A Preliminary Study to Delineate Irreversible Electroporation From Thermal Damage Using the Arrhenius Equation

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Intense but short electrical fields can increase the permeability of the cell membrane in a process referred to as electroporation. Reversible electroporation has become an important tool in biotechnology and medicine. The various applications of reversible electroporation require cells to survive the procedure, and therefore the occurrence of irreversible electroporation (IRE), following which cells die, is obviously undesirable. However, for the past few years, IRE has begun to emerge as an important minimally invasive nonthermal ablation technique in its own right as a method to treat tumors and arrhythmogenic regions in the heart. IRE had been studied primarily to define the upper limit of electrical parameters that induce reversible electroporation. Thus, the delineation of IRE from thermal damage due to Joule heating has not been thoroughly investigated. The goal of this study was to express the upper bound of IRE (onset of thermal damage) theoretically as a function of physical properties and electrical pulse parameters. Electrical pulses were applied to THP-1 human monocyte cells, and the percentage of irreversibly electroporated (dead) cells in the sample was quantified. We also determined the upper bound of IRE (onset of thermal damage) through a theoretical calculation that takes into account the physical properties of the sample and the electric pulse characteristics. Our experimental results were achieved below the theoretical curve for the onset of thermal damage. These results confirm that the region to induce IRE without thermal damage is substantial. We believe that our new theoretical analysis will allow researchers to optimize IRE parameters without inducing deleterious thermal effects.
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1 Introduction

The cell membrane is essentially a nonconductive barrier that protects the inside of the cell from the outside, controlling transport into and out of the cell through ion channels and the membrane's resting potential. Electroporation is a molecular tool and an electromechanical method used to introduce polar molecules into a cell through the cell membrane via intense and short electrical fields [1]. For the past 3 decades, when the field of electroporation has become dominated by reversible electroporation applications, irreversible electroporation (IRE) was viewed as an undesirable side effect. However, for the past few years IRE has begun to emerge as an important minimally invasive nonthermal ablation technique in its own right for tumors and arrhythmogenic regions in the heart [2–9]. IRE's ability to create complete and predictable cell ablation with sharp transition between normal and necrotic tissues, while sparing neighboring blood vessels and connective tissue, is of great advantage in numerous medical applications.

IRE had been studied primarily only to define the upper limit of electrical parameters that induces reversible electroporation. Thus, the line that delineates IRE from conventional thermal damage is poorly defined. There have been experimental studies on viability of cells following electric pulses. Some of these proposed that empirical relationships connect the probability of cell death to pulse strength, duration, or number of pulses [10–12]. There is a great degree of disagreement since some studies find correlation between cell death and the total energy delivered by the pulse [11,13], while others do not [14,15], and yet others correlate cell death with the total charge [12]. The mechanism of cell death is still not well understood but it has been postulated that it is via necrosis in IRE presumably due to loss of homeostasis as opposed to supraporation, which is via apoptosis [14]. Martin et al. and Tropea and Lee [16,17] extensively investigated the thermal effects that may be induced by high-voltage electroporation pulses as a mechanism of cell death during electroporation. Tropea and Lee [17] investigated tissue damage in high-voltage electrical trauma and estimated the extent of heat mediated cellular injury using the Arrhenius equation. Three possible mechanisms for tissue damage in skin were proposed by Martin et al. [16]. The damage results from long exposure time to temperatures exceeding a threshold or a phase change in the stratum corneum (SC) lipids or water [16].

In order to aid planning IRE treatments, several theoretical studies have focused on computing electrical and thermal conditions in the treated tissue [18–21]. Given the electrode configuration and tissue properties, the models predict the electric field in the tissue, pulse-induced transmembrane potential, and rise in temperature. Typically, a threshold value of E or V_m has to be assumed based on experimental measurements to estimate the extent of tissue destroyed by the pulse [2]. In this study, we achieved IRE on THP-1 human monocyte cells experimentally and quantified the percentage of dead cells in the sample. We adapted the heat transfer transient conduction solution for a finite slab with convection boundary conditions to determine the thermal damage due to Joule heating [26]. Assuming that the convection boundary condition is equivalent to an infinite fin, this model is analogous to the configuration in our study in which heat is dissipated by the electrodes. Our experimental results were accomplished below this theoretical curve, confirming that the IRE region is substantial and that the energy delivered by the electric pulses is not sufficient to generate thermal damage. We believe that our new theoretical analysis will allow researchers to optimize IRE parameters without inducing thermal damage.

2 Materials and Methods

2.1 Experimental. To detect IRE, we conducted in vitro studies on THP-1 human monocyte cells ($\sim 1.5 \times 10^6$ cells/ml) suspended in 1X Dulbecco's phosphate buffered saline (PBS) solu-

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tion (Teknova, Hollister, CA). Samples of 200 μl were placed into cuvettes with embedded electrodes separated by 2 mm (Model 620, Harvard Apparatus, Holliston, MA), which are compatible with a housing system for electroporation (Model 630B Safety Stand, BTX Harvard Apparatus, Holliston, MA). Pulses were delivered by a BTX electroporator (ECM 830, BTX Harvard Apparatus, Holliston, MA) with electric fields between 1000 V/cm and 7500 V/cm and several pulse durations (Table 2). The percentage of dead cells was determined using 0.4% Trypan Blue (Sigma-Aldrich, St. Louis, MO), which labels membrane compromised cells blue.

After IRE, the 200 μl sample was removed from the cuvette and mixed at a 1:1 ratio with Trypan Blue. Cells were labeled 5 min after the delivery of the electric pulse to ensure that the cells were not stained as a result of reversible electroporation since it has been postulated in Refs. [27,28] that the pore resealing time is in the order of seconds. To quantify cell death we used 10 μl of the stained solution placed on a hemacytometer (Bright-Line, Hausser Scientific, Horsham, PA) under an inverted light microscope (Leica DMI 6000B, Leica Microsystems, Bannockburn, IL) equipped with a color camera (Leica DFC 420).

2.2 Analytical Model. The temperature of the cell solution increases due to Joule heating during electroporation. The heat transfer solution to transient conduction in a finite slab was used to determine the temperature profile versus position and time. This model is similar to the electrode geometry used in our study in which the electrodes serve as infinite fins that dissipate the heat away from the cell solution. The exact solution is given as a Fourier series in Refs. [24,26],

$$\frac{T-T_0}{T_i-T_0}=\sum_{i=1}^{\infty} C_i e^{-\beta_i^2 \alpha t/L^2} \cos\left(\frac{\beta_i x}{L}\right) \tag{1}$$

where T is the temperature at time t, T_i is the initial temperature, T_0 is the room temperature, α is the thermal diffusivity, L is the half thickness of the slab, and x is the distance from the centerline of the slab. C_i is calculated from

$$C_i=\frac{4\ \sin\beta_i}{2\beta_i+\sin(2\beta_i)} \tag{2}$$

and the constants β_i are calculated from the following equation:

$$\beta_i \tan(\beta_i)=h_0 L/k \tag{3}$$

where k is the thermal conductivity of the buffer and h_0 is the equivalent heat transfer coefficient assuming that the stainless steel electrodes act as infinite fins in free convection as previously described in Ref. [22]. To determine the temperature we used the first term of the Fourier series in the exact solution which is accurate within 1% since the dimensionless time $t^*=\alpha t/L^2$ is greater than 0.2 under our conditions [24,29]. We also assumed a homogeneous temperature throughout the domain equal to the temperature at the centerline of the sample to be conservative ($x=0$). Thus the exact solution (Eq. (1)) can be rewritten as

$$\frac{T-T_0}{T_i-T_0}=C_1 e^{-\beta_1^2 \alpha t/L^2} \tag{4}$$

The temperature rise due to Joule heating, $\Delta T=T_i-T_0$, of cell solutions or homogeneous tissue for the parallel plate electrode configuration is given in Ref. [12],

$$\Delta T=\frac{\sigma\cdot E^2}{\rho\cdot c_p}\cdot t_p \tag{5}$$

where $\sigma\cdot E^2$ is the heat generated per unit volume, E is the applied electric field, t_p is the pulse length, and σ, ρ, and c_p are the electrical conductivity, density, and heat capacity of the buffer, respectively [23] (Table 1). The value for the electrical conductivity was measured experimentally using a SevenGo pro conductivity meter (SevenGo pro, Mettler Toledo, Switzerland). The values for the physical properties of the cell solution (PBS) are taken from the literature [23] and the activation energy and the frequency factor in Eq. (6) from Ref. [25].

Table 1 Physical properties used in the analysis of IRE and thermal damage of THP-1 cells suspended in PBS solution and tissue

Property	Symbol	Value	Units	Ref.
Electrical conductivity	σ_{PBS}	1.4	S m^{-1}	–
	σ_{tissue}	0.2	S m^{-1}	[22]
Density	ρ	1000	kg m^{-3}	[23]
Heat capacity	c_p	4200	J kg^{-1} K^{-1}	[23]
Thermal diffusivity	α	1.34×10^{-7}	m^2/s	[24]
Frequency factor	ζ	1.19×10^{35}	s^{-1}	[25]
Activation energy	E_a	2.318×10^{5}	J mol^{-1}	[25]
Universal gas constant	R	8.314	J K^{-1} mol^{-1}	–

Thermal damage occurs when cells or tissues are exposed to a temperature higher than physiological temperature for an extended period of time. Thermal damage, Ω, is described and quantified by the Arrhenius rate equation proposed by Henriques [16,17,30] as follows:

$$\Omega=\int \zeta\cdot e^{-E_a/RT}dt \tag{6}$$

where ζ is the frequency factor, E_a is the activation energy, R is the universal gas constant, and T is the absolute temperature in kelvins. By substituting Eqs. (4) and (5) into Eq. (6), the thermal damage for a cell solution between two parallel plate electrodes can be estimated as

$$\Omega(t',t_p,E)=\int_0^{t'}\Psi(t_p,E)dt=\int_0^{t'}\zeta\exp\left(-\frac{E_a}{R\left[\frac{\sigma\cdot E^2}{\rho\cdot c_p}\cdot t_p C_1\exp\left(-\frac{\beta_1^2\alpha t}{L^2}\right)+T_0\right]}\right)dt \tag{7}$$

where t' is the heat dissipation time after electroporation. The heat dissipation is dependent on the physical properties of the cell solution, cell type, pulse parameters, and electrode configuration.

It has been shown that $\Omega=0.53$ is the threshold for burn injuries in blood-perfused skin [31,32]. We have adapted the Arrhenius equation, which traditionally has been used to study burn injuries in skin and transdermal drug delivery using electroporation, to investigate therapeutic IRE. The onset of thermal damage ($\Omega=0.53$) [31,32] is shown in Fig. 1 as a function of the electric field strength, E, and pulse duration, t_p, in cell solutions and tissue. The plots were developed using WOLFRAM MATHEMATICA 6.0 for students (Champaign, IL). The effect of the electrical conductivity of PBS and tissue on the onset of thermal damage is investigated and shown in Fig. 1. The percentage of cell death, K, due to IRE is also shown in Fig. 1 and is defined as

$$K=\left(1-\frac{c(t)}{c(t_0)}\right)\cdot 100\% \quad 0\le K\le 100 \tag{8}$$

where $c(t)$ is the concentration of viable cells at time t and $c(t_0)$ is the concentration of the control. In this definition a value of $K=0$ represents 0% cell death and $K=100$ represents 100% cell death.

3 Results and Discussion

Figure 1 shows the onset of thermal damage curve because of Joule heating effect during and after electroporation for THP-1

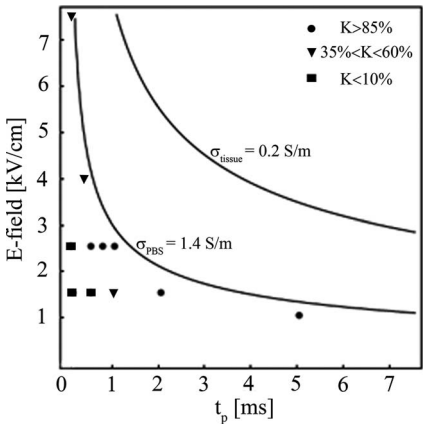

Fig. 1 Theoretical onset of thermal damage curve, $\Omega=0.53$ (upper limit of IRE) as a function of the IRE electrical pulse parameters (magnitude and duration) for cells suspended in PBS and tissue. The pulse parameters for the in vitro experiments were chosen to prevent thermal damage and the corresponding percentage of THP-1 cell death (K) is represented by circle, triangle, and squares.

cells suspended in PBS as well as tissue. These curves indicate that cells suspended in media or PBS are more susceptible to thermal damage compared with tissue. This occurs due to less Joule heating produced during electroporation in tissue with lower electrical conductivity in comparison with PBS. Thus, the IRE pulse parameters used for cells suspended in PBS are conservative when applied in tissue. These curves of thermal damage onset might be the upper limit boundary of a nonthermal IRE single pulse treatment. We conducted several experiments with the pulse parameters from the region below the PBS (cell solution) curve, (experimental data in Fig. 1) within our equipment capabilities in order to investigate the extent of cell death through IRE in this region. The percentages of cell death with the corresponding standard deviations are given in Table 2. These results show that by choosing the appropriate pulse parameters, one can achieve significant cell death by nonthermal IRE. In four of the pulse parameters investigated, we achieved at least 88% cell death without exceeding the thermal damage threshold.

These results are based on a highly conductive buffer solution (PBS). We can infer that the electrical conductivity of the cell solution or tissue has great influence on the onset of thermal damage. For the same experimental conditions, buffers with lower electrical conductivities would generate lower Joule heating in the sample. Consequently, IRE pulses with higher electric fields or longer durations could be still used prior to the onset of thermal damage. Future studies will incorporate the activation energies and frequency factors for the entire range of cell types into our new theoretical expression in order to predict the upper limit of IRE.

Table 2 Percentage of dead cells after one single IRE pulse for several electric field strengths and pulse durations with the corresponding standard deviations ($n=3$)

E-field (V/cm)	Pulse duration (ms)	$K=(1-c(t)/c(t_0))$ (%)	Stand. dev. (%)
1000	5.00	93.0	2.0
1500	0.10	4.3	0.6
1500	0.50	6.4	1.5
1500	1.00	34.8	2.2
1500	2.00	91.8	4.1
2500	0.10	10.0	2.3
2500	0.50	81.9	10.8
2500	0.75	88.7	4.3
2500	1.00	88.4	2.4
4000	0.35	53.4	8.5
7500	0.10	49.9	23.2

Figure 2 shows the temperature profile and the function $\Psi(t_p,E)$ for the cases in which minimum ($\Omega=0.53$) and third-degree burns ($\Omega=10{,}000$) due to Joule heating occur. The temperature of the cell solution returns to room temperature independent of the initial temperature within 30 s. The function $\Psi(t_p,E)$ returns to zero within this time frame as well due to the effect of heat dissipation through the electrodes. Therefore, we used a time, $t'=30$ s, to evaluate thermal damage integral in Eq. (7).

The thermal damage delineation from the potential IRE region for cells suspended in PBS (Fig. 3(*a*)) and tissue (Fig. 3(*b*)) is given as a function of the electrical pulse parameters (magnitude and duration). These plots can be used to select appropriate pulse parameters for nonthermal IRE treatments using parallel plate electrodes. The potential IRE regions for cells suspended in PBS are narrower than that for tissue due to its higher electrical conductivity since more Joule heating is generated for the same pulse parameters. In tissue, blood perfusion and a larger volume are other factors that help dissipate the heat after IRE. The boundary between reversible electroporation and no effects on the cell membrane has been investigated and is not included in this study [33,34].

This analytical model provides preliminary tools for researchers to model multiple pulses and assess the thermal damage. The

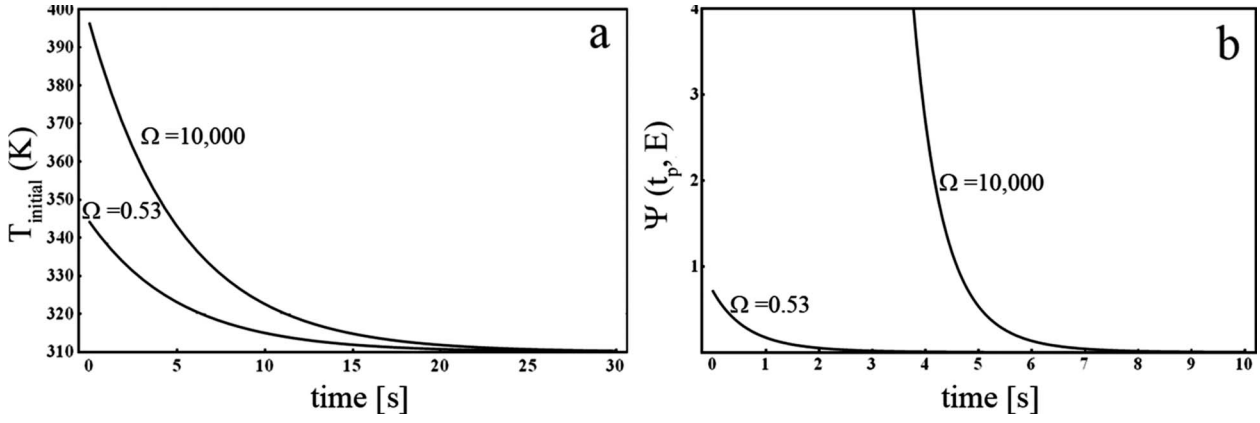

Fig. 2 (*a*) Temperature profile and (*b*) the function $\Psi(t_p,E)$ versus time for $\Omega=0.53$ (onset of thermal damage) and $\Omega=10{,}000$ (third-degree burn injury) [31,32]. The thermal damage magnitude, Ω, is the area under the $\Psi(t_p,E)$ curve (Eq. (7)).

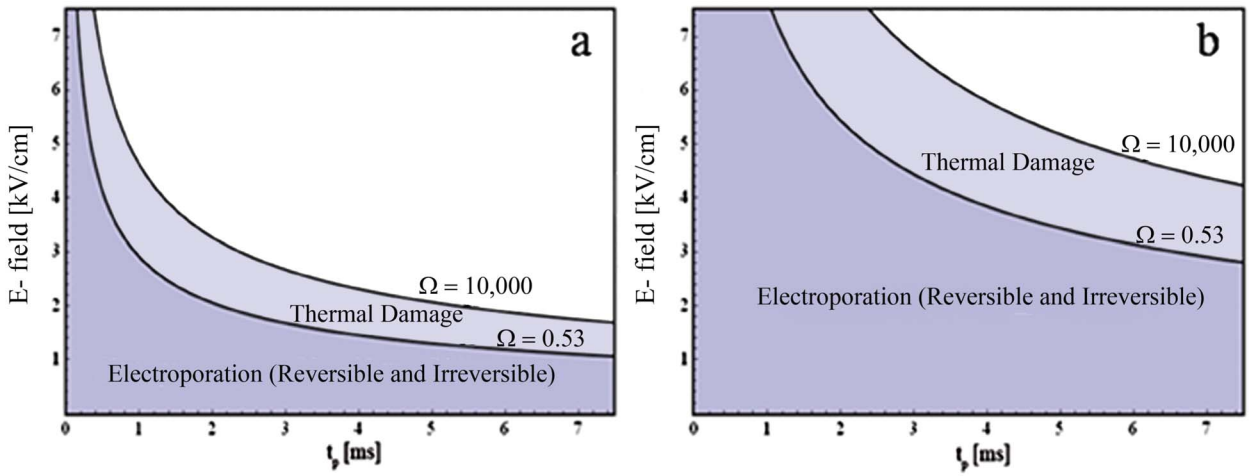

Fig. 3 Thermal damage delineation from the area of potential irreversible electroporation as a function of the IRE electrical pulse parameters (magnitude and duration) for (*a*) cells suspended in PBS and (*b*) tissue. Ω=0.53 represents the onset of thermal damage and Ω=10,000 represents a third-degree burn injury in skin [31,32]. Note that the onset of electroporation is not depicted.

number and frequency of the pulses are some other factors that need to be considered in subsequent studies. The proposed single IRE pulse analytical model might be used for multiple IRE studies considering the heat dissipation time after each pulse.

The research on IRE presents a new challenge to scientists studying biothermal fields. The study of how electrical fields can be applied to living tissue to induce IRE damage to the cell membrane without causing any thermal effects is an emerging area of research. The goal of this study was to express the upper bound of IRE (onset of thermal damage) theoretically as a function of physical properties of the cell solution and the pulse parameters. We believe that our new theoretical analysis will allow researchers to optimize IRE parameters without inducing thermal damage.

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