The literature suggests that the initial studies could have been as early as the 18th century (Nollet 1754; Biedermann 1898; Fuller 1898; Rockwell 1903; Frankenhaeuser and Widen 1956). However, it was not until 1967 that Sale and Hamilton demonstrated the non-thermal lethal effect of high electrical fields on organisms (Hamilton and Sale 1967; Sale and Hamilton 1967; Sale and Hamilton 1968; Rubinsky 2007). They concluded that the damage to the cell membrane occurs when the transmembrane potentials of around 1 V are reached. This result (threshold) is based on the theoretical potential of an insulating sphere in a conducting medium in an analysis that has become a classic in the field of electroporation (Hamilton and Sale 1967; Sale and Hamilton 1967; Sale and Hamilton 1968; Rubinsky 2007).

For decades, IRE has been studied extensively within *in vitro* cellular systems, in particular the food industry for sterilization and preprocessing of food (Doevenspeck 1961; Toepfl, Mathy et al. 2006). IRE has also been considered an effective means to destroy both gram positive and gram negative bacteria and amoebae with regards to water decontamination for biofouling control (Schoenbach, Peterkin et al. 1997; Rowan, MacGregor et al. 2000; Joshi and Schoenbach 2002; Vernhes, Benichou et al. 2002).

Another context in which IRE has been studied is in the delayed cell damage in high-voltage accidents (Lee and Kolodney 1987; Lee 2005) and the post-electric-shock arrhythmias during defibrillation (Jones, Proskauer et al. 1980). Lee et al. showed that electrical injury is attributed to thermal damage as well as IRE in superposition (Lee and Kolodney 1987; Lee 2005). It had also been observed in medical applications involving thermal ablation using electrical fields that the electrosurgical tools also induce electroporation and that coagulation may be due to electrofusion of the membranes (Belov 1978).

IRE is currently being studied as part of a family of non-thermal methods to ablate tissue with electrical pulses, which includes electrochemotherapy (ECT) (Mir, Orlowski et al. 1991; Mir, Glass et al. 1998; Mir 2001; Marty, Sersa et al. 2006; Mir, Gehl et al. 2006; Al-Sakere, André et al. 2007) and supra-poration (Beebe, White et al. 2003; Deng, Schoenbach et al. 2003; Gowrishankar and Weaver 2006; Al-Sakere, André et al. 2007). ECT is a relatively new minimally invasive tissue ablation technique that employs reversible electroporation pulses to facilitate the penetration of non-permeant or low-permeant drugs, such as
bleomycin or cisplatin, into cells. A major advantage of ECT is that it selectively kills only rapidly dividing cells, such as tumor cells. In tissue ablation, ECT is a safe and highly efficient method to introduce non-permeable cancer drugs into malignant cells and is currently used to treat cutaneous and subcutaneous tumors in humans (Mir, Orlowski et al. 1991; Belehradek, Domenge et al. 1993; Mir, Glass et al. 1998; Gothelf, Mir et al. 2003; Sersa, Cemazar et al. 2003; Al-Sakere, André et al. 2007). However, this method requires the combination of chemical agents with an electric field, which IRE does not.

Supra-poration is achieved by means of nanosecond electrical pulses in the tens of nanoseconds range and field strengths of 40-80 kV/cm (Beebe, White et al. 2003; Deng, Schoenbach et al. 2003; Al-Sakere, André et al. 2007). In supra-poration, cell death is not a consequence of the irreversible cell membrane permeabilization as in IRE, but the probable result of Ca^{2+} ions released inside the cells (Beebe, White et al. 2003; Al-Sakere, André et al. 2007). R. Nuccitelli et al. (Nuccitelli, Pliquett et al. 2006) described antitumor effects in mice using this technique.

The new techniques based on the non-thermal delivery of electric pulses, namely ECT, IRE and supra-poration, have inherent advantages and disadvantages for tissue ablation. It is quite likely that each will find appropriate uses in modern medicine, separately or in combination.

**THEORY OF IRREVERSIBLE ELECTROPORATION**

The natural transmembrane potential is on the order of 70 mV in healthy cells. If the potential drop across the membrane is made to exceed approximately 1 V by the action of an applied electric field, structural rearrangement of the lipid bilayer occurs, creating permanent aqueous pathways or pores for ions and macromolecules to pass through, i.e. electroporation (Sale and Hamilton 1967).

The typical formula to approximate the induced transmembrane potential ($V_m$) resulting from an applied electric field for a cell in suspension is:

$$V_m = \lambda r E_a \cos(\theta) \left[ + \left( \frac{f}{f_s} \right)^2 \right]^{0.5}$$

(1)

where $\lambda$ is the shape factor of the cell (1.5 for spherical cells), $r$ is the radius of the cell, $E_a$ is the applied electric field, $\theta$ is the angle between electric field and the vector from the cell center to any point on its surface, $f_s$ is approximately equal to the frequency where the beta dielectric dispersion occurs (below which the cell membrane charge is in step with the electric field) and $f$ is the frequency of the assumed sinusoidal $E_a$ (Lee, Zhang et al. 2000). This results from the simplifying model of a cell as a resistor (intra- and extra-cellular path-resistance) in series with a capacitor (membrane capacitance). For most cases, the transient terms can be neglected because the electroporation pulse (100 µs - 50 ms) is much larger than the membrane charging time (about 1 µs for spherical cells about 10 µm in diameter) (Weaver 2000).

Despite studies, relatively little is known about the mechanism by which IRE causes cell death. There have been numerous experimental studies on cell viability following the delivery of an electric pulse (Hulsheger and Niemann 1980; Gabriel and Teissie 1995; Lubicki and Jarayam 1997; Krassowska, Nanda et al. 2003). Yet, there are disagreements within the
literature. There is an ongoing debate whether IRE-induced cell death is caused by: (1) cell membrane rupture (Weaver 1995; Weaver 1995), (2) excessive leakage through pores (Hoffman 1989; Weaver 1995; Weaver 1995), or (3) thermal damage to cells (Kekez, Savic et al. 1996). The three proposed mechanisms have different scaling laws that relate the strength (E) and duration (d) of the threshold electric pulse for cell death. In particular, for rupture: \[ \ln(d) \sim \frac{1}{E^2} \] for leakage: \[ d \sim \frac{1}{E} \] and for thermal damage: \[ d \sim \frac{1}{E^2} \] (Krassowska and Filev 2007). Some studies find a correlation between cell death and the total energy delivered by the pulse (Okino, Tomie et al. 1992; Kekez, Savic et al. 1996), while others do not (Schoenbach, Peterkin et al. 1997; Vernhes, Cabanes et al. 1999); and yet others correlate cell death with the total pulse charge (Krassowska, Nanda et al. 2003). Despite the varying mechanism theories, we present guidelines for applying IRE to treat pathologic tissues and planning medical treatments.

**NUMERICAL MODELING FOR TREATMENT PLANNING**

In tissue, there are a number of conditions that determine the extent of electroporation, such as tissue type and temperature, as well as a number of pulse parameters, including duration, number, shape, and repetition rate. However, for a given set of conditions, the primary parameter affecting the degree of electroporation is the local electric field strength (Miklavcic, Beravs et al. 1998; Davalos, Otten et al. 2002). Therefore, in order to design protocols for an IRE procedure, the electric field distribution, which is dependent on the procedure’s specific electrode-tissue geometry, pulse amplitude, and tissue conductivity distribution, must be determined. Furthermore, to verify that a specific protocol does not induce thermal effects, the temperature distribution can be calculated from the electric field distribution, the electric pulse parameters, and tissue electrical and thermal properties. Knowledge of the electric field and temperature distribution enables surgeons and researchers to reliably predict the results of an IRE procedure. This insight enables surgeons to plan and optimize the electrode geometry and voltage parameters for varying types of tissue and heterogeneities to:

- Ensure treatment of the entire region, especially when multiple applications are necessary
- Minimize applied voltages in order to reduce charge delivered
- Visualize where potential thermal damage may occur to surrounding tissues
- Reduce treatment time, invasiveness, and number of procedures
- Superimpose medical images to plan treatment of the appropriate region

**Models:**

To illustrate how the IRE treated area/volume depends on the electrode configuration and applied voltage, two electrode types are analyzed as examples, as depicted in Figure 2. Case A shows single 2-mm diameter bipolar electrode, and case B shows two 1-mm diameter
monopolar electrodes, separated by a distance of 10 mm. The red regions are energized and the black ones are set to ground.

![Diagram of electric field and temperature distributions in tissue](image)

**Electric Field Distribution:**

The methods used to generate the electric field and temperature distributions in tissue are similar to the ones described by Edd and Davalos (Edd and Davalos 2007). The electric field distribution associated with the electric pulse is given by solving the Laplace equation:

\[ \nabla \cdot (\sigma \nabla \phi) = 0 \]  

(2)

where \( \sigma \) is the electrical conductivity of the tissue and \( \phi \) is the electrical potential (Edd and Davalos 2007). Boundary conditions most often include surfaces where electric potential is specified, as in the case of a source or sink electrode, or surfaces that are electrically insulating, as on the free surfaces of the tissue, for example. The electrical boundary condition along the tissue that is in contact with the energized electrode is \( \phi = V_0 \). The
electrical boundary condition at the interface of the other electrode is \( \Gamma_{i} \). The remaining boundaries are treated as electrically insulating:

\[
\Gamma_{i} = \frac{\partial \mathbf{E}}{\partial n} = 0
\]

The models are fully defined and readily solvable using a numerical method once an appropriate set of boundary conditions and the properties of the tissue are defined. The computations were performed with a commercial finite element package (FEMLab, Comsol AS, Stockholm, Sweden). The analyzed domain extends far enough from the area of interest (i.e. the area near the electrodes) that the electrically and thermally insulating boundaries at the edges of the domain do not significantly influence the results in the treatment zone.

The models for the two treatment relevant electrode geometries outlined above may be seen in Figure 3. A voltage of 2000 V was placed on the energized electrode, and the resulting electric field distribution in tissue with an electric conductivity of \( \sigma = 0.2 \, \text{S} \cdot \text{m}^{-1} \) has been mapped out in the three Cartesian planes (y-z, z-x, and x-y). Based on these models, a general distribution pattern can be seen. It is important to remember that this is a visualization of how the field strength disperses, and that the values of the field seen in the legend will vary with the voltage applied to the energized electrode. Thus, applying 4000 V or 1000 V to the energized electrode will result in identical electric field contours of significantly larger and smaller strengths, respectively. It is also important to note that the two needle array of Case B has a larger exposed surface area than the bipolar electrode of Case A, contributing to its larger volume of high electric field regions.

From the images in Figure 3, it can be clearly seen that the strongest electric fields will occur directly beside the electrodes as two distinct ellipses that will connect at the center, forming a “peanut” shape, before expanding into an ellipse. For the bipolar electrode of Case
A, the greatest fields occur in concentric rings (Figure 3C) expanding out from the electrode, indicating that inserting this electrode directly into the targeted tissue would yield the most symmetric results. The monopolar needle array has the greatest fields immediately around the conducting surfaces and then expanding inwards between them, suggesting this arrangement would work better when placed slightly on opposing outer regions of the targeted tissue.

**Joule Heating:**

Joule heating refers to the heat generation rate per unit volume caused by an electric field. As described in (Davalos, Rubinsky et al. 2003), the joule heating source term is evaluated by solving the Laplace equation for the potential distribution associated with an electrical pulse. The associated joule heating rate per unit volume, \( \dot{q} \), from an electric field, is the square of the local electric field magnitude, \( |\nabla \phi| \), times the electrical conductivity of the tissue:

\[
\dot{q} = \sigma |\nabla \phi|^2
\]  

(4)

A convenient equation to estimate the increase in temperature (\( \Delta T \)) of homogeneous tissue for the parallel plate configuration from the joule heating is:

\[
\Delta T = \frac{\sigma}{\rho c_p} |\nabla \phi|^2 \Delta t,
\]  

(5)

where \( \Delta t \) is the total duration of the pulses, \( \rho \) is density and \( c_p \) is specific heat of the tissue (Krassowska, Nanda et al. 2003). This equation assumes no heat dissipation between the pulses, and no fringe effects at the electrode edge. Furthermore, this equation assumes that the biological properties are uniform and the contributions from blood flow, metabolic heat generation, and electrode heat dissipation are negligible.

To account for these other effects, the Pennes bioheat equation is often used to assess tissue heating associated with thermally relevant procedures, because it accounts for the dynamic processes that occur in tissues, such as blood perfusion and metabolism. Blood perfusion is an effective way to dissipate (take away) heat contrary to metabolic processes which generate heat in the tissue. Modifying this equation to include the joule heating term gives the equation the following form:

\[
\nabla \cdot (k \nabla T) - w_b c_b (T - T_a) + q'' + \sigma |\nabla \phi|^2 = \rho c_p \frac{\partial T}{\partial t}
\]  

(6)

where \( k \) is the thermal conductivity of the tissue, \( T \) is the temperature above the arterial temperature (\( T_a = 37^\circ C \)), \( w_b \) is the blood perfusion per unit volume, \( c_b \) is the heat capacity of the blood, \( q'' \) is the metabolic heat generation, \( \rho \) is the tissue density, and \( c_p \) is the heat capacity of the tissue. However, it has been suggested that these factors have a negligible contribution to the overall temperature distribution as compared with joule heating (Davalos,
Rubinsky et al. 2003). Therefore, we have neglected the blood perfusion and metabolic heat generation terms in our models.

Several thermal boundary conditions can be employed to study the heat exchange between the electrodes and the tissue (Davalos, Rubinsky et al. 2003; Becker and Kuznetsov 2006; Becker and Kuznetsov 2007). In these models, the electrodes were considered as infinite fins, \( h_f = 15 \frac{W}{m^2 \cdot K} \) as described in (Davalos, Rubinsky et al. 2003), which dissipate heat from the tissue through the electrodes to the environment. However, in other studies, the boundaries are taken to be adiabatic to predict the maximum temperature rise in the tissue:

\[
\frac{\partial T}{\partial n} = 0
\]

\( (7) \)

**COMMON PHYSICAL PROPERTIES:**

For all the models, we used the physical properties of homogeneous liver tissue to provide insight, but the properties can be easily adapted for other tissues. The values of the liver tissue heat capacity \( (c_p = 4 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{K}^{-1}) \), electrical conductivity \( (\sigma = 0.2 \text{ S} \cdot \text{m}^{-1}) \), thermal conductivity \( (k = 0.5 \text{ W} \cdot \text{m}^{-1} \cdot \text{K}^{-1}) \), and density \( (\rho = 1000 \text{ kg} \cdot \text{m}^{-3}) \) used in the models are taken from the literature (Swarup, Stuchly et al. 1991; Deng and Liu 2001; Davalos and Rubinsky 2008). The tissue temperature is assumed to be initially the same as the physiological temperature \( (37 \degree \text{C}) \).

**Temperature Distribution:**

An additional comparison of numerical models explored between the two electrode geometries depicted in Figure 2 may be seen in Figure 4 using the joule heating term to observe changes in temperature. The images represent the electric field and temperature distributions of the two geometries at time \( t = 50 \mu\text{s} \), during the application of a single IRE pulse.

From Figure 4, it can be seen that large volumes of tissue may be treated with IRE-relevant electric fields (A) and (C); maintaining the same shape of distribution observed in Figure 3. Parts (B) and (D) shows the thermal effects and where they are most prevalent, which is at the edges of the energized surfaces. It should be noted that, although the thermal effects have been depicted to help visualize their distribution, the maximum temperature found at the end of the 50 \( \mu\text{s} \) pulse was only 43\( \degree \text{C} \). This temperature is well below the range of thermal lesioning or scarring, typically taken to be 50\( \degree \text{C} \) (Diller 1992). Therefore, this figure, demonstrates the significantly large volumes of tissue that may be treated by IRE without the occurrence of any considerable thermal damage. This is in accord with previously published studies (Davalos, Otten et al. 2002; Davalos, Mir et al. 2005; Edd, Horowitz et al. 2006; Al-Sakere, André et al. 2007; Onik, Mikus et al. 2007). Higher energy applications (multiple pulses or longer pulses) will increase the temperature change. However, for most clinical application purposes, the volume of tissue undergoing thermal damage will typically
never exceed 5% of the volume of tissue ablated by IRE. Further thermal damage assessment is possible by using the Pennes bioheat equation, calculating the equivalent thermal dose from an IRE treatment, or by assessing the thermal effects using the thermal damage equation.

Figure 4. Electric field and temperature distributions for the bipolar (A,B) and monopolar electrodes (C,D). Parts A and C depict the electric field for IRE-relevant ranges from 650 to 1000 V/cm while B and D show the temperature region from 37°C to 43°C.

Equivalent Thermal Dose:

For procedures involving time varying temperatures, thermal damage can be assessed by calculating the amount of time it would take to equivalently damage the tissue as if it was held at a constant temperature, typically 43°C (Sapareto and Dewey 1984; Becker and Kuznetsov 2006; Al-Sakere, André et al. 2007; Becker and Kuznetsov 2007; Edd and Davalos 2007; Davalos and Rubinsky 2008). The following expression is the duration necessary to hold the tissue at 43°C to result in an equivalent thermal dose:

\[
T_{43} = \sum_{t=0}^{t_{\text{final}}} R^{(43-T_t)} \Delta t
\]

where \( T_t \) is the average temperature during \( \Delta t \) with \( R = 0.25 \) when \( T_t \leq 43°C \) and \( R = 0.5 \) when \( T_t > 43°C \) (Sapareto and Dewey 1984; Damianou, Hynynen et al. 1993).

Figure 5 shows the equivalent thermal dose curves for the two monopolar electrode configuration described in Case B of Figure 2. The thermal dose was calculated for an eighty pulse (50 μs pulse length) IRE treatment at a frequency of 1 pulse per second using 1500, 2000 and 2500 V as the input voltage. Thermal doses were calculated along the electrode-
electrode axis extending 10 mm to the left and right from the middle of the electrodes, as shown at the bottom of Figure 5. The highest thermal doses occurred at the electrode tissue interface because these locations experienced the maximum electric fields. However, at 2 mm from the interface the thermal dose decreases by a factor of 10 due to heat diffusion to areas of lower temperature. Increasing the voltage results in more joule heating, as described in Equation 5. The 500 V intervals examined exhibited thermal doses that increased by roughly an order of magnitude each. Nevertheless, the thermal doses remained well below a typical thermal damage threshold of $t_{43} = 120$ min that has been found for common soft tissues (Damianou, Hynynen et al. 1993; Ho, Ju et al. 2007). This shows that the temperature increase generated by the entire IRE procedure is not responsible for the tissue death.

**Figure 5.** Thermal dose curve along the electrode-electrode axis for eighty (50 µs) pulses at a frequency of 1 Hz for 1500, 2000 and 2500 V.

**Thermal Damage Equation:**

An additional assessment of thermal effects is thermal damage. Since thermal damage is a function of temperature and duration at elevated temperatures, the negligible heating associated with these case studies is emphasized by the fact that an electroporation pulse is typically a very small fraction of a second long (Al-Sakere, André et al. 2007; Becker and Kuznetsov 2007; Edd and Davalos 2007). One of the distinguishing features of IRE is that it does not induce thermal damage (Tropea and Lee 1992; Davalos, Mir et al. 2005; Lee and Despa 2005). The thermal damage can be calculated to assess whether a particular set of
voltage parameters will induce thermal effects in addition to IRE. Thermal damage occurs when cells or tissues are exposed to a temperature higher than the physiological temperature for an extended period of time. If the period of exposure is long, thermal damage can occur at temperatures as low as 42°C. However, 50°C is generally chosen as the target temperature (Diller 1992). Thermal damage, $\Omega$, is quantified by the Arrhenius type equation:

$$\Omega = \int \zeta \cdot e^{-E_a/RT} \, dt$$  \hspace{1cm} (9)

where $\zeta$ is the frequency factor, $E_a$ is the activation energy, $R$ is the universal gas constant, and $T$ is the temperature (Henriques and Moritz 1947; Diller 1992; Rylander, Feng et al. 2005; Feng, Tinsley Oden et al. 2008).

**SPECIAL CONSIDERATIONS**

**Heterogeneous Tissue:**

In an IRE treatment, if the conductivity distribution in the targeted region is homogenous, the results in Figure 6 can be applied directly to estimate the size of the treated region as a function of electrode geometry and applied voltage. Exposing the entire tissue to the electric field magnitude necessary to achieve cell death is important. However, there can be factors that would make the targeted domain heterogeneous, such as the presence of large blood vessels, multiple tissue types, or tissues with anisotropic properties, such as muscle. Three electric field contour levels (500, 750 and 1000 V/cm) are used to illustrate the effects of heterogeneity in the electric field distribution. Under these circumstances, the reader would need to understand these guidelines when making their own model to match their specific procedure. Figures 6 - 8 shows the tissue treated with a 2000 V (50 µs pulse length) IRE pulse using the same dimensions as Case B in Figure 2. It is important to note that different tissues may have different electric field thresholds to cause IRE. Figure 7 shows the electric field distribution for an IRE procedure in which the electrical conductivity of the surrounding tissue ($\sigma = 0.1$ S·m$^{-1}$) is half the magnitude of the treated tissue ($\sigma = 0.2$ S·m$^{-1}$). The same electric field contour levels (500, 750 and 1000 V/cm) that were used in the homogeneous tissue discussion were used in this analysis. Having two different electrical conductivities affects the electric field distribution, so knowledge of the physical properties of the tissue is important for more accurate predictions. The treated area by IRE is increased when the surrounding tissue to the region of interest has a smaller electrical conductivity. For example, an electric field of 750 V/cm covers the entire region of interest but was not sufficient to treat the homogeneous tissue. This scenario can occur in mammary tumors in which the fat surrounding the tissue has lower electrical conductivity than the tumor itself.
Figure 6. Electric field [V/cm] distribution using a 2000 V (50 µs) IRE pulse in homogeneous tissue ($\sigma_{\text{target}} = \sigma_{\text{surrounding}}$). The black line outlines the area to be treated with IRE.

Figure 7. Electric field [V/cm] distribution using a 2000 V (50 µs) IRE pulse in heterogeneous tissue. The black line outlines the area to be treated with IRE ($0.5 \cdot \sigma_{\text{target}} = \sigma_{\text{surrounding}}$).

Figure 8 shows the electric field distribution for an IRE procedure in which the electrical conductivity of the surrounding tissue ($\sigma = 0.4 \text{ S·m}^{-1}$) is twice the magnitude of the treated
tissue ($\sigma = 0.2 \text{ S}\cdot\text{m}^{-1}$). The same electric field contour levels (500, 750 and 1000 V/cm) that were used in the two previous examples were used in this analysis. The treated area by IRE is reduced when the surrounding tissue to the region of interest has a larger electrical conductivity. For example, an electric field of 500 V/cm is now required to cover the entire region of interest which is lower than was needed to treat the homogeneous tissue. These results are to give insight to the reader about the influence of the electrical conductivity in the electric field distribution in heterogeneous tissue. For more information on these effects, other studies of electric fields on heterogeneous tissue can be found in (Miklavcic, Beravs et al. 1998; Edd and Davalos 2007; Esser, Smith et al. 2007).

Figure 8. Electric field [V/cm] distribution using a 2000 V (50 µs) IRE pulse in heterogeneous tissue. The black line outlines the area to be treated with IRE ($2 \cdot \sigma_{\text{target}} = \sigma_{\text{surrounding}}$).

**Dynamic Properties During Electroporation:**

Researchers have shown that there is a change in tissue conductivity during and after pulsing, as a result of electroporation (Bhatt, Gaylor et al. 1990; Davalos, Otten et al. 2002; Pavlin and Miklavcic 2003; Davalos, Otten et al. 2004; Miklavcic, Sel et al. 2004). The electrical conductivity of tissue during electroporation increases as a result of electroporation (Lee, Zhang et al. 2000; Pavlin and Miklavcic 2003; Davalos, Otten et al. 2004; Miklavcic, Sel et al. 2004). These changes can be readily incorporated into the reader’s numerical models (Davalos, Otten et al. 2002; Davalos, Otten et al. 2004). Since the conductivity
changes during IRE, it provides an active means for the physician to monitor the procedure by measuring the change in current. This also allows imaging of the irreversibly electroporated tissue with electrical impedance tomography to verify treatment success (Davalos, Otten et al. 2002; Davalos, Otten et al. 2004; Lee, Loh et al. 2007).

**Temperature Dependent Properties:**

If it is necessary to take into consideration the thermal effects from a treatment, then other tissue properties such as the mass density, heat capacity and thermal conductivity are needed. If these properties cannot be directly measured, the properties of the tissue can be taken from the literature, for example from (Duck 1990). It should be noted that the thermal and electrical conductivities of biological tissues are dependent on temperature and their dependence can be found in literature and incorporated into the models if necessary. Since IRE produces negligible heating, the change in conductivity is not usually significant. For example, thermal and electrical conductivities increase by about 0.25% and 1.5% per degree Celsius rise in temperature, respectively, in liver (Duck 1990).

**CONCLUSION**

This chapter introduced the field of non-thermal IRE and its potential to ablate large tissue volumes. The advantages of IRE over other focal ablation techniques lay within its ability to ablate tissue through a non-thermal mechanism. This method preserves the extracellular matrix, nerves, major blood vessels, and other sensitive tissues, enhancing treatment outcome. Furthermore, the ablation area can be predicted using numerical modeling for accurate treatment planning, and application of the procedure can be monitored in real-time using ultrasound. This ablation of the targeted areas exhibits rapid lesion creation and resolution, prompting the repopulation of the region with healthy cells. Though treatment success is not dependent upon the immune system, a tumor specific immune response capable of helping to destroy any residual micro-metastases occurs, decreasing the chances of recurrence. These aspects, in conjunction with short treatment times and the minimally invasive nature of treatment administration, show strong potential for using IRE as an effective tissue ablation modality for the improved treatment of many localized tissue pathologies. This chapter then focused on applying the basic principles involved in IRE therapies to facilitate accurate treatment planning for clinical therapies.

**REFERENCES**


